ICOPA XVI Montréal 2026

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ICOPA 2026 Abstracts Book



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Invited Speaker Abstracts

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IS001

KEYNOTE
THE RISE OF PARASITIC ILLNESS IN THE CLIMATE CRISIS AND NEW APPROACHES TO THEIR CONTROL
08-16-2026 6:00 PM - 7:00 PM

THE RISE OF PARASITIC ILLNESS IN THE CLIMATE CRISIS AND NEW APPROACHES TO THEIR CONTROL

Peter Hotez
Baylor College of Medicine, Pediatrics, National School Of Tropical Medicine, Houston, United States of America

Historically, Texas and the Southern U.S., Europe, and other countries in the Northern Hemisphere suffered from high rates of parasitic infections and other neglected tropical diseases. Now, because of modern 21st century forces such as climate change, deforestation, and urbanization, we are seeing the return of these illnesses. This is especially apparent in some temperate climates or even high-income countries, including Texas and the Gulf Coast and Southern Europe. Thermotolerant hookworm larvae in the soil could ensure that hookworm anemia becomes a dominant helminthiasis in Africa, whereas schistosomiasis could undergo paradoxical declines in East Africa but increases at higher elevations. In response, a climate health program is underway at the NSTM focused on the metagenomics analysis of arthropod vectors to reveal unexpected pathogens. In addition, a program of developing new vaccines for parasitic diseases is in progress. In a controlled human infection model (CHIM), a recombinant protein Na-GST-1 vaccine on aluminum with a CpG oligonucleotide adjuvant resulted in high levels of protective immunity and is being accelerated as one of the first human helminth vaccines. Similarly, new vaccines are under development for Chagas disease, schistosomiasis, and tick-borne illnesses using both protein and mRNA platforms. Because of the complexities of adding new stand-alone vaccines to pediatric immunization schedules, efforts are underway to examine how these vaccines could be co-administered with other interventions. There is also urgency to understand how to best advocate for vaccines in a complex health policy framework that considers MDA, cutbacks to USAID, and rising anti-science activism.

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IS002

PLENARY SESSION
UNTANGLING ZOONOTIC SCHISTOSOMIASIS IN A CHANGING WORLD
08-17-2026 8:30 AM - 9:30 AM

UNTANGLING ZOONOTIC SCHISTOSOMIASIS IN A CHANGING WORLD

Joanne Webster
Royal Veterinary College, University of London, Pathobiology And Population Sciences, London, United Kingdom

Zoonoses, diseases transmitted between animals and humans, pose a critical global health threat. Increased migration, changing agricultural practices and climate, all enhance the spread of infectious agents among humans and animals, facilitating co-infection and genetic exchange, and on occasion, even creating new genotypes. Schistosomiasis is a major Neglected Tropical Disease, with over 250 million people, and untold billions of animals, currently infected. Despite over two decades of human mass drug administration with praziquantel, and new WHO targets aiming towards elimination as a public health problem by 2030, schistosomiasis remains prevalent in certain regions and even emerging or re-emerging in others. Whilst animal hosts, particularly livestock, have long been acknowledged as zoonotic reservoirs for *Schistosoma japonicum* across Asia, elsewhere any zoonotic component of schistosomiasis transmission and its implications for disease control has, until recently, been largely ignored. This is true of both *S. mansoni*, but also notably, *S. haematobium*, which was assumed to be an exclusively human infection – and thus amenable to elimination by targeting treatment of humans alone. Here I will present some of the research from my group examining the role of animals, both wildlife and domestic, on potential zoonotic transmission and ultimately schistosomiasis persistence. This work raises profound epidemiological and evolutionary implications regarding the One Health control strategies needed, as well as for pathogen transmission dynamics in general in our rapidly changing world.

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IS003

TOPIC 4: HOST IMMUNITY, MOLECULAR INTERACTIONS, AND MICROBIOME
HELMINTH REGULATION OF INFLAMMATORY DISEASES
08-17-2026 10:00 AM - 12:00 PM

INTERACTION OF ASCARIS WITH GUT MICROBES, BACTERIAL COINFECTION AND HOST IMMUNE RESPONSES.

Susanne Hartmann
Freie Universität Berlin, School Of Veterinary Medicine Insitute Of Immunology, Berlin, Germany

Soil-transmitted helminths (STH) are among the most common infections worldwide, affecting humans, animals and the environment alike. Within STH infections, *Ascaris* is the most abundant, with approximately 800 million people being globally infected with *Ascaris lumbricoides*. *Ascaris suum* in pigs and *Ascaridia galli* in chickens are highly prevalent in our important farm animals. We study *A. suum* infections in their natural host, the pig. These pathogens reside in their host's gut, living in close contact with the host gut microbiota and harbouring bacteria within their own intestines. Questions such as whether and how *Ascaris* modulates the host gut microbiome and what the nematode microbiome is like and where it is derived from will be discussed. Furthermore, coinfections represent the natural situation such as *Ascaris* and non-typhoid *Salmonella* spp. infections are highly prevalent in low- and middle-income countries. Both pathogens are also extremely widespread in commercial pig farming, posing a serious zoonotic threat to human health. Information on the consequences of an acute *A. suum* infection for the control of a Salmonella Typhimurium challenge in pigs will be discussed. The talk will illustrate the impact on innate cells, such as macrophages and natural killer cells, and their modulated effector functions, as well as antibody responses. Finally, a new graduate school funded by the German Research Foundation on the topic of "One Health Approach to Soil-Transmitted Helminths" will be presented.

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IS004

TOPIC 4: HOST IMMUNITY, MOLECULAR INTERACTIONS, AND MICROBIOME
HELMINTH REGULATION OF INFLAMMATORY DISEASES
08-17-2026 10:00 AM - 12:00 PM

CHRONIC INFLAMMATION INDUCED BY ASCARIS SUUM SHAPES THE TUMOR MICROENVIRONMENT IN BREAST CANCER

Lilian Bueno
Federal University of Minas Gerais, Parasitology, Belo Horiozonte, Brazil

Helminth infections profoundly modulate host immunity, but their effects on cancer progression remain controversial and highly dependent on the inflammatory context. Here, we investigated how distinct infectious doses of Ascaris suum affect pulmonary metastasis in the 4T1 breast cancer model. While our previous study showed that low-dose infection (250 eggs) accelerated tumor progression, high-dose infection (2500 embryonated eggs) markedly reduced tumor growth, metastatic dissemination, and pulmonary tissue damage. High-dose infection induced intense leukocyte recruitment, particularly eosinophils, which emerged as essential mediators of tumor control. Experiments using GATA1-deficient mice demonstrated that eosinophil absence abolished the protective phenotype, increased mortality, and maintained elevated TGF-β levels, indicating that eosinophils are critical for limiting immune-suppressive pathways. Unlike low-dose infection, in which tumor cells encountered a pre-established Th2/regulatory environment permissive to tumor growth, high-dose infection generated a rapid inflammatory response that prevented formation of a pro-tumorigenic niche. High-dose infection was associated with increased TNF and IFN-γ, reduced VEGF expression, and the emergence of IL-17-expressing eosinophils, suggesting activation of an eosinophil-driven anti-tumor axis. Myeloid populations also displayed increased CD54 and CX3CR1 expression, markers linked to vascular patrolling and anti-metastatic activity. Collectively, our findings demonstrate that the impact of Ascaris suum infection on cancer progression is strongly dependent on infectious dose and immune kinetics. High-dose infection promotes a rapid eosinophil-dependent inflammatory program that reshapes the pulmonary microenvironment toward tumor suppression and reduced metastasis.

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IS005

TOPIC 4: HOST IMMUNITY, MOLECULAR INTERACTIONS, AND MICROBIOME
HELMINTH REGULATION OF INFLAMMATORY DISEASES
08-17-2026 10:00 AM - 12:00 PM

TAENIA CRASSICEPS: FROM COLITIS AND COLORECTAL CANCER IMPACT TO POTENTIAL BIOTHERAPEUTIC ISOLATION

Luis I Terrazas, Cuauhtemoc Del Angel Sanchez-Barrera, Monica G. Mendoza-Rodriguez, Yadira Ledesma-Soto, Miriam Rodriguez-Sosa
Universidad Nacional Autonoma de Mexico (UNAM), Unidad De Investigación En Biomedicina, Tlalnepantla, Mexico

The immunomodulatory capacity of helminth parasites to suppress inflammatory responses has prompted investigations into their therapeutic potential in inflammatory-mediated diseases. *Taenia crassiceps* in its larval form has demonstrated significant immunomodulatory effects in several experimental autoimmune and inflammatory diseases. A prior infection improved the outcomes of these diseases. Given the impracticality of using live parasite infections as therapeutic interventions, we aimed to determine whether administering *T. crassiceps*-excreted/secreted molecules (TcES) could mitigate experimental colitis and colorectal cancer (CRC). Using >50KD TcES after the onset of colitis, we found reduced oxidative stress and protein damage in the colon, along with increased formation of adherens junctions and enhanced E-cadherin and β-catenin expression, which are crucial for maintaining epithelial barrier function and intestinal homeostasis. As exacerbated colon inflammation is associated with the onset and development of CRC, we tested the impact of TcES on CRC. After the colon tumors had established, treatment with TcES alone or in combination with 5-fluorouracil (5FU) was administered. While single TcES significantly reduced tumor load, TcES+5FU therapy yielded better results in reducing colon tumor burden by modulating the P53 and P21 pathways, confirmed by *in vitro* assays. During host-helminth interactions, the parasite acquires host proteins. TcES displayed abundant mouse IgGs. Following IgGs removal and fractionation of TcES into neutral and cationic components, the latter demonstrated the most potent anticancer properties, with strong cytostatic effects by downregulating Cyclin D1 and Ki67, inducing cell-cycle arrest, and effectively disrupting the normal kinetics of the RAF-ERK-MAPK signaling cascade, halting cancer cell proliferation. PAPIIT-DGAPA-UNAM IV200425-IN207526.

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IS006

TOPIC 4: HOST IMMUNITY, MOLECULAR INTERACTIONS, AND MICROBIOME
HELMINTH REGULATION OF INFLAMMATORY DISEASES
08-17-2026 10:00 AM - 12:00 PM

DISSECTING HOST AND PARASITE CONTRIBUTIONS TO DISEASE REGULATION IN HELIGMOSOMOIDES BAKERI INFECTION

Constance Finney
University of Calgary, Biological Sciences, Calgary, Canada

Helminths are increasingly recognized as potent regulators of inflammatory disease, with their effects emerging from a dynamic interplay between host immunity and parasite-driven modulation. Using the murine nematode *Heligmosomoides bakeri*, we investigate how these interactions shape intestinal inflammation across infection contexts. Our work reveals that host responses to infection are not inherently protective, but instead generate a continuum from resistance to pathology. In chronic and repeated ("trickle") infections, sustained type 2 immune responses reduce parasite burden but drive intestinal inflammation, remodelling, and scarring, highlighting a fundamental trade-off between parasite control and tissue integrity. In contrast, co-infection with the inflammatory intestinal parasite *Toxoplasma gondii* disrupts this balance, resulting in impaired granuloma formation, dysregulated immune responses, and increased worm burdens, demonstrating that disease outcomes are highly context-dependent. Critically, these host-driven processes occur alongside active parasite-mediated modulation. *H. bakeri* secretes immunoregulatory molecules that suppress key inflammatory pathways and reshape host immune function, enabling parasite persistence while constraining excessive inflammation. Defining the diversity, timing, and context-dependent expression of these molecules remains an active area of investigation, particularly how they respond to differing inflammatory environments. Together, these findings show that helminth infection outcomes cannot be explained by host or parasite factors in isolation, but instead arise from their integration. This has important implications for helminth-based therapies, emphasizing the need to understand how helminths and their derived molecules differentially influence host immunity under distinct inflammatory conditions, such as homeostasis versus diseases like Crohn's.

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IS007

TOPIC 4: HOST IMMUNITY, MOLECULAR INTERACTIONS, AND MICROBIOME
VACUOLAR PARASITES: THRIVING WITHIN A HOST CELL
08-17-2026 10:00 AM - 12:00 PM

USING CRISPR TO DISSECT TOXOPLASMA'S ARSENAL FOR HOST MANIPULATION

Jeroen Saeij
University of California Davis, Pathology, Microbiology & Immunology, Davis, United States of America

The success of the parasite *Toxoplasma gondii* is largely attributed to its ability to establish a non-fusogenic parasitophorous vacuole. While this sheltered life provides a physical barrier against cytosolic innate immune sensors, the human host has evolved specialized "vacuole seekers" to monitor this niche. Primary among these is the E3 ubiquitin ligase RNF213, which identifies and targets the vacuolar surface to initiate a cell autonomous immune response. To maintain and modify its intracellular environment, the parasite must export an array of effector proteins onto and across the vacuolar membrane. This required secretion creates a molecular signature that host sensors like RNF213 have evolved to recognize. We used a CRISPR screen in human cells stimulated with IFNgamma to identify parasite factors that determine host detection. By comparing parasite fitness in wild type cells versus those lacking RNF213, we identified a critical complex of dense granule proteins and specialized chaperones. These factors are important for the modification of the vacuolar membrane, but they simultaneously act as the primary triggers for RNF213 recruitment. Our study also identified parasite effectors that have evolved to counteract this host surveillance. These effectors prevent RNF213 recruitment, allowing the parasite to maintain its niche even in the presence of IFNgamma induced seekers. Our findings demonstrate that RNF213 functions as a specialized seeker to detect the activity of parasite effectors. This mechanism represents an example of effector triggered immunity, where the metabolic requirements of niche maintenance provide the very signals used by the host to overcome the parasite's sheltered life.

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IS008

TOPIC 4: HOST IMMUNITY, MOLECULAR INTERACTIONS, AND MICROBIOME
VACUOLAR PARASITES: THRIVING WITHIN A HOST CELL
08-17-2026 10:00 AM - 12:00 PM

A GLOBAL MAP OF APICOMPLEXAN CELLULAR ARCHITECTURE THROUGH IN SITU CROSSLINKING MASS SPECTROMETRY

Sebastian Lourido, Simon Butterworth
Whitehead Institute, Cambridge, United States of America

Protein–protein interactions underpin nearly all cellular processes, yet systematic definition of these networks remains limited outside a small number of model organisms. Most technologies to map protein interactions are low-throughput or poorly suited to capturing interactions in their native cellular context. Despite extensive efforts in select model systems, the composition and architecture of essential protein complexes in many divergent lineages remain poorly characterized. Here we developed a high-coverage crosslinking mass spectrometry (XL-MS) framework to define the proteome-wide interactome of the model apicomplexan parasite *Toxoplasma gondii*. From 29,624 crosslinked peptide pairs, we defined a network of 2,859 protein–protein interactions and integrated these data with structural modelling to resolve interaction interfaces. We identified and validated previously unrecognized components of essential protein complexes, including a structurally distinct ATP synthase subcomplex containing a highly divergent, apicomplexan-specific α subunit that is essential for parasite fitness. Beyond revealing unexpected diversification of core mitochondrial machinery, these findings provide a general strategy to define the molecular architecture of divergent organisms and represent a foundational resource for hypothesis generation, structural inference, and discovery of lineage-specific vulnerabilities in pathogen biology.

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IS009

TOPIC 4: HOST IMMUNITY, MOLECULAR INTERACTIONS, AND MICROBIOME
VACUOLAR PARASITES: THRIVING WITHIN A HOST CELL
08-17-2026 10:00 AM - 12:00 PM

THE LYSOSOME SENSING PATHWAY IS ESSENTIAL FOR THE INTRACELLULAR DEVELOPMENT OF THE HUMAN PATHOGEN LEISHMANIA

Dan Zilberstein[1], Renana Fischer-Weinberger[1], Ramu Dandugudumula[1], Roni Nitzan Koren[1], Peter Myler[2]
[1]Technion-Israel Institute of Technology, Biology, Haifa, Israel, [2]Center for Global Infectious Disease, Seattle, United States of America

Leishmania are intracellular parasitic protozoa that reside and proliferate within the phagolysosomes of mammalian macrophages. During a sand fly's (the vector) blood meal, extracellular metacyclic (virulent) promastigotes are injected subcutaneously into the host. Parasites are then phagocytosed by nearby macrophages. Once inside the phagosome, parasites trigger fusion with primary lysosomes, forming infected phagolysosomes. Within the acidic phagolysosome, promastigotes differentiate into amastigotes, the intracellular form of *Leishmania*. We discovered that invading promastigotes sense the combined high temperature (relative to the vector) and acidic pH (typical of phagolysosomes) and transduce these cues via a lysosome-sensing pathway (LSP) that initiates their differentiation into amastigotes. My talk will provide an update on LSP and the molecular mechanisms underlying their role in the intracellular development of *Leishmania* amastigotes.

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IS010

TOPIC 4: HOST IMMUNITY, MOLECULAR INTERACTIONS, AND MICROBIOME
VACUOLAR PARASITES: THRIVING WITHIN A HOST CELL
08-17-2026 10:00 AM - 12:00 PM

PLASMODIUM LIVER STAGE: SILENTLY MAKING A BANG

Maria Mota
GIMM- Gulbenkian Institute For Molecular Medicine, Group Leader & Ceo, Lisbon, Portugal

"PLASMODIUM LIVER STAGE: SILENTLY MAKING A BANG" Under natural transmission settings, malaria infections commence with the inoculation of hepatotropic sporozoites (spz) by infected Anopheles mosquitoes. Once inside hepatocytes, spz undergo replication, giving rise to thousands of blood-infectious merozoites and initiating the symptomatic phase of the disease. Despite the traditional focus on the blood stage of infection for understanding malaria pathophysiology, recent evidence suggests a critical role for the liver stage (LS) in shaping host responses.
Our research challenges the notion of the LS as immunologically silent, revealing its influence on subsequent blood stage (BS) infection and disease outcomes. By utilizing innovative experimental models decoupling liver and blood stages, we demonstrated that signals from the LS significantly impact the host immune response. Notably, this crosstalk between parasite developmental stages triggers the activation of distinct subsets of γδ T cells, with IL-17-mediated stress erythropoiesis emerging as a key protective mechanism against severe malaria manifestations.
Building upon these findings, our work underscores the importance of considering the LS in malaria research and intervention strategies. By elucidating the intricate interplay between Plasmodium developmental stages and host immunity, we aim to uncover novel therapeutic targets and enhance our understanding of malaria pathogenesis. Through interdisciplinary collaborations and cutting-edge methodologies, we strive to harness the potential of the LS in mitigating the global burden of malaria and advancing towards the ultimate goal of eradication.

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IS011

TOPIC 1: PARASITE ECOLOGY, BIODIVERSITY, AND TAXONOMY
PROTISTS – NEGLECTED MEMBERS OF THE MICROBIOME
08-17-2026 10:00 AM - 12:00 PM

A GUT COMMENSAL PROTOZOAN MODIFIES MULTI-ORGAN DISEASE SEVERITY

Arthur Mortha
University of Toronto, Immunology, Toronto, Canada

The gut microbiota controls a vast variety of physiological processes. Bacteria are at the center of these investigations, leaving other microbial members of the microflora neglected and understudied. The identification that eukaryotic members of the gut microbiota are well equipped to thrive in the intestinal environment and have significant impact on the host's physiology making these microbes an intriguing subject of investigations. The identification of gut commensal protozoa as members of the gut microbiota further increased our understanding of this microbial ecosystem. Here, we will discuss the role and biology of the protozoan microbe *Tritrichomonas musculus* in controlling intestinal and extra-intestinal immune and tissue homeostasis through host-microbe regulation, microbe-microbe interactions and interorgan communication. We will highlight the beneficial and detrimental impacts of these host-protozoa interactions on intestinal and extra-intestinal diseases and reflect on the relevance of these findings for human health.

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IS012

TOPIC 1: PARASITE ECOLOGY, BIODIVERSITY, AND TAXONOMY
PROTISTS – NEGLECTED MEMBERS OF THE MICROBIOME
08-17-2026 10:00 AM - 12:00 PM

THE ROLE OF THE HUMAN GUT MICROBIOME AND BLASTOCYSTIS WITH DIET AND HOST HEALTH

Francesco Asnicar
University of Trento, Department Cibio, Trento, Italy

Diet impacts human health, influencing body adiposity and the risk of developing cardiometabolic diseases. The gut microbiome is a key player in the diet-health axis, but while its bacterial fraction is widely studied, the role of micro-eukaryotes, including Blastocystis, is underexplored. We performed a global-scale analysis on 56,989 metagenomes and showed that human Blastocystis exhibits distinct prevalence patterns linked to geography, lifestyle, and dietary habits. Blastocystis presence defined a specific bacterial signature and was positively associated with more favorable cardiometabolic profiles and negatively with obesity ($p < 1e{-}16$) and disorders linked to altered gut ecology ($p < 1e{-}8$). In a diet intervention study involving 1,124 individuals, improvements in dietary quality were linked to weight loss and increases in Blastocystis prevalence ($p = 0.003$) and abundance ($p < 1e{-}7$). Our findings suggest a potentially beneficial role for Blastocystis, which may help explain personalized host responses to diet and downstream disease etiopathogenesis.

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IS013

TOPIC 1: PARASITE ECOLOGY, BIODIVERSITY, AND TAXONOMY
PROTISTS – NEGLECTED MEMBERS OF THE MICROBIOME
08-17-2026 10:00 AM - 12:00 PM

GIARDIA-MICROBIOME INTERACTIONS IN THE GUT: FROM HEALTH TO DISEASE

André Buret
University of Calgary, Biological Sciences, Calgary, Canada

Giardiasis causes diarrhea, post-infectious irritable bowel syndrome, and growth and cognitive deficits in children from resource-limited settings. Recent findings demonstrate that parasite cysteine proteases lyse the gut mucus barrier and disrupt human microbiota biofilms (1). Mucus glycosylation is disrupted, and infected tissues exhibit increased expression of mucin-modifying enzymes—including mucin glycosyltransferases, sialyltransferases, and fucosyltransferases—and high tissue fucose levels, alterations known to influence the gut microbiota. These glycosylation changes are driven by the microbiota dysbiosis induced by the infection. *Giardia* promotes the release of invasive bacterial pathobionts from commensal microbiota communities (2). Microbiota biofilms disrupted by *Giardia* increase pathogenic pro-inflammatory cytokines in the gut of germ-mice (2). *Giardia* trophozoites release extra-cellular vesicles that directly increase epithelial adhesion and swimming motility in commensal enterobacteria (3). The effects require intact small RNA within the vesicles. Proteomic analyses of the vesicles revealed a high abundance of RNA-binding proteins and RNA chaperones (3), explaining at least in part the resistance of these vesicles as they transit through the gastrointestinal tract. These discoveries have unveiled a sophisticated trans-kingdom dialogue between *Giardia* and the resident microbiota and characterize hitherto unrecognized pathogenic mechanisms in the gut (4). The findings position *Giardia* as a key regulator of gut microbial ecology and barrier function, and as a powerful model system to help design novel therapeutic strategies. References 1. Beatty et al, Int. J. Parasitol. 2017; 2. Fekete et al, Gut Microbes 2024; 3. Siddiq et al, J. Extracell. Biol. 2023; 4.Buret et al, Trends Parasitol. 2026

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IS014

TOPIC 1: PARASITE ECOLOGY, BIODIVERSITY, AND TAXONOMY
PROTISTS – NEGLECTED MEMBERS OF THE MICROBIOME
08-17-2026 10:00 AM - 12:00 PM

TRACING THE PATH FROM INFECTION TO COEXISTENCE

Katerina Jirku
Biology Centre of the Czech Academy of Sciences, Institute Of Parasitology, České Budějovice, Czech Republic

The microbiome era is changing how intestinal protists are interpreted. Organisms historically viewed mainly through the lens of infection, pathogenicity and eradication are increasingly being recognized as common, silent and context-dependent members of host-associated ecosystems. This shift does not negate their clinical relevance, but expands the questions we ask about their biology. *Blastocystis* provides a useful model for this conceptual shift precisely because it resists simple categorization. It is globally widespread, genetically diverse, common in gut-healthy humans, present across a broad range of animal hosts and associated with different microbiome states. At the same time, it has been interpreted as a potential pathogen, a commensal and a marker of gut eubiosis. This ambiguity makes it a valuable model for moving beyond the simple question of whether it is pathogenic or harmless, and toward a more ecological interpretation of intestinal protists. The One Health perspective extends this interpretation beyond the individual host, showing that intestinal protists cross host boundaries, carry hidden diversity and enter microbial networks shaped by contact, environment and exposure. Yet presence is not function, and association is not mechanism. Experimental models are therefore essential for testing whether protists merely reflect the state of the gut ecosystem or actively contribute to it. Within this broader framework, the key question is no longer whether a gut protist is inherently harmful or harmless, but under which ecological, microbial and host conditions its presence becomes harmful, neutral or potentially beneficial.

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IS015

TOPIC 2: TRANSMISSION DYNAMICS, EPIDEMIOLOGY, AND GLOBAL HEALTH
CLINICAL PARASITOLOGY IN A MOLECULAR WORLD
08-17-2026 10:00 AM - 12:00 PM

THE FUTURE OF PARASITIC DISEASE DIAGNOSIS: HARNESSING NEW MOLECULAR INSIGHTS AND AI-BASED ANALYTICS

Kenneth Gavina
Indiana University School of Medicine, Pathology And Laboratory Medicine, Indianapolis, United States of America

Manual microscopy and morphological identification has long served as the diagnostic gold standard in parasitology. However, in the past few decades advances in laboratory techniques have revolutionized how we diagnose parasite infections, particularly with the use of molecular diagnostics. The use of syndromic multiplex real-time PCR panels and next-generation sequencing technologies have greatly enhanced our ability to identify parasitic infections with increased sensitivity and specificity. In this session, attendees will get a high-level overview of current commercial syndromic panels, targeted nucleic-acid amplification tests, and next-generation sequencing methodologies, discuss their advantages and limitations, and gain an understanding of how these modern tools can be operationalized within the clinical laboratory to improve our standard-of-care methodologies for parasitic disease management.

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IS016

TOPIC 2: TRANSMISSION DYNAMICS, EPIDEMIOLOGY, AND GLOBAL HEALTH
CLINICAL PARASITOLOGY IN A MOLECULAR WORLD
08-17-2026 10:00 AM - 12:00 PM

GI PARASITE DETECTION AND ARTIFICIAL INTELLIGENCE...THE FUTURE WAS YESTERDAY!

Marc Couturier, Marc Couturier
MaineHealth/ Tufts University, Nordx Microbiology, Scarborough, United States of America

Artificial intelligence and machine learning algorithms have shown value in various disciplines of pathology, with diagnostic parasitology actually leading the field within microbiology. This session will explore two such advents of AI in clinical parasitology that are in use. Such applications include the trichrome stain and the wet mount examination, each for stool preparations. These technologies are rapidly evolving and allowing for faster, easier, more sensitive detection of gastrointestinal parasites.

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IS017

TOPIC 2: TRANSMISSION DYNAMICS, EPIDEMIOLOGY, AND GLOBAL HEALTH
CLINICAL PARASITOLOGY IN A MOLECULAR WORLD
08-17-2026 10:00 AM - 12:00 PM

DIGITAL PCR IN DIAGNOSTIC PARASITOLOGY: USING QIACUITY TO MONITOR CHANGES IN PARASITEMIA IN PATIENTS WITH CHRONIC CHAGAS DISEASE

Yvonne Qvarnstrom
CDC: Centers for Disease Control and Prevention, Diagnostic Parasitology Team, Atlanta, United States of America

Digital PCR (dPCR) is a new molecular tool for diagnostic parasitology that offers highly sensitive and precise quantification of nucleic acids. Unlike quantitative real-time PCR, which requires standard curves for absolute quantification, dPCR partitions the sample into thousands to millions of individual reactions and scores partitions as positive or negative. This partition-based counting permits direct absolute target quantification. In parasitology, dPCR can address several diagnostic challenges. Many parasitic infections are characterized by low parasite burdens, and the analytical sensitivity of dPCR can enhance detection of e.g., *Plasmodium* spp. in low-density malaria and *Trypanosoma cruzi* in chronic Chagas Disease. Because dPCR is more tolerant of PCR inhibitors, it can improve detection of parasites in complex matrices such as stool. It is also well suited for longitudinal monitoring of parasite load, e.g., during treatment. This presentation will include a short overview of digital PCR applications in diagnostic parasitology, with a special focus on parasitemia monitoring in Chagas Disease patients.

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IS018

TOPIC 2: TRANSMISSION DYNAMICS, EPIDEMIOLOGY, AND GLOBAL HEALTH
CLINICAL PARASITOLOGY IN A MOLECULAR WORLD
08-17-2026 10:00 AM - 12:00 PM

SOIL TRANSMITTED HELMINTHS: NEW APPROACHES FOR AN OLD PARASITE

Joel Barratt
Emory University School of Medicine, Pathology And Laboratory Medicine, Atlanta, United States of America

The term "soil-transmitted helminths" (STHs) conveniently groups taxonomically disparate nematode genera united by a single ecological trait: transmission via contaminated soil. However, this umbrella term obscures fundamentally distinct infection mechanics across distantly related taxa. While *Ascaris* and *Trichuris* are acquired via ingestion of embryonated eggs, hookworms and *Strongyloides* infect hosts through active larval skin penetration. Although these parasites have co-evolved with humans for millennia, a comprehensive understanding of their life cycles and true genetic diversity is a relatively recent development. High-throughput genomic sequencing reveals that classical morphological classifications significantly underestimated STH complexity. Sanger and next-generation sequencing (NGS) have redefined STH evolutionary history, uncovered complex population structures, and identified previously unrecognized zoonotic and cryptic species complexes hidden within long-established taxonomic groupings. This presentation highlights critical recent advances in STH molecular epidemiology, focusing on how hidden genetic diversity can inform and refine mass drug administration (MDA) and elimination strategies, illustrating that seemingly academic taxonomic exercises can have substantial programmatic value. A particular emphasis is placed on the implications of sympatric cryptic species and host-associated parasite populations for transmission networks, zoonotic reservoirs, and elimination strategy design. The presentation also considers how morphologically defined species complexes often encompass multiple genetically, and perhaps antigenically, distinct populations, raising important questions regarding their impact on diagnostic efficacy. Ultimately, integrating genomic surveillance into public health frameworks and diagnostic development is presented as essential for maximizing the success of infection control and elimination programs.

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IS019

TOPIC 5: DIAGNOSTICS, HOST-PARASITE INTERACTION AND EMERGING TECHNOLOGIES
VIRUSES IN PARASITES: HIDDEN PASSENGERS OR FACILITATORS OF VIRULENCE?
08-17-2026 10:00 AM - 12:00 PM

DIVERSITY OF RNA VIRUSES IN TRYPANOSOMATIDAE

Vyacheslav Yurchenko[1], Alexei Kostygov[2]
[1]University of Ostrava, Life Science Research Centre, Ostrava, Czech Republic, [2]University of Ostrava, Ostrava, Czech Republic

Protists of the family Trypanosomatidae are intensively studied parasites. This group unites etiological agents of severe (yet, neglected) tropical diseases with a plethora of monoxenous and dixenous species exhibiting diverse and unusual biological features. In addition to their medical importance, these flagellates have also emerged as hosts of a remarkable diversity of RNA viruses. Since the first description of Leishmania RNA viruses (LRVs) in the late 20th century, the pace of viral discovery in trypanosomatids has increased dramatically, particularly after the recognition that LRVs can exacerbate the pathology of leishmaniasis and adoption of the next generation sequence-based screening techniques. Subsequent analysis of monoxenous species and previously neglected dixenous taxa has uncovered nearly forty putative viral species across multiple trypanosomatid genera, representing seven lineages of double-stranded, positive-sense, and negative-sense RNA viruses. In one case, extreme sequence divergence required structural phylogenetic analysis to resolve the virus's evolutionary placement. In addition, some trypanosomatid genomes contain viral remnants (endogenous viral elements), providing evidence of past infections. The observed diversity indicates repeated viral acquisitions from diverse donors, shaped by ecological exposure, the feeding habits of insect hosts, and parasite population structure. Following initial acquisition, viruses often spread to additional trypanosomatid species, giving rise to parasite-specific viral lineages. Together, these findings refine our understanding of virus–trypanosomatid evolution and inform targeted searches for yet-undiscovered viral diversity.

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IS020

TOPIC 5: DIAGNOSTICS, HOST-PARASITE INTERACTION AND EMERGING TECHNOLOGIES
VIRUSES IN PARASITES: HIDDEN PASSENGERS OR FACILITATORS OF VIRULENCE?
08-17-2026 10:00 AM - 12:00 PM

GENOMIC AND EVOLUTIONARY DIVERSITY OF LEISHMANIA (VIANNIA)–LRV1 AND ITS IMPACT ON PARASITE FITNESS AND DISEASE

Elisa Cupolillo[1], Mayara Santana[2], Lilian Cantanhêde[1]
[1]Oswaldo Cruz Foundation, Oswaldo Cruz Institute, Rio de Janeiro, Brazil, [2]Oswaldo Cruz Foundation, Rio de Janeiro, Brazil

The presence of virus-like particles in *Leishmania* was first demonstrated in the late 1970s in a non-pathogenic species of humans; however, only about ten years later was the molecular characterization of a virus found in *L. guyanensis* performed, leading to the identification of *Leishmania* RNA Virus 1 (LRV1). Since then, our understanding of the *Leishmania*–LRV1 association has expanded considerably, particularly after studies demonstrated that the virus modulates the severity of mucocutaneous leishmaniasis. Based on these findings, our group investigated the frequency of LRV1 in patients with cutaneous and mucosal leishmaniasis and observed a high prevalence in the latter group. We also showed that patients positive for LRV1 in cutaneous lesions were more likely to develop recurrent disease, suggesting a role for the virus in parasite dissemination. In addition, parasites infected with LRV1 persisted longer in sand flies and experimentally infected mice, exhibiting increased parasite loads compared with virus-free parasites. In Brazil, LRV1 appears to be restricted to the Amazon region, where several *Leishmania* species harbor species-specific viral lineages, although sporadic host-shifts events occur. Experimental transfer of LRV1 through exosomes revealed a fitness advantage for recipient parasites. Together, these findings support a Matryoshka Model of LRV1, in which the effects of a viral endosymbiont extend from the parasite to the host, vector, and ultimately the ecosystem.

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IS021

TOPIC 5: DIAGNOSTICS, HOST-PARASITE INTERACTION AND EMERGING TECHNOLOGIES
VIRUSES IN PARASITES: HIDDEN PASSENGERS OR FACILITATORS OF VIRULENCE?
08-17-2026 10:00 AM - 12:00 PM

VIRUSES OF CARCINOGENIC TREMATODES ARE RISK FACTORS AND DIAGNOSTIC TARGETS

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Since the launch of the Parasite Microbiome Project in 2019, and thanks to high throughput sequencing technologies, our knowledge of the taxonomy of viruses of parasites, and role of parasites in virus evolution has been expanding dramatically. The discovery of a diverse virome in parasitic flatworms and its transmission to the vertebrate host prompts reconsideration of the etiology of helminth infection and associated diseases. As a first step to investigate the role of viruses in trematode pathogenesis we chose to focus our attention on carcinogenic trematodes.The International Agency for Research on Cancer (IARC) categorizes only three eukaryotic parasites as group 1 carcinogens: the blood fluke *Schistosoma haematobium* and the liver flukes *Opisthorchis viverrini* and *Clonorchis sinensis*, and viruses associated with all three species were characterized. Focusing on *O. viverrini*, we observed virus persistence at discrete life stages, viral tropism for the fluke tegument, and provided molecular and serological evidence of parasitized hosts exposure to viral RNA and proteins. Serological analyses confirmed seroconversion of *O. viverrini*-infected residents, and revealed that antibody titer and avidity against fluke viruses increase in individuals with hepatobiliary diseases. Random forest models discriminate efficiently between liver-fluke infected participants without disease from those with periductal fibrosis or cholangiocarcinoma.These studies suggest that viruses of flukes are not passive passengers and may indeed contribute to parasite-associated diseases, including cancer.

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IS022

TOPIC 5: DIAGNOSTICS, HOST-PARASITE INTERACTION AND EMERGING TECHNOLOGIES
VIRUSES IN PARASITES: HIDDEN PASSENGERS OR FACILITATORS OF VIRULENCE?
08-17-2026 10:00 AM - 12:00 PM

TRICHOMONADS: TO HAVE OR NOT TO HAVE DSRNA VIRUS

Jan Tachezy
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Trichomonasvaginalis virus (TVV), a member of the Totiviridae family discovered in 1985, was the first double-stranded (ds)RNA virus reported in parasitic protists. To date, four species (TVV1-4) have been found in clinical samples, while the fifth (TVV5) has been reconstructed from transcriptomic data. In addition, two related dsRNA viruses (TTV1 and TTV3) have been found in *Trichomonas tenax*. Nonsegmented dsRNA TVV/TTV genomes, ranging from 4.6-4.9 kbp, consist of two overlapping open reading frames coding for the capsid protein (CP) and RNA-dependent RNA polymerase (RdRp). The RdRp is produced as a C-terminal fusion with CP via a -2 ribosomal frameshift. The virion forms an icosahedral capsid composed of 120 subunits, forming channels, which may facilitate viral genome release. TVV is a non-lytic virus that is transmitted vertically during cell replication. Electron microscopy showed that TVV can be released from trichomonad cells within extracellular vesicles; however, whether this enables horizontal transmission remains unresolved. Interest in TVV stems from its high global prevalence in *T. vaginalis* (about 47%) and its potential role in modulating parasite–host interactions. TVV presence has been associated with enhanced proinflammatory host responses, altered drug susceptibility, changes in exosomal cargo, and modulation of virulence factor expression. However, disentangling virus-specific effects from strain-dependent variation has been challenging. Recent advances include the generation of isogenic TVV-positive and TVV-negative *T. vaginalis* strains using nucleoside analogs that inhibit RdRp and suppress viral replication. These isogenic strains showed no difference in metronidazole susceptibility, suggesting that previously observed variability may reflect strain-specific rather than virus-driven effects.

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IS023

TOPIC 3: RESISTANCE AND DEVELOPMENT OF DRUGS AND VACCINES
PROTOZOAN PARASITES AND THE AMR FRONTIER: FROM BENCH TO CONTROL STRATEGIES
08-17-2026 10:00 AM - 12:00 PM

NEW INSIGHTS INTO METRONIDAZOLE RESISTANCE IN MICROAEROPHILIC/ANAEROBIC PARASITES

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Metronidazole and other drugs from the 5-nitroimidazole class are widely used in the treatment of microaerophilic and anaerobic infections. 5-nitroimidazoles are prodrugs which require reduction of their nitro group in order to exert toxicity. Importantly, most microraerophiles and anaerobes are susceptible to metronidazole, whereas organisms with an aerobic metabolism are not. This has been commonly ascribed to the presence of highly reductive enzymes in microaerophilic/anaerobic cells which are able to reduce metronidazole and other 5-nitroimidazoles to their reactive state. Although such factors have been identified aplenty over the last decades, recent research suggests that the bulk of metronidazole reduction in anaerobic/microaerophilic cells might be attributable to non-enzymatic reduction by ferrous iron (Fe^{2+}) and thiols. Ferric iron (Fe^{3+}) is unreactive and can be safely stored in cells. However, it is poorly soluble and not easily mobilized. In contrast, Fe^{2+} can be readily used for the synthesis of iron-containing proteins but is highly reactive with reactive oxygen species (ROS) and many other molecules. Consequently, Fe^{2+} levels are very low and carefully controlled in aerobic cells. In microaerophilic and anaerobic cells, however, little intracellular oxygen is present, rendering the storage of Fe^{2+} relatively safe. Reduction of Fe^{3+} to Fe^{2+} can be achieved by many reductants, most rapidly, however, by $FMNH_2$ or $FADH_2$. Reduction of FMN and FAD to their respective reduced forms, in turn, is catalyzed by flavin reductases. In our previous work, we discovered the loss of flavin reductase activity as an important factor contributing to metronidazole resistance in several microaerophilic pathogens.

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IS024

TOPIC 3: RESISTANCE AND DEVELOPMENT OF DRUGS AND VACCINES
PROTOZOAN PARASITES AND THE AMR FRONTIER: FROM BENCH TO CONTROL STRATEGIES
08-17-2026 10:00 AM - 12:00 PM

STRONGER... OR JUST STRANGER? FITNESS TRADE-OFFS IN DRUG-RESISTANT LEISHMANIA

Christopher Fernandez-Prada
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Drug resistance in *Leishmania* is often framed as a gain in fitness under treatment pressure, yet resistant parasites may become not simply stronger, but biologically stranger. In this presentation, we explore the fitness trade-offs associated with drug resistance and resensitization in *Leishmania*, integrating longitudinal phenotyping with multi-omics analyses of both parasites and their extracellular vesicles (EVs). Using antimony resistance as an archetype, we show that resistance is accompanied by profound remodeling of parasite physiology, including altered mitochondrial and respiratory function, shifts in infectivity, and changes in stress-response pathways. Resistant parasites also release EVs with distinct proteomic, lipidomic, and metabolomic signatures, enriched in virulence-associated factors and determinants linked to drug resistance. These vesicles are not passive byproducts: they actively reshape recipient parasites and host macrophages, promoting survival, immune modulation, and traits consistent with persistence or quiescence. Importantly, withdrawal of drug pressure does not result in a simple return to the ancestral susceptible state. Instead, resensitized populations emerge with novel phenotypic and therapeutic profiles, diverging from both wild-type and resistant parasites. This transition is associated with metabolic rewiring and, unexpectedly, with collateral resistance to clinically relevant drugs such as miltefosine and amphotericin B, despite no direct exposure. Together, these findings reveal drug resistance as a dynamic evolutionary process marked by trade-offs, compensatory adaptation, and intercellular communication. From a One Health perspective, such plasticity is particularly concerning at the human-animal interface, where treatment practices in dogs and cats may contribute to shifting and poorly predictable selection landscapes.

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IS025

TOPIC 3: RESISTANCE AND DEVELOPMENT OF DRUGS AND VACCINES
PROTOZOAN PARASITES AND THE AMR FRONTIER: FROM BENCH TO CONTROL STRATEGIES
08-17-2026 10:00 AM - 12:00 PM

LEISHMANIA UNDER DRUG PRESSURE: LINKING TREATMENT FAILURE AND TRANSMISSION

Guy Caljon
Laboratory for Microbiology, Parasitology and Hygiene, University of Antwerp, Department Of Biomedical Sciences, Infla-med Research Centre Of Excellence, Antwerp, Belgium

Treatment failure in visceral leishmaniasis is increasingly recognized as a multifactorial process involving the parasite's adaptative capabilities, sanctuary niches in the mammalian host and transmission through the sand fly vector. Yet, the mechanisms underlying persistence, relapse and spread remain incompletely understood. Using integrated infection models in rodents and sand flies, we investigated how parasite adaptations arising under antileishmanial drug pressure influence both treatment outcome and transmission potential. Repeated drug exposure induced phenotypic and genetic changes associated with reduced drug susceptibility. At the same time, bone marrow stem cells were identified as a critical sanctuary niche supporting persistent infection and parasite quiescence, enabling survival during treatment and acting as a source of relapse. We next aimed to understand how such traits affecting survival under drug pressure impact the transmission dynamics beyond the vertebrate host by assessing parasite fitness in sand fly vectors. While some resistance-associated alterations impaired parasite development in sand flies, other adaptive and quiescence-associated phenotypes exhibited high infectivity and efficient transmissibility. The persistence of these traits after vector passage highlights their potential to propagate despite therapeutic intervention. Together, these findings demonstrate that treatment failure and transmission are intimately connected processes. Integrating vertebrate and vector infection models provides new insights into how parasites can adapt and spread after treatment failure, underscoring the risk of relapses for the emergence of adaptive parasite traits.

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IS026

TOPIC 3: RESISTANCE AND DEVELOPMENT OF DRUGS AND VACCINES
PROTOZOAN PARASITES AND THE AMR FRONTIER: FROM BENCH TO CONTROL STRATEGIES
08-17-2026 10:00 AM - 12:00 PM

ANTI-MALARIAL EFFICACY AND RESISTANCE MONITORING OF ARTEMISININ BASED COMBINATION (CTA) IN BURKINA FASO FROM 2017 TO 2023 : RESULTS FROM TWO PREVIOUS THERAPEUTIC EFFICACY STUDIES

Adama Gansane
Institut National de Sante Publique, Ouagadougou, Burkina Faso

Background Artemisinin-based combination therapies (ACTs) are the first lines treatment for uncomplicated *Plasmodium falciparum* malaria in Burkina Faso. Regular monitoring of therapeutic efficacy is critical to detect early signs of drug resistance and inform national malaria control programme. **Methods** We synthesized data from two consecutive Therapeutic Efficacy Studies (TES) conducted in patients aged 6 months to 12 years with uncomplicated *P. falciparum* malaria between 2017 and 2023 across sentinel sites (Nanoro, Gourcy, and Niangoloko) with varying malaria transmission intensities. Patients received either artemether-lumefantrine (AL) or dihydroartemisinin-piperaquine (DP) or artesunate-pyronaridine (AS-Pyr). The primary endpoint was the PCR-corrected Adequate Clinical and Parasitological Response (ACPR) at Day 28 (AL) and Day 42 (AS-Pyr, DP). Molecular markers of resistance to artemisinin derivatives and partner drugs were also analysed. **Results** In the 2017–2018 TES, AL and DP failed to reach the World Health Organization (WHO) recommended ≥90% efficacy threshold in Nanoro and Gourcy, with PCR-corrected ACPR rates dropping as low as 74% and 76% for AL respectively. In contrast, both drugs maintained high efficacy in Niangoloko. The 2020–2021 TES confirmed persistent sub-optimal AL efficacy, particularly in areas with shorter, seasonal malaria transmission. No pfk13 mutation was observed. **Conclusion** The confirmed decline in AL efficacy below the 90% threshold in multiple regions of Burkina Faso signals a critical challenge for malaria control. Because this clinical failure is driven by partner-drug selection rather than pfk13-mediated artemisinin resistance, an immediate review and diversification of the national first-line antimalarial policy are urgently required to contain further resistance;

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IS027

TOPIC 2: TRANSMISSION DYNAMICS, EPIDEMIOLOGY, AND GLOBAL HEALTH
THE UNDERDESCRIBED BURDEN OF MALE GENITAL SCHISTOSOMIASIS (MGS)
08-17-2026 10:00 AM - 12:00 PM

THE ROLE OF ZOONOTIC SCHISTOSOMES IN MALE GENITAL SCHISTOSOMIASIS

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[1]Liverpool School of Tropical Medicine, Medical Parasitology, Liverpool, United Kingdom, [2]Kamuzu University of Health Sciences, Blantyre, Malawi

In this presentation I review the recently completed HUGS (Hybridisation in UroGenital Schistosomiasis) project's findings in Malawi with specific reference to the diagnostics of male genital schistosomiasis (MGS). Our work on MGS started several years ago during the PhD studies of Dr Seke Kayuni who showed that many men on the shoreline of Lake Malawi have overt MGS. Expanding upon these findings we show how infections with *Schistosoma mattheei* can also account for MGS, in part, which uniquely links zoonotic transmission within a wider sexual reproductive health agenda. Using real-time PCR assays I discuss the advantages of molecular diagnostics against standard light microscopy of semen. Looking ahead, MGS needs to be brought out of the shadows of schistosomiasis control and placed firmly in parallel context to the public health advances being made to control female genital schistosomiasis.

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IS028

TOPIC 2: TRANSMISSION DYNAMICS, EPIDEMIOLOGY, AND GLOBAL HEALTH
THE UNDERDESCRIBED BURDEN OF MALE GENITAL SCHISTOSOMIASIS (MGS)
08-17-2026 10:00 AM - 12:00 PM

UNRECOGNIZED CONSEQUENCE OF A PREVALENT NTD AMONG SUB SAHARAN AFRICA MEN - THE BURDEN OF MGS IN MALAWI

Sekeleghe Kayuni[1,2,3]
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INTRODUCTION: Male genital schistosomiasis (MGS) is a chronic complication of a prevalent neglected tropical disease (NTD), urogenital schistosomiasis, caused by *Schistosoma haematobium* eggs entrapped in the genital tract. Despite its first description in 1911, MGS is still underreported, misdiagnosis and mismanagement. Furthermore, zoonotic and hybrid schistosomiasis are poorly described though emerging as public health concern in Sub-Saharan Africa. We describe the unrecognised burden of MGS in schistosomiasis-endemic areas of Malawi. ***METHODS:*** Following written informed consent, recruitment, questionnaires interviews, urine and semen were collected and analysed to determine schistosome infection using parasitological and molecular techniques, also to detect human, zoonotic or hybrid schistosomes. ***RESULTS:*** Longitudinal study conducted in 2018 among fishermen along southern Lake Malawi shores of Mangochi district (median age: 31.0 years), detected MGS in 10.4% (n=114) on semen microscopy and 26.5% (n=65) on real-time PCR. In 2023, a longitudinal hybrid study in Nsanje and Mangochi districts (median age: 31.0 years) revealed MGS in 50.0% men (n=22) on microscopy and 72.7% on PCR, detecting *S. haematobium, S. mattheei*, *S. mansoni* and *S. haematobium-S. mattheei* hybrid. A 2025 cross-sectional study among 60 men accessing sexual and reproductive health services (median age: 32.5 years) detected MGS in 19 men (35.2%) by semen microscopy and 33 men (61.1%) by PCR, showing *S. haematobium*, *S. mansoni* and *S. mattheei*. ***CONCLUSION:*** MGS is prevalent in Malawi, caused by various schistosomes which poses a challenge in diagnosis, management and control interventions. Increased awareness among local communities and healthcare workers, improved diagnostic capacity and disease management should be advocated to curb misdiagnosis and mismanagement.

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IS029

TOPIC 2: TRANSMISSION DYNAMICS, EPIDEMIOLOGY, AND GLOBAL HEALTH
THE UNDERDESCRIBED BURDEN OF MALE GENITAL SCHISTOSOMIASIS (MGS)
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DIAGNOSTIC APPROACHES FOR MALE GENITAL SCHISTOSOMIASIS: LESSONS LEARNED FROM A DIAGNOSTIC EVALUATION STUDY IN ZAMBIA

Bronwyn Neufeld[1,2], Sergio España-Cueto[3], Isaac Mshanga[4], Rhoda Ndubani[4], Fiona Allan[5], Lisette Van Lieshout[6], Bonnie Webster[7], Maina Cheeba[4], Lucas Cunningham[8], Tegwen Marlais[9], Shinjiro Hamano[10], Sekeleghe Kayuni[11], Russell Stothard[8], Musonda Simwinga[4], Kwame Shanaube[4], Amaya Bustinduy[1]
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Male genital schistosomiasis (MGS) is a neglected tropical disease which results from the deposition of *Schistosoma haematobium* eggs within the male genital tract and reproductive organs. MGS is underreported partly due to diagnostic challenges and a lack of awareness within health systems. The aim of this pilot study was to evaluate the performance of two diagnostic tests on semen (egg microscopy and *Schistosoma* ITS-2 qPCR) using samples collected from men across three communities in Zambia. 156 men were recruited into the study after community sensitization and obtaining written informed consent. Of these, 155 (99.4%) completed a symptom questionnaire, 156 (100%) provided urine, and 47/156 (30.1%) provided semen. Microscopy was performed on semen for the detection of *S. haematobium* eggs, and *Schistosoma* ITS2 qPCR was performed on DNA extracted from semen. The performance of each diagnostic test for MGS (semen microscopy, ITS2-qPCR) was assessed using a composite reference standard (CRS), defined as being positive by either test. The overall prevalence of MGS by either diagnostic across all sites was 12.8% (6.4% [3/47] by semen microscopy and 12.8% [6/47] by semen qPCR). Against the CRS, the sensitivity of semen microscopy was 50.0% (95% CI: 11.8-88.2) and the sensitivity of semen qPCR was 100.0% (95% CI: 54.1-100.0). This pilot study presents the first data on MGS in Zambia and is the first evaluation of MGS diagnostics using a composite reference standard. We show that qPCR on semen is a highly sensitive diagnostic

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method compared to microscopy. Further optimization of diagnostic tools for MGS, particularly for use in resource-constrained settings, is needed to facilitate enhanced case detection.

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IS030

TOPIC 2: TRANSMISSION DYNAMICS, EPIDEMIOLOGY, AND GLOBAL HEALTH
THE UNDERDESCRIBED BURDEN OF MALE GENITAL SCHISTOSOMIASIS (MGS)
08-17-2026 10:00 AM - 12:00 PM

ASSESSMENT OF MORBIDITY ASSOCIATED WITH MALE GENITAL SCHISTOSOMIASIS IN ZAMBIAN MEN BY POINT-OF-CARE ULTRASONOGRAPHY

Sergio España-Cueto[1,2], Bronwyn Neufeld[3,4], Isaac Mshanga[5], Rhoda Ndubani[6], Lucas Cunningham[7], Fiona Allan[2,8], Lisette Van Lieshout[9], Bonnie Webster[10], Maina Cheeba[5], Tegwen Marlais[11], Shinjiro Hamano[12], Silvia Roure[1], Roger Paredes[1], Sekeleghe Kayuni[13,14,15], Russell Stothard[7,16], Kwame Shanaube[6], Amaya Bustinduy[2]
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Male genital schistosomiasis (MGS) remains a neglected manifestation of urogenital schistosomiasis, with limited evidence on its associated morbidity in endemic communities. While parasitological and molecular tools can support infection detection, the structural consequences of MGS in the male urogenital tract remain insufficiently characterized. Point-of-care ultrasonography offers a feasible, non-invasive approach to assess schistosomiasis-associated morbidity in field settings, but its role in MGS evaluation is still poorly defined. This presentation will focus on the use of portable ultrasonography to assess urogenital morbidity potentially associated with MGS. It will briefly review previous ultrasound-based studies in urogenital schistosomiasis, discuss the challenges of defining imaging markers for male genital involvement, and explore how ultrasound may complement laboratory-based diagnostic approaches by documenting organ-level morbidity. Preliminary findings will be presented from the SchistoM pilot study conducted in southern Zambia. Adult men from three communities with differing schistosomiasis endemicity underwent clinical assessment, urine and semen testing, serology, and point-of-care ultrasound. Ultrasound examinations assessed the urinary

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tract and male genital organs, including bladder, kidneys, prostate, seminal vesicles and testes. Among 156 recruited men, 136 underwent portable ultrasound. Frequent abnormalities were observed, including bladder wall thickening, bladder polyps, renal findings, prostate calcifications, hydrocele and testicular abnormalities. These findings support the feasibility of integrating point-of-care ultrasonography into MGS field studies and highlight its potential value for characterizing morbidity beyond infection detection. Larger studies are needed to validate ultrasound markers of schistosomiasis-associated male urogenital morbidity and inform standardized imaging protocols for endemic settings.

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IS031

TOPIC 5: DIAGNOSTICS, HOST-PARASITE INTERACTION AND EMERGING TECHNOLOGIES TROPICAL HELMINTHOLOGY- NEW INSIGHTS INTO DIAGNOSIS AND CONTROL OF HELMINTHS IN TROPICAL REGIONS- SPONSORED BY THE INTERNATIONAL FEDERATION FOR TROPICAL MEDICINE
08-17-2026 10:00 AM - 12:00 PM

HARNESSING NEXT-GENERATION MOLECULAR TECHNOLOGIES TO STRENGTHEN SCHISTOSOMIASIS CONTROL

Skye Macgregor[1], Veronika Toth[1], Chika Zumuk[1], Thomas Egwang[2], Malcolm Jones[3], Hong You[1]
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Background and Aims Control of the neglected tropical disease schistosomiasis remains a major challenge due to inadequate diagnostics, the sole reliance on a single anthelminthic, threat of resistance, and the urgent need for vaccines. This presentation will report on molecular technologies we are developing to advance molecular diagnostics, accelerate drug discovery and vaccine development to improve disease control and reduce pathology.
Methods We report on three areas of laboratory advances: a CRISPR-Cas12/13 platform combining Recombinase Polymerase Amplification and Cas12/13 detection for field diagnosis of *schistosomes* and helminth coinfections; single-cell *in-situ* spatial technology to investigate cellular transcriptomic profiles involved in host-parasite interactions in schistosomiasis; and use of mRNA vaccines in experimental studies against *S. japonicum*.
Results CRISPR-based diagnostics can detect schistosomiasis and co-infecting helminths, with qPCR-level sensitivity/specificity, while being field-friendly, and requiring no specialized equipment/expertise. Spatial analysis shows schistosome-induced fibrosis developed a complex microenvironment with immune and hepatic stellate cell interactions; ten cell clusters—including hepatocytes, fibroblasts, macrophages, and T cells—are identified in granulomas. We show that mice vaccinated with an mRNA construct encoding *S. japonicum* paramyosin presented significant protection, reducing total and female worm burdens by 54% and 66%, and liver egg burden and granuloma area by 76% and 83% against controls. Immune responses characterized by increased Tfh cells, germinal centre B cells, and IL-21 response may correlate with enhanced antibody production.
Conclusions Our next-generation approaches—encompassing CRISPR-based diagnostics, single-cell spatial analysis for drug discovery and mRNA vaccine technology have the potential to address key challenges in schistosomiasis.

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TOPIC 5: DIAGNOSTICS, HOST-PARASITE INTERACTION AND EMERGING TECHNOLOGIES TROPICAL HELMINTHOLOGY- NEW INSIGHTS INTO DIAGNOSIS AND CONTROL OF HELMINTHS IN TROPICAL REGIONS- SPONSORED BY THE INTERNATIONAL FEDERATION FOR TROPICAL MEDICINE
08-17-2026 10:00 AM - 12:00 PM

INNOVATIVE DIAGNOSTIC TECHNOLOGIES FOR SCHISTOSOMIASIS ELIMINATION: LESSONS FROM SENEGAL

Doudou Sow
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Despite significant progress in schistosomiasis control, timely and accurate diagnosis remains a major challenge, particularly in low-endemic settings and among underserved populations such as women and preschool-aged children. Conventional parasitological methods often fail to detect low-intensity infections and do not adequately identify female genital schistosomiasis (FGS), a neglected manifestation associated with severe reproductive health consequences. Over the past decade, our research group in Senegal has evaluated innovative diagnostic approaches aimed at improving schistosomiasis detection across the human–parasite–snail interface. At the human level, we demonstrated the feasibility of molecular diagnosis of urogenital schistosomiasis using non-invasive and minimally invasive specimens, including urine, cervical cytobrushes, and cotton swabs, thereby improving the diagnosis of FGS and infections in preschool-aged children, school-aged children, women of reproductive age, and pregnant women. These approaches provide enhanced sensitivity while remaining compatible with field implementation. Beyond human diagnosis, our work has contributed to a better understanding of transmission through molecular characterization of *Schistosoma* species and their intermediate snail hosts. We have also evaluated the application of MALDI-TOF mass spectrometry for rapid identification of freshwater snails, geographical source attribution, and detection of *Schistosoma* infections in intermediate hosts, providing new opportunities for precision surveillance. This presentation will synthesize evidence generated from these complementary studies and discuss how integrating molecular diagnostics, innovative sampling strategies, proteomic technologies, and digital health tools can transform schistosomiasis surveillance and case management. Our experience illustrates how combining emerging technologies with implementation research can bridge the gap between laboratory innovation and routine public health practice.

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08-17-2026 10:00 AM - 12:00 PM

CHANGING DYNAMICS OF SCHISTOSOMIASIS TRANSMISSION IN CAMEROON: IMPLICATIONS FOR DIAGNOSIS AND CONTROL

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Over the past four decades, schistosomiasis transmission in Cameroon has undergone profound epidemiological, ecological and evolutionary changes that challenge conventional paradigms of disease transmission and control. While preventive chemotherapy has substantially reduced morbidity in many endemic communities, persistent transmission hotspots, recurrent reinfections and the emergence of new epidemiological patterns highlight the increasing complexity of the disease. This presentation synthesizes evidence from four decades of longitudinal field studies and recent molecular investigations to describe the changing dynamics of schistosomiasis transmission in Cameroon. It highlights temporal changes in prevalence and infection intensity, shifts in the distribution of *Schistosoma* species and intermediate host snails, and the emergence of hybridization between human and livestock schistosomes, illustrating the growing importance of One Health approaches in understanding transmission. The presentation will also illustrate the influence of human – environmental change on transmission landscapes. These evolving dynamics have important implications for diagnosis, surveillance and control. Conventional parasitological diagnostics are becoming increasingly inadequate in low-transmission settings and where hybrid parasites circulate, underscoring the need for more sensitive diagnostics, molecular xenomonitoring, precision mapping and integrated surveillance. Sustainable control will require adaptive, multidisciplinary and One Health strategies that combine preventive chemotherapy with environmental management, snail surveillance and investigation of potential animal reservoirs. The Cameroonian experience provides valuable lessons for understanding the evolving epidemiology of schistosomiasis and informing resilient elimination strategies across Africa.

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IS034

TOPIC 5: DIAGNOSTICS, HOST-PARASITE INTERACTION AND EMERGING TECHNOLOGIES TROPICAL HELMINTHOLOGY- NEW INSIGHTS INTO DIAGNOSIS AND CONTROL OF HELMINTHS IN TROPICAL REGIONS- SPONSORED BY THE INTERNATIONAL FEDERATION FOR TROPICAL MEDICINE
08-17-2026 10:00 AM - 12:00 PM

THE CHANGING EPIDEMIOLOGY OF SCHISTOSOMIASIS IN AFRICA: HIGHLIGHTING HYBRID, ZOONOTIC AND MIXED SPECIES INFECTIONS IN MALAWI

Russell Stothard[1], Janelisa Musaya[2]
[1]Liverpool School of Tropical Medicine, Department Of Tropical Disease Biology, Liverpool, United Kingdom, [2]Kamuzu University of Health Sciences, Parasitology, Blantyre, Malawi

The epidemiology of schistosomiasis in Africa is changing due to a combination of natural or manmade factors. Schistosomes experience many populational selection pressures although their sexual and reproductive biology are particularly special and highly dynamic. If given sufficient opportunity, certain schistosome species can hybridize and give rise to progeny that have either abrupt genomic changes or exhibit more gradual genetic introgressions. Sometimes these introgressed variants can also develop in local intermediate host snails. The importance and impact of such short- and long-term dynamics will be dicussed focusing on the results of a recently completed 4-year study entitled HUGS - Hybridisation in UroGenital Schistosomiasis conducted in Malawi. Here, two study communities, some 2,500 individuals, in Mangochi and Nsanje, were followed closely with preventive chemotherapy offered annually then bi-annually. Advanced molecular methods of diagnostics were applied. Surveillance techniques also included clinical examinations for male and female genital schistosomiasis. Zoonotic inputs, particularly those from *Schistosoma mattheei,* revealed ongoing hybridisation with *Schistosoma haematobium*, as well as triple infections with *Schistosoma mansoni*. Such infections were mainly found in children, inclusive of pre-school-aged individuals. Such evolving and changing schistosome infection dynamics of multiple parasite species challenge, not only traditional diagnostics but also the prospects for gaining transmission control in this lacutrine and riparian settting.

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IS035

PLENARY SESSION
WHEN IS A PARASITE A PROBLEM?
08-17-2026 1:00 PM - 2:00 PM

WHEN IS A PARASITE A PROBLEM?

Kevin Lafferty
U.S. Geological Survey, Western Ecological Research Center, Santa Barbara, United States of America

Medical doctors and veterinarians are tasked with treating parasites that infect humans or host species with utilitarian or aesthetic value to society. In this context, parasites are framed as problems. However, a parasite's perceived societal impact depends on the disease it causes and how the affected host species is valued. Although the number of parasites of concern to humans may vary, only 13% of parasites and 6% of host–parasite links in a given ecosystem (e.g., a kelp forest food web) might be considered "problematic." These figures suggest that most parasites and infectious diseases are relatively inconsequential. Among parasitologists, there is a common expectation that parasites, and their impacts, increase under environmental stress as ecosystems degrade globally. However, reports of disease have not consistently increased in response to human-driven environmental change, indicating that the factors influencing parasitism are more complex. Thus, the expectation that parasites inherently create problems, and that the diseases they cause are worsening, appears to be the exception rather than the rule.

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IS036

TOPIC 4: HOST IMMUNITY, MOLECULAR INTERACTIONS, AND MICROBIOME
PHAGOCYTOSIS IN HOST-PARASITE INTERACTION
08-17-2026 2:10 PM - 4:10 PM

IMPLICATION OF THE ANNEXIN 1/FPR AXIS IN LEISHMANIAL EXOSOME-MEDIATED LEISHMANIA SKIN PATHOLOGY

Martin Olivier
Research Institute of the McGill University Health Centre, Infectious Diseases And Immunology In Global Health Program, Montréal, Canada

Nano-sized extracelular vesicles named Exosomes are produced by the protozoan parasite *Leishmania* (LeishEXO) and are now well-established drivers of virulence, though mechanisms underlying their exacerbation of experimental leishmaniasis remain elusive. Expression of Annexin A1 (ANXA1), a protein implicated in exosome-mediated pathologies and viral internalization, has been shown to correlate with cutaneous leishmaniasis severity. Given ANXA1's regulation of myeloid cells – the canonical hosts for *Leishmania* – we studied the potential role of ANXA1 and its receptor FPR2 in exerting LeishEXO's effects. Using *in vivo* and *in vitro* ANXA1$^{-/-}$ models, we established that ANXA1 deficiency prohibits LeishEXO-mediated hyperinflammation and myeloid cell infection, with minimal alterations to adaptive and innate immune phenotypes. FPR2 blockade with WRW4 similarly inhibited leishmanial hyperpathogenesis, while direct activation of the receptor with WKYMVm enhanced infection and recapitulated the LeishEXO-mediated phenotype. This research describes LeishEXO's utilization of the ANXA1/FPR2 axis to facilitate parasitic internalization and pathogenesis, which may be leveraged in the development of therapeutics for leishmaniasis.
Research in Olivier Laboratory is supported by grants from the Canadian Institute of Health Research (CIHR/IRSC) and the National Science and Engineering Research Council (NSERC/CRSNG).

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TOPIC 4: HOST IMMUNITY, MOLECULAR INTERACTIONS, AND MICROBIOME
PHAGOCYTOSIS IN HOST-PARASITE INTERACTION
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CROSSTALK BETWEEN NEUTROPHILS AND DENDRITIC CELLS DURING LEISHMANIA INFECTION

Claudia Brodskyn[1,2], Amanda Lago[2], Gabriela Duarte[2]
[1]Instituto Nacional de Ciencia e Tecnologia em Mucosa e Pele, Minas Gerais, Brazil, [2]Instituto Gonçalo Moniz FIOCRUZ Bahia, Laboratory Of Interaction Parasite-host And Epidemiology, Salvador, Brazil

Introduction:
Neutrophils are among the first immune cells recruited during Leishmania infection and interact with dendritic cells (DCs), influencing parasite survival, antigen presentation, and T-cell activation. However, how infected neutrophils modulate DC-driven T-cell responses during *Leishmania spp*. infection remains poorly understood. **Methods:**
Human neutrophils were infected with *L. amazonensis* or *L. braziliensis* and cocultured with DCs. Infection rates, parasite burden, neutrophil death, DC phenotypic markers, and cytokine production were evaluated by microscopy, flow cytometry, and ELISA. DCs from selected conditions were used in T-cell priming assays to assess downstream responses. **Results:**
Neutrophils showed 50–60% infection rates and initiated apoptosis within 30 min, whereas necrosis was detected at 24 h. After 18 h, DCs cocultured with infected neutrophils exhibited higher parasite burden than directly infected DCs. Direct infection increased CD86 expression, whereas coculture reduced CD86 levels. CD80 upregulation occurred only in DCs cocultured with *L. braziliensis*–infected neutrophils, while HLA-DR remained unchanged. Cocultures increased IL-1β production for both species. TNF-α and IL-6 increased only in DCs directly infected with *L. amazonensis*, whereas IL-10 increased selectively in cocultures with *L. amazonensis*. Infected neutrophils did not produce IL-1β or IL-10. *L. amazonensis* modulated CD4+ T-cell memory subpopulations, while CD8+ T cells were unchanged. Increased IL-2, TNF-α, and IFN-γ production was observed in coculture at 24 and 48 h for both species. **Conclusion:**
Infected neutrophils modulate DC activation, and cytokine responses in a species-dependent manner, leading to altered CD4+ T-cell memory differentiation and enhanced cytokine production with different patterns, depending on Leishmania specie..

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TOPIC 4: HOST IMMUNITY, MOLECULAR INTERACTIONS, AND MICROBIOME
PHAGOCYTOSIS IN HOST-PARASITE INTERACTION
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LIFE INSIDE THE PHAGOCYTE: METAL HOMEOSTASIS AND ANTIOXIDANT STRATEGIES OF LEISHMANIA

Ana Tomás[1,2], Margarida Duarte[2], Teresa Leão[2]
[1]ICBAS, University of Porto, Pathology And Molecular Immunology, Porto, Portugal, [2]i3S, University of Porto, Molecular Parasitology, Porto, Portugal

Macrophages deploy diverse antimicrobial strategies, including the production of reactive oxygen and nitrogen species and, in many host-pathogen systems, the restriction of essential nutrient metals. Nonetheless, *Leishmania* parasites such as *L. infantum* survive and replicate within macrophage phagolysosomes of different tissues, implying the existence of adaptations that counteract host-imposed stresses. To identify these adaptations and probe the magnitude and timing of phagocyte-imposed oxidative and metal-related stresses during infection, we exploited *L. infantum* mutants lacking key components of antioxidant and metal homeostasis pathways. The core antioxidant system of *Leishmania* comprises three peroxiredoxin-family peroxidases. Unexpectedly, mutants lacking these enzymes remained viable, likely through metabolic rewiring that compensates for the loss of intrinsic antioxidant defenses, although they exhibited increased susceptibility to exogenous oxidants. Despite this heightened sensitivity, the mutants remained fully infective, challenging the view that oxidative stress constitutes a major obstacle during early phagocytosis. The impact of peroxidase deficiency emerged only later in infection. A similar picture emerged from the analysis of zinc homeostasis. Loss of the major zinc transporter, a protein required only under zinc-limiting conditions, had little impact on early macrophage infection, suggesting that zinc availability is sufficient at this stage. However, the inability of these mutants to persist in the spleen revealed that efficient zinc acquisition becomes critical for long-term survival *in vivo*. Together, these findings highlight the remarkable adaptability of *Leishmania* and reveal that phagocyte-imposed oxidative and metal-related stresses become most consequential during *L. infantum* persistence rather than during the establishment of infection.

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TOPIC 4: HOST IMMUNITY, MOLECULAR INTERACTIONS, AND MICROBIOME
PHAGOCYTOSIS IN HOST-PARASITE INTERACTION
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LEISHMANIA INFANTUM INFECTION ENHANCES HUMAN DENDRITIC CELL MIGRATION IN 2D AND 3D ENVIRONMENTS: IMPLICATIONS FOR DISEASE VISCERALIZATION?

Carla Polyana Bernardes[1], Amanda Rebouças[1], Yasmin Luz[1], Flavio Henrique Santos[2], Albert Schriefer[3], Patricia Veras[1], Leonardo Farias[2], Washington Dos Santos[2], Claudia Brodskyn[1], Valéria Borges[1], Juliana Menezes[1]
[1]Fiocruz Bahia, Laboratory Of Host-parasite Interaction And Epidemiology, Salvador, Brazil, [2]Instituto Gonçalo Moniz, Salvador, Brazil, [3]Federal University of Bahia, Salvador, Brazil

Leishmaniasis results in a wide spectrum of clinical manifestations, ranging from skin lesions to disseminated lesions in internal organs. While the ability of Leishmania-infected host cells to migrate may be important to lesion distribution and parasite dissemination, the underlying mechanisms and the role of host cells remain poorly understood. The present study aimed to investigate the effects of infection by different strains of Leishmania associated with diverse forms of disease presentation on 2D and 3D migration of human dendritic cells (hDCs). We evaluated cellular migration, adhesion complex formation, podosome development, actin polymerization, and CCR7 expression following infection of hDCs with *L. amazonensis*, *L. braziliensis*, or *L. infantum*. Since intrinsic factors of the parasite are important for maintaining the infection in the host and that lipophosphoglycan (LPG) plays a key role in the *Leishmania*-host cells interaction, we also examined the role of this molecule in hDC migration. Our findings show that *L. infantum*, but not *L. braziliensis* or *L. amazonensis*, infection enhances hDC migration in both 2D and 3D environments, correlating with increased adhesion complex formation, podosome activation, actin dynamics, and CCR7 expression. Furthermore, migration of hDC infected with *L. infantum* Δlpg2 was significantly reduced compared to those infected with wild-type or complemented Δlpg2 strains. These results suggest that *L. infantum* infection promotes dendritic cell migration by altering actin dynamics and the expression of proteins involved in plasma membrane-extracellular matrix interactions, and that this enhanced migration is dependent on LPG, indicating a potential association between dendritic cell behavior and disease visceralization.

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IS040

TOPIC 3: RESISTANCE AND DEVELOPMENT OF DRUGS AND VACCINES
SCHISTOSOMIASIS VACCINE CANDIDATES AND VACCINE DEVELOPMENT
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UPDATE ON HUMAN CLINICAL TRIALS OF SCHISTOSOMIASIS VACCINE, SCHISTOSHIELD(R)

Afzal Siddiqui
Texas Tech University Health Sciences Center, Immunology And Molecular Microbiology, Lubbock, United States of America

World Health Organization estimates that over 200 million people are currently infected and additional 800 million are at risk of acquiring the infection with schistosome parasites. Schistosomiasis carries pronounced mortality and high morbidity. Existing infection control measures have not been sufficient in reducing the parasite transmission, morbidity and disease burden. Currently, there is no vaccine available for human use to prevent or treat schistosomiasis. Over the last three decades, our team has followed a systematic and methodical approach to develop a Sm-p80-based vaccine as a viable and effective schistosomiasis vaccine for humans, called SchistoShield®. A Phase 1, Open-Label, Dose-Escalation Trial to Evaluate the Safety, Reactogenicity, and Immunogenicity of the Sm-p80 + GLA-SE (SchistoShield®) Vaccine in Healthy Adults (45 volunteers) has just been completed. A Phase 1b, Multicenter, Randomized, Placebo-controlled, Observer-blinded, Dose escalation Study to Evaluate the Safety, Tolerability and Immunogenicity of the Sm-p80 + GLA-SE (SchistoShield®) candidate vaccine in healthy adults in Madagascar and Burkina Faso (120 volunteers) was also recently completed. These vaccine trials have confirmed that in humans, SchistoShield® is well-tolerated, safe with dose escalation and 100% of vaccinated individuals seroconverted, exhibiting strong, vaccine-induced humoral and T cell responses. SchistoShield® has the potential to benefit hundreds of millions of people in geographical areas ranging all across sub-Saharan Africa and parts of Asia/South America where schistosomiasis is endemic.

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TOPIC 3: RESISTANCE AND DEVELOPMENT OF DRUGS AND VACCINES
SCHISTOSOMIASIS VACCINE CANDIDATES AND VACCINE DEVELOPMENT
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SMTSP2 VACCINES

Athos Silva De Oliveira[1], Sevan Alwan[2], Philip Loverde[2], Ramiz Shaheed[2], Rakesh Adhikari[1], Peter Hotez[3], Maria Elena Bottazzi[3], Jeroen Pollet[1]
[1]Baylor College of Medicine, Pediatrics Tropical Medicine Center For Vaccine Development, Houston, United States of America, [2]University of Texas at San Antonio, Dept. Of Chemistry, San Antonio, United States of America, [3]Baylor College of Medicine, Tropical Medicine, Houston, United States of America

Schistosoma mansoni remains a major cause of chronic morbidity worldwide, and effective vaccines are needed to complement existing control strategies. Our vaccine program has focused on *Sm*-TSP-2, a conserved tegumental tetraspanin identified as a leading antigen based on its unique recognition in putatively immune individuals and its robust preclinical protection. As a recombinant protein vaccine, *Sm*-TSP-2 has advanced into clinical development, where it has been shown to be well tolerated and capable of eliciting strong antigen-specific IgG responses in adults living in endemic areas. Building on this foundation, we expanded into nucleic acid–based technologies to assess whether alternative antigen presentations can further enhance protective immunity. Using a modular mRNA design strategy, we generated constructs encoding either full-length *Sm*-TSP-2 or its large extracellular loop (EC2) in secreted, membrane-anchored, or cytosolic forms. In mice, the secreted and membrane-anchored EC2 constructs induced the strongest antigen-specific antibody responses. These constructs, along with full-length *Sm*-TSP-2 mRNA, significantly reduced adult worm and hepatic egg burdens relative to controls. The membrane-anchored EC2 design yielded the greatest protection, reducing worm and egg burdens by 66.7% and 66.9%, respectively, with histopathology confirming smaller hepatic granulomas. Notably, the protective efficacy of the mRNA constructs was comparable to that achieved with the recombinant *Sm*-TSP-2-EC2 protein vaccine. Together, these data demonstrate that Sm-TSP-2 is a robust cross-platform antigen and highlight extracellular EC2 presentation as a key driver of protective immunity. This presentation will integrate preclinical and clinical findings with new mRNA platform data to outline next steps in advancing *Sm*-TSP-2-based vaccines for schistosomiasis.

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TOPIC 3: RESISTANCE AND DEVELOPMENT OF DRUGS AND VACCINES
SCHISTOSOMIASIS VACCINE CANDIDATES AND VACCINE DEVELOPMENT
08-17-2026 2:10 PM - 4:10 PM

SM14 SCHISTOSOMIASIS VACCINE CLINICAL TRIALS IN BRAZIL AND AFRICA

Miriam Tendler
Oswaldo Cruz Foundation (Fiocruz), Lpdvac (anti-helminth Vaccine Research And Development Laboratory, Rio de Janeiro, Brazil

Schistovac, the Sm14-based Schistosomiasis Vaccine, has been under development for more than four decades by a network of collaborators spearheaded by Fiocruz, with the leadership and coordination of Dr. Miriam Tendler. An unprecedented innovation from an endemic country, the Fiocruz vaccine represents a fundamental shift in technology transfer dynamics, as it establishes a South-South scientific collaboration in global health to create a tool well-positioned to break the continuous cycle of parasitic reinfection. Following successful Phase I trials in Brazil and three subsequent Phase II trials in hyperendemic regions of Senegal, the vaccine has now advanced to Phase III in Senegal river delta region In this presentation, Dr. Tendler will provide an overview of the initiative and detail the trial about to start in the Senegal River Delta region. In partnership with the Centre de Recherche Biomédicale Espoir Pour La Santé (CRB-EPLS), this randomized, placebo-controlled study with 1500 school-aged children paves the way for implementing Sm14 as an innovative public health tool for long-term transmission control.

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IS043

TOPIC 3: RESISTANCE AND DEVELOPMENT OF DRUGS AND VACCINES
INNOVATIVE TECHNOLOGIES FOR UNRAVELLING AMR IN PARASITES
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THE EVOLVING ROLE OF TARGETED DEEP SEQUENCING ANTHELMINTIC RESISTANCE MUTATION DISCOVERY AND SURVEILLANCE

John Gilleard
Faculty of Veterinary Medicine, University of Calgary, Canada, N, Canada

Parasitic nematode control has been largely dependent on blanket administration of anthelmintic drugs in both humans and animals for many decades. However, this approach is increasingly unsustainable due to the emergence of anthelmintic drug resistance and concerns regarding environmental impacts. Consequently, more targeted evidence-based approaches are needed which in turn require better diagnostics and surveillance. High-throughput sequencing technologies now offer unprecedented opportunities to transform how parasitic nematode infections are detected, monitored, and managed and high-quality reference genomes are foundational for the development of the tools needed. In this talk I will discuss our work using metabarcoding and targeted sequencing to characterize complex parasitic nematode communities with an emphasis on anthelmintic drug resistance diagnostics, surveillance and management and the opportunities to leverage genomic data to enhance these approaches. I will discuss our continued development of nemabiome metabarcoding and deep sequencing tools and how we are using them to direct evidence-based parasite control as well as support the development of better molecular diagnostic tests. Examples will draw from projects on gastrointestinal nematodes of ruminant livestock, the canine hookworm *Ancylostoma caninum* and Human Soil Transmitted Helminths (STH).

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TOPIC 3: RESISTANCE AND DEVELOPMENT OF DRUGS AND VACCINES
INNOVATIVE TECHNOLOGIES FOR UNRAVELLING AMR IN PARASITES
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NEW MASS SPECTROMETRY-BASED APPROACHES TO UNRAVEL TREATMENT FAILURE IN CHAGAS DISEASE

Laura-Isobel Mccall
San Diego State University, Chemistry And Biochemistry, San Diego, United States of America

Treatment failure in parasitic diseases can be divided into parasitological treatment failure, where treatment is unable to clear all parasites, and clinical treatment failure, where symptoms do not fully resolve, with or without parasite persistence. Chagas disease is caused by *Trypanosoma cruzi* parasites. Depending on the study, up to 40% of individuals treated with the current standard-of-care antiparasitics benznidazole or nifurtimox remain*T. cruzi*-positive at the end of the treatment, representing parasitological treatment failure. In parallel, some patients still progress to worsening disease or death, even if all parasites are successfully cleared. Thus, both clinical and parasitological treatment failure are major concerns in Chagas disease. We have been addressing both using spatially resolved liquid chromatography-tandem mass spectrometry and single-cell mass spectrometry (SCMS). Using SCMS and low-input liquid chromatography-mass spectrometry, we are quantifying intracellular benznidazole levels in cells that clear *T. cruzi,* in comparison to cells that fail to be cured. With regards to clinical treatment success vs failure, we showed that restoration of parasite-induced metabolic alterations provides an alternative readout of treatment success. In contrast, an inability to restore tissue metabolism supports a mechanism for clinical treatment failure. We observed delayed metabolic restoration with benznidazole, compared to a combined benznidazole and immunomodulator treatment. Certain chemical classes were more resistant to post-treatment restoration. Thus, mass spectrometry is providing insight into mechanisms of parasitic disease treatment failure and paths for improved interventions.

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TOPIC 3: RESISTANCE AND DEVELOPMENT OF DRUGS AND VACCINES
INNOVATIVE TECHNOLOGIES FOR UNRAVELLING AMR IN PARASITES
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GENOMIC SCREENS FOR DRUG RESISTANCE STUDIES IN LEISHMANIA

Marc Ouellette[1], Fereshteh Fani[2], Raouia Fakhfakh[2]
[1]Université Laval, Centre De Recherche En Infectiologie Du Centre De Recherche Du Centre Hospitalier Universitaire De Québec, Québec, Canada, [2]Université Laval, Quebec, Canada

Throughout the years the laboratory has developed a number of dominant positive and dominant negative genomic screens for accelerating our understanding of the mode of action and antimicrobial resistance mechanisms of anti-leishmanial drugs. These screens have contributed significantly to our understanding of resistance to antimonials, miltefosine, and amphotericin B. In this presentation we will describe the application of recent screens on resistance mechanisms to the veterinary purine analogue prodrug allopurinol and to the second line aminoglycoside drug paromomycin in Leishmania infantum. Mutations in glycosomal PEX proteins present in resistant mutants were proven by gene transfection and gene editing to produce resistance to allopurinol. Since purine metabolism is within the glycosome our current hypothesis is that allopurinol activation is deficient in the PEX mutants. Gene transfections and gene editing have also shown that mutations in two kinases (of the STE and CAMK family) found in paromomycin resistant mutants produce resistance to paromomycin but also to all other translational inhibitors tested that are active against Leishmania. Those two kinases are epistatic and we are currently investigating the potential targets of these kinases and how they contribute to the translational machinery. Whole genome screens are helpful to identify relevant pathways involved in mode of action and resistance mechanisms to drugs in Leishmania.

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TOPIC 5: DIAGNOSTICS, HOST-PARASITE INTERACTION AND EMERGING TECHNOLOGIES
PATHOGENESIS OF PLACENTAL MALARIA
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REGULATION OF CHONDROITIN SULFATE A IN RESPONSE TO PLACENTAL MALARIA

Rebecca Reif[1], Melisa Gualdron Lopez[2], Olga Maria Agudelo[3], Samuel Chenge[4], Kimberly Martin[5], Amanda Maestre[3], Francis Kobia[6], Bernard Kanoi[4], Jesse Gitaka[4], Eliana Arango[7], Michael Hawkes[8], Denise Hemmings[1], Stephanie Yanow[5]
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Placental malaria poses a significant threat to child health, causing low birthweight and other poor birth outcomes. During infection with *Plasmodium falciparum*, infected red blood cells (iRBCs) accumulate in the placenta through interactions between a parasite surface antigen and chondroitin sulfate A (CSA) expressed on placental receptors. We examined the fetal response to placental malaria by quantifying CSA in placental tissues from participants with and without placental malaria from a high transmission (Kenya) and a low transmission (Colombia) setting. Placental malaria was diagnosed by PCR and/or histopathology and tissues fixed in formalin and embedded in paraffin. Immunofluorescent staining was performed with antibodies to CSA and quantified using an AI-assisted tissue classifier that segments the placenta into structural compartments. In both cohorts, CSA was significantly increased in the villous core, on the syncytiotrophoblast (ST), and in the intervillous spaces (IVS) of placentas with malaria. Importantly, increased CSA on the ST was inversely associated with infant birthweight. CSA levels were associated with active infections, and we replicated this observation *ex vivo* in placental explant-iRBC co-cultures. In this model, only iRBCs that could adhere to CSA receptors on the explant tissues induced higher levels of CSA. Our findings indicate that iRBC binding to placental receptors induces a fetal response that upregulates CSA levels. Understanding how changes to CSA impact birthweight can help identify interventions to prevent poor birth outcomes from placental malaria.

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TOPIC 5: DIAGNOSTICS, HOST-PARASITE INTERACTION AND EMERGING TECHNOLOGIES
PATHOGENESIS OF PLACENTAL MALARIA
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SYNCYTIOTROPHOBLAST COMPROMISE IN PLACENTAL MALARIA

Julie Moore
University of Florida, Infectious Diseases And Immunology, Gainesville, United States of America

Placental malaria caused by *Plasmodium falciparum* is characterized by inflammation, hypercoagulation, oxidative stress and damage and dysfunction to the syncytiotrophoblast (STB), the key interface between mother and fetus in the villous placenta. Detailed molecular understanding of the dynamics and key components of the maternal response to malaria infection, and how these impact the STB remain poorly understood. In this lecture, we will review current understanding of the origins and mechanisms of STB damage in placental malaria, with a focus on oxidative stress and regulated cell death pathways.

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TOPIC 5: DIAGNOSTICS, HOST-PARASITE INTERACTION AND EMERGING TECHNOLOGIES
PATHOGENESIS OF PLACENTAL MALARIA
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MATERNAL IMMUNE RESPONSE TO P. FALCIPARUM: INSIGHTS FOR VACCINE DEVELOPMENT AGAINST MALARIA IN PREGNANCY

Bernard Kanoi
Mount Kenya University, Centre For Research In Infectious Diseases, Thika, Kenya

Pregnancy-associated malaria (PAM), caused by *Plasmodium falciparum*, poses serious risks to maternal and neonatal health in endemic regions. Our team is conducting a series of studies to better understand immune responses in PAM and identify targets for improved diagnostics and vaccines. Using protein microarrays, we profiled antibody responses to hundreds of *P. falciparum* antigens in pregnant women, revealing gravidity-dependent patterns and highlighting novel antigens associated with multigravida immunity. We are also evaluating PfCSA-L, a conserved protein co-expressed with VAR2CSA, as a potential vaccine candidate due to its strong and consistent antibody recognition. To address the lack of diagnostic tools for detecting placental malaria during pregnancy, we applied proximity extension assays (PEA) to maternal plasma samples. We identified differentially expressed host proteins-including IL-20, FCGR2B, and HO-1-that may serve as biomarkers for PAM. Additionally, we are examining the effects of PAM on placental immune regulation. While macrophage numbers remained stable, infected placentas showed reduced expression of STAT-6 and ANG-1, suggesting altered immune function. These integrated studies provide insights into PAM immunopathogenesis and point to promising avenues for improving diagnosis and prevention of malaria in pregnancy.

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TOPIC 2: TRANSMISSION DYNAMICS, EPIDEMIOLOGY, AND GLOBAL HEALTH
NEGLECTED TROPICAL DISEASES IN A CHANGING WORLD
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NTDS OUT OF THE TROPICS: THE CASE OF ITALY [IN-NTD NETWORK]

Adriano Casulli
EU Reference Laboratory for Parasites (EURL-P) - EU Reference Laboratory for Public Health on Helminths and Protozoa (EURL-PH-HP). ISTITUTO SUPERIORE DI SANITA', Rome, Italy

WHO currently focuses on a diverse group of 21 Neglected Tropical Diseases (NTDs), mainly infectious, caused by virus, bacteria, fungi, parasites (protozoa and helminths), and toxins (snake bite envenoming), all of global public health importance. Although NTDs are most prevalent in low-income countries, they also occur in upper–middle-income countries, including several in Europe. This study provides a quantitative assessment of the most relevant endemic and imported NTDs in Italy and introduces the Italian Network on NTDs (IN-NTD), an alliance of scientific societies, research institutes, foundations, universities, and non-profit organizations committed to combating NTDs. National Hospital Discharge Records (HDRs) were analyzed to quantify major endemic or imported NTDs among residents in Italy over the period 2001-2024. Approximately 49,000 cases were identified with a primary diagnosis of one of the selected NTDs, corresponding to approximately 73,000 hospitalizations. The three most frequently reported NTDs were echinococcosis, leishmaniasis, and schistosomiasis, with 15,218, 7,167, and 4,237 cases, respectively. In terms of hospitalizations, these diseases accounted for 25,131, 14,707, and 5,334 records, respectively. NTDs in Italy exemplify how globalization (including human mobility and the trade of animals and food products) and climate change are reshaping the epidemiology of infectious diseases, posing increasing health challenges beyond tropical and subtropical regions. The Italian Network on NTDs (IN-NTD) was established as a coordinated platform bringing together scientific societies, research institutes, foundations, universities, and non-profit organizations, with the aim of supporting national and international efforts toward the control of these poverty-driven diseases.

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IS050

TOPIC 2: TRANSMISSION DYNAMICS, EPIDEMIOLOGY, AND GLOBAL HEALTH
NEGLECTED TROPICAL DISEASES IN A CHANGING WORLD
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NEW DRUGS AGAINST NTDS - UP FOR PARADIGM SHIFTS IN THE CONTROL OF NTDS [DNTD NETWORK]

Achim Hoerauf[1], Andrea Schiefer[1], Kenneth Pfarr[1], Linda Batsa Debrah[2], Alex Debrah[3], Ute Klarmann-Schulz[1], Samuel Wanji[4], Marc Hübner[1]
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The successful paradigm shift in treating Human African Trypanosomiasis (sleeping sickness) with purely oral regimens demonstrates how innovative therapeutics can accelerate the elimination of neglected tropical diseases. A similar breakthrough is urgently needed for onchocerciasis and other filarial infections. Reaching the WHO elimination targets for onchocerciasis and lymphatic filariasis is severely hindered by the lack of a safe, short-course macrofilaricidal—adult worm killing—drug. While optimized microfilaricidal strategies, such as the triple-drug therapy (IDA) for lymphatic filariasis and moxidectin for onchocerciasis, have significantly improved control efforts, eliminating adult worms remains the critical bottleneck, especially for the endgame scenario. Driven by initiatives funded by the Gates Foundation, initially the AWOL consortium and subsequently the Macrofilaricidal Drug Accelerator program, preclinical research led to the identification of promising clinical candidates. Targeting the endosymbiotic *Wolbachia* bacteria has emerged as a highly effective alternative. Up to date six drug candidates have successfully transitioned into clinical development. However, two of those candidates were terminated following phase 1 (AWZ1066) and phase 2 (Flubentylosin) clinical trials, respectively, being in line with the high attrition rate known in general during drug development. The repurposed veterinary anthelmintics emodepside and oxfendazole are currently assessed as pan-nematode drug candidates for onchocerciasis and soil-transmitted helminth infections in phase 2 clinical trials. Corallopyronin A and fusidic acid present two novel *Wolbachia*-targeting antibiotics, which are currently prepared for phase 1 and phase 2 clinical trials, respectively. This development offers a historic opportunity to close the therapeutic gap and secure the sustainable elimination of filarial infections.

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IS051

TOPIC 1: PARASITE ECOLOGY, BIODIVERSITY, AND TAXONOMY
ENDOSYMBIOTIC ASSOCIATIONS WITHIN PROTISTS: ECOLOGICAL, EVOLUTIONARY AND FUNCTIONAL IMPACTS
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PROTISTS THAT BENEFIT THEIR HOSTS: THE NUTRITIONAL SYMBIONTS OF TERMITES

Gillian Gile
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The eukaryotic superphylum Metamonada includes well-known parasites, such as *Trichomonas vaginalis*, many animal gut commensals, and some mutualists, such as those that help termites digest wood. Symbiosis with termites initiated dramatic changes in cell morphology in several metamonad lineages, including size increases, multiplication of nuclei, and increase in flagellar number to the hundreds or thousands. The speciation rate of termite-symbiotic metamonads also appears to be orders of magnitude greater than the rate in non-termite metamonads, though investigator bias may have magnified the difference. We are interested in the diversity and evolutionary history of termite-symbiotic Metamonada, especially coevolution with their hosts, evolution of wood digestion, and symbiosis with diverse bacterial lineages.

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IS052

TOPIC 1: PARASITE ECOLOGY, BIODIVERSITY, AND TAXONOMY
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SUBCELLULAR PROTEOMICS OF NOVYMONAS ESMERALDAS DEMONSTRATES THE PROTEOMIC ROLE IN ESTABLISHING ENDOSYMBIOSES WITHIN A PARASITIC CONTEXT

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The protist *Novymonas esmeraldas* parasitises the hindguts of the insect *Niesthrea vicentii*, with the assistance of the symbiont bacteria *Ca*. Pandoreaea novymonadis. This internal bacterium supplies its protist host with certain metabolites such as heme and purines, and thus the two organisms form a mutually beneficial relationship to mediate successful parasitism. This association is judged as one recently established, with bacterial number per cell varying dramatically. To better explore this endosymbiotic relationship, we analysed this protist through LOPIT-DC (Localisation of Organelle Proteins via Isotope Tagging through Differential Centrifugation). An analysis of fractional protein distribution of host and endosymbiont, revealed novel organelle relationships, including bacterial contact with the membranes of both the mitochondrion and glycosomes of the host cell. In turn we identified a selection of host proteins specifically targeted to the bacteria, which presumably assist the bacteria with certain metabolic processes no longer encoded within its reduced proteome. Such knowledge helps advance our understanding of how such endosymbiont relationships are initially established, and how the host cell begins the process of gaining control over its bacterial residents.

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MAPPING ORGANELLE INNOVATION: SPATIAL PROTEOMICS AND THE EVOLUTION OF HOST CONTROL OVER ENDOSYMBIONTS

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The transformation of bacterial endosymbionts into genetically integrated organelles required profound structural, physiological, and genetic innovations. Recently established endosymbiotic systems therefore provide rare opportunities to investigate intermediate stages of organelle evolution and the molecular mechanisms underlying host–endosymbiont integration. Two emerging models, the cercozoan *Paulinella chromatophora* and the trypanosomatid *Angomonas deanei*, offer complementary insights into these processes. In *Paulinella*, chromatophores represent the only known case of cyanobacterial endosymbionts evolving into genetically integrated photosynthetic organelles outside the Archaeplastida lineage, characterized by the import of hundreds of nuclear-encoded proteins. Using localization of organelle proteins by isotope tagging following differential ultracentrifugation (LOPIT-DC), we generated the first spatial proteome of a rhizarian organism and uncovered key aspects of chromatophore subcompartmental organization. In parallel, *A. deanei*, a genetically tractable model system, has recently been shown to exert nuclear control over bacterial endosymbiont division. Our work identifies and characterizes previously unknown nucleus-encoded components of this dual-origin division machinery, supporting the existence of a novel evolutionary intermediate between endosymbiont and organelle. Together, these systems provide powerful experimental platforms to dissect the cellular and evolutionary mechanisms driving organelle biogenesis and host control over endosymbiotic integration.

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METABOLIC OUTSOURCING AND GENOME REDUCTION IN A MURINE GUT PROTIST WITH ENDOSYMBIONTS

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Genome reduction in microbial eukaryotes is often associated with metabolic dependency, but the mechanisms underlying this process remain unclear. A murine gut tritrichomonad, *Tritrichomonas casperi*, exhibits a markedly reduced genome relative to its sister species *T. musculus*. Intracellular bacterial endosymbionts are present within *T. casperi* but absent from *T. musculus*, and genome-resolved analyses identify a compact bacterial genomes uniquely associated with the host. Endosymbiont-derived transcripts are detected across host cell populations, indicating persistent intracellular activity. Metabolic reconstruction reveals that key biosynthetic pathways, including nucleotide and cofactor metabolism, are encoded by the endosymbiont but absent from the host genome. These findings support a model in which essential functions are outsourced to intracellular partners, providing a mechanistic framework for symbiosis-associated genome contraction in gut protists.

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TOPIC 4: HOST IMMUNITY, MOLECULAR INTERACTIONS, AND MICROBIOME
FRIEND, FOE, OR FELLOW TRAVELLER? BLASTOCYSTIS AND HOST MICROBIOME INTERACTIONS
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PHYLOGENOMIC ANALYSES OF BLASTOCYSTIS SUBTYPES

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Blastocystis is a strictly anaerobic microbial eukaryote belonging to Stramenopiles. Recently, *Blastocystis* has attracted considerable attention in the scientific community, as it is one of the most common eukaryotic inhabitants of the gut microbiome, including that of humans. However, its specific ecological role in the microbiome and its clinical status as a mutualist, commensal, or parasite of humans remain unclear, with studies often producing conflicting results. *Blastocystis* has a uniquely simple morphology and, while other forms exist, the most commonly observed one is the vacuolar form — a round cell with a very large central vacuole. This makes any inference of taxonomy using morphology impossible and describing new species based on host have been sometimes applied in the past. However, single-gene phylogenetics has shown that isolates often span multiple hosts, and a novel way of tracing *Blastocystis* diversity was implemented: so-called subtypes (STs). The original definition held that up to 96% identity at the nucleotide level along the entire SSU rRNA gene justifies designation as a novel subtype. Here we use phylogenomics of all genome data available in GenBank to reconstruct a “species” tree. We used the state-of-the-art phylogenomic software PhyloFisher to assemble the dataset, explored the tree structure of *Blastocystis* isolates, and compared it with SSU rRNA data to validate STs as a useful tool for delineating lineages of *Blastocystis*.

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FROM COMMENSAL TO CULPRIT? IMMUNE MODULATION AND EPITHELIAL RESPONSES TO BLASTOCYSTIS

Kevin Tan
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Blastocystis, a prevalent protist within the human gut microbiota, is increasingly recognised as a key modulator of gut health and disease progression. Emerging evidence positions *Blastocystis* as an ecosystem engineer of the gut microbiome, capable of reshaping microbial community structure and host–microbe interactions. This talk will explore the dual roles of *Blastocystis* subtypes, which can either exacerbate gut inflammation through disruption of the epithelial barrier or confer protection by enhancing barrier integrity and promoting anti-inflammatory immune responses. The influence of *Blastocystis* on the gut–brain axis will also be examined, with particular attention to associations between colonisation and cognitive and behavioural changes mediated by shifts in gut metabolite profiles. Collectively, these findings underscore the growing recognition of *Blastocystis* as an active participant in microbiome dynamics — far from a mere protistan bystander.

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MAPPING THE BLASTOCYSTIS FOOTPRINT: POPULATION-SCALE METAGENOMES AND MICROBIOME CONTEXT

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The incidence of cardiometabolic diseases is increasing globally, and both poor diet and the human gut microbiome have been implicated[1]. However, the field lacks large-scale, comprehensive studies exploring these links in diverse populations[2]. Here, in over 34,000 US and UK participants with metagenomic, diet, anthropometric and host health data, we identified known and yet-to-be-cultured gut microbiome species associated significantly with different diets and risk factors. We developed a ranking of species most favourably and unfavourably associated with human health markers, called the ‘ZOE Microbiome Health Ranking 2025’. This system showed strong and reproducible associations between the ranking of microbial species and both body mass index and host disease conditions on more than 7,800 additional public samples. In an additional 746 people from two dietary interventional clinical trials, favourably ranked species increased in abundance and prevalence, and unfavourably ranked species reduced over time. In conclusion, these analyses provide strong support for the association of both diet and microbiome with health markers, and the summary system can be used to inform the basis for future causal and mechanistic studies. It should be emphasized, however, that causal inference is not possible without prospective cohort studies and interventional clinical trials.

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TOPIC 4: HOST IMMUNITY, MOLECULAR INTERACTIONS, AND MICROBIOME
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MAKING SENSE OF BLASTOCYSTIS IN THE CLINIC: DIAGNOSTICS, SUBTYPES, AND SYMPTOM CONTEXT

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Although *Blastocystis* was first described in the early twentieth century, investigations into its relationship with human health and disease have gained substantial momentum last decades. Widely detected in humans worldwide, this enigmatic protist continues to present unresolved questions regarding its true clinical significance in humans. The lack of a standardized diagnostic algorithm — from microscopy to advanced molecular techniques — has considerably limited interstudy comparability and consensus. Due to its remarkable genetic diversity — with at least 45 subtypes — a definitive characterization of the relationship between Blastocystis subtypes and clinical manifestations has yet to be established. The frequent detection of *Blastocystis* in both asymptomatic and symptomatic individuals has fueled ongoing debate as to whether this organism should be regarded primarily as a commensal component of the intestinal microbiota rather than a true pathogen. Intriguingly, recent gut microbiome studies have provided emerging evidence supporting its potential role as a biomarker of intestinal eubiosis and a healthy gut ecosystem. Nevertheless, many critical questions remain unanswered regarding its ecological interactions, functional modulation, and overall impact within the intestinal microbiome. A comprehensive understanding of the complex clinical and ecologic role of *Blastocystis* will likely require large-scale, prospective, longitudinal case–control studies supported by accessible advanced molecular methodologies and integrative multi-omics approaches. Collaborative cohort studies conducted within a global framework may not only help unravel the clinical mysteries surrounding *Blastocystis*, but may also reveal its potential balancing, modulatory, and perhaps even restorative functions within the gut ecosystem, thereby opening new perspectives for microbiome-based therapeutic strategies.

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TOPIC 5: DIAGNOSTICS, HOST-PARASITE INTERACTION AND EMERGING TECHNOLOGIES
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ADVANCED ORGANOID INFECTION MODELS IN GIARDIA PATHOGENESIS

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Background and aims
Giardia duodenalis colonizes the small intestine and induces epithelial barrier dysfunction, malabsorption, and diarrhea. Although infections are common, the mechanisms underlying host epithelial injury remain incompletely understood. Human duodenal organoid-derived monolayers (ODMs) provide an advanced and physiologically close system to study host-parasite interactions. **Methods**
Epithelial barrier integrity was monitored using transepithelial electrical resistance (TEER)-measurements as well as analysis of tight junction components. Human ODM transcriptional responses to *Giardia* infection were captured by RNA sequencing. The role of individual factors in barrier dysfunction was investigated through the generation of CRISPR-knockouts in both organoids and parasites. **Results**
ODMs reproduced key physiological properties of the duodenal epithelium and displayed a response to *Giardia* infection that followed a sequential pathogenic cascade. RNA sequencing revealed that cAMP/PKA/CREB signaling is a key step in this sequence that eventually leads to loss of epithelial barrier homeostasis. TNF-alpha transcripts were highly induced but, surprisingly, in the respective gene knockout-ODMs, kinetics of barrier function loss remained unaltered. Physical prevention of parasite attachment and the use of attachment-impaired quadruple knockout parasites identified the role of parasite attachment in epithelial barrier breakdown. **Conclusions**
Advanced human organoid-derived infection models recapitulate *Giardia*-induced epithelial dysfunction and provide a powerful platform for mechanistic studies of host-parasite interactions. **References** *Holthaus, D., et al., Dissection of Barrier Dysfunction in Organoid-Derived Human Intestinal Epithelia Induced by Giardia duodenalis. Gastroenterology, 2022. Hagen, K.D., et al., Giardia's domed ventral disc architecture is essential for attachment and contributes to epithelial barrier disruption. Mol Biol Cell, 2025.*

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TOPIC 5: DIAGNOSTICS, HOST-PARASITE INTERACTION AND EMERGING TECHNOLOGIES
UNRAVELLING PAHTOGENESIS OF GIARDIASIS FROM IN VITRO AND IN VIVO PERSPECTIVE
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IN VITRO AND IN VIVO MODEL TO DISSECT THE IMPACT OF GIARDIAVIRUS ON GIARDIASIS

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Giardia duodenalis is a protozoan parasite able to infect the upper-small intestine of mammals causing giardiasis, a diarrheal disease, with symptoms ranging from asymptomatic to acute or chronic. The molecular mechanisms governing these clinical manifestations are yet to be fully understood. Among factors potentially modulating giardiasis outcomes, the parasite infection with *Giardia lamblia virus* (GLV), a dsRNA non-enveloped virus of the family *Totiviridae,* must be considered. Here we summarize our *in vitro* infection studies to dissect the role of GLV on *G. duodenalis*/host interaction by using WBC6, infected or not with two different GLV strains, on long term (72h) bi-culture enterocytes monolayer of human CaCo2/TC7 and HT29 tumor-derived cell lines. We observed that Giardia-induced intestinal barrier alterations are well replicated in this *in vitro* model (e.g. TEER decrease, Papp increment, disruption of tight junction, differential response at gene level) and that GLV infection mitigates in a viral strain-dependent fashion enterocytes response. The *in vitro* observations were also complemented *in vivo* using a well-established gerbil model of giardiasis (28 days) showing similar GLV dependent effect on both intestinal epithelium and intestinal trophozoite load. Our data provides robust experimental evidence pointing on GLV infection as a factor potentially attenuating giardiasis outcome opening a new frontier for biocontrol of giardiasis.

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TOPIC 5: DIAGNOSTICS, HOST-PARASITE INTERACTION AND EMERGING TECHNOLOGIES
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MODELS OF GUT MICROBIOTA BIOFILMS AND HOST -GIARDIA INTERACTIONS

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Gut microbiota predominantly live as adherent biofilms rather than free-living microbes. Integrity of these polymicrobial communities are at the core of gut and extraintestinal homeostasis. Here we present findings that demonstrate how *Giardia* disrupts the intestinal mucus barrier and gut microbiota biofilms to cause disease. *Giardia* cysteine proteases, particularly cathepsin B, directly degrades the major intestinal mucin protein MUC2 (1), while activation of Proteinase-Activated Receptor-2 (PAR-2) on goblet cells induces hypersecretory mucus responses that lead to mucus depletion (2). The infection disrupts mucus glycosylation in a microbiota dysbiosis -dependent manner (3). *Giardia* breaks down microbiota biofilms and facilitates invasion of otherwise non-pathogenic commensal bacteria (4). These pathobionts trigger tissue inflammatory responses in germ-free mice (4). In *Caenorhabditis elegans,* feeding *Giardia* with non-pathogenic *E. coli* altered the expression of over 170 genes in the bacteria, induced colonization by the bacteria in the nematode intestine, and was lethal to the worm (5). *Giardia* extracellular vesicles alone promote the adhesion and transepithelial invasion of otherwise commensal Enterobacteriaceae while inhibiting their biofilm formation (6). The findings reveal a novel trans-kingdom communication disrupting host-microbial homeostasis within the gut ecosystem to cause disease.

References: 1. Amat C et al. Am. J. Pathol. 2017; 2. Fekete E et al. Int. J. Parasitol. 2022; 3. Fekete E et al. Gut Microbes. 2024 16(1):2412676; 4. Beatty J et al. Int. J. Parasitol. 2017; 5. Gerbaba T et al, Am. J. Physiol. 2015; 6. Siddiq A et al, J. Extracell. Biol. 2023

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IS062

TOPIC 5: DIAGNOSTICS, HOST-PARASITE INTERACTION AND EMERGING TECHNOLOGIES
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EPITHELIAL REMODELING UPON GIARDIA INFECTION: HYPOXIC RESPONSE AND TUFT CELL MODULATION

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Giardia duodenalis is a prevalent intestinal protozoan that disrupts epithelial barrier integrity, alters mucus composition, contributes to microbiota dysbiosis, and reshapes mucosal immunity. Increasing evidence suggests that epithelial adaptation to parasitic infections extends beyond immune signaling and involves metabolic and sensory remodeling of the intestinal mucosa. Here, we present evidence of epithelial responses to *Giardia* infection: hypoxia signaling and tuft cell response. Hypoxia-inducible factor (HIF) is a central regulator of epithelial adaptation to low oxygen tension, coordinating cellular metabolism, barrier maintenance, and inflammation. Using human intestinal epithelial cells infected with *G. duodenalis* GS/M, we identified a transient epithelial hypoxic signature during infection under normoxic conditions. Infection induced time-dependent stabilization of HIF-1α and upregulated HIF target genes associated with cellular stress responses (*VEGFA, ANKRD37, GADD45A*) and glycolytic metabolism (*HK2, LDHA*). Metabolomic analyses revealed altered glycolytic intermediates (glucose-6-phosphate, pyruvate, and lactate), indicating metabolic reprogramming during infection. We also examined the contribution of tuft cells in the epithelial response against *Giardia* in a murine model of giardiasis. Infection induced region- and time-dependent increases in tuft and goblet cell numbers within the small intestine, accompanied by elevated expression of tuft cell markers and chemosensory receptors and goblet cell-associated genes. Interestingly, tuft cell-deficient (*Pou2f3-/-*) mice exhibited reduced parasite burden and decreased goblet cell counts, suggesting that tuft cells may facilitate aspects of *Giardia* colonization. Together, hypoxic responses and tuft cell-mediated pathways during giardiasis reveal epithelial remodeling involving metabolic adaptation and sensory epithelial signaling. Understanding these adaptations may provide insight into parasite persistence and barrier dysfunction.

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TRANSLATING CONSEQUENCES OF GIARDIA ON EARLY DEVELOPMENT: FROM BENCH TO BEDSIDE

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Early childhood Giardia infection associates with impaired child growth and potential cognitive and other developmental sequelae. These associations, however, are uncoupled from diarrhea or other typical giardiasis manifestations. They also co-occur with childhood malnutrition, multiple intestinal pathogen exposures, and environmental enteric dysfunction (EED). The underlying pathogenesis leading to these longterm sequelae are poorly understood. Through the use of experimental models linked with epidemiological risk factors, investigations are identifying key pathways that underly Giardia-mediated growth impairment. Emerging from this work are diet-dependent nutrient-metabolic and intestinal disruptions that are driven by complex interactions with intestinal microbiota in the undernourished host.

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PLENARY SESSION
ACCELERATING ANTIBIOTIC DISCOVERY WITH AI
08-18-2026 8:30 AM - 9:30 AM

ACCELERATING ANTIBIOTIC DISCOVERY WITH AI

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For a century, antibiotic discovery relied on labor-intensive “dirt mining,” a trial-and-error paradigm too slow to keep pace with escalating resistance. Over the past decade, our laboratory has helped replace this approach with digital discovery, using artificial intelligence (AI) models to mine the code of life itself. Although AI’s impact on biology and medicine is only beginning to emerge, we have shown that antibiotics can be designed with AI, yielding molecules with strong efficacy in preclinical models. By mining the human proteome, we uncovered thousands of novel antimicrobial peptides that constitute a new branch of immune effectors. We then hypothesized that similar compounds might be found across evolution. This AI-driven approach led to the discovery of molecules from extinct organisms such as Neanderthals and the woolly mammoth, launching the field of molecular de-extinction. To explore the full tree of life, we then turned to the global microbiome, identifying nearly one million new antibiotic molecules made freely available to encourage worldwide collaboration. By computationally analyzing thousands of microbial and human metagenomes, we discovered a vast array of new agents, including prevotellin-2. Most recently, we digitally mined archaea, identifying a new class of antibiotics called archaeasins. Finally, I will introduce our latest AI models—APEX, ApexGo, ApexDuo, and ApexOracle—which enable sequence-to-function prediction, antibiotic optimization, and rapid response solutions. Collectively, our efforts have dramatically accelerated discovery, reducing the time to identify preclinical candidates from years to hours. I believe AI-enabled advances will help control antibiotic resistance, infectious outbreaks, and future pandemics.

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IS065

TOPIC 5: DIAGNOSTICS, HOST-PARASITE INTERACTION AND EMERGING TECHNOLOGIES ADVANCEMENTS IN HELMINTH GENOMICS
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ASSEMBLY MATTERS: HOW THE BUILD CHANGES BIOLOGY

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If genomes are our primary lens on parasite biology, then the way we build them determines what we are able to see. Genome assembly is often treated as a technical prelude to biology. Here, we argue that it is itself a biological decision, because assembly choices determine which genes and genomic structures are visible, and which are effectively lost to downstream analysis. Using multiple assemblies of three nematode genomes, we show that assembler choice alone alters gene family composition, including families central to immunomodulation and anthelmintic discovery. These differences arise despite similar global assembly metrics, indicating that standard benchmarks do not capture biologically meaningful variation. Extending this, we demonstrate that even trivial perturbations to input data can change biological conclusions. Shuffling FASTQ read order has little impact on global assembly statistics, but alters the recovered repertoire of highly conserved genes. This raises concerns about the stability of gene family analyses built on a single assembly and, more broadly, about the reproducibility of comparative genomics. These observations sit alongside broader inconsistencies in structural variant detection. Our benchmarking studies show that tool performance varies with variant class, coverage, and data type, while even read order can influence variant calls in long-read pipelines. Together, these results emphasise a simple point: assemblies and downstream analyses are not neutral. The way we build genomes shapes the biology we infer from them, and we need to treat that choice with the same care as any experimental design.

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IS066

TOPIC 5: DIAGNOSTICS, HOST-PARASITE INTERACTION AND EMERGING TECHNOLOGIES ADVANCEMENTS IN HELMINTH GENOMICS
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HIGH-QUALITY CHROMOSOMAL GENOMES FOR ASCARIDS AND HOOKWORMS

Erik Andersen
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To map and discover the genes that cause drug resistance, underlie targets for vaccine research, or influence host-pathogen interactions, we must understand the gene content and the complete chromosomal extent of a genome. Numerous projects in each of these areas have been stymied by draft genomes that comprise many discontigous fragments and incomplete gene models. Very recent progress in long-read genome sequencing along with high-throughput DNA contact maps have enabled the construction of highly contiguous genomes. Some even stretch from telomere to telomere without any gaps. These genomes combined with long-read RNA sequencing can be used to make high-quality gene model annotations to defined the gene content of a species. We will present our progress focused on ascarids that infect poultry and rodents and on hookworms that infect dogs. These parasite species occupy experimentally tractable niches that can enable drug resistance gene mapping, optimization of targets for vaccine research, and host-pathogen interactions.

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IS067

TOPIC 5: DIAGNOSTICS, HOST-PARASITE INTERACTION AND EMERGING TECHNOLOGIES ADVANCEMENTS IN HELMINTH GENOMICS
08-18-2026 10:00 AM - 12:00 PM

A MULTI-STAGE SINGLE CELL ATLAS FOR THE MODEL HELMINTH HAEMONCHUS CONTORTUS

Sarah Buddenborg[1], Viacheslav Osokin[1], Anna Bewsher[2], Alison Morrison[3], Dave Bartley[3], Stephen Doyle[2]
[1]McGill University, Institute Of Parasitology, Sainte-Anne-de-Bellevue, Canada, [2]Wellcome Sanger Institute, Cambridge, United Kingdom, [3]Moredun Research Institute, Edinburgh, United Kingdom

Identifying and understanding the genetic mechanisms underpinning key life-history traits in helminths offers the potential to advance our knowledge of fundamental parasite-specific life processes and discover novel drug targets. However, helminth research has primarily focused on bulk RNA-seq of a limited number of species and life-cycle stages, providing only a homogeneous view of gene expression. This approach has limited our understanding of helminths in an evolutionary context as well as the complexities of sex- and stage-specific transcriptional responses. Single-cell and single-nuclei transcriptomics is a revolutionary technology that enables the identification of cell types, cellular pathways, and molecular targets relevant to the development, growth, and spread of parasitic worms. We generated a cell atlas from whole transcriptomes of ~200,000 nuclei from *Haemonchus contortus* infective larvae (iL3), exsheathed L3 (exL3), 10-day female and male L4 larvae, and female and male adult worms. We identified 22 major tissue-type clusters within our data including anterior and posterior intestine, muscle, neuronal, and germinal. A select number of clusters, including neuronal and muscle, were sub-clustered to achieve greater definition of cell-types. Infective larvae and exsheathed larvae show remarkable homogeneity cell transcriptional states and lower transcriptional activity, compared to L4 and adult stages, likely reflecting their reduced metabolic and developmental activity. This atlas also provides a opportunity for target-based drug discovery at the single-cell level. Together, these data reveal new insights into parasite biology and prioritize novel targets and drugs for functional assays.

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IS068

TOPIC 5: DIAGNOSTICS, HOST-PARASITE INTERACTION AND EMERGING TECHNOLOGIES ADVANCEMENTS IN HELMINTH GENOMICS
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FIGHTING LITTLE DRAGONS TOOTH AND NAIL: THE TRIALS, TRIBULATIONS, AND SUCCESSES OF APPLYING POPULATION GENETICS TO A PARASITE ON THE VERGE

Elizabeth Thiele[1], Jessica Ribado[2], Maryann Delea[3], Robert Arthur[4], Henry Johnston[4], Alexandra Sack[3], Yujing Zhao[3], Kathryn Schaber[3], Obiora Eneanya[3], Adam Weiss[3]
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Guinea worm disease, or dracunculiasis, is a neglected tropical disease caused by the parasitic nematode *Dracunculus medinensis*. Until recently, it was believed that Guinea worm disease was primarily a disease of humans. However, detection of emergent worms amongst domesticated dogs in Chad in 2012 and subsequent confirmation of the worms as *D. medinensis* substantiated historical accounts that non-human animals can also serve as definitive hosts for Guinea worm. Intensified surveillance in the other remaining Guinea worm-endemic countries also found that non-human transmission is not limited to Chad, with laboratory-confirmed Guinea worm infections having since been detected in domesticated and/or wild mammals in all currently endemic countries. Early population genetic analyses found no evidence that transmission of Guinea worms was isolated by host species, suggesting that animals and humans both pose transmission risk to the other. Moreover, the amount of nucleotide variation observed in both whole genome and targeted mitochondrial genome sequencing was consistent with the conclusion that transmission among domestic animals (particularly dogs) was neither a new nor rare occurrence. To garner a more complete understanding of the extent of Guinea worm transmission and population dynamics, as well as to assist Guinea worm eradication programs with evaluating the scope of their surveillance efforts, the decision was made to attempt sequencing on every Guinea worm detected by national surveillance networks, resulting in over 20,000 worms having been processed to date. Emerging results and the lessons learned from this exhaustive sampling and sequencing effort will be presented here.

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IS069

TOPIC 4: HOST IMMUNITY, MOLECULAR INTERACTIONS, AND MICROBIOME
INFLAMMASOMES AND INNATE IMMUNITY TO PROTOZOAN PARASITES
08-18-2026 10:00 AM - 12:00 PM

VECTOR-SPECIFIC ACTIVATION OF INFLAMMASOMES SUPPORT LEISHMANIA ESTABLISHMENT AFTER TRANSMISSION BY SAND FLY BITES

Eva Iniguez[1], Pedro Cecilio[1], Somaditya Dey[1,2], Tiago Serafim[1], Serena Doh[1], Jesus Valenzuela[1], Shaden Kamhawi[1]
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Phlebotomine sand flies transmit leishmaniasis with unparalleled efficiency. Previously, we have demonstrated that bites from a *Leishmania*-infected sand fly initiate a vector-specific immune response from the host. These include an early inflammasome-dependent production of IL-1β by neutrophils, which sustains cellular infiltration at the bite site and creates an acute host inflammatory milieu. This inflammation is later controlled by the induction of heme oxygenase-1 specifically in response to prolonged bleeding during vector feeding, with participation from skin-residing iron recycling macrophages. These two events contribute to parasite establishment and enhanced virulence of vector-transmitted parasites. Adding to the complexity of this specific vector-induced host immune response, we recently found that the alarmin IL-33 is also abundant at the bite site within two hours of *L. mexicana*- or *L. major*-infected bites but is absent following needle challenge. Intriguingly, we also detect a p40 NT-GSDMD fragment resulting from noncanonical processing of GSDMD by yet-to-be-identified cells at the bite site indicative of pore formation for IL33 release. Skin cells collected at 6 hours post-infected bite establish that a “damaged” population of erythrocytes and neutrophils are positive for IL-33. Live imaging by intravital microscopy shows swarming neutrophils rapidly engulfing erythrocytes which may account for IL-33+ neutrophils. We are currently working on elucidating the cellular source/s of IL-33 and the p40 NT-GSDMD fragment, and determining the impact of having IL-33, associated with type 2 immunity, in abundance at the bite site on shaping long-term pathogenicity of sand fly-initiated leishmaniasis.

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IS070

TOPIC 4: HOST IMMUNITY, MOLECULAR INTERACTIONS, AND MICROBIOME
INFLAMMASOMES AND INNATE IMMUNITY TO PROTOZOAN PARASITES
08-18-2026 10:00 AM - 12:00 PM

NLRP3 INFLAMMASOME AS A PATHOGEN SENSOR FOR INVASIVE ENTAMOEBA HISTOLYTICA

Kris Chadee
University of Calgary, Microbiology, Immunology And Infectious Diseases, Calgary, Canada

BACKGROUND: *Entamoeba histolytica* (*Eh*) is an extracellular protozoan parasite of the human colon, which occasionally breaches the intestinal barrier. Eradicating ameba that invades is essential for host survival. A defining but uncharacterized feature of amebic invasion is direct contact between ameba and host cells. This event corresponds with a massive pro-inflammatory response. To date, pathogen recognition receptors (PRR) that are activated by contact with viable *Eh* are unknown. METHODS: We quantified inflammasome activation in macrophages using well-defined pharmacological inhibitors of the NLRP3 pathway and in macrophages that were genetically deficient for inflammasome components. Mechanistic studies investigated if the danger signal ATP could trigger the inflammasome by blocking the P_2X_7 receptor and by enzymatic depletion of extracellular ATP. Inflammasome activation was visualized in real-time using confocal microcopy with cell-permeable caspase-1 probes. RESULTS: Here we show that the innate immune system responds in a qualitatively different way to contact with viable *Eh* versus soluble ligands produced by viable or dead ameba. This unique *Eh* Gal-lectin contact-dependent response in macrophages was mediated by activation of the inflammasome. Soluble native Gal-lectin did not induce inflammasome activation, but was sufficient for transcriptional priming of the inflammasome and non-inflammasome-dependent pro-inflammatory cytokine release. CONCLUSIONS: These results suggest that the inflammasome is a pathogenicity sensor for invasive *Eh* and identify for the first time a PRR that specifically responds to contact with intact parasites in a manner that accords with scale immune response to parasite invasion.

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IS071

TOPIC 1: PARASITE ECOLOGY, BIODIVERSITY, AND TAXONOMY
INTERNATIONAL WORKSHOP FOR ARCTIC PARASITOLOGY VII: WHY WE SHOULD CONTINUE TO CARE ABOUT PARASITES AT THE POLES
08-18-2026 10:00 AM - 12:00 PM

PARASITES ON THE MOVE: A HITCHHIKER'S GUIDE TO CARIBOU AND REINDEER ACROSS A CHANGING ARCTIC

Rebecca Berg
Ilisimatusarfik University, Greenland Center For Health Research, Nuussuaq, Greenland

Rapid environmental change in the Arctic is altering the conditions under which parasites, hosts, and vectors interact, creating new opportunities for parasite transmission and persistence. While climate warming is often highlighted as a key driver of parasite emergence, changes in host distributions, migration patterns, species introductions, and community composition are also influencing parasite dynamics across the Arctic. In this context, parasites can be viewed as hitchhikers, travelling through Arctic ecosystems as host populations shift, species interactions change, and new ecological connections emerge. This presentation explores how a changing Arctic influences parasite dynamics in caribou and reindeer. Drawing on examples from Greenland and across the circumpolar North, I will discuss how endemic parasite systems may respond to environmental change, how host movements and community composition influence parasite distributions, and how changing ecological conditions may create new opportunities for parasite transmission and persistence. Particular attention will be given to parasite groups that exemplify different transmission pathways in Arctic ecosystems, including gastrointestinal helminths, vectorborne nematodes, and meatborne apicomplexan parasites. Together, they illustrate links between wildlife health, ecosystem processes, food security, and One Health perspectives. By considering parasites as both indicators and participants of ecological change, this talk aims to provide a contemporary overview of the challenges and opportunities facing Arctic parasitology in a rapidly changing North.

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TOPIC 1: PARASITE ECOLOGY, BIODIVERSITY, AND TAXONOMY
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08-18-2026 10:00 AM - 12:00 PM

ZOONOTIC PARASITES IN THE ARCTIC – ONE HEALTH PERSPECTIVES

Pikka Jokelainen
Statens Serum Institut, One Health And International Collaborations, Copenhagen, Denmark

Zoonotic parasites circulate in the Arctic at the interfaces of animals, humans, food systems, and rapidly changing environments. Climate-driven, environmental and societal changes may influence parasite distribution, persistence, transmission dynamics, and exposure pathways. Examples from Arctic parasitology illustrate the importance of integrated One Health surveillance, interdisciplinary research, and cross-sectoral collaboration, as well as attention to local traditions and knowledge systems alongside scientific approaches.

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IS073

TOPIC 1: PARASITE ECOLOGY, BIODIVERSITY, AND TAXONOMY
INTERNATIONAL WORKSHOP FOR ARCTIC PARASITOLOGY VII: WHY WE SHOULD CONTINUE TO CARE ABOUT PARASITES AT THE POLES
08-18-2026 10:00 AM - 12:00 PM

WHERE DID ARCTIC PARASITES COME FROM?

Antti Oksanen
Finnish Food Authority, Oulu, Finland

Endoparasites of homeothermic animals live in a rather stable environment at around 38 °C. However, free-living stages on the ground and developing larvae in vectors face the challenges of the different zones of the world. In the tropics, heat and drought, and correspondingly in the Arctic, cold may devastate parasites in their infancy. Many parasites have global distribution, but there are also some arctic terrestrial mammal parasites having their closest relatives in the tropics, while the temperate regions would be generally devoid of them. The reindeer/caribou (*Rangifer tarandus*) is a circumpolar cervid species present in most of the inhabitable areas of the Arctic and Subarctic. Its parasite fauna is rich and varied, consisting of species with relatives in other cervids in the temperate zone. Reindeer also harbour the cyst-forming coccidian genus *Besnoitia* and the pentastomid *Linguatula*, which are generally tropical parasites. The ongoing climate change and human actions have introduced vector-borne nematodes to emerge in reindeer populations. Parasites in the arctic/subarctic reindeer have various origins. The speciation of *Rangifer* occurred during the Pleistocene, Ice Age, when most of the current reindeer population ranges were covered by ice. Reindeer co-existed with many animal species/genera currently limited to the tropics. Hypothesis is that host switches took place from hosts now regarded as tropical, and perhaps also vice versa. Most probably, molecular phylogenetic studies will soon shed light on the currently dim origin of arctic parasites.

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IS074

TOPIC 1: PARASITE ECOLOGY, BIODIVERSITY, AND TAXONOMY
INTERNATIONAL WORKSHOP FOR ARCTIC PARASITOLOGY VII: WHY WE SHOULD CONTINUE TO CARE ABOUT PARASITES AT THE POLES
08-18-2026 10:00 AM - 12:00 PM

DETECTING, TRACKING AND MITIGATING EMERGING WILDLIFE PATHOGENS IN THE ARCTIC

Rachele Vada[1], Russell Akeeagok[2], Association Kugluktuk Angoniatit[3], Committee Olokhaktomiut Hunters And Trappers[4], Organization Ekaluktutiak Hunters And Trappers[5], Fabien Mavrot[1], Mitch Campbell[6], Dayna Goldsmith[1,7], Hadjira Hamou[8], Naima Jutha[9], Lisa-Marie Leclerc[2], Paul Macdonald[10], Amanda Melin[8,11,12], Danielle Nowosad[1], Jamie Rothemburger[1,7], John Soghigian[1], Brian Stevens[13,14], Susan Kutz[1]
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The Community-Based Wildlife Health Surveillance (CBWHS) program is a partnership that has, for over a decade, brought together researchers, government bodies, and Indigenous communities in the Canadian Arctic to monitor the health of Arctic ungulates, mainly caribou (*Rangifer tarandus*) and muskoxen (*Ovibos moschatus*). Through the leadership and participation of Indigenous harvesters and community Hunters and Trappers organizations, it has allowed for early detection of and rapid response to emerging pathogens. Outputs inform decision-making processes on caribou and muskox conservation and inform public health guidelines for wildlife harvesters. In late summer 2023, harvesters in Kugluktuk, Nunavut, identified a novel disease syndrome in caribou, later attributed to filarioid nematodes. It raised urgent concerns for food safety and security. The network enabled a rapid and coordinated response to diagnose the cause of the disease and evaluate the food safety risk. This rapidly restored confidence in country food. Earlier, in 2016, the same network facilitated the detection of one of the first cases of brucellosis in muskoxen, prompting expanded serological surveillance and revealing a broader circulation of the pathogen than previously recognised. The effectiveness of this CBWHS program rests on three core pillars: (i) a well-established and efficient communication network among partners, (ii) trust built within communities and among all partners,

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and (iii) access to robust and rapid diagnostic tools. In a rapidly changing, climate-sensitive ecosystem, this model highlights how deeply integrated partnerships—grounded in local knowledge and supported by scientific capacity—are essential for early detection, rapid response, and effective mitigation of wildlife diseases.

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IS075

TOPIC 3: RESISTANCE AND DEVELOPMENT OF DRUGS AND VACCINES
ANTHELMINTHICS: CURRENT DRUGS AND VACCINES
08-18-2026 10:00 AM - 12:00 PM

NEXT-GENERATION TREATMENTS FOR SOIL-TRANSMITTED HELMINTHS

Jennifer Keiser
Swiss Tropical and Public Health Institute, Allschwil, Switzerland

Millions of people are infected with soil-transmitted helminths, resulting in anaemia, malnutrition, growth stunting, and cognitive deficits. The current control strategy of the World Health Organization to reduce the burden and morbidity of these infections is preventive chemotherapy, i.e. the administration of the two benzimidazoles (albendazole and mebendazole) using single, oral doses to at risk populations, mainly children without prior diagnosis. Both benzimidazoles are highly efficacious against *Ascaris lumbricoides*, but only albendazole is efficacious against hookworm, and both drugs are unsatisfactory against *Trichuris trichiura* infections. Drug combinations as albendazole-ivermectin and albendazole-moxidectin result in superior efficacy against *T. trichiura*, but not in all settings. The newly discovered *Trichuris incognita* might be responsible for the varying efficacy. Therefore, there is a need to engage in research and development for new anthelminthic drugs. In this presentation I will give an overview of promising new drugs under development for soil-transmitted helminthiasis, including emodepside, oxfendazole and oxantel pamoate.

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TOPIC 3: RESISTANCE AND DEVELOPMENT OF DRUGS AND VACCINES
ANTHELMINTHICS: CURRENT DRUGS AND VACCINES
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TRANSIENT RECEPTOR POTENTIAL CHANNELS AND ANTHELMINTIC RESISTANCE

Jonathan S. Marchant
Medical College of Wisconsin, Cell Biology, Neurobiology & Anatomy, Milwaukee, United States of America

Clinical treatment of schistosomiasis relies heavily on praziquantel (PZQ), such that the continued efficacy of this drug is paramount for control and elimination strategies. An obvious threat to this strategy is the emergence of resistance to PZQ, which now has been widely deployed in mass drug administration campaigns. Is PZQ resistance here, near, or is there nothing to fear? This has been a recurring topic of debate. A relevant development has been the identification and validation of the parasite target of PZQ. PZQ activates a transient receptor potential ion channel named $TRPM_{PZQ}$ in parasitic flatworms. The identification of $TRPM_{PZQ}$ has sharpened focus in understanding potential molecular mechanisms through which ‘target-based’ or ‘non-target based’ resistance to PZQ could emerge. Such mechanisms can now be the focus for surveillance in natural worm populations, and new chemotherapeutic vulnerabilities prioritized with the goal of delivering novel classes of therapeutics. In this talk, I will discuss ongoing efforts to understand schistosome sensitivity to PZQ at the molecular level, evaluate trajectories that could attenuate PZQ efficacy (and also how to circumvent them), and integrate this insight with community-wide efforts working to monitor PZQ efficacy in the field.

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IS077

TOPIC 3: RESISTANCE AND DEVELOPMENT OF DRUGS AND VACCINES
ANTHELMINTHICS: CURRENT DRUGS AND VACCINES
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A REVERSE VACCINOLOGY APPROACH TO CONTROL SCOUR WORMS IN SHEEP

Alasdair Nisbet[1], Tom Mcneilly[1], Dan Price[1], David Smith[1], Stewart Burgess[1], Collette Britton[2], Sam Duncan[2], Tatum Scharkie[3], Nicholas Andronicos[3]
[1]Moredun Research Institute, Edinburgh, United Kingdom, [2]University of Glasgow, Glasgow, United Kingdom, [3]University of New England, Armidale, Australia

Trichostrongylid scour worms, *Trichostrongylus* and *Teladorsagia,* are not blood-feeding parasites so the "parasite gut antigen" approach used for *Haemonchus contortus* is unsuitable. Instead, vaccines need to be developed from analysis of naturally acquired immunity and host-parasite interactions. The project described here is a 5-year project across 3 partners integrating current, multi-institutional expertise in small ruminant parasitology, immunology and vaccine formulation with activities in three major subprograms. The first subprogram establishes how acquired immunity develops and operates early after parasites are ingested and established. We are now 3 years into this programme and have developed the serological, histological and transcriptomic tools and datasets required to understand the immunological effector landscape that we will need to recreate with a vaccine. The second subprogram focusses on antigen discovery. This has involved the use/generation of chromosome-level genome assemblies for both species from which to produce datasets of predicted excretory/secretory protein antigens, alongside the transcriptomic and proteomic resources to verify and augment these predictions. From this combined resource of secreted antigen candidates, we have produced large-scale peptide arrays and probed them with immune sera from both parasite species. The data from these arrays, as well as the data from *in silico* predictions of antigenicity, stage-specific RNA and protein expression, host-parasite interaction studies and RNAi screening have been fed into the prediction of the best antigens. In the final subprogram**,** we have determined the optimal vaccine formulations, combined with candidate parasite antigens, which will actually generate those protective responses in the challenge studies planned for 2027.

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IS078

TOPIC 3: RESISTANCE AND DEVELOPMENT OF DRUGS AND VACCINES
ANTHELMINTHICS: CURRENT DRUGS AND VACCINES
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THE TOXIFICATION OF A COMMERCIAL NEMATICIDE BY WORM AND HUMAN CYTOCHROME P450S

Peter Roy
University of Toronto, Molecular Genetics, Pharmacology & Toxicology, Toronto, Canada

Tioxazafen is a nematicide that was withdrawn from the market because handlers purportedly developed rashes after working with seeds coated with a tioxazafen-laced cocktail. In the first half of the talk, I will show that tioxazafen is bioactivated into toxic products by nematode and human cytochrome P450s. Through systematic analyses, we found that bioactivated tioxazafen disrupts proteosome function, leading to the accumulation of the NFR1 transcription factor ortholog SKN-1A in the nematode *C. elegans*, which in turn drives a 'bounce-back' transcriptional up-regulation of proteosome components. Genetic upregulation of the *C. elegans* proteasome supresses tioxazafen's lethality, indicating that proteotoxicity is a key contributor to death. A survey of human P450s using a yeast-based assay revealed that skin-expressed CYP1A1 toxifies tioxazafen and may account for the reaction to tioxazafen-coated seeds. We also found that rabbit CYP1A1 fails to bioactivate tioxazafen, which may explain the pre-market failure to detect robust adverse skin reactions. Our work highlights vulnerabilities in pre-market toxicological assays. In the second half of the talk, I will show how we have extended our yeast-based P450 assays to investigate the ability of all *C. elegans*, Human and mosquito P450s to toxify small molecules in a massively parallelled fashion. In this way, we are able to identify candidate pro-nematicides, pro-insecticides and tools to study human P450s in a highly efficient manner.

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IS079

TOPIC 2: TRANSMISSION DYNAMICS, EPIDEMIOLOGY, AND GLOBAL HEALTH
EMERGING ISSUES IN CLINICAL GIARDIASIS: FROM PARASITE RESISTANCE TO CHRONIC DISEASE
08-18-2026 10:00 AM - 12:00 PM

ONE HEALTH IN GIARDIASIS

Marco Lalle
Istituto Superiore di Sanità, Infectious Diseases, Rome, Italy

The flagellated protozoan *Giardia duodenalis* is responsible of giardiasis, a worldwide distributed gastrointestinal infection of mammals, including humans. Giardiasis can present with a wide range of clinical manifestations from asymptomatic to acute diarrhea, it can become chronic or eventually lead to post-infection sequelae, such as irritable bowel syndrome. Recurrent infections in children and young animals have been clearly linked with growth faltering. *G. duodenalis* displays considerable genetic variation with eight (A to H) major genetic groups, or assemblages, being identified. Whereas, assemblages A and B have been found in both humans and animals, assemblages C to H are mostly host-specific, and rarely reported in humans. Additional genetic variability and geographical origin of isolates within assemblage A and B have been also associated to clinical manifestations and treatment refractory infections. Infection occurs trough ingestion of infectious cysts and multiple transmission routes are possible, from direct (person-to person, animal-to-person, and animal-to-animal) to indirect contact (ingestion of contaminated water and food). Consequently, drivers of exposure to zoonotic assemblages are numerous, including environmental contamination, amplified infection spread in wildlife, and cross-transmission from companion animals and livestock to humans. In this scenario, to understand the complex epidemiology of giardiasis, a One Health approach is necessary. The increasing application of highly informative molecular techniques combined with more sensitive detection methods and integrated epidemiological studies are helping to address zoonotic transmission, environmental persistence, and the implications of Giardia's genetic diversity across humans, animals, and water sources.

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IS080

TOPIC 1: PARASITE ECOLOGY, BIODIVERSITY, AND TAXONOMY
THE ECOLOGY OF WILDLIFE MALARIA IN A CHANGING WORLD
08-18-2026 10:00 AM - 12:00 PM

SPATIOTEMPORAL VARIATION IN AVIAN HAEMOPARASITE-HOST INTERACTIONS

Jenny Dunn[1], Daliborka Stankovic[2], Kasun Bodawatta[3]
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Wildlife Malaria and other blood parasites of wildlife are globally widespread and highly prevalent across all host taxa. The diversity and host impacts of wildlife malaria parasites, as well as spatiotemporal trends in their distribution are of key importance when understanding how climate change will impact host species. Here, I present results from a 7-year study on avian haemoparasite-host interactions, highlighting temporal variation in the prevalence and persistence of generalist parasites suggesting that host-parasite interactions in wildlife are not necessarily stable. I also present a global-scale analysis conducted by Working Group 5 of the Wildlife Malaria Network (WIMANET) in compiling data from host-parasite networks across the world, which enables us to scale-up community-level studies of host-parasite interactions to identify general trends and patterns, with implications for the impacts of wildlife malaria under environmental change.

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IS081

TOPIC 1: PARASITE ECOLOGY, BIODIVERSITY, AND TAXONOMY
THE ECOLOGY OF WILDLIFE MALARIA IN A CHANGING WORLD
08-18-2026 10:00 AM - 12:00 PM

HAEMOSPORIDIAN INFECTIONS IN LITHUANIAN AVIFAUNA: INSIGHTS FROM MUSEUM SPECIMENS.

Mélanie Duc[1], Monika Montvidaitė[2], Giedrė Pakeltytė[3], Dalia Tumaitė[3], Alžbeta Šujanova[4]
[1]State Scientific Research Institute Nature Research Centre, Vilnius, Lithuania, [2]Vilnius University Life Science Centre, Vilnius, Lithuania, [3]Kaunas Tadas Ivanauskas Museum of Zoology, Kaunas, Lithuania, [4]Institute of Zoology, Slovak academy of Sciences, Bratislava, Slovak Republic

Avian haemosporidian parasites (Haemosporida) are cosmopolitan and mainly studied through their blood stages in passerines and birds of prey. Many bird species remain understudied due to legal restrictions, conservation status and logistical constraints associated with live capture. Additionally, host-vector-parasite associations are changing, with newly or emerging prevalences, underscoring the need for broader surveillance across host taxa. This study aims to assess the prevalence and diversity of avian haemosporidian infections in Lithuanian avifauna.

Avian tissue samples were obtained opportunistically from museum specimens during routine preparation for collections. Collected organs were preserved in ethanol for molecular analyses and fixed in formalin for future histological investigations. Genomic DNA was extracted from tissues, and haemosporidian parasites were detected using nested PCR targeting the cytochrome b gene barcoding region.

Samples from 237 specimens processed in 2022 - 2025 represented 96 bird species from 39 families. Haemosporidian infections were detected in 106 individuals belonging to 50 host species, including six host species for which no haemosporidian infections have previously been reported in the MalAvi database.

Our results expand current knowledge of haemosporidian host range and infection diversity in avian wildlife and highlight the value of museum specimens for parasite surveillance. Collaborations with museums enable access to a broader range of host species and provide a complementary approach for monitoring parasite diversity and potential disease risks in changing environments.
Grant P-PD-23-029 from the Research Council of Lithuania (LMTLT) is acknowledged.

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IS082

TOPIC 1: PARASITE ECOLOGY, BIODIVERSITY, AND TAXONOMY
THE ECOLOGY OF WILDLIFE MALARIA IN A CHANGING WORLD
08-18-2026 10:00 AM - 12:00 PM

WHEN THE PLANET WARMS, PARASITES MOVE: BAT MALARIA IN THE ANTHROPOCENE

Alexandra Corduneanu[1], Alejandro Cabezas-Cruz[1], Angélique Foucault-Simonin[1], Aron Peter[2], Attila Sandor[3]
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Climate change is reshaping the distribution and dynamics of infectious diseases worldwide, influencing host–parasite interactions and facilitating the spread of pathogens into new regions. Bat malaria, caused by Polychromophilus parasites, represents an emerging model for understanding how environmental change affects parasite ecology in wildlife. As global temperatures rise and ecosystems are altered during the Anthropocene, shifts in bat distribution, vector abundance, and transmission dynamics may promote the expansion of malaria parasites across new geographic and ecological boundaries. This study explores the diversity, distribution, and ecological drivers of bat malaria, highlighting how climate change may influence parasite movement and host–vector interactions. Understanding these dynamics is essential for predicting future patterns of parasite emergence and for assessing broader impacts on wildlife health and ecosystem stability.

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IS083

TOPIC 1: PARASITE ECOLOGY, BIODIVERSITY, AND TAXONOMY
THE ECOLOGY OF WILDLIFE MALARIA IN A CHANGING WORLD
08-18-2026 10:00 AM - 12:00 PM

EFFECTS OF HUMAN FOREST USE ON HAEMOSPORIDIAN PREVALENCE

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Human-driven forest-modification is reshaping host–vector–parasite interactions worldwide, yet quantitative evidence on how land-use/land-cover changes influences avian haemosporidian dynamics remains limited. Working Group 4 of WiMaNet CA22108 contributes essential data compilation and an AI-based machine learning pipeline to assess white blood cell counts in relation to land use. Here we present the effects of human forest use on haemosporidian prevalence using two complementary case studies: Zalon Taung (2019) and Bhutan (2025). Both studies compare natural or near-natural forests with human-used or disturbed habitats. From both sites, training images were used to enable automated, fast, and harmonized parasite detection and white blood cell counts (WBC). These images are essential for the training database and for integrating standardized sampling with the machine-learning-assisted data processing “MLAD” pipeline, which is part of WiMaNet Working Group 4 (WG4AI). The machine learning pipeline is currently under advanced training; however, the analyses already provide both empirical ecological insights and methodological advances relevant to WiMaNet Working Group 4 objectives, including scalable data integration, cross-study comparability, and AI-assisted parasite detection. Preliminary results indicate consistent shifts in parasite prevalence associated with forest use intensity. In Bhutan, disturbed forests showed higher infection frequencies, with increased prevalence of Haemoproteus and Leucocytozoon compared to natural habitats, suggesting altered vector exposure or host community restructuring. Comparable trends from Zalon Taung reinforce the hypothesis that anthropogenic habitat modification modifies transmission dynamics rather than uniformly increasing or decreasing infection risk.

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TOPIC 4: HOST IMMUNITY, MOLECULAR INTERACTIONS, AND MICROBIOME
BIOLOGY OF PROTOZOAN PARASITE EXTRACELLULAR VESICLES: FROM BENCH TO THE FIELD
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EXTRACELLULAR VESICLES RELEASED BY TRYPANOSOMA CRUZI AS PRIMARY MEDIATORS OF MODULATION OF THE HOST IMMUNE RESPONSE

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Chagas disease, caused by *Trypanosoma cruzi*, remains a major public health problem affecting millions worldwide. Extracellular vesicles (EVs) released by trypomastigote forms of the parasite play key roles in host–parasite communication and immune modulation, yet their effects on human monocyte–macrophage biology remain incompletely understood. This study investigated how *T. cruzi*-derived EVs interact with and modulate human THP-1 monocytes and macrophages. EV uptake was monitored using PKH26 labeling combined with confocal microscopy and ImageStream flow cytometry, while functional effects were assessed through cytokine profiling, viability assays, activation marker expression, and morphological analyses by scanning electron microscopy. EVs were rapidly internalized by host cells, triggering NF-κB pathway activation within minutes and promoting monocyte differentiation into macrophage-like cells with increased expression of CD14, CD16, CD80, CD86, CD69, and HLA-DR. EV exposure enhanced intracellular parasite load and induced dynamic immunomodulation characterized by early pro-inflammatory cytokine production followed by a later anti-inflammatory profile. the interaction increased EV uptake capacity, cellular complexity, and apoptosis. Together, these findings demonstrate that *T. cruzi* EVs actively regulate host innate immune responses by influencing cellular differentiation, activation pathways, and inflammatory balance, highlighting their relevance as potential biomarkers and therapeutic targets in Chagas disease.

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TOPIC 4: HOST IMMUNITY, MOLECULAR INTERACTIONS, AND MICROBIOME
BIOLOGY OF PROTOZOAN PARASITE EXTRACELLULAR VESICLES: FROM BENCH TO THE FIELD
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EXTRACELLULAR VESICLES AS NOVEL BIOMARKERS OF HIDDEN PARASITIC INFECTIONS

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Cryptic infections represent a major challenge for malaria elimination efforts targeting *Plasmodium vivax*. Traditionally, these infections have been associated with dormant liver stages (hypnozoites), responsible for relapses and sustained parasite transmission. More recently, increasing evidence indicates that the spleen also acts as an important reservoir accumulating a substantial proportion of parasite biomass, particularly in asymptomatic individuals. Both scenarios contribute to the persistence of hidden parasite reservoirs, hampering malaria elimination strategies. Therefore, the identification of reliable biomarkers (BMKs) capable of detecting these cryptic carriers is urgently needed. In recent years, extracellular vesicles (EVs) have emerged as promising biomarker sources. EVs are double-membrane nanovesicles released by all cells and present in biological fluids. They carry a molecular cargo composed of proteins, nucleic acids, metabolites, and lipids reflecting the physiological state and cellular origin. Here, we present the application of a novel isolation approach based on strong anion exchange (SAX) magnetic beads that enables EV purification from minimal sample volumes, followed by proteomic characterization and the identification of novel biomarkers associated with cryptic liver-stage and asymptomatic *P. vivax* infections. The study includes EVs isolated from supernatants of experimentally infected human hepatic cells, from plasma samples from patients experiencing *P. vivax* relapses and from asymptomatic patients. Funding: MICINN (PID2022-142908OB-I00), AGAUR (2021 SGR 01554), Fundación Ramón Areces and US NIH (R01AI146590)

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CROSS-KINGDOM CONVERSATIONS: THE ROLE OF EXTRACELLULAR VESICLES IN MICROBIOME–GIARDIA INTERACTIONS

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Extracellular vesicles (EVs) emerged as key mediators of intercellular communication within the intestinal ecosystem, facilitating signaling between microbes, parasites, and the host. *Giardia duodenalis* releases EVs that contribute to parasite pathophysiology, yet their role in shaping microbiome interactions remains poorly understood. We explored the role of EVs in the bidirectional communication between *Giardia* and gut microbiota. We investigated whether *Giardia* EVs modulate the behavior of Enterobacteriaceae. EVs derived from *G. duodenalis* NF were isolated using membrane affinity methods and characterized by nanoparticle tracking analysis and transmission electron microscopy. Multiomic analyses, including RNA-Seq and LC-MS proteomics, characterized EV cargo. Functional assays demonstrated that *Giardia* EVs exert bacteriostatic effects on *Escherichia coli* and *Enterobacter cloacae*, while enhancing bacterial swimming motility and adhesion to intestinal epithelial cells. EV exposure also reduced biofilm formation by *E. coli* HB101. Mechanistic studies revealed that EV-associated RNAs, rather than proteins, mediated alterations in bacterial motility and biofilm dynamics. RNA-Seq identified abundant small RNAs (e.g. endo-siRNAs), whereas proteomic analyses revealed enrichment of translation-associated pathways and RNA chaperone networks. We also explored the reciprocal effects of microbiota-derived EVs on *Giardia*. EVs from selected small intestine bacteria (e.g. *Lactobacillus* spp.) were isolated using tangential flow filtration and size-exclusion chromatography and were evaluated for direct anti-*Giardia* activity by assessing parasite growth, survival, and metabolic activity *in vitro*. These studies aim to determine whether microbiota-derived EVs can exert anti-parasitic effects and counteract parasite-induced epithelial dysfunction. Collectively, these findings support the concept that EVs are central mediators of microbiome–parasite communication within the gut.

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ORGAN AND ORGAN-ON-CHIP STUDIES OF CRYPTIC ERYTHROCYTIC INFECTIONS IN HUMAN MALARIA

Nuria Sima[1], Marian Muskus[2], Robert Benaiges Fernandez[3], Alvaro Lopez Malizia[4], Daniela Grases[4], Marta Chiodin[1], Jessica Molina-Franky[5], Maria Fernanda Yasnot[2], Lauro Sumoy[3], Eduard Porta[4], Carmen Fernandez-Becerra[6], Hernando Del Portillo[3,5]
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Intercellular communication mediated by extracellular vesicles (EVs) plays an important role in the pathophysiology of *Plasmodium vivax* and solid evidence indicates that this species evolved cryptic niches in the bone marrow (BM) and spleen. To address the ethical and technical challenges of studying malaria infections in these erythropoietic tissues, we have implemented a BM-on-a-chip mimicking a 3D functional unit of the perisinusoidal niche where erythroid cells interact with parasites. We have performed single-cell RNA seq to determine cell-cell interactions mediated by EVs and to study parasite migration into the bone marrow compartment. Moreover, we are generating a spatial map of the human spleen during cryptic intrasplenic infections caused by malaria. To achieve this objective, we are first generating a spatial atlas of the human spleen from spleen ruptures of normal individuals. After ethical approval, we have obtained paraffin blocks with high quality RNA from ten human spleens. We have profiled tissues with the 10x Xenium platform using the 5K Pan-Tissue and Pathways panels plus a customized panel. This research could shed light on these cryptic erythrocytic niches of *P. vivax* allowing the identification of parasite-specific signaling pathways for new therapeutic strategies to contribute to its elimination.

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TOPIC 3: RESISTANCE AND DEVELOPMENT OF DRUGS AND VACCINES
SCHISTOSOME IMMUNOBIOLOGY AND VACCINE DEVELOPMENT: MULTIDISCIPLINARY RESEARCH PERSPECTIVE
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DISSECTING THE MECHANISMS THROUGH WHICH MATERNAL INFECTION IMPACTS PASSIVE TRANSFER OF MATERNAL IMMUNITY

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Maternal immunization is an increasingly used strategy to protect neonates and infants from early childhood infection before they can be vaccinated themselves. Maternal infections have been implicated in the reduced transfer of maternal immunization induced antibodies to offspring, potentially, leaving offspring more susceptible to infection. Maternal schistosomiasis impacts 10s of millions of women of reproductive age annually. Experiments in animal models including those in our lab have provided evidence that chronic maternal *S. mansoni* infection leads to the transfer of pathogen antigen specific antibodies to offspring. This exposure serves to modulate the immune response to subsequent postnatal immunization with early childhood vaccines. We have identified both a cytokine and cell intrinsic mechanism for reduced peripheral immunity to early life vaccination in offspring. We have identified that offspring born to schistosome infected mothers are also more susceptible to early life influenza infection. With our new model of maternal immunization during acute and chronic schistosome infection we have identified that maternal infection suppresses the maternal immune response to influenza vaccination. Maternal influenza immunization of control uninfected dams transfers maternal B and T cells to offspring, creating a microchimeric state that correlates with an accelerated germinal center response to influenza infection at 25 days of age. Maternal schistosomiasis reduces this cellular transfer, and offspring are not protected from morbidity or mortality.

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SCHISTOSOME IMMUNOBIOLOGY AND VACCINE DEVELOPMENT: MULTIDISCIPLINARY RESEARCH PERSPECTIVE
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REGULAR MASS DRUG ADMINISTRATION OF PZQ TO BOOST ANTI-SCHISTOSOME IMMUNITY: PRECLINICAL AND CLINICAL EVIDENCES

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Background: Schistosomiasis remains a significant public health challenge in endemic regions, leading to substantial morbidity, in the absence of a functional vaccine until date. While regular mass drug administration (MDA) of praziquantel (PZQ) is a cornerstone of schistosomiasis control programs in endemic areas, emerging evidence suggests that its benefits, not fully harnessed, may extend beyond mere parasite killing, orchestrating a chemotherapeutically-induced immunity in treated hosts. We aimed to address this possibility. **Methods:** We examined schistosomiasis in school-aged children from affected areas who received different cumulative numbers of annual PZQ treatments. We monitored reinfection rates and health effects, like anemia, cognitive impairment and liver fibrosis. A mouse model was also used to analyse, beyond association, the likelihood of causality in how the numbers of infection-PZQ treatment cycles might affect susceptibility to reinfection, pathologies and immune responses. **Results:** Analysis of data and samples from SAC showed that regular use of PZQ in endemic area activated more rapidly arginine/proline metabolism and increased protective IgE levels and type-2 cytokines in these SAC. This lowered the chance of subsequent high parasite levels (AOR = 0.16) and improved hemoglobin (AOR = 2.58) and academic performance (AOR = 2.39). Similarly, In mouse models, repeated cycle of infection/treatments boosted immune responsiveness, particularly of the type-2 arm (parasite-directed IgE and IgG1, Arginase-1) and translated into increased resistance to reinfection. **Conclusion** The study shows that regular

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treatment with PZQ can improve protective immune responses in children from schistosomiasis-endemic areas, reducing the risk of reinfection and associated sequelae

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SCHISTOSOME IMMUNOBIOLOGY AND VACCINE DEVELOPMENT: MULTIDISCIPLINARY RESEARCH PERSPECTIVE
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IMMUNE RESPONSES IN CONTROLLED HUMAN SCHISTOSOME INFECTION

Emma Houlder[1], Meta Roestenberg[1], Jan Pieter Koopman[1], Marijke Langenberg[1], Marie-Astrid Hoogerwerf[1], Jacqueline Janse[1], Koen Stam[1], Cornelis Hokke[2], Maria Yazdanbakhsh[2], Emmanuella Driciru[3], Emilie Velders[1], Jeroen Sijtsma[1], Govert Van Dam[4], Paul Corstjens[5], Friederike Sonnet[1], Stan Hilt[5], Alison Elliott[6], Angela Van Diepen[7]
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Schistosomiasis is a neglected tropical disease affecting over 200 million people, with no available vaccine. Our understanding of clinical and immunological outcomes schistosomiasis is limited to studies of inherently heterogenous natural (endemic) infection, or murine infection models. At LUMC, NL, we have developed a controlled human Schistosoma mansoni infection model as a platform for vaccine and therapeutic testing. This model provides an opportunity for in-depth longitudinal analyses of schistosome immunology. In our initial single-sex dose escalation studies using male or female cercariae, we revealed early symptom-related inflammatory and Th1 responses changing to Th2 by week 8 post-infection. To extend this model, and ask if partial immunity develops with multiple infections, a repeat (3x) infection model was performed. Notably, no development of protection from infection was observed. Mixed Th1/Th2/regulatory worm-specific T cell responses peaked in the secondary infection cycle, remaining elevated until final treatment. Specific antibodies to an array of schistosome proteins and glycans were elevated over the course of repeat infection. In the second infection cycle, a minority of participants were accidentally exposed to female cercariae, with potential egg-production and higher Th2 responses, as well as antibodies to specific antigens. Current work is focusing on understanding initial innate responses, and how they direct the eventual quality of antigen-specific adaptive (T cell/antibody) responses between participants. Together, the novel data from the controlled human infection model has provided significant insights into the clinical and immunological aspects of *S. mansoni* infection.

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WHAT COVID19 VACCINE EFFICACY STUDIES ON THE AFRICAN CONTINENT HAVE TAUGHT US FOR SCHISTOSOMIASIS VACCINE DEVELOPMENT AND EVALUATION

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The SARS-CoV-2 pandemic serves as a critical blueprint for vaccine development in populations with complex pre-existing immunity. This synthesis applies lessons from COVID-19 to three pillars of schistosomiasis vaccine research: antigen discovery, effectiveness evaluation, and efficacy assessment. First, SARS-CoV-2 highlights the impact of immunological imprinting. With up to 31.9% of pre-pandemic African samples showing cross-reactive antibodies, schistosome antigen discovery must account for existing immune landscapes. Modern schistosome pipelines now integrate reverse vaccinology and immunomics with cohort studies of naturally resistant individuals to identify truly protective targets. Second, evaluating vaccine effectiveness requires moving beyond simple seroconversion. Just as background immunity complicated COVID-19 data, schistosomiasis-endemic populations possess antibody profiles shaped by age and chronic infection. Defining context-specific correlates of protection is essential for accurate measurement. Finally, real-world efficacy must consider dynamic exposure. Because schistosome immunity develops gradually through repeated infection and treatment (Praziquantel), optimal vaccine impact likely depends on strategic integration with existing mass drug administration programs. This picture is further complicated by the impact of praziquantel treatment on natural host immune responses against schistosomiasis. Several cohort studies show that PZQ accelerates the rate of development of ant-schistosome immune responses. By addressing these immunological nuances, we can transition from traditional models to more sophisticated, context-aware vaccine strategies for endemic settings.

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PLENARY SESSION
CELL-COMMUNICATION IN MALARIA: THE PARASITE'S SOCIAL MEDIA NETWORK
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CELL-COMMUNICATION IN MALARIA: THE PARASITE'S SOCIAL MEDIA NETWORK

Neta Regev-Rudzki
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Parasites have evolved sophisticated strategies to manipulate their hosts and promote survival. The malaria parasite *Plasmodium falciparum* (*Pf*), one of the deadliest human pathogens, exploits secreted extracellular vesicles (EVs) as a key communication and virulence mechanism. We found that while residing within red blood cells, *Pf* secretes EVs loaded with functional cargo, including RNA, genomic DNA, and even an active 20S proteasome complex, each playing a role in modulating host systems. Remarkably, we also identified the transfer of three parasitic mRNAs into human monocytes, where they rapidly localize to the nucleus and interfere with the splicing machinery, suppressing target protein expression. We further reveal a mechanism for *Pf*-EV entry into host immune cells, demonstrating that uptake is governed by both membrane deformability and cholesterol levels. Together, our findings demonstrate an EV-mediated communication network that enables *Pf* to reshape host responses at molecular and biophysical levels, highlighting new avenues for targeting parasite virulence.

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PARASITE METABOLISM: FUNDAMENTAL AND TRANSLATIONAL APPLICATIONS
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TOO MUCH OF A GOOD THING: METABOLIC DISEASE AS A COST OF CHRONIC INFLAMMATION DURING TOXOPLASMA INFECTION

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Toxoplasma gondii has a long evolutionary relationship with human and rodent hosts that can be leveraged to understand host physiology and disease pathology. *Toxoplasma* invades the small intestine, leading to host recognition of commensal bacteria, which is necessary for protective immunity. *Toxoplasma* infects hosts for life and is controlled by chronic, low-grade inflammation. Capitalizing on the observation that protozoa often cause wasting disease in humans and livestock, we established that *Toxoplasma* infection of mice is a robust model of cachexia. Cachexia is defined as the loss of >5% muscle mass in under 6 months accompanied by inflammation, weakness, fatigue, anorexia or fat loss. There are ~9 million people with cachexia, and cachexia contributes to 2 million deaths annually, yet the molecular mechanism(s) of cachexia are unknown. To understand causal relationships between infection and inflammation driving metabolic dysbiosis in cachexia, we have employed a combination of mouse genetic tools, 16S profiling of commensal microbiota and chemical cartography, in collaboration with Dr. Laura-Isobel McCall. These data implicate sustained changes in colon microbial communities as drivers of metabolic dysbiosis in chronic cachexia. The long-term goal of this work is to leverage *Toxoplasma* infection to assess interventions that improve cachexia pathology without increasing susceptibility to infection.

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PARASITE METABOLISM: FUNDAMENTAL AND TRANSLATIONAL APPLICATIONS
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OUT OF THE MOUTHS OF BABES: TOWARDS NON-INVASIVE BREATH-BASED DIAGNOSTICS FOR PLASMODIUM INFECTION

Audrey Odom John
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The most common and sensitive rapid diagnostic tests (RDTs) for malaria due to *Plasmodium falciparum* infection detect the exported parasite protein HRP2. Implementation of RDTs for malaria diagnosis has contributed to improved malaria control; unfortunately *P. falciparum* strains that fail to produce HRP2 have emerged. HRP2-negative parasite lines cause false-negative malaria testing, and thus new clinical diagnostics are required. We have previously identified volatile organic compounds (VOCs) produced by *P. falciparum* in culture, which are known mosquito attractants. We find that these same compounds are enriched in the breath of children with symptomatic falciparum malaria, when compared to febrile and healthy controls. Additional breath biomarkers have been shown to reproducibly distinguish children with and without malaria in independent cohorts, providing strong proof-of-concept for development of a “malaria breathalyzer.” Here we report the extension of this work to examine the feasibility of breath-based screening for asymptomatic *P. falciparum* infection. We employ a case-control study design, with parallel enrollment in two independent sites (Blantyre, Malawi and Chipata, Zambia) to allow for rapid biomarker discovery and validation. Infection status has been determined by qPCR, and breath and skin volatile samples were obtained for two-dimensional gas chromatography-mass spectrometry (GCxGC-MS) analysis. Ongoing analyses include biomarker discovery and associations with clinical metadata, including asexual and sexual parasitemia.

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PARASITE METABOLISM: FUNDAMENTAL AND TRANSLATIONAL APPLICATIONS
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THE IMPACT OF LEISHMANIA ON MACROPHAGE IMMUNOMETABOLISM

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Leishmania parasites replicate within host macrophages and have evolved strategies to suppress the antimicrobial responses that would otherwise eliminate them. Using mass-spectrometry–based untargeted metabolomics, we examined metabolic changes in macrophages infected with *L. mexicana* promastigotes or amastigotes, alongside controls exposed to heat-killed parasites. Infection with live parasites prevented the macrophage from adopting the aerobic glycolytic metabolic program characteristic of an M1 inflammatory response. Targeted isotopomer tracing further revealed that the parasites actively acquired and catabolised host immunometabolites, including arginine, tryptophan, itaconate and propionate. Together, these findings indicate that Leishmania manipulates host immunometabolism both by blocking macrophage activation and by diverting key metabolites, thereby promoting parasite survival within an otherwise hostile intracellular niche.

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CONFRONTING ANTHELMINTIC RESISTANCE: MECHANISMS, MONITORING & MITIGATION IN MULTICELLULAR PARASITES
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BETA-TUBULIN AND BEYOND: MECHANISMS OF NEMATODE BENZIMIDAZOLE RESISTANCE

Erik Andersen
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For almost four decades, we have known that benzimidazole (BZ) drug resistance is caused by mutations in beta-tubulin genes based on foundational studies in the free-living nematode *Caenorhabditis elegans* and the parasitic helminth *Haemonchus contortus*. The experimental tractability of *C. elegans* facilitated the discovery and validation of BZ resistance alleles from parasitic worms, trade-offs in fitness and drug resistance, the focal tissue and cell type where BZs likely act to poison nematodes, and the evolutionary genetics of BZ resistance in parasitic worms. Our recent research is focused on two areas. First, I will present our progress defining the genetic complexity that underlies BZ resistance across diverse parasitic nematode species. Second, I will present data on BZ resistance genes independent of beta-tubulin and our strategies to discovery additional BZ resistance mechanisms. Because parasitic nematode species vary in BZ resistance over several orders of magnitude, additional genes must underlie resistance to this drug class. The discovery of these genes can improve molecular diagnostics to prolong the usefulness of this WHO Essential Medicine.

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TOPIC 3: RESISTANCE AND DEVELOPMENT OF DRUGS AND VACCINES
CONFRONTING ANTHELMINTIC RESISTANCE: MECHANISMS, MONITORING & MITIGATION IN MULTICELLULAR PARASITES
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WHAT CAN WE LEARN FROM MODELLING ANTHELMITIC RESISTANCE?

Hannah Vineer
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Anthelmintic resistance threatens the sustainable control of gastrointestinal nematodes in ruminant livestock worldwide, and this threat is compounded by climate variability that alters parasite transmission and challenges existing control practices. Field trials evaluating strategies to slow resistance while maintaining effective parasite control are time consuming and expensive. Modelling, by contrast, can rapidly explore a wide range of scenarios to, predict how resistance develops under different management regimes and climates. Decades of research have allowed us to develop increasingly detailed evolutionary-epidemiological models that capture both parasite dynamics and resistance evolution in response to the environment and interventions. Here we consider what such models have taught us and where they can take us next. Large-scale simulations linking weather-dependent epidemiology to resistance evolution show that phenotypic measures such as faecal egg count reduction tests can miss fine-scale changes in resistance allele frequency, that drug pharmacokinetics strongly influence outcomes, and that trade-offs between resistance management and parasite control depend on the genetic basis of resistance. Importantly, key uncertainties remain, including the genetic basis of resistance and the dominance of resistance alleles. Rather than being barriers, these gaps are tractable questions that modelling is well suited to explore. By incorporating climate explicitly into these simulations, we can ensure predictions reflect climate-related uncertainty, producing robust, sustainable guidance for managing resistance in a changing world.

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ASSESSING ANTHELMINTIC RESISTANCE IN HUMAN STH IN THE FIELD

Luc Coffeng[1], Bruno Levecke[2]
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Control of infection and morbidity due to soil-transmitted helminths (STH) still relies heavily on mass drug administration with benzimidazoles donated by the pharmaceutical industry. Experiences from veterinary parasitology and modelling studies for human helminthiasis suggest that after roughly two decades of annual deworming of hundreds of millions of children, we should be vigilant for emerging benzimidazole resistance in STH. In this session, we will review the state of the art of pharmacovigilance for STH in research and practice, covering diagnostics, survey design (including key differences for trials vs. monitoring & evaluation), cost-efficiency, the WHO guidelines (which are currently being revised), and potential new strategies using molecular approaches and environmental sampling (e.g., latrines). Importantly, we will discuss how detection of emerging drug resistance in human STH is currently impeded by suboptimal pharmacovigilance strategies which lead to overestimation of drug efficacy, and how to address this.

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TOPIC 3: RESISTANCE AND DEVELOPMENT OF DRUGS AND VACCINES
CONFRONTING ANTHELMINTIC RESISTANCE: MECHANISMS, MONITORING & MITIGATION IN MULTICELLULAR PARASITES
08-18-2026 2:10 PM - 4:10 PM

GENOME-WIDE APPROACHES TO ANTHELMINTHIC RESISTANCE IN GASTROINTESTINAL NEMATODES

James Cotton
University of Glasgow, School Of Biodiversity, One Health And Veterinary Medicine, Glasgow, United Kingdom

The spread of resistance to anthelminthic drugs represents a threat to our ability to control parasitic worms in livestock and could ultimately affect treatment of human helminth infections. Understanding the genetic basis of resistance would allow us to track its spread and adapt management strategies to slow this spread, but for some classes of anthelminthics this understanding has remained elusive. With many collaborators, we have been working with gastrointesintal nematodes (GINs) of small ruminants as a model for anthelminthic resistance in nematodes. Armed with chromosome-level reference genomes for the parasite *Haemonchus contortus*, and more recently for many other GIN species, we have attempted to go beyond genetic approaches based on assumptions about gene function to use unbiased, genome-wide approaches to identify the genetic basis of resistance and study its evolution. Both genetic crosses and population genetic analyses have identified a single major locus for ivermectin resistance in *Haemonchus contortus*, that appears to be widely distributed around the world. It has been challenging to identify a causal variant in this large QTL. An apparently completely different locus is under selection in the related worm *Teladorsagia circumcincta* in both farm populations and in experimental settings, where we have been able to exploit knowledge of this QTL to quantify the strength of selection for resistance induced by different anthelminthic treatment regimes. I'll discuss some possible reasons why we have so far failed to pinpoint the genetic basis of ivermectin resistance and some ideas about how we might be able to do so.

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TOPIC 5: DIAGNOSTICS, HOST-PARASITE INTERACTION AND EMERGING TECHNOLOGIES
PARASITIC INFECTIONS AND CIRCADIAN RHYTHMS
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CIRCADIAN CLOCKS AND IMMUNE RESPONSE IN MALARIA AND LEISHMANIASIS

Nicolas Cermakian[1,2], Priscilla Carvalho Cabral[1], Sebastián Boy Waxman[3,4], Sophia Stegeman[3], Martin Olivier[5,6]
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Malaria and leishmaniasis are diseases caused by insect vector-borne infections with parasites *Plasmodium spp.* and *Leishmania spp.*, respectively. Infection with both parasites is associated with an inflammatory component that is central to disease progression and outcome. Since various immune functions show circadian rhythms, we wondered whether *Plasmodium* and *Leishmania* infections are regulated by host circadian clocks. In *Leishmania major*-infected mice, we found that time of infection impacted the inflammatory response and parasite load. These circadian rhythms were lost in mice lacking clock function in immune cells. Similarly, in a mouse model of cerebral malaria (with mice infected with *Plasmodium berghei ANKA*), disease progression and parasite load varied according to time of infection, and following systemic clock desynchronization. Parasite load was also impacted by a loss of plasma glucose rhythm. In both experimental leishmaniasis and cerebral malaria, circadian rhythms can be traced down to the cellular level, with rhythms of infection, signaling pathway activation, and cytokine secretion in cultured macrophages. Lastly, since circadian rhythms were found in other unicellular eukaryotes (including *Plasmodium spp.*), we aimed to find out whether this is the case in *Leishmania*. *L. major* promastigotes were subjected to temperature cycles alike those experienced by the parasite in its insect vector, and the transcriptome and metabolome investigated under alternating temperatures or constant temperature. Interestingly, transcripts and metabolites, involved in pathways related to response to reactive oxygen species and metabolism, were found to display ~24 hour rhythms.

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TOPIC 5: DIAGNOSTICS, HOST-PARASITE INTERACTION AND EMERGING TECHNOLOGIES PARASITIC INFECTIONS AND CIRCADIAN RHYTHMS
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DEATH AT SUNSET: MECHANISMS DRIVING ROBUST DAILY TIMING OF DEATH IN ENTOMOPHTHORA MUSCAE-INFECTED FRUIT FLIES

Carolyn Elya
Harvard University, Molecular And Cellular Biology, Cambridge, United States of America

Many parasites are known to manipulate the behavior of their animal hosts, but how this occurs remains poorly understood. My research program leverages the lab tractable "zombie fruit fly" system – consisting of *Drosophila melanogaster* infected with the behavior manipulating fungal pathogen *Entomophthora muscae* – to investigate mechanisms of parasitic host manipulation at the level of molecules, genes, cells and circuits. On their final day of life, infected flies perform the “zombie” behaviors characteristic of *E. muscae* infection: they climb to a high location (a behavior known as “summiting”), extend their proboscises, and raise their wings in a pose that facilitates spore dispersal. Intriguingly, death by *E. muscae* always occurs at sunset. I will share our recent work integrating the fruit fly toolkit with fungal genomics and transcriptomics to understand the mechanism by which this eerie punctuality emerges.

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TOPIC 5: DIAGNOSTICS, HOST-PARASITE INTERACTION AND EMERGING TECHNOLOGIES PARASITIC INFECTIONS AND CIRCADIAN RHYTHMS
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CALLING TIME ON DISEASE IN AQUACULTURE: CHRONOBIOLOGICAL INTERACTIONS OF SALMONIDS AND THEIR PARASITES

Amy Ellison[1], Miku Kawahara[1], Lauren Chapman[2], Jiyeon Park[2], Marlene Lorgen-Ritchie[2], Rhi Hunt[3], Jo Cable[3], David Wilcockson[4], Alexander Papadopulos[1], Jason Holland[2], Sam Martin[2]
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Understanding how biological rhythms shape host–parasite interactions is emerging as an important avenue in animal health research. Circadian rhythms regulate diverse aspects of host physiology, including immune function, metabolism, and behaviour, while parasites and pathogens also exhibit rhythmic patterns in activity, infectivity, and transmission. However, these interacting rhythms are rarely considered together, despite their potential importance in determining disease susceptibility and health outcomes. In this presentation, I explore how circadian processes influence health and welfare in salmonids and aquaculture systems through their effects on host–parasite interactions. Modern salmonid aquaculture frequently manipulates photoperiod to enhance growth and control development, yet the consequences of these altered light environments for immune rhythmicity and disease susceptibility remain poorly understood. I present evidence that salmonid mucosal immune activity and commensal microbiota exhibit daily rhythmicity, which can be disrupted under manipulated light regimes. Parasite activity and gene expression similarly exhibit rhythmicity, suggesting that infection success may depend on alignment between host and parasite biological timing. In addition, infection itself can disrupt host behavioural and physiological rhythms, indicating a complex bidirectional relationship between circadian regulation and disease processes. Together, these findings highlight the importance of considering time as a fundamental component of animal health biology. Integrating chronobiology into parasitology offers new opportunities to better understand infection dynamics, improve animal welfare, and develop temporally informed intervention strategies in managed animal systems.

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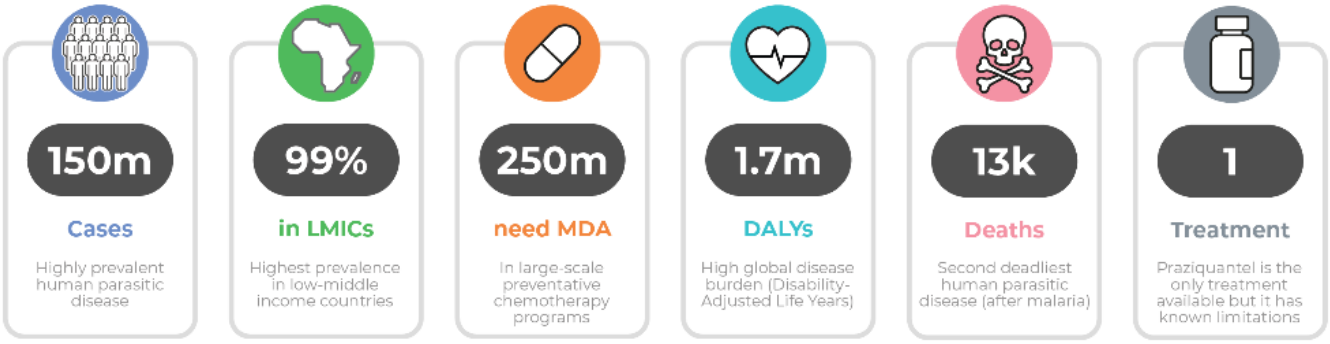
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TOPIC 3: RESISTANCE AND DEVELOPMENT OF DRUGS AND VACCINES
ANTI-PARASITE DRUG DISCOVERY
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ANTI-SCHISTOSOMAL DRUG DISCOVERY - CURRENT STATUS AND FUTURE PROSPECTS.

Beatriz Baragana
University of Dundee, Drug Discovery Unit, Faculty Of Life Science, Dundee, United Kingdom

Schistosomiasis is caused by infection with trematode worms from the genus, *Schistosoma,* infecting ~150 million people globally. Acute symptoms include abdominal pain, inappetence and diarrhoea whereas chronic or repeated infections can lead to gastrointestinal bleeding, liver disease, kidney failure, bladder cancer, and infertility. Schistosomiasis imposes an enormous economic and long-term health burden.



Praziquantel (PZQ) is the only approved treatment for schistosomiasis and is used in preventative chemotherapy (PC) programs in endemic areas. Approximately 250 million people require annual PC with a single dose of PZQ. However, cure rates are suboptimal and PZQ-based treatment will not be sufficient to deliver the WHO goal of schistosomiasis elimination by 2030. PZQ lacks efficacy against juvenile parasites, and this is likely to contribute to its inability to effect complete cures, despite repeated dosing. The reliance on this single therapeutic for disease control represents a serious vulnerability. New, effective anti-schistosomals with novel mechanisms of action are urgently required. To identify a PZQ replacement, to offset attrition, and to provide options for future combination therapies, a robust pipeline of anti-schistosomal compounds is required. However, since the approval of PZQ in 1980, drug discovery for schistosomiasis has been limited. Efforts to develop new therapeutics have been confounded by limited investment, a lack of robustly validated drug targets and high attrition rates. As a result, the current drug development pipeline is sparse, with few compounds progressed beyond early lead identification. In my talk, I will outline our strategy to accelerate progression of new leads towards preclinical candidate selection.

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TOPIC 3: RESISTANCE AND DEVELOPMENT OF DRUGS AND VACCINES
ANTI-PARASITE DRUG DISCOVERY
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CURRENT DRUG DISCOVERY PIPELINES FOR CHAGAS DISEASE AND LEISHMANIASIS.

Jadel Kratz
Drugs for Neglected Diseases initiative - DNDi, Discovery, Geneva, Switzerland

DNDi and partners are advancing a patient-centered R&D strategy to deliver safer, simpler, and affordable treatments for Chagas disease and leishmaniasis, leveraging open science and collaborative networks. For Chagas disease, DNDi is pursuing a dual track: optimizing benznidazole use through shorter regimens while progressing novel chemical entities. Recently, the pipeline for Chagas disease has been populated with new candidates arising from global pharma and biotechs, providing interesting monotherapy and combinations options to move into proof of concept studies in patients. DNDi is also addressing a critical translational barrier—the lack of early cure markers—through biomarker innovation such as the MultiCruzi assay to accelerate decision-making during the clinical stages of development. For leishmaniasis, the current global portfolio reflects the positive results of discovery efforts over the last decade across disease forms: LXE408 completed Phase II recruitment in India and Ethiopia, with cutaneous leishmaniasis studies initiating in Latin America. DNDI-6899 advanced into Phase I multiple-ascending dose evaluation. These together exemplify a maturing pipeline of new mechanisms and oral regimens. The talk will highlight pipeline status across both diseases, emphasizing how DNDi and collaborators—from academia to industry consortia and regional platforms—are building a robust, diverse candidate flow while confronting enduring challenges: complex parasite biology, biomarker validation, operational hurdles in endemic settings, and sustainable financing. Concrete examples from DNDi's portfolio will illustrate solutions that are moving candidates efficiently from bench to bedside.

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TOPIC 3: RESISTANCE AND DEVELOPMENT OF DRUGS AND VACCINES
ANTI-PARASITE DRUG DISCOVERY
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MALARIA DRUG DISCOVERY ON THE AFRICAN CONTINENT.

Lyn-Marié Birkholtz[1], Richard Amewu[2], Amanda Rousseau[3]
[1]Stellenbosch University,, Biochemistry, Stellenbosch, South Africa, [2]University of Ghana, Chemistry, Accra, Ghana, [3]University of the Witwatersrand, Molecular Sciences Institute, johannesburg, South Africa

Malaria remains a major health challenge in Africa, with few innovations forthcoming from locally driven solutions. However, strategic initiatives (Grand Challenges African Drug Discovery Accelerator, GC ADDA.org & Africa Centre for Therapeutics Innovation, acti.org.za) are driving new antimalarial discovery campaigns. Parallel screening of the Medicines for Malaria Venture (MMV) Pandemic Response Box yielded several novel hits with activity across asexual blood stages (ABS), gametocytes, and liver stages of *Plasmodium falciparum*, which formed the starting points for hit-to-lead optimisation campaigns. Chemical synthesis and solubility analysis were followed by triaging compounds through a screening cascade, testing them against drug-sensitive *P. falciparum* asexual blood stages. Active compounds (<1 µM) were cross-validated for toxicity (HepG2) and life-cycle stage profiling, including immature and mature gametocytes. All active compounds were profiled for phase I & II ADME profiling. Resistance risk was assessed using the multistrain, MIR, and AreBar assays. Mode-of-action was investigated through several complementary cellular and biochemical assays. The hit-to-lead campaign of MMV'558 (with enhanced activity against late-stage gametocytes (<100 nM), compared to ~900 nM against ABS), improved ABS activity and solubility with its isoquinoline scaffold (<100 nM) with no toxicity concerns. The series exhibited low resistance risk (MIR 8) and no cross-reactivity with known antimalarial compounds in the AreBar assay. The series has a novel, stage-specific mode of action, implicating Cu binding and inducing toxic cupropotosis in the parasites, which is associated with mitochondrial respiration and is independent of kinase signalling and hemozoin formation. This study delivers multistage active preclinical antimalarial candidates developed in Africa.

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TOPIC 3: RESISTANCE AND DEVELOPMENT OF DRUGS AND VACCINES
ANTI-PARASITE DRUG DISCOVERY
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REPLENISHING THE MALARIA DRUG DISCOVERY PIPELINE.

James Duffy
Medicines for Malaria Venture, Drug Discovery, Geneva, Switzerland

Malaria continues to impose a substantial burden on high-risk populations, particularly children and pregnant women in Africa. At present, only two regimens (sulfadoxine-pyrimethamine and sulfadoxine-pyrimethamine in combination with amodiaquine) are used as chemoprevention to protect tens of millions of women and children across the continent. Expanding the protection for this underserved patient population in the face of emerging drug resistance, while also simplifying implementation, underscores the urgent need for new medicines. Addressing this challenge required the malaria R&D community to rethink our discovery and development strategies to identify novel drug candidates with the potential for an extended duration of protection. Acting on this strategic prioritization, MMV and our partners are replenishing the malaria drug pipeline with a new generation of long-acting oral and injectable preventive chemotherapies. This presentation will describe learnings and highlight key advances made towards these goals.

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TOPIC 1: PARASITE ECOLOGY, BIODIVERSITY, AND TAXONOMY
UNDERSTANDING THE SEXUAL BIOLOGY OF PARASITIC PROTISTS
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CONSERVED MEIOTIC PROTEINS AND GAMETE FUSOGENS INVOLVED IN SEXUAL MATING IN LEISHMANIA

Carol Catta Preta
University of São Paulo, Parasitology, São Paulo, Brazil

Sexual reproduction in *Leishmania* has long been inferred from population genomics and experimental hybridization, yet the cell biology behind it remains essentially unknown. This talk asks how much of the conserved eukaryotic sexual toolkit *Leishmania* retains as functional machinery, and where it diverges from it. I will present functional genetic evidence that core meiotic genes, *HOP1* and *SPO11*, are required for hybrid formation in sand flies, and that their loss shifts hybrids toward polyploidy, a signature of failed reductional division. REC8, which makes this division reductional by retaining centromeric cohesion, is absent across Kinetoplastida. The kinetochore itself is also unconventional, likely of independent evolutionary origin, and incorporates axial-element proteins repurposed from the meiotic synaptonemal complex. How reductional division is actually achieved in this lineage therefore remains unresolved. A meiosis-like genetic exchange would also require cell-cell fusion. I will show that *Leishmania* carries two divergent and ancestral fusogens HAP2, with an unexpected functional asymmetry: one behaves as a canonical, unilaterally required fusogen, while the other is obligately required in both parents. Together, this evidence points to a sexual cycle confirmed by biparental, Mendelian inheritance and genome-wide recombination, yet only partly conserved mechanistically, and still missing direct observation of the gamete itself.

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TOPIC 1: PARASITE ECOLOGY, BIODIVERSITY, AND TAXONOMY
UNDERSTANDING THE SEXUAL BIOLOGY OF PARASITIC PROTISTS
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FERTILITY AND MOSQUITO TRANSMISSION IN A MALARIA PARASITE

Oliver Billker
Umeå University, Molecular Biology, Umeå, Sweden

Malaria is caused by protozoan parasites of the genus *Plasmodium,* which is transmitted between hosts by a mosquito vector, causing more than half a million deaths annually, with 70% of deaths occurring in African children under the age of seven. Malaria parasites are divergent eukaryotes with a complex life cycle, whose asexual replication in erythrocytes is responsible for disease, but whose transmission by mosquitoes depends entirely on sexual reproduction. In a tractable rodent model, we have scaled up the targeted disruption of parasite genes to the point of enabling genome scale genetic screens at different life cycle stages. This has revealed how gene essentiality has evolved differently in different parts of the genome. It has also allowed us map how parasite metabolism is reorganised as the parasite moves between different hosts and tissues, exposing changing drug vulnerabilities throughout the life cycle. Sexual reproduction is essential for malaria parasites to infect mosquitoes, and our screens identify hundreds of parasite genes involved in the process. An analysis of fertility genes leads us to propose new potential targets for transmission blocking interventions among some of the unique aspects of parasite sex. It also reveals unexpected, conserved aspects of developmental regulation, sperm biogenesis and gamete fusion that may represent ancestral mechanisms present already close to the last eukaryotic common ancestor.

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TOPIC 1: PARASITE ECOLOGY, BIODIVERSITY, AND TAXONOMY
UNDERSTANDING THE SEXUAL BIOLOGY OF PARASITIC PROTISTS
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GENETIC EVIDENCE FOR SEXUALITY IN AMOEBOZOA

Yonas Tekle
Spellman College, Biology, Atlanta, United States of America

Abstract Sexual reproduction is a fundamental feature of eukaryotic life, yet its presence and evolutionary history in microbial lineages such as Amoebozoa remain incompletely understood. This study investigates the genetic basis of sexuality in Amoebozoa by examining the presence, conservation, and expression of key meiosis-related genes across diverse taxa. Using comparative genomic and transcriptomic approaches, we identify homologs of core meiotic genes—including those involved in homologous recombination, chromosome pairing, and cell cycle regulation—in multiple amoebozoan lineages previously considered asexual. Notably, we observe substantial variation in the number and composition of core meiosis genes across taxa. This heterogeneity suggests that Amoebozoa may utilize diverse sexual pathways and recombination strategies rather than a single conserved meiotic program. Such variation likely reflects lineage-specific adaptations of the sexual toolkit and may be associated with differences in ecological context, life history, or the presence of cryptic sexual cycles. Despite this variability, many meiosis-related genes are broadly conserved, providing strong genetic evidence that the capacity for sexual processes is widespread within Amoebozoa, even when sexual stages have not been directly observed. Phylogenetic analyses further indicate that these genes are ancestrally derived and have been maintained under selective constraint, supporting the hypothesis that sexuality was present in the last common ancestor of the group. Together, these findings challenge assumptions of widespread asexuality in Amoebozoa and highlight the evolutionary persistence and diversification of sexual mechanisms in microbial eukaryotes.

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TOPIC 1: PARASITE ECOLOGY, BIODIVERSITY, AND TAXONOMY
PARASITE RESILIENCE IN A CHANGING WORLD
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GLOBAL CHANGE AND ECOSYSTEM STRESS: THREATS TO PARASITES?

David Marcogliese
Department of Fisheries and Oceans Canada, Saint Andrews, Canada

Background and aims: Parasites, like free-living organisms, are susceptible to environmental stressors, be they abiotic or biotic, natural or anthropogenic. In this era of global change, major threats to biodiversity in aquatic habitats include climate change, contaminants, eutrophication, acidification, hydrological changes, habitat loss, exploitation and invasive species, among a number of others. **Methods:** Overall effects of some of the most important anthropogenic stressors on parasites in aquatic ecosystems will be reviewed and summarized, with some examples provided. Stressors discussed include climate change, contaminants, eutrophication and invasive species. **Results:** In general, effects of environmental stressors on parasite populations and communities are negative. Trophically-transmitted parasites with complex life cycles tend to decline, while directly transmitted ectoparasites actually may increase in abundance. Examples of case studies on spottail shiners (*Notropis hudsonius*) exposed to municipal effluents in the St. Lawrence River will be presented to illustrate the varying effects of contaminants, eutrophication, and invasive species on parasites of this fish host in this local ecosystem. Results demonstrate that these stressor effects can be modulated or masked by natural environmental conditions. **Conclusions:** Varying effects of the same stressor on hosts and parasites, on the different parasite life cycle stages and on host–parasite interactions render specific predictions problematic. When examining anthropogenic effects, it also is important to account for other natural environmental stressors acting simultaneously in the ecosystem under consideration.

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TOPIC 1: PARASITE ECOLOGY, BIODIVERSITY, AND TAXONOMY
PARASITE RESILIENCE IN A CHANGING WORLD
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EFFECTS OF WILDFIRE ON AQUATIC HOST-PARASITE INTERACTIONS

Daniel Preston[1], Emma Svatos[2], Landon Falke[3]
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Background and Aims: Wildfires are increasing in frequency and intensity, but how they affect aquatic host-parasite interactions remains poorly understood. Our objectives were to quantify how wildfires in 2020 affected parasites and their aquatic hosts in two study systems: nematodes infecting mayflies in mountain streams in Colorado and trematodes infecting snails in temperate forested streams in Oregon. Methods: 'Before-After-Control-Impact' or 'Space-for-Time' survey designs were used to quantify aquatic hosts, parasite communities, and environmental variables in relation to wildfire disturbance. In the Colorado study, we documented interactions between nematodes, mayflies, and predatory trout. In the Oregon study, we measured trematode community structure and measures of infection abundance and prevalence before and after fire. Results: Effects of wildfire varied between the two systems. In Colorado, we observed short-term declines in mayfly hosts following fire, which led to host density-mediated declines in parasite abundance. Recovery of hosts and parasites occurred within two years. In Oregon, we found that host snails and their trematode communities were resilient to wildfire effects and remained relatively stable over the course of the study. Conclusions: The responses of aquatic host-parasite interactions to wildfire disturbance likely reflected natural history differences in the study systems, including the complex life cycles of trematodes vs. direct life cycles of nematodes; the longevity of the focal aquatic hosts (relatively long for snails and short for mayflies); and the magnitude of fire disturbance to the stream environment (generally higher in Colorado than Oregon). These findings help to integrate parasites and disease into our understanding of disturbance ecology.

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TOPIC 1: PARASITE ECOLOGY, BIODIVERSITY, AND TAXONOMY
PARASITE RESILIENCE IN A CHANGING WORLD
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THERMAL THRESHOLDS AND THE FATE OF DIGENEAN PARASITES

Christopher Blanar, Briana Zaffiro, Eloy Martinez
Nova Southern University, Fort Lauderdale, United States of America

Background and aims: Recent studies suggest that parasite biodiversity is declining, particularly among specialist taxa and/or parasites with complex life cycles whose transmission depends on multiple hosts and intact ecological interactions. Proposed drivers include declining host populations, habitat alteration or loss, pollution, and climate change. Ongoing experimental work and traits-based attempts to link climate change to decreased diversity suggest that parasites (especially transmission stages) might have narrow climatic tolerances. But do parasites have limited climatic tolerances, i.e. are they less tolerant than their hosts or free living relatives? Here, we use a metabolic approach to study this question. Methods: We used respirometry to assess metabolism (as oxygen consumption rates) in isolated Bivesiculid cercariae at a range of temperatures (20-40C), including upper values consistent with current broader trends of record-high global sea-surface temperatures. We then compared our results with published metabolic data from parasites and free-living taxa. Results: Cercarial mean oxygen consumption rates ranged from 3.3-45 nL/h, peaking at 35C and decreasing sharply beyond that point. These values are comparable to those reported from other parasitic taxa; interestingly, they are not extraordinarily low compared with many free-living invertebrates, e.g. planaria, entomopathogenic nematodes. Conclusions: This analysis confirms the strong temperature sensitivity of parasite metabolism and suggests that parasite metabolic activity remains robust at environmentally relevant temperatures. We discuss the significance of these results in the context of projected temperature increases and energetic costs imposed by parasites on hosts.

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TOPIC 1: PARASITE ECOLOGY, BIODIVERSITY, AND TAXONOMY
PARASITE RESILIENCE IN A CHANGING WORLD
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UNDERSTANDING PARASITE RESILIENCE IN THE FACE OF GLOBAL CHANGE: INSIGHTS FROM LONG-TERM DATA SYNTHESES

David Thieltges[1,2], Cyril Hammoud[2], Juan Antonio Balbuena[3], Isabel Blasco Costa[4], Katie O'Dwyer[5], Tomáš Scholz[6], Chris Selbach[7,8], Bernd Sures[9,10,11], Rachel Paterson[12]
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Background: Global biodiversity is undergoing unprecedented declines in the face of global change. While overarching trends have been well documented in free-living species, patterns in parasite resilience or sensitivity to anthropogenic changes remain largely unexplored. We aim here to examine the long-term trends in parasite populations and communities documented to date and their links to anthropogenic drivers. We also assess the methods used to characterise long-term changes, while outlining key limitations and considerations for future research. Methods: We present a global systematic literature review on long-term trends of eukaryotic parasites of wild animals in terrestrial and aquatic ecosystems, categorized into four types of data acquisition: (a) long-term monitoring; (b) snapshot resampling; (c) literature-based research; and (d) natural history collection-based studies. Results: No consistent trends in parasite infection intensity or diversity emerged from the analyses. Many parasite populations and communities did not show any trend, while in other cases the proportions of population decreases and increases were roughly equal. Where significant temporal shifts were observed, they were frequently associated with anthropogenic disturbances, although such interpretations were seldom substantiated by inferential statistical analyses. Conclusions: Overall, our findings highlight that many parasite populations seem to show resilience to ongoing global change. However, limited data and research biases constrain our broader

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understanding of long-term parasite trends. We therefore call for the integration of large-scale parasite monitoring into existing ecological programmes, the development of scalable biomonitoring tools, and greater use of historical data and museum collections to further explore parasite resilience in a changing world.

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TOPIC 4: HOST IMMUNITY, MOLECULAR INTERACTIONS, AND MICROBIOME
HOST-PATHOGEN INTERACTIONS DURING CRYPTOSPORIDIUM INFECTION
08-18-2026 2:10 PM - 4:10 PM

DMLT ADJUVANTATION POTENTIATES HUMORAL AND TH1/TH17 RESPONSES TO CRYPTOSPORIDIUM PARVUM ANTIGENS

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Background and aims
Cryptosporidiosis is the second most common cause (after rotavirus) of moderate to severe diarrhea in children and immunocompromised individuals. *C. parvum* oocysts release sporozoites when ingested, infecting and replicating in the intestinal epithelial cells. The only drug approved by the US Food and Drug Administration for the treatment of Cryptosporidiosis is nitazoxanide. This drug is not effective in treating malnourished children and immunocompromised individuals. Surface-expressed antigens represent promising vaccine targets due to their accessibility to host immune recognition. This study aimed to assess the immunogenic potential of three C. parvum surface protein (GP45, GP900, and P23) administered individually and in combination as vaccine candidates in C57BL/6 mice.

Methods
Recombinant GP45, GP900, and P23 proteins were expressed and purified, and administered to C57BL/6 mice using a standard immunization schedule. Following vaccination, immune responses were evaluated by cytokine quantification assays to assess antigen-induced cellular and ELISA to assess humoral immune activation.

Results
All recombinant proteins elicited detectable immune responses when administered individually and in combined formulations. Increased production of interferon-gamma (IFN-γ) and interleukin-12 (IL-12) was observed, indicating induction of Th1-type cellular immunity. In addition, elevated levels of interleukin-4 (IL-4) were detected, suggesting activation of Th2-associated immune responses. Adjuvanted groups demonstrated a marked increase in antigen-specific IgG and IgA responses, which were minimal or undetectable in unadjuvanted controls. In parallel, cytokine analysis revealed elevated levels of interferon-γ (IFN-γ) and interleukin-17 (IL-17), indicating the induction of a balanced Th1 and Th17 immune profile.

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Conclusion

This study demonstrates that immunization with *Cryptosporidium parvum* antigens elicits significantly enhanced immune responses when administered with DmLT adjuvantation. Adjuvanted formulations induced robust antigen-specific IgG and IgA responses, alongside elevated IFN-γ and IL-17 production, reflecting a balanced Th1 and Th17 immune profile. In contrast, unadjuvanted antigen administration resulted in minimal immunogenicity. Importantly, the use of combined antigen formulations further improved immune responses compared to individual antigen immunization, suggesting a synergistic effect that may better mimic the complexity of natural infection. Collectively, these findings highlight the critical role of adjuvantation and antigen combination in driving both systemic and mucosal immunity, supporting their potential application in the rational design of effective vaccines against *C. parvum*.

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TOPIC 4: HOST IMMUNITY, MOLECULAR INTERACTIONS, AND MICROBIOME
HOST-PATHOGEN INTERACTIONS DURING CRYPTOSPORIDIUM INFECTION
08-18-2026 2:10 PM - 4:10 PM

INVESTIGATING IEC-INTRINSIC CONTROL OF CRYPTOSPORIDIUM INFECTION

Ryan Pardy
INRS, Centre Armand-frappier Santé Biotechnologie, Laval, Canada

Cryptosporidium spp. are apicomplexan parasites that are restricted to infecting intestinal epithelial cells (IEC). While infection in immunocompetent individuals is self-limiting, patients with T cell deficiencies experience severe and chronic diarrheal disease. In addition, there are no approved medications in Canada to treat this infection, and the only US FDA-approved drug (Nitazoxanide) is ineffective in immunocompromised individuals. As such, understanding the factors that maintain chronic infection by preventing parasite clearance is an important knowledge gap for the development of novel treatment strategies. Within the small intestine, *Cryptosporidium* infection triggers the production of multiple types of interferon (IFN). Local immune cells produce IFN-γ, which is crucial for parasite control. In addition, infected IEC release both IFN-α/β and IFN-λ, which signal to infected and nearby bystander IEC. While IFN-λ moderately reduces parasite burden, IFN-α/β are thought to support parasite replication by limiting the ability of IEC to respond to IFN-γ. We previously demonstrated that in immunocompromised, IFN-γ KO mice, treatment with recombinant IFN-γ during infection causes a robust and transient reduction in parasite burden. However, when this same approach was applied to Rag2 KO mice, a model for chronic *Cryptosporidium* infection, IFN-γ treatment had no effect on parasite burden. In contrast, blocking IFN-γ during chronic infection dramatically increased parasite replication. Together, these data suggest IEC are limited in their capacity to respond to continuous IFN-γ stimulation. Understanding how IEC balance pathogen control and pro-apoptotic effects of IFN-γ could inform approaches that target the pathogen while limiting intestinal inflammation and pathology.

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TOPIC 4: HOST IMMUNITY, MOLECULAR INTERACTIONS, AND MICROBIOME
HOST-PATHOGEN INTERACTIONS DURING CRYPTOSPORIDIUM INFECTION
08-18-2026 2:10 PM - 4:10 PM

PHYLOGENOMIC RECONSTRUCTION OF CRYPTOSPORIDIUM GENOMES CAPTURED FROM HUMAN AND ANIMAL HOSTS IDENTIFIES EXTENSIVE GENETIC HETEROGENEITY

Eliza Alves-Ferreira[1], Asis Khan[1], Mabel Tettey[1], Fumiaki Ihara[1], Helena Vogel[1], Senyo Botchie[2], Irene Ayi[3], Mattie Pawlowic[4], Guy Robinson[5], Rachel Chalmers[6], Hernan Lorenzi[7], Michael Grigg[1]
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Cryptosporidium causes diarrhea and high mortality in young children in Africa and southern Asia. Low oocyst counts in stool often limit whole-genome sequencing from clinical samples. Here, we enriched *Cryptosporidium* genomic DNA directly from stool samples using 74,973 RNA baits (CryptoCap_75K; 8.2 Mb) derived from gene-rich areas (100% coding capacity) within the *Cryptosporidium parvum* genome. We validated this method to produce genome assemblies of *C. parvum* and *C. hominis* with sufficient depth and breadth of coverage using as few as 25 oocysts. We next showed that *C. parvum* baits efficiently capture highly divergent *Cryptosporidium* species, including *C. canis, C. meleagridis,* and *C. ubiquitum*. To minimize reference bias, we designed a dual-mapping strategy that integrated reads aligned to both *C. parvum* and *the divergent Cryptosporidium spp.* to capture lineage-specific variation. When applied against DNA extracted from 26 *C. ubiquitum* fecal samples, we identified three *C. ubiquitum* groups, A and B exclusive to humans, with B showing evidence of admixture, whereas C was zoonotic. Phylogenetic networks, haplotypes, and admixture analyses identified Group-specific polymorphisms that were clustered within discrete genomic regions, suggesting conserved haploblocks associated with host adaptation. TES-Seq was compatible with archived, frozen samples and could distinguish multiple genomes in both natural and experimentally mixed clinical samples at high resolution. This capture-based genomics approach provides high-resolution insights into *Cryptosporidium* population genetics and lays the groundwork for studies of zoonotic transmission, anthroponosis, and GWAS directly from clinical material.

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TOPIC 2: TRANSMISSION DYNAMICS, EPIDEMIOLOGY, AND GLOBAL HEALTH
THE HELMINTH ELIMINATION IN THE LOWER MEKONG (HELM) RESEARCH PROGRAM
08-18-2026 2:10 PM - 4:10 PM

SPATIAL DISTRIBUTION OF MAJOR HELMINTHS ACROSS THE LOWER MEKONG BASIN

Apiporn Suwannatrai[1], Kinley Wangdi[2], Angela Cadavid Restrepo[3], Archie C. A. Clements[4], Somphou Sayasone[5], Virak Khieu[6], Banchob Sripa[7], Darren Gray[8]
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Background and aims *Opisthorchis viverrini* (OV) and soil-transmitted helminth (STH) infections remain major neglected tropical diseases in the Lower Mekong Basin. Understanding the geographical distribution and determinants of mono- and co-infection is essential for designing targeted control strategies. We investigated the spatial epidemiology of OV infection, STH infection, and OV-STH co-infection in Thailand and Laos. **Methods** Data were obtained from cross-sectional surveys of 15,465 individuals from 524 villages across Thailand and Laos. Participants were classified into four infection categories: no infection, OV infection, STH infection, and OV-STH co-infection. Environmental, socioeconomic, and demographic variables were evaluated as potential predictors. Bayesian multinomial geostatistical models incorporating spatial random effects were fitted to identify risk factors and generate spatial predictions of infection risk. **Results** Substantial geographical heterogeneity was observed in the distribution of mono- and co-infection. OV infection was associated with higher altitude, male sex, and older age, whereas normalized difference vegetation index (NDVI) and nighttime light intensity were negatively associated with infection risk. In contrast, STH infection was positively associated with NDVI, soil moisture, and male sex. Co-infection was more spatially focal and was associated primarily with altitude. Spatial prediction identified OV hotspots in northeastern Thailand and central to southern Laos, whereas hotspots of STH infection and co-infection were concentrated in Laos. **Conclusions** OV infection, STH infection, and co-infection were influenced by distinct environmental, socioeconomic, and demographic factors. The resulting risk maps provide an evidence base for geographically targeted surveillance and integrated helminth control programs across the Lower Mekong Basin.

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TOPIC 2: TRANSMISSION DYNAMICS, EPIDEMIOLOGY, AND GLOBAL HEALTH
THE HELMINTH ELIMINATION IN THE LOWER MEKONG (HELM) RESEARCH PROGRAM
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PREVALENCE OF HELMINTHIASES IN CAMBODIA VIA NOVEL MOLECULAR DIAGNOSTICS

Visal Khieu[1,2,3], Peter Odermatt[1,2], Mary Mationg[4,5], Catherine Gordon[4,6,7,8], Rekol Huy[3], Virak Khieu[3], Darren Gray[4,6]
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Background: *Opisthorchis viverrini* and soil-transmitted helminth (STH) infections remain important public health concerns in Cambodia. Owing to the limited sensitivity of conventional diagnostic methods, the true burden is likely underestimated. We assessed *O. viverrini* and STH infections in endemic sites in northern Cambodia at baseline and 12 months after treatment using microscopy and quantitative real-time PCR (qPCR). **Methods:** A total of 585 participants provided fecal samples at baseline and 521 at 12-month follow-up. Samples were examined using the modified formalin-ethyl acetate concentration technique (MFECT), Kato-Katz (KK; follow-up only), and qPCR. Infection prevalence was compared across methods, and the relationship between egg-per-gram (EPG) counts and qPCR cycle threshold (Ct) values was assessed. **Results:** At baseline, qPCR detected higher proportions of single (57.3%) and mixed infections (15.7%) than MFECT (38.8% and 13.2%, respectively). *O. viverrini* prevalence was substantially higher by qPCR than MFECT (70.3% vs. 45.0%). Although hookworm prevalence was similar between methods (15.7% vs. 15.2%), qPCR identified *Necator americanus* (13.5%) as the predominant species, followed by *Ancylostoma duodenale* (2.6%). At follow-up, qPCR continued to detect higher *O. viverrini* prevalence than MFECT and KK (58.0% vs. 32.4% and 32.8%, respectively) and identified *N. americanus* (2.9%), *A. ceylanicum* (1.9%), and *A. duodenale* (1.3%). Ct values were strongly inversely associated with *O. viverrini* EPG counts. **Conclusions:** qPCR revealed substantially higher detection of *O. viverrini*, hookworm, and mixed infections than microscopy. Persistent *O. viverrini* infection at 12 months suggests ongoing transmission, highlighting the value of molecular diagnostics for surveillance and control programmes in Cambodia.

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TOPIC 2: TRANSMISSION DYNAMICS, EPIDEMIOLOGY, AND GLOBAL HEALTH
THE HELMINTH ELIMINATION IN THE LOWER MEKONG (HELM) RESEARCH PROGRAM
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TRANSMISSION OF HOOKWORMS AND OPISTHORCHIS VIVERRINI INFECTIONS ACROSS HUMANS, FISH, AND DOMESTIC ANIMALS IN RURAL LAO COMMUNITIES: A ONE HEALTH PERSPECTIVE

Vanthanom Savathdy[1], Peter Odermatt[1], Mary Mationg[2], Matthew Kelly[3], Somphou Sayasone[4], Darren Gray[2]
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Please note that the title has been updated to reflect the final data analysis and findings. Updated title: Transmission of hookworms and *Opisthorchis viverrini* infections across humans, fish, and domestic animals in rural Lao communities: a one-health perspective. **Background:** *Opisthorchis viverrini* and hookworm infections are significant public health concerns in Lao People's Democratic Republic (Lao PDR). Sustainable transmission reduction has not been achieved with current control strategies, highlighting the need to better understand prevailing transmission patterns. **Methods:** A cross-sectional study was conducted in Savannakhet province in Lao PDR. Demographic characteristics, and sanitation and hygiene practice questionnaires were obtained to collect data. Infection prevalence in fish and domestic animals were examined, and associated risk factors were also accessed in human infections. **Results:** Among participants, 43.0% and 50.7% had heard about intestinal worms and *O. viverrini*, respectively. Hookworms and *O. viverrini* prevalence in humans were 29.4% and 45.7% respectively. Village levels hookworms prevalence showed a moderate negative correlation between humans and domestic animals ($r = -0.55$), while *O. viverrini* prevalence showed a moderate positive correlation between humans and cyprinoid fish ($r = 0.45$); neither was not significant ($p > 0.05$). Age ≥18 years was significantly associated with both infections ($p < 0.05$). Raw fish consumption was a significant associated with *O. viverrini* infection (aOR 1.8, 95% CI: 1.4–2.2, $p < 0.001$). **Conclusion:** High prevalences of hookworms and *O. viverrini* infections persist in rural Lao communities, indicating ongoing local transmission. Scalable integrated interventions combining health education, improved sanitation, and mass drug administration targeting adult populations are needed to interrupt transmission cycle of these parasitic diseases.

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TOPIC 2: TRANSMISSION DYNAMICS, EPIDEMIOLOGY, AND GLOBAL HEALTH
THE HELMINTH ELIMINATION IN THE LOWER MEKONG (HELM) RESEARCH PROGRAM
08-18-2026 2:10 PM - 4:10 PM

HELM CLUSTER RANDOMISED TRIAL RESULTS IN LAO PDR, CAMBODIA, AND THAILAND

Mary Lorraine Mationg[1,2], Gail William[3], Archie C. A. Clements[4], Matthew Kelly[2], Donald Stewart[5], Catherine Gordon[6], Kinley Wangdi[7], Sirikachorn Tangkawattana[8], Apiporn Suwannatrai[9], Vanthanom Savathdy[10,11], Visal Khieu[11,12,13], Sangduan Wannachart[14], Suji O'Connor[2], Simon Forsyth[3], Peter Odermatt[11,12], Donald Mcmanus[6], Somphou Sayasone[10], Virak Khieu[13], Banchob Sripa[14], Darren Gray[1]
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Soil-transmitted helminths (STH) and *Opisthorchis viverrini* (OV) are among the most prevalent helminths in the Lower Mekong Basin. Mass drug administration (MDA) remains the primary approach to controlling these infections. This strategy, however, does not prevent reinfection, thereby limiting progress towards elimination. Supplementary measures such as improved water, sanitation, and hygiene (WASH), culturally appropriate health education, and treatment of reservoir hosts are required to achieve sustainable control and eventual elimination. The Helminth Elimination in the Lower Mekong Basin (HELM) is a multi-country cluster randomised controlled trial conducted in 18 communities across Cambodia, Laos and Thailand. The study evaluates whether combining ALB (targeting STH)/ PZQ (targeting OV) treatment with a multi-component elimination package comprising the Magic Glasses health education program and the Lawa Model, an integrated One Health approach to OV control, compared with ALB/PZQ treatment alone, is more effective in reducing transmission and advancing the elimination of these infections in the Lower Mekong Basin. Parasitological and knowledge, attitude and practices (KAP) assessments were conducted among individuals aged 5-75 years at baseline and at follow-up (12 months after the delivery of the intervention). STH and OV infection levels were evaluated using the modified formalin-ethyl acetate concentration technique. KAP data was collected using a questionnaire. Differences in infection

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prevalence and KAP outcomes between the study arms were analysed using generalised linear mixed models to account for clustering. This presentation reports the impact of the HELM intervention across the three countries and discusses its implications for regional helminth elimination strategies.

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PLENARY SESSION
WHAT NEXT FOR MALARIA VACCINES?
08-19-2026 8:00 AM - 9:00 AM

WHAT NEXT FOR MALARIA VACCINES?

Adrian Hill
University of Oxford, Jenner Institute, Oxford, United Kingdom

Malaria is one of the major killers of children with about 500,000 deaths a year particularly affecting sub-Saharan African. Until relatively recently no malaria vaccine had been deployed despite over 115 years of research efforts. But in the last few years two malaria vaccines have been licensed, more candidates show potential to join these now deployed products, and multi-component multi-stage vaccines are showing credible potential, not only to substantially reduce deaths, but to target elimination of malaria from large numbers of countries. Most recently, advances in structural vaccinology have allowed specific domains and subdomains of conserved plasmodial proteins to be expressed on nanoparticles allowing the required level and durability of antibody responses to be achieved. Recent progress with trials of the high efficacy R21/Matrix-M vaccine will be reviewed succinctly to illustrate these points, and prospects for the feasibility of malaria elimination with next-generation multi-stage vaccines discussed. Several key advances have allowed this rapid recent progress: greater translational capacity for vaccine design, development and licensure by non-profit groups and universities; better quantitative comparative data on immunogen types; ongoing emergence of outstanding African clinical trial teams; and appreciation that ultra-low-cost vaccines can be supplied if manufacturing scale exceeds 100 million doses annually. In parallel, outstanding progress with development of two long-acting injectable drugs by Medicines for Malaria Ventures (C Donini et al.) suggests that combined use of vaccines and long-acting injectable drugs should offer a rapid route to progress. Possible sites, strategies and funding options for such an approach will be highlighted.

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TOPIC 4: HOST IMMUNITY, MOLECULAR INTERACTIONS, AND MICROBIOME
CANADIAN PARASITOLOGY NETWORK SYMPOSIUM
08-19-2026 9:10 AM - 11:10 AM

LIFE HISTORY OF EXPOSURE TO SAND FLY VECTOR BITES DETERMINES DISEASE SEVERITY FOLLOWING SUBSEQUENT SAND FLY TRANSMITTED LEISHMANIA INFECTION

Nathan Peters
University of Calgary, Microbiology, Immunology, And Infectious Diseases, Calgary, Canada

Pre-exposure to insect-vector bites can impact vector transmitted infections. However, our knowledge of immunity primed by pre-exposure, how it changes with repeated exposure, and how a cumulative life-history of exposure impacts vector-transmitted disease, is limited. Short-term exposure to the bites of uninfected sand flies, the insect vector for *Leishmania*, activated $CD4^+$ T helper (Th)-1 cells. However, upon repeated exposure, the response diversified to include multiple $CD4^+$ subsets, Interleukin (IL)-5 production, and a Type-4b delayed-type hypersensitivity (DTH) response involving eosinophil infiltration, altered monocyte maturation, and enhanced disease upon subsequent *Leishmania* transmission. DTH was T cell-dependent, employed the Th2 signaling molecule STAT6, and was ablated in eosinophil deficient or anti-IL-5 treated mice. These results demonstrate the life history of pre-exposure to vector blood feeding influences the severity of a vector-transmitted disease.

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TOPIC 4: HOST IMMUNITY, MOLECULAR INTERACTIONS, AND MICROBIOME
CANADIAN PARASITOLOGY NETWORK SYMPOSIUM
08-19-2026 9:10 AM - 11:10 AM

PRESERVING ORGAN FUNCTION DURING INTESTINAL HELMINTH INFECTION

Irah King
Research Institute of the McGill University Health Centre, Microbiology And Immunology, Montreal, Canada

Type 1 immunity mediates host defense through pathogen elimination, but whether this pathway also impacts tissue function is unknown. Here we demonstrate that rapid induction of IFNg signaling coordinates a multi-cellular response that is critical to limit tissue damage and maintain gut motility following infection of mice with a tissue-invasive helminth. IFNg production is initiated by antigen-independent activation of lamina propria $CD8^+$ T cells following recognition of the microbiota during helminth-induced barrier invasion. IFNg acted directly on intestinal stromal cells to recruit neutrophils that limited parasite-induced tissue injury. IFNg sensing also limited the expansion of smooth muscle actin-expressing cells to prevent pathological gut dysmotility. Importantly, this tissue-protective response did not impact parasite burden, indicating that IFNg supports a disease tolerance defense strategy. Our results have important implications for managing the pathophysiological sequelae of post-infectious gut dysfunction and chronic inflammatory diseases associated with stromal remodelling.

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TOPIC 4: HOST IMMUNITY, MOLECULAR INTERACTIONS, AND MICROBIOME
CANADIAN PARASITOLOGY NETWORK SYMPOSIUM
08-19-2026 9:10 AM - 11:10 AM

TRICHINELLA SPIRALIS INFECTION INDUCES NEUROIMMUNE REMODELING AND CLINICAL REMISSION IN A MOUSE MODEL OF MULTIPLE SCLEROSIS

Lisa Osborne
University of British Columbia, Microbiology & Immunology, Vancouver, Canada

The central nervous system (CNS) is under constant immunosurveillance and influenced by immune-related effector molecules, including type 2-associated cytokines. Long-lasting type 2 immunity elicited by intestinal helminth infections can modify immune responses and wound repair locally and in peripheral tissues, but direct effects of helminth infection on the CNS are poorly understood. Here, we explore whether naturally-evoked type 2 immune responses can modify neuroimmune interactions for therapeutic gain in a mouse model of multiple sclerosis. Chronic infection with the helminth *Trichinella spiralis* (*Ts*) remodelled the neuroimmune landscape, including establishment of a robust population of CNS-resident T helper 2 cells, which subsequently minimized CNS inflammation and demyelination during experimental autoimmune encephalomyelitis (EAE). Clinical remission could be achieved with prophylactic or therapeutic infection, was *Stat6*-dependent, and adoptive transfer of Th2 cells promoted remission in the absence of overt infection. These findings highlight the potential for harnessing type 2 immunity to modify outcomes of neuroinflammation and neurodegeneration.

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TOPIC 4: HOST IMMUNITY, MOLECULAR INTERACTIONS, AND MICROBIOME
NOVEL APPROACHES IDENTIFY PROTECTIVE IMMUNE MECHANISMS IN EXPERIMENTAL MALARIA LIVER AND BLOOD-STAGE INFECTIONS
08-19-2026 9:10 AM - 11:10 AM

MALARIA MRNA VACCINATION: HARNESSING CD8 T CELL IMMUNITY AGAINST THE LIVER STAGE

Lauren Holz[1], Ariane Lee[1], Taylah Phabmixay[1], Jordan Minnell[2], John Mamum[2], Sarah Draper[3], Rebecca Mackenzie[2], Jochem Vink[2], Anton Cozijnsen[4], Dhilshan Jayashinghe[5], Stephanie Gras[5], Geoffrey Mcfadden[4], Gavin Painter[3], Ian Hermans[2], William Heath[1]
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With the identification of liver tissue-resident memory T (Trm) cells as crucial effectors for the control of liver stage *Plasmodium* infections, we previously developed a simple lipoplex (LPX) mRNA-based vaccine to prevent malaria. Trm cell formation and protection from sporozoite challenge was only achieved through the addition of an agonist for type I Natural Killer T (NKT) cells, and intravenous administration. Building on this work, we developed a novel lipid nanoparticle (LNP)-based mRNA vaccine platform that induces both antibody responses and liver Trm cells following intramuscular administration, resulting in durable protection in preclinical models. This LNP platform has been validated using numerous *Plasmodium* liver stage antigens and MHC-I backgrounds, and we are in the process of developing a multivalent vaccine that targets the sporozoite and liver stages, with potential for application to malaria endemic regions.

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TOPIC 4: HOST IMMUNITY, MOLECULAR INTERACTIONS, AND MICROBIOME
NOVEL APPROACHES IDENTIFY PROTECTIVE IMMUNE MECHANISMS IN EXPERIMENTAL MALARIA LIVER AND BLOOD-STAGE INFECTIONS
08-19-2026 9:10 AM - 11:10 AM

EFFECTOR MECHANISMS OF ANTIBODY MEDIATED PROTECTION AGAINST MALARIA SPOROZOITE INFECTION.

Fidel Zavala
Johns Hopkins Bloomberg School of Public Health, Molecular Microbiology And Immunology, Baltimore, United States of America

Protection against infection by Plasmodium falciparum sporozoites is mediated in part by antibodies that recognize the repeated (NPNA)3 epitopes of the circumsporozoite protein (CSP). To evaluate the role that the antibody Fc region plays in neutralizing sporozoite infectivity, we assess the protective efficacy of Fab fragments obtained from protective human monoclonal antibodies and use antibodies bearing the LALA and KA mutations known to reduce Fc functions. To determine the impact that multiple-antibody binding to CSP on protection, we use sporozoites from genetically modified P. berghei strains expressing a CSP containing different numbers of (NPNA)3 epitopes. Our results indicate that Fab fragments and Fc-mutated antibodies have a protective efficacy comparable to intact antibodies, indicating a limited role, if any, of Fc functions in sporozoite in this model system. We also determine that antibody binding to multiple adjacent epitopes and the establishment of homotypic interactions between bound antibodies may not be necessary for protection, as binding to one epitope is sufficient to strongly reduce liver burden while only two separate epitopes are needed to achieve sterile protection.

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TOPIC 4: HOST IMMUNITY, MOLECULAR INTERACTIONS, AND MICROBIOME
NOVEL APPROACHES IDENTIFY PROTECTIVE IMMUNE MECHANISMS IN EXPERIMENTAL MALARIA LIVER AND BLOOD-STAGE INFECTIONS
08-19-2026 9:10 AM - 11:10 AM

METABOLIC REGULATION OF ANTI-MALARIAL IMMUNITY

Noah Butler, Taylen Nappi, Regina Antonetti, Peng Shao, Shuyu Xian, Eric Taylor
University of Iowa, Iowa City, United States of America

Progressive malaria elicits severe perturbations to host physiology that include hypoglycemia, hyperlactatemia, and lactic acidosis. We are using experimental models to investigate how malaria parasite-associated metabolic flux influences CD4 helper T cell responses that are essential for host resistance to infection. We hypothesize that parasite-specific CD4 T cell differentiation and function are suboptimal due to altered nutrient/metabolite abundance uniquely established during malaria, which may contribute to the lack of sterilizing immunity that is observed in humans who are repeatedly exposed to Plasmodium parasites. We applied genetic and pharmacological analyses to investigate how T cells acquire and utilize lactate as both an energy source and an instructive signal that shapes their differentiation and protective functions. Our findings reveal CD4 T cell subset-specific dependence on glycolytic metabolism, with some subsets exhibiting metabolic plasticity and access alternative means of energy production that includes lactate oxidation. These findings establish key rationale to explore cellular metabolism as a mechanism to promote durable long-lived memory against Plasmodium infection.

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TOPIC 4: HOST IMMUNITY, MOLECULAR INTERACTIONS, AND MICROBIOME
NOVEL APPROACHES IDENTIFY PROTECTIVE IMMUNE MECHANISMS IN EXPERIMENTAL MALARIA LIVER AND BLOOD-STAGE INFECTIONS
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TEMPORALLY OVERLAPPING MECHANISMS DIVERSIFY AND SAFEGUARD B CELL RESPONSES DURING EXPERIMENTAL MALARIA

Ashraful Haque, Oliver Skinner, Saba Asad
University of Melbourne, Microbiology & Immunology, Melbourne, Australia

Naive B cells amplify and diversify their responses when activated by cognate antigen, via *Myc*-dependent clonal expansion, immunoglobulin class switch recombination (CSR), phenotypic variation, and somatic hypermutation (SHM). Whether these mechanisms act combinatorially *in vivo* to diversify clonal responses to a single pathogen remains unclear. Since diversity in the antigenic targets, functional classes, and production kinetics of parasite-specific antibodies influences immunity to malaria, we test here whether individual B cell clones diversify over time during *Plasmodium* infection and treatment. During the first week of infection, amid widespread Type I Interferon (IFN)-mediated bystander activation, CSR initiates soon after *Myc* up-regulation, and overlaps partially with clonal expansion, resulting in isotype variegation amongst clones. During the second week of infection, expanded clones that seed germinal centres (GC) bifurcate into extra-follicular plasmablasts, exhibit isotype variegation, and initiate SHM, revealing substantial intra-clonal diversification. Over the following month, GC clones exhibit SHM at approximately four mutations per week, with IgG mutational diversity and IgM^+ cells also preserved in GCs over time. Anti-malarial intervention does not impede SHM, but instead exerts quantitative limits on GC size, plasma cell emergence, circulating IgG levels, and protection against re-infection. Finally, contemporaneous B cell development relocates from bone marrow to spleen during infection and treatment. Thus, multiple temporally overlapping mechanisms combine *in vivo* to amplify, diversify, and safeguard humoral immune responses.

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TOPIC 3: RESISTANCE AND DEVELOPMENT OF DRUGS AND VACCINES
ANTHELMINTIC RESISTANCE: DEVELOPMENT, MECHANISMS AND MONITORING
08-19-2026 9:10 AM - 11:10 AM

HOOKWORM RESISTANCE IN DOGS: THE FACTS, THE MYTHS AND THE REALITY

Jan Slapeta
University of Sydney, Sydney School Of Veterinary Science, Sydney, Australia

Anthelmintic resistance in canine hookworm (*Ancylostoma caninum*) is no longer hypothetical, it is measurable, trackable and increasingly unavoidable. This presentation draws primarily on our work in the Australian context, while critically engaging with the rapidly growing global literature and extends beyond *A. caninum* to consider the often-overlooked *Uncinaria stenocephala*, a neglected comparator that challenges assumptions about how and where resistance emerges. The facts are clear. Benzimidazole resistance-associated β-tubulin SNPs, particularly F167Y and Q134H, are now widespread in dog populations, often at high frequency and associated with reduced drug susceptibility. Longitudinal data from shelter dogs in Sydney demonstrate sustained transmission of resistant genotypes, highlighting the realistic potential for dissemination into the broader companion animal population. Parallel surveys of community dogs in remote central Australia, enabled by ambient DESS preservation, extend these observations into under-sampled populations with minimal veterinary intervention. In contrast, *U. stenocephala*, more prevalent in cooler climates, provides an important counterpoint: it circulates within a substantial wildlife reservoir particularly ubiquitous fox populations. Yet, despite detection of putative resistance-associated mutations, convincing phenotypic resistance remains unconfirmed. The myths arise where evidence is limited. While multidrug-resistant isolates have been characterised, robust population-level data and validated phenotypic assays, particularly for macrocyclic lactones, remain scarce, fuelling both overstatement and complacency. The reality is that we can no longer assume efficacy of routine anthelmintic treatments. Continued reliance on empirical deworming without verification risks selecting for resistance while obscuring its detection. Moving from assumption to measurement through routine, quantitative parasitological diagnostics is essential to safeguard parasite control.

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ANTHELMINTIC RESISTANCE: DEVELOPMENT, MECHANISMS AND MONITORING
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FROM AWARENESS TO ACTION: PREVENTING THE SPREAD OF DIROFILARIA IMMITIS RESISTANCE

Anastasia Diakou
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Dirofilaria immitis (heartworm) primarily affects canids and felids and occasionally humans. Infection in pets is prevented by administering macrocyclic lactones (MLs). MLs in a variety of pharmaceutical formulations are a safe and effective tool against heartworm disease. Since the early 2000s, reports of suspected ML-resistant *D. immitis* strains have emerged in the USA, and a few years later, resistant strains from the Lower Mississippi region were genetically and clinically confirmed. Ongoing research in this field demonstrates that resistance has intensified in the areas where it first appeared, spreading over a wider geographic range within the USA. Recently, ML-resistant isolates have been genetically identified in Canada. To date, the circulation of resistant strains has not been confirmed in Europe and Australia. Nevertheless, a case of a dog infected with an ML-resistant strain in Louisiana, imported into Italy, is of particular importance. Similar cases are highly likely to have occurred elsewhere. The dissemination of resistant strains from the USA and the potential independent emergence of resistance represent a major challenge. It is critical to inform veterinarians and pet owners and to attempt limiting the spread of the problem through: (a) MLs administration in accordance with the guidelines of the American Heartworm Society and the European Society for Dirofilariosis and Angiostrongylosis; (b) combined use of effective microfilaricidal agents with antibiotics against the endosymbiotic *Wolbachia* in microfilaraemic animals and mosquito repellents; (c) annual heartworm testing, even in animals under preventive treatment; and (d) thorough investigation of suspicious cases, in order to detect resistant strains.

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MOLECULAR DIAGNOSTICS OF ANTHELMINTIC RESISTANCE VETERINARY PARASITES; CURRENT STATUS AND FUTURE DIRECTIONS

John Gilleard
Faculty of Veterinary Medicine, University of Calgary, Canada, N, Canada

Parasitic nematode control has been dependent on blanket administration of anthelmintic drugs in livestock and companion animals for many decades. However, this approach is increasingly unsustainable due to the emergence of anthelmintic drug resistance and concerns regarding environmental impacts. Consequently, more targeted evidence-based approaches are needed which in turn will require better diagnostics and surveillance. High-throughput sequencing technologies are revolutionizing both our understanding of the genetic basis of anthelmintic resistance and the detection and monitoring of the causal mutations in field populations. In this talk, I will discuss current status and potential future directions in the application of metabarcoding and deep amplicon sequencing to anthelmintic drug resistance diagnostics, surveillance and sustainable management. I will also discuss how these approaches can be used in molecular epidemiological studies of anthelmintic resistance to provide insights into how anthelmintic emerges and spreads in parasite populations in the field. I will also discuss how the current genetic evidence for strongylid gastrointestinal nematodes supports a model in which anthelmintic resistance emerges from a from a relatively small number of origins in a region and subsequently spreads by migration. This has important implications both for molecular diagnostics and the prevention/mitigation of resistance emergence. Examples will drawn from projects on gastrointestinal nematodes of ruminant livestock and the canine hookworm *Ancylostoma caninum*.

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MACROCYCLIC LACTONE RESISTANCE: MOLECULAR INSIGHTS AND THE PATHWAY FORWARD

Anne Lespine
Univ Toulouse, ENVT, INRAE, Intheres, Toulouse, France

Nematodes have a major impact on animal welfare and productivity. Macrocyclic lactones (MLs), broad-spectrum anthelmintics effective at low doses, have become the cornerstone of nematode control. However, the rapid emergence of resistance to these treatments is now severely compromising their efficacy. Accumulating evidence supports a complex, polygenic architecture that allow the nematodes to survive chemical stress, involving multiple pathways rather than a single determinant. We examine advances in ML resistance, highlighting the main mechanisms involved by integrating data from experimentally selected ML-resistant *Caenorhabditis elegans* strains with findings from the parasitic nematode *H. contortus*. In *C. elegans*, ML resistance has been associated with variants in genes involved in amphid development, likely impairing the detection and uptake of xenobiotics; and to altered expression of genes of detoxification, whose modulation may impact drug efficacy by lowering their concentration at target sites. A model has been proposed in which early P-glycoproteins activation aids worms survival by effluxing drugs during selection, facilitating emergence of more stable resistance genes. Studies in *H. contortus* have provided new insights by linking genomic variation and gene expression to resistance phenotypes and treatment history. Importantly, several independent and in-depth studies converge on a region on chromosome 5, which has been identified as a key signature of ML resistance in field populations. Together, these findings provide a solid foundation for identifying novel molecular targets and for the development of sustainable solutions for controlling helminth infections.

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GIARDIA GENETICS AND CELL BIOLOGY
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GIARDIA SURFACE ANTIGENS- IMPORTANT FACTORS IN HOST-PARASITE INTERACTIONS

Emma Stenlund, Anjeela Bhetwal, Staffan Svärd
Uppsala University, Department Of Cell And Molecular Biology, Uppsala, Sweden

Background and aims: *Giardia* surface antigens are mainly made-up of variant-specific surface proteins (VSPs). To evade the host's immune system, *Giardia* can periodically "switch" the VSP expressed on its surface. This spontaneous switching acts as an adaptation mechanism that allows the parasite to persist and cause chronic or repeated infections. In addition to VSPs, alpha-giardins are also found on the parasite surface and they serve as early antigens during infections. Our aim was to study the regulation and function of VSPs and alpha-1 giardin during host-parasite interactions. **Methods:** Recombinant VSP protein, human enteroids,TLR2 and -4 test cells, CRISPR/CAS mutagenesis in the parasite,proteomics, microscopy, single cell and bulk transcriptomics. **Results:** The extracellular, variable portion of the classical VSP TSA417 (GL50803_113797) was added to human enteroids and gene expressions changes were studied using RT-PCR and RNA sequencing. Contrary to earlier studies the VSP proteins did not induce any up-regulation of human cytokines. Nor did they activate TLR2 and 4. Single cell transcriptomic analyses showed that trophozoites express more than one VSP per cell on the RNA level. The stage of the cell cycle, as well as the small RNA associated proteins dicer and argonaute affect expression. CRISP/CAS mutagenesis of alpha-1 giardin affect the growth rate and protein secretion, which results in a decrease of the disruption of human brush border enzymes. **Conclusions:** VSP proteins are mainly involved in antigenic variation as a way to escape adaptive immune responses. Alpha-1 giardin is involved in the secretion of virulence factors and it is needed in the disruption of brush border enzymes.

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PROTEIN TRAFFICKING MECHANISMS AT THE GIARDIA PARASITE-HOST INTERFACE

Carmen Faso
University of Bern, Institute Of Cell Biology, Institute For Infectious Diseases, Mcid, Bern, Switzerland

Molecular communication at the Giardia-host interface necessarily involves peripheral endocytic compartments or peripheral vacuoles. These are feeding organelles about 50 nm below the plasma membrane, which receive extracellular material and have been more recently implicated in releasing it too. We focus on these organelles as an exciting example of how eukaryotic subcellular compartments have evolved along distinct evolutionary trajectories in species with extreme lifestyles, such as parasitism.
We study these organelles in terms of their morphology, dynamics, molecular machineries and function as hubs for protein trafficking in a eukaryote with only 5 identified subcellular compartments. In my presentation, I aim to share with you data generated by my team members on molecular machineries at PECs/PVs and their possible function in both endo- and exo- cytosis. Finally, I shall place our own research efforts into the broader picture of a recently established international collaboration, aimed at shedding light on adaptations to parasitisim using the lineage Fornicata as an example.

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GIARDIA GENETICS AND CELL BIOLOGY
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CYST WALL FORMATION IN GIARDIA: CELLULAR MECHANISMS AND EVOLUTIONARY ORIGINS

Lenka Arbonová[1], Luboš Voleman[1], Martina Vinopalová[1], Vit Dohnálek[1], Courtney Stairs[2], Monika Wisniewska[3], Martin Kolisko[3], Pavel Dolezal[4]
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Encystation is essential for transmission of the intestinal parasite *Giardia intestinalis*. The cyst wall is composed of cyst wall proteins (CWPs) and a β-1,3-N-acetylgalactosamine homopolymer known as giardan, yet the structural roles of these components remain poorly understood. Using CRISPR/Cas9-mediated gene knockouts, microscopy, proteomics, and evolutionary analyses, we investigated the contribution of both components to cyst wall formation. Deletion of the dominant cyst wall protein CWP1 resulted in exposure of polysaccharide fibers and disruption of cyst wall integrity, whereas disruption of giardan synthesis by deletion of UDP-N-acetylglucosamine 4-epimerase (UAE) produced “naked” cysts lacking detectable wall material. These results demonstrate that giardan forms the primary structural scaffold of the cyst wall, while CWPs organize and stabilize this polysaccharide framework. Proteomic analysis of ΔUAE cells identified a highly divergent membrane glycosyltransferase as a strong candidate for the elusive cyst wall synthase (CWS). The protein is specifically induced during encystation and localizes in close association with encystation-specific vesicles. Comparative analyses across Metamonada further suggest that both CWPs and the giardan synthesis pathway originated in the common ancestor of Fornicata and Barthelonids. Together, our data define the complementary roles of protein and polysaccharide components in *Giardia* cyst wall assembly and identify a strong candidate for the enzyme responsible for giardan polymerization.

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GIARDIA GENETICS AND CELL BIOLOGY
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A DIVERGENT TATA-BINDING PROTEIN FROM GIARDIA REVEALS UNEXPECTED DNA RECOGNITION

Janet Yee[1], Melanie Marlow[2], Shelby Bryan[2]
[1]Trent University, Biology, Peterborough, Canada, [2]Trent University, Biochemistry And Molecular Biology Department, Peterborough, Canada

The TATA-binding protein (TBP) is a critical transcription factor in eukaryotes, originally identified for its ability to bind TATA-box sequences within promoter regions of double-stranded DNA (dsDNA). The TBP homolog in *Giardia intestinalis* (gTBP) lacks three of the four conserved phenylalanine residues essential for dsDNA binding in other eukaryotes, and structural modeling predicts that gTBP has a significantly narrower DNA-binding pocket than other TBPs. To better understand these unique features, our laboratory has been investigating gTBP using electrophoretic mobility shift assays (EMSAs), a high-throughput DNA-binding assay, and *in silico* DNA sequence analyses. Our findings reveal that gTBP preferentially binds single-stranded DNA (ssDNA) over dsDNA and exhibits two distinct binding modes. In the A mode, gTBP forms multimers that bind ssDNA sequences containing four or more consecutive guanine residues, which may adopt G-quadruplex secondary structures that can regulate gene expression, maintain telomeres, and modulate DNA replication and genome stability in other eukaryotes. CD and ThT analyses of selected Giardia gene promoters showed that they can adopt G4 structures. In the B mode, gTBP binds as a monomer to flexible DNA regions characterized by low base-stacking energies. Genome-wide analysis of Giardia revealed that promoter regions of many protein-coding genes, as well as tRNA and rRNA genes, contain low base-stacking energy sequences, suggesting that gTBP may have evolved to recognize DNA structural features rather than specific nucleotide motifs. Collectively, these findings broaden our understanding of transcriptional regulation in reduced eukaryotic systems and provide novel insights into the function of TBPs.

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ONE HEALTH APPROACH TO HELMINTH TRANSMISSION AND CONTROL - EXPERIENCES FROM TROPICAL MEDICINE RESEARCH CENTERS IN INDIA AND PERU
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DEVELOPING POC DIAGNOSTICS FOR SOIL TRANSMITTED HELMINTHS FOR SURVEILLANCE PROGRAMS

Nils Pilotte[1], Megan Fris[1], Malathi Manuel[2], Sitara Ajjampur[3]
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Recent years have seen a significant increase in the use of molecular diagnostics to support soil-transmitted helminth (STH) monitoring efforts. Assays using molecular methods, such as real-time PCR, result in improved sensitivity and specificity of detection when compared with traditionally employed coproscopic techniques. However, performing such methods at scale remains challenging, with instrumentation and infrastructure requirements preventing widespread adoption of molecular testing in some settings. Because of such challenges, the coupling of crude DNA extraction procedures with field-friendly molecular diagnostic approaches presents an increasingly attractive option as these methods provide the benefits of molecular tools while allowing for point-of-collection-based testing. Accordingly, we now describe the development of a platform for STH detection which couples recombinase polymerase amplification, with a FAM-labeled endonuclease IV probe, a biotinylated dead Cas9 enzyme, and a lateral flow detection strip. Preliminary testing of assays developed for the detection of both *Ascaris* spp. and *Necator americanus* demonstrated the ability to effectively detect pathogen signal from both analytical and clinical samples, with *Ascaris* spp. detection mirroring sensitivities seen by real-time PCR. Given these findings, further development and testing of these assays is warranted, as demonstration of their performance and utility will expand the reach of molecular testing, enabling its use in regions formerly unable to accommodate molecular diagnostics at scale.

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STATISTICAL AND GEOSPATIAL MODELS TO UNDERSTAND FASCIOLA HEPATICA EPIDEMIOLOGY IN THE HIGHLANDS OF PERU

Melinda Tanabe (Wright)
University of Texas Medical Branch, Department Of Internal Medicine, Galveston, United States of America

Geographic Information Systems (GIS) have been part of modern spatial analysis since the 1960s, when Roger Tomlinson first used computers to analyze geographic data. Since then, GIS has expanded rapidly across government, academia, and the private sector with advances in computing power and digital cartography. Today, GIS tools are embedded in everyday life—from GPS navigation and digital maps to election mapping and real estate visualization. In infectious diseases, the use of GIS gained major visibility during the COVID-19 pandemic, accelerating its adoption alongside satellite imagery, remote sensing, and machine learning. In this talk, we will review the fundamentals of GIS and its applications in infectious disease research. We will highlight its use in the Highlands of Peru and discuss key considerations when applying geospatial approaches to the complex life cycle of *Fasciola hepatica*, a zoonotic trematode requiring freshwater snails as intermediate hosts, with final hosts becoming infected through metacercariae in aquatic vegetation or water. Particular attention will be given to environmental determinants such as altitude, vegetation, water sources, and livestock prevalence that shape transmission patterns. Finally, we will explore how a One Health framework can integrate human, animal, and environmental data to improve understanding of transmission dynamics and guide targeted control strategies.

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DIAGNOSTIC AND ENVIRONMENTAL SURVEILLANCE TOOLS TO INFORM FASCIOLIASIS CONTROL IN PERU

Martha Fernandez-Baca[1], Melinda Tanabe (Wright)[2], Alejandro Castellanos[2], Miguel Cabada[2]
[1]Universidad Peruana Cayetano Heredia, Sede Cusco - Tropical Medicine Institute, Cusco, Peru, [2]University of Texas Medical Branch, Department Of Internal Medicine, Galveston, United States of America

Fascioliasis, caused by the zoonotic trematode *Fasciola hepatica*, is a globally distributed disease affecting both livestock and humans. Infection causes major economic losses in the livestock industry and remains an important yet underrecognized public health problem. The Andean highlands of Peru are among the most endemic regions worldwide, where environmental conditions sustain parasite transmission. However, the true prevalence of human infection is likely underestimated due to the limited sensitivity of conventional diagnostic methods. This presentation will review recent advances in diagnostic and environmental surveillance tools for fascioliasis within a One Health framework, recognizing the interconnected roles of humans, snails, and the environment in transmission. Recent innovations in molecular diagnostics have enabled the detection of parasite DNA in non-traditional samples, expanding surveillance beyond conventional stool microscopy. Topics will include PCR-based detection of *F. hepatica* DNA in human fecal samples and snail intermediate hosts, including the use of portable mini-PCR platforms suitable for resource-limited settings. Additional approaches will include urine PCR, environmental DNA detection in water sources, and serological screening using the recombinant FhCL1 antigen. Particular emphasis will be placed on how these emerging tools may improve sensitivity compared with conventional microscopy, strengthen epidemiological surveillance, and support early detection and fascioliasis control in endemic regions.

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TOPIC 4: HOST IMMUNITY, MOLECULAR INTERACTIONS, AND MICROBIOME
HOST-DIRECTED IMMUNOTHERAPY OF PARASITIC INFECTIONS
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USING CRISPR TO SCREEN FOR STAGE-SPECIFIC GENES REQUIRED FOR TOXOPLASMA TRANSMISSION TO ADVANCE VACCINE DEVELOPMENT

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Transmission of *Toxoplasma gondii* in nature is highly dependent on the parasite's sexual cycle, which occurs exclusively in felids. Infected cats shed high numbers of infectious oocysts capable of causing toxoplasmosis outbreaks in animals and humans. The molecular pathways regulating sexual stage development remain largely unknown. To identify genes essential for oocyst formation, we performed a forward genetic signature-tag mutagenesis screen using CRISPR/Cas9 to generate a library of uniquwly barcoded *T. gondii* strains, each lacking a single gene predicted to be highly expressed during the parasite's sexual stages. We used previous RNA-Seq datasets to identify sexual stage-specific transcripts, including surface antigen genes related to *Plasmodium* 6-CYS proteins, flagellar genes, ApiAP2 transcriptional factors, and families of merozoites-specific secreted proteins (Families A-D). To identify genes affecting oocyst formation, we pooled *T. gondii* knock-out (KO) strains, infected mice, then challenged cats with brain cysts to perform an input/output screen that identified sexual stage-specific genes critical for oocyst formation. Mouse brains were harvested 50 d.p.i. and divided into an input sample and a cat inoculation sample. MiSeq analysis identified two genes, IFT88 and SRS15, whose knockout strains showed reduced or absent oocyst production in cats, respectively. When assayed individually through cats, neither IFT88- nor SRS15-KO strains produced oocysts. Complementation of IFT88 restored oocyst production. Cats vaccinated with either IFT88- or SRS15-KO strains were protected against *Toxoplasma* rechallenge. These findings demonstrate the potential of a live attenuated oral vaccine to block oocyst shedding and reduce *Toxoplasma* transmission through contaminated food and water.

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LONG PENTRAXIN 3 (PTX-3) AS A THERAPEUTIC TARGET FOR CUTANEOUS LEISHMANIASIS

Jude Uzonna
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Cutaneous leishmaniasis (CL) is a major neglected tropical disease affecting millions worldwide, yet treatment options remain limited and no licensed vaccine is currently available. A growing body of evidence indicates that host immune regulatory pathways play critical roles in determining disease outcome and may represent promising therapeutic targets. Long pentraxin 3 (PTX3), a soluble pattern-recognition molecule involved in inflammation, tissue repair, and wound healing, has recently emerged as an important regulator of immune responses in infectious and inflammatory diseases. We previously demonstrated that PTX3 expression is elevated in human and experimental CL and correlates with disease severity. Genetic deletion of PTX3 confers enhanced resistance to both primary and secondary *Leishmania* infection, characterized by reduced lesion development, lower parasite burdens, increased effector memory T-cell responses, and augmented IL-17-producing CD4+ T-cell immunity. Importantly, neutralization of IL-17 abolishes this enhanced protection, identifying the PTX3–IL-17 axis as a critical pathway regulating anti-*Leishmania* immunity. Beyond infectious diseases, PTX3 has also emerged as a therapeutic target in cancer models, where modulation of PTX3-dependent pathways can enhance protective immune responses and improve disease outcomes. Collectively, these findings position PTX3 as a potential immune checkpoint that limits protective immunity during CL. We propose that therapeutic inhibition of PTX3 may represent a novel host-directed treatment strategy capable of enhancing endogenous anti-parasitic immune responses, promoting parasite clearance, and improving long-term protective immunity

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THE IMMUNOTHERAPEUTIC POTENTIAL OF STEM-LIKE CD4^{+} T CELLS IN LEISHMANIA INFECTION

Simona Stäger
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The discovery of antimicrobial drugs has significantly reduced the burden of infectious diseases. However, the growing emergence of antimicrobial resistance, coupled with the slow pace of new drug development, represents a major global health challenge. Visceral leishmaniasis (VL), a chronic parasitic disease, is similarly affected by these limitations. In this context, host-directed therapy (HDT) has emerged as a promising complementary or alternative approach to conventional antimicrobial treatments. Protective immunity against VL relies on T helper 1 (Th1) CD4 T cells, which promote the leishmanicidal activity of macrophages, the primary host cells targeted by the parasite. However, the mechanisms required to sustain and enhance Th1 responses during chronic infection remain poorly understood, particularly as these cells progressively become dysfunctional. We will discuss our recent findings using a murine model of VL, where we identified a unique subset of CD4 T cells exhibiting stem cell-like properties. These cells expand during chronic infection and retain the capacity to self-renew and differentiate into protective Th1 cells as well as other CD4 T cell subsets. Notably, adoptive transfer of these stem-like CD4 T cells into recipient mice significantly reduced parasite burden, highlighting their potential as a novel therapeutic strategy for VL. Further characterization of the mechanisms regulating their expansion, persistence, and differentiation may support the development of innovative HDT-based approaches aimed at restoring durable antiparasitic immune responses.

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HIGH DIMENSIONAL ANALYSIS OF IMMUNE RESPONSES DURING CONTROLLED HUMAN MALARIA AND HELMINTH INFECTIONS

Maria Yazdanbakhsh
Leiden University Medical Center, Leiden University Center For Infectious Diseases, Leiden, Netherlands

Prevention of infectious diseases through vaccination is one of the greatest achievements of medicine, yet there is growing realisation that vaccine immunogenicity and efficacy varies greatly across populations in high- versus low/middle- income countries (LMIC) and in urban- versus rural areas within one country. There are few vaccines against parasitic infections that show high efficacy, this might in part be due to their develpment and the first testing of the vaccine in the so called Global North where parasitic infections are not highly prevalent. Understanding host parasite interaction at the immunological level can help better vaccine development but it should take into account the geographical settting. Controlled human infection models have become an important part of vaccine development pipeline. They provide the opportunity to study human immune responses to parasites in great depth and to test vaccines rapidly. Moreover, if controlled human infection models are set up in endemic areas, they can unravel immunological pathways that underlie naturally acquired immunity (NAI) to parasites. Combining high dimensional cellular immunological analysis with controlled human malaria or hookworm infection models that took place in Europe and in Africa, have allowed us to identify immunological markers that are associated with NAI to malaria and to hookworm infections. In addition, analysing samples from trials testing vaccination with attenuated sporozoites or hookworm infective larvae have identified networks of interplay between specific innate and adaptive immune cells that align with the development of vaccine-induced immunity.

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NON-HUMAN PRIMATE MALARIA AND THE RISK OF ZOONOTIC TRANSMISSION IN A CHANGING ENVIRONMENT
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THE EMERGENCE OF PLASMODIUM KNOWLESI MALARIA IN SABAH, MALAYSIA

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Malaysia has achieved significant success in controlling human malaria, reporting no indigenous cases since 2018. However, zoonotic malaria, particularly that caused by *Plasmodium knowlesi*, presents an ongoing public health challenge. In 2021, 3,575 *P. knowlesi* cases were reported nationwide, with Sabah alone accounting for 61.3% of these infections. Here we highlight the critical need to understand the complex transmission dynamics of zoonotic *Plasmodium* species, involving macaque hosts, *Anopheles* vectors, and humans. Since 2008, Malaysia has recorded over 25,795 cases of *P. knowlesi* malaria and 61 associated deaths. The persistent high incidence in Sabah underscores the need for targeted interventions. While the exact reasons for the surge in *P. knowlesi* cases remain unclear, there are several potential contributing factors. The proposed control measures that will be discussed includes: (i) developing point-of-care diagnostics and proactive surveillance; (ii) implementing intervention strategies based on advanced data analytics; (iii) fostering inter-sectoral cooperation; and (iv) engaging and empowering communities. Further research is crucial to effectively implement these measures and ultimately control zoonotic malaria in Malaysia. We emphasize the importance of a comprehensive approach that includes assessing *Plasmodium* prevalence in human, mosquito, and macaque populations.

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BENCH TO BEDSIDE: CLINICAL PERSPECTIVES OF MANAGING PLASMODIUM KNOWLESI MALARIA WITHIN RESOURCE-LIMITED SETTINGS IN SABAH, MALAYSIA

Cassandra Binti Abot
Ehime University, Parasitology, Ehime, Japan

Plasmodium knowlesi is now the predominant cause of human malaria in Malaysia, with the state of Sabah (Malaysian Borneo), contributing a substantial proportion of cases. As a zoonotic infection maintained in macaque reservoirs and transmitted by forest-fringe mosquito vectors, *P. knowlesi* presents clinical, diagnostic, and public health challenges distinct from human-only malaria species. It's 24-hour erythrocytic cycle can lead to rapid parasite multiplication, with severe disease developing from initially non-specific symptoms. Drawing from clinical and laboratory experiences in Sabah, we examine the management of *P. knowlesi* malaria in resource-constrained and geographically complex settings, including the practical realities of diagnosis, severe disease recognition, treatment, follow-up and prophylaxis across rural communities. Particular attention is given to the interface between clinical laboratory capacity, bedside decision-making, and public health responses, where operational constraints can directly shape patient care. From a Sabahan perspective, we consider how local realities may inform the use of emerging, repurposed, and novel approaches, and discuss practical bench-to-bedside priorities for supporting zoonotic malaria control in endemic settings.

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NON-HUMAN PRIMATE MALARIA AND THE RISK OF ZOONOTIC TRANSMISSION IN A CHANGING ENVIRONMENT
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ZOONOTIC MALARIA IN SRI LANKA: A POTENTIAL THREAT DURING POST-MALARIA ELIMINATION PHASE

Nadira Karunaweera[1], Sadeeka Karunarathne[2], Vishmi Uduwela[3], Rupika Rajakaruna[3], Priyanka De Silva[3], Ashoka Dangolla[4], Rajika Dewasurendra[1], Osamu Kaneko[5]
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Non-human primate malaria (NHPM) has emerged in Southeast Asia as a potential zoonotic threat. In 2022, the WHO revised its certification criteria for malaria elimination, requiring assurance that humans face no risk from NHPM. Sri Lanka received WHO malaria elimination certification in 2016; however, there is no recent documentation of NHPM despite historical reports of simian malaria parasites with zoonotic potential, including *P. cynomolgi* and *P. inui*, in local toque monkey populations. This study examined the NHPM parasites in toque macaques, *Macaca sinica,* in Sri Lanka. Ninety-four blood samples were collected into EDTA-coated tubes from macaques in selected areas of the Central and North Central provinces with intense human–monkey interactions. Giemsa–stained blood smears were prepared, and DNA was extracted using a commercial kit, followed by PCR-based molecular screening and gene sequencing. A nested PCR targeting the 18S ribosomal RNA (rRNA) gene was used to identify the *Plasmodia* species. Fifty-five out of a total of 94 macaques were positive for malaria (58.5%), which included 34/52 (65%) from the Central Province, and 21/42 (50%) from the North-Central Province. Further analysis of *Plasmodium*-positive samples indicated the presence of *P. cynomolgi* 89% (49/55). Further studies on NHPM species identification are ongoing. This study provides evidence of a high prevalence of NHPM in macaques from both provinces studied to date. Although the WHO has not set a numerical threshold of zoonotic malaria cases that would revoke certification, these results highlight the need to closely monitor the risk of human infection from zoonotic malaria in Sri Lanka.

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NON-HUMAN PRIMATE MALARIA AND THE RISK OF ZOONOTIC TRANSMISSION IN A CHANGING ENVIRONMENT
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CYTOADHESION OF ERYTHROCYTES INFECTED WITH ZOONOTIC MALARIA PARASITES

Osamu Kaneko
Institute of Tropical Medicine, Nagasaki University, Department Of Protozoology, Nagasaki, Japan

Plasmodium parasites confer adhesive properties on infected erythrocytes, which, together with erythrocyte invasion, contributes to malaria pathogenesis. In *Plasmodium falciparum*, the most extensively studied malaria parasite, infected erythrocytes adhere to both vascular endothelial cells and uninfected erythrocytes (rosetting). These cytoadhesions are mediated by PfEMP1, ligands encoded by a multigene family, which bind to various host receptors. In contrast, our work on the zoonotic malaria parasite *Plasmodium knowlesi* has shown that infected erythrocytes adhere to vascular endothelial cells through a SICA (Schizont-Infected Cell Agglutination) protein (Chuang et al., 2022). We have also obtained evidence suggesting that parasite-derived ligands are involved in rosetting, and I would like to discuss these findings in this symposium.

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PLASMODIUM SIMIUM: BIRTH AND EVOLUTION OF A ZOONOTIC MALARIA PARASITE

Nathalia De Albuquerque[1,2], Winni Ladeia[1], Helena De Andrade[3], Priscila T. Rodrigues Rodrigues[4], Thaís De Oliveira[5], Joana Da Silva[6], Marcelo Ferreira[1]
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Plasmodium vivax is hypothesized to have arrived with colonizers from Portugal and Spain as vivax malaria was endemic in southern Europe until mid-1900. Once in America, a subpopulation of *P. vivax* underwent anthroponosis, adapting to Neotropical platyrrhine monkeys and originating a sister species, *P. simium*. *P. simium* infects humans as well, making it unique for studying host-shift in malaria parasites as it went through both anthroponosis and zoonosis. Here, we analyzed samples collected in the 1950s from malaria patients in the Sado Valley, Portugal — one of the last regions with autochthonous transmission in Europe at that time. We identified a patient infected with *P. simium*, a parasite restricted to South America, highlighting the historical exchange of malaria parasites between the Americas and Portugal.

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HOW SERIOUS IS THE MENACE OF ZOONOTIC MALARIA?

Georges Snounou
CNRS/Institut Pasteur, Ecologie Et Emergence Des Pathogènes Transmis Par Les Arthropodes, Paris, France

Over the past 20 years cases of zoonotic malaria have been recorded throughout Southeast Asia. This concerns principally infection by *Plasmodium knowlesi*, a parasite that naturally infect macaques that are now the prevalent cause of malaria throughout Malaysia, but especially in the states of Sarawak and Sabah in Borneo where they occur in large numbers. The infection by this species can lead to severe clinical symptoms and death of not treated promptly. Confirmed sporadic cases have also been reported for another macaque parasite species, *P. cynomolgi*. PCR detection suggests that other species might also infect humans. Zoonotic malaria poses a challenge to malaria eradication programmes. In this presentation, I will review recent epidemiological observations and confront them to what is known on the biology and natural history of these parasites, as well as to what remains to be investigated, in order to appraise the threat that zoonotic malaria might pose in the future.

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PARASITIC EOSINOPHILIA: MECHANISMS, DILEMMAS, AND DIAGNOSTIC PERSPECTIVES
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IMMUNOBIOLOGY OF EOSINOPHILIA IN PARASITIC INFECTIONS

Thomas Nutman
National Institute of Allergy and Infectious Diseases, Laboratory Of Parasitic Diseases, Bethesda, United States of America

Eosinophils are granulocytic leukocytes that occupy a central position in type 2 immune responses, where they link innate sensing of tissue damage to adaptive responses against parasitic helminths. Differentiation, survival, and trafficking of eosinophils are tightly controlled by IL-5 and eotaxins, which drive the marked blood and tissue eosinophilia characteristic of worm infection. In many helminth infections, especially with tissue-migrating nematodes, eosinophils accumulate at parasite niches and can mediate larvicidal activity through release of cytotoxic granule proteins, reactive oxygen species, and lipid mediators. Eosinophils also serve as rapid sources of type 2 cytokines reinforcing Th2 polarization and promoting antibody responses that may opsonize helminth parasites for antibody-dependent killing. Beyond direct effector functions, eosinophils have antigen-presenting capacity and can localize to lymphoid tissues during infection, thereby shaping T-cell responses. More recently, eosinophils have been shown to have important immunoregulatory and tissue-repair roles, in which eosinophil-derived IL-4, IL-13 and other mediators that promote alternative macrophage activation, support metabolic remodeling of infected tissues, and can paradoxically enhance worm growth and chronicity. Consequently, eosinophils can be either protective—contributing to larval killing and reduced burdens—or pathogenic, driving immunopathology and fibrosis, depending on helminth species, life-cycle stage, tissue niche, and the broader cytokine milieu

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WHEN EOSINOPHILIA SPEAKS AND WHEN IT DOESN'T: PARASITIC AND NON-PARASITIC TRIGGERS

Harsha Sheorey[1], Abhishek Mewara[2], Thomas Nutman[3], Blaine Mathison[4]
[1]St Vincent's Hospital Melbourne, Microbiology, Melbourne, Australia, [2]Postgraduate Institute of Medical Education & Research, Medical Parasitology, Chandigarh, India, [3]National Institute of Allergy and Infectious Diseases, Laboratory Of Parasitic Diseases, Bethesda, United States of America, [4]ARUP Laboratories Adjunct Instructor, Department of Pathology, University of Utah, Technical Director Of Parasitology, Technical Operations Infectious Diseases, Salt Lake City, United States of America

Eosinophilia is a condition in which the eosinophil count in the peripheral blood exceeds 0.5×10^9/L (500/µL). **Hypereosinophilia** is an elevation in an individual's circulating blood eosinophil count above 1.5×10^9/L (1,500/µL). The hypereosinophilic syndrome is a sustained elevation in this count above 1.5×10^9/L (1,500/µL) that is also associated with evidence of eosinophil-based tissue injury. Eosinophils usually account for less than 7% of the circulating leukocytes and play a key role in immune defense. Eosinophilia can result from a variety of conditions: 1. Allergic reactions: Seasonal allergies, asthma, or drug reactions. 2. Parasitic infections: Especially helminth infections. 3. Autoimmune diseases: Inflammatory bowel disease, vasculitis, sarcoidosis. 4. Blood disorders: Certain leukemias or other hematologic malignancies. 5. Medications: Some drugs can trigger eosinophilia or syndromes like DRESS (Drug Reaction with Eosinophilia and Systemic Symptoms). It is important to ascertain the cause as the managment of cases differs. Clinical presentation and a thorough history can give clues to help in diagnosis of the trigger. However, some investigations are necessary to confirm these which will be discussed. Non-parasitic causes will be briefly mentioned in this talk. The focus will however be on parasitic (mainly helminths) causes and what investigations should be carried out to come to a definitive diagnosis so specific treatment can be started. Limitations of certain diagnostic tests will be discussed.

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TISSUE PATHOLOGY IN PARASITIC EOSINOPHILIA

Blaine Mathison
ARUP Laboratory, Parasitology And Fecal Testing, Salt Lake City, United States of America

Many parasititc diseases present with eosinophilia. In my talk, I will focus on the morphologic identification of select parasites that elicit an eosinophilic response. Examples will include eosinophilic granulomas, Splendore-Hoeppli phenomenon, and parasites that may elicit eosinophilia even though eosinophils may not be present at or around the parasitic entity.

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CLINICAL SCENARIOS AND DIAGNOSTIC APPROACH FOR PARASITIC EOSINOPHILIA

Abhishek Mewara
Postgraduate Institute of Medical Education & Research, Medical Parasitology, Chandigarh, India

Peripheral eosinophilia is a common but often underexplored clinical problem. Its causes are diverse, including allergy, drug reactions, malignancy, autoimmune disease, etc. However, in endemic settings and migrant populations, parasitic infections remain an important and sometimes overlooked cause. In scenarios such as immunosuppressed patients being considered for corticosteroids, asthma-like illness in filariasis-endemic regions or migrants, unexplained hypereosinophilia, fever or abdominal pain with hepatobiliary exposure, paediatric pica, and migratory skin or soft-tissue disease after travel, parasitic causes should be actively considered. This talk will focus on the approach to patients with eosinophilia, drawing on individual clinical scenarios and cross-sectional and prospective cohorts from North India, highlighting frequent helminth seropositivity among immunosuppressed eosinophilic patients, with *Strongyloides* and *Toxocara* predominating, multiple seropositivities being common, and severe eosinophilia, barefoot walking, pica, animal contact, and organ involvement serving as useful triage variables. An eosinophilic patient requires a comprehensive evaluation, including clinical presentation and pattern of organ involvement, followed by grading of the absolute eosinophil count, and careful assessment of geography, travel and migration, food and water exposure, barefoot soil contact, animal contact, immunosuppression, drug history, organ involvement, etc. Stool microscopy is useful only for selected intestinal helminths and may miss early tissue migration, intermittent larval shedding, tissue-dwelling parasites, or infections in immunosuppressed, thus diagnostic testing must be scenario-based. Serology, antigen detection, molecular assays, imaging, tissue or bronchoalveolar sampling, and therapeutic response all have roles, but require cautious interpretation. The goal must be a rational, risk-based approach that avoids both missed parasitic disease and indiscriminate testing.

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TOPIC 4: HOST IMMUNITY, MOLECULAR INTERACTIONS, AND MICROBIOME
FISH PARASITES VS FISH IMMUNITY: INNOVATIVE ADAPTATIONS ON BOTH SIDES
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DEVELOPING A MODEL FOR MICROSPORIDIAN INFECTION IN FISH

Mariam Abdulsayed[1], Noah Rogozynski[1], Yubing Chen[1], Nguyen Vo[2], Brian Dixon[1]
[1]University of Waterloo, Biology, Waterloo, Canada, [2]Wilfrid Laurier University, Health Studies, Waterloo, Canada

Spraguea americanus, is an obligate intracellular microsporidian neuroparasite that is endemic to the American anglerfish, *Lophius americanus*. We have attempted to develop a suitable *in vitro* model involving rainbow trout (*Oncorhynchus mykiss*) cell lines to study *S. americanus* infections but have not been able to deem rainbow trout a primary host—a host that can accommodate a parasite's complete life cycle and reproductive processes. To bridge this methodological gap, this study aims to not only establish a monkfish brain cell line but to characterize monkfish brain cells and observe the interactions between them and *S. americanus* spores. These interactions will be compared to that of RTBrain, a rainbow trout brain cell line. While this is a work in progress, current findings suggest that monkfish brain cells are capable of internalizing spores at a greater rate than RTBrain cells: 64.3% were internalized compared to 8.7%, three days post-infection ($\alpha = 0.05$). Data also suggest that culture medium obtained from flasks that have been housing monkfish brain cells for several months can induce rapid germination in spores within minutes after inoculation, without the presence of cells. Additionally, heat-inactivating this culture medium was found to not affect the amount of germination observed ($\alpha = 0.05$), suggesting that a non-protein component is responsible for triggering germination and propagating infection. As this study progresses, the goal is to fully establish the first monkfish brain cell line, to characterize a *S. americanus* infection, and to document *S. americanus*' life cycle in monkfish brain cells.

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INFLUENCE OF SEA LICE ON VIRAL INFECTION DYNAMICS IN ATLANTIC SALMON

Fabio Zanuzzo[1], Donnie Sonier[2], Brian Dixon[2]
[1]Onda, Aquatic Research, Victoria, Canada, [2]University of Waterloo, Biology, Waterloo, Canada

Sea lice infestation in combination with Cardiomyopathy Syndrome (CMS) caused by Piscine myocarditis virus (PMCV), or Heart and Skeletal Muscle Inflammation (HSMI), caused by Piscine orthoreovirus (PRV), represent some of the most relevant co-infection scenarios in Atlantic salmon aquaculture. However, limited information is available on the dynamics of these pathogen interactions within the same host and their consequences for fish health. From both industry and research perspectives, it is important to understand whether, and through which mechanisms, sea lice infestation may increase susceptibility to viral infections, and conversely, how viral infections may influence parasite outcomes. Our research addresses these knowledge gaps and provides findings relevant to both academic research and commercial production. Our results to date show that sea lice infestation significantly influenced early PRV replication and may subsequently affect disease progression. However, this effect was dependent on the order of pathogen infection. Comparatively, sea lice failed to impact PMCV load in the heart, when lice infestation occurred at the peak of viral replication. Despite this, we found sea lice altered the regulation of innate anti-viral and anti-inflammatory genes, and cytokine concentrations in the heart during PMCV infection. In a related study, because CMS and HSMI are associated with cardiac inflammation and potential reductions in cardiac performance, we evaluated standard metabolic rate (SMR), maximum metabolic rate (MMR), critical thermal maximum (CTmax), and relative ventricular mass (RVM) in Atlantic salmon infected with PRV-1b and PMCV. Although PRV-1b and PMCV infections impact cardiac function, neither virus significantly altered SMR, MMR, or thermal oxygen-use performance under acute warming conditions. Collectively, these findings highlight the importance of considering parasite co-infection when evaluating animal welfare, host responses, and the development of effective fish health management strategies.

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SCUTICOCILIATOSIS IN TWO CRITICALLY ENDANGERED SPECIES OF HANDFISH – CHALLENGES COMMON WITH OUTBREAKS IN OTHER HOST SPECIES

Barbara Nowak[1,2], Rachelle Hawkins[3], Khattapan Jantawongsri[1], Mai Dang[1], Andrew Thompson[4], Rebecca Van Gelderen[2], Jemina Stuart-Smith[1], Andrew Trotter[1], Nathan Bott[2]
[1]University of Tasmania, Institute For Marine And Antarctic Studies, Hobart, Australia, [2]RMIT University, School Of Science, Melbourne, Australia, [3]Seahorse World, Beauty Point, Australia, [4]Animal Health Laboratories, Kings Meadows, Australia

Background and aims Scuticociliatosis is caused by free-living histophagous ciliates. It is common in mariculture and captive fish. Handfish (Brachionichthyidae) is the most threatened marine teleost family and a captive breeding program was established in 2017. Recent review of the submissions from spotted handfish (*Brachionichthys hirsutus* (Lacepède, 1804)) and red handfish (*Thymichthys politus* (Richardson, 1844)) from a public aquarium showed that scuticociliatosis was common (37.5% of diagnosis) in both juvenile and adult spotted handfish and predominant in 2018- 2019 (6 out of 7 cases) (Nowak et al 2025). **Methods** Dead or moribund fish were submitted fixed in formalin and processed for histology. For identification of the scuticociliates, DNA extraction was followed up by PCRs to determine the presence of SSU rRNA of scuticociliates and sequencing of amplicons. Archival samples of handfish from a research facility were reviewed to identify scuticociliatosis outbreaks. **Results** Scuticociliatosis diagnosis was based on detection of the parasite in histological samples, in some cases evidence of systemic infection, but sometimes it appeared to be secondary or possibly postmortem colonisation. Molecular analysis confirmed DNA of two scuticociliates: *Porpostoma notata* and *Uronema*. Recently, the first case of scuticociliatosis was diagnosed in red handfish at the public aquarium. **Conclusions** The main challenges for diagnosis and management will be discussed. These include limited sampling, mortalities being the only samples and the presence of multiple invasive species of scuticociliates. Most of those challenges are common with other species affected by this disease. We propose guidelines for future diagnosis and monitoring of scuticociliatosis.

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DEVELOPMENT OF A LIVE ATTENUATED VACCINE AGAINST LEISHMANIA: PROGRESS THROUGH HUMAN CLINICAL TRIALS
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LEISHMANIASIS: AN OVERVIEW OF THE DISEASE, GLOBAL BURDEN, IMMUNITY, AND UNMET NEEDS FOR VACCINES AND IMMUNE MONITORING TOOLS

Shinjiro Hamano
Department of Parasitology, Institute of Tropical Medicine (NEKKEN), Nagasaki University, Nagasaki, Japan

Leishmaniasis is a neglected tropical disease caused by *Leishmania* parasites, affecting more than 12 million people worldwide and placing hundreds of millions at risk. Clinical manifestations range from cutaneous lesions to severe or fatal visceral disease. Despite its global burden, disease control remains constrained by the lack of licensed vaccines and robust immune-monitoring tools, particularly in endemic, low-resource settings and among vulnerable populations. Protective immunity is primarily mediated by IFN-γ–producing $CD4^+$ T cells, which activate macrophages to eliminate intracellular parasites. Natural infection and historical leishmanization show that durable immunity in humans can be achieved. Major unmet needs remain, including: (i) safe vaccines that recapitulate naturally acquired immunity; (ii) standardized tools to quantify cellular immune responses; (iii) harmonized correlates of protection and endpoints; and (iv) validated controlled human infection models (CHIM) to accelerate clinical development and comparative vaccine evaluation across regions. Gene-edited live attenuated parasites, including centrin-deficient strains, now provide a credible, translationally viable path toward safe and effective vaccines. The Leishmanin Skin Test, which captures IFN-γ–dependent delayed-type hypersensitivity, may serve as a practical tool for immune assessment, endpoint harmonization, and transmission monitoring at both individual and population levels. Manufacturing, regulatory, and equitable access considerations continue to shape implementation. Recent advances in genetic engineering and translational research offer opportunities to address these challenges. This presentation will summarize current understanding of burden and immunity, define key unmet needs, and introduce talks on live-attenuated vaccine platforms, immune monitoring tools, cGMP manufacturing, CHIM approaches, and planned clinical trials in Kenya and Brazil.

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ADVANCEMENT IN LEISHMANIA VACCINE DEVELOPMENT: ROLE IN GLOBAL ELIMINATION OF LEISHMANIASIS

Hira Nakhasi
U.S. Food and Drug Administration (FDA), Us Department Of Health And Human Services, Silver Spring, United States of America

Leishmaniasis is a neglected tropical disease caused by infection with the Leishmania parasite following transmission by an infected sand fly. It results in different clinical presentations ranging from cutaneous leishmaniasis (CL), that can lead to physical disfigurement, to visceral leishmaniasis (VL; Kala-azar) which is fatal if not treated. Millions of people worldwide are at risk of developing leishmaniasis, with an estimated incidence of over 200,000 cases of CL, and over 50,000 cases of VL each year. Although progress has been made in reducing VL burden in Southeast Asia through the elimination program, there are numerous recent outbreaks of CL and VL reported from other countries in Africa and South America and in non-endemic areas. To achieve global elimination of leishmaniasis, new tools are required, including better surveillance and a safe and effective vaccine. Asymptomatic infection or treatment of VL patients confers life-long immunity in most cases. Moreover, the stability of the Leishmania parasite genome indicates that the development of an effective vaccine against leishmaniasis might be possible using a live attenuated vaccine strategy. Our laboratory has developed a genetically modified live attenuated dermotropic Leishmania vaccine (Leishmania major Centrin gene deleted parasite, LmCen-/-) which is safe and effective against both cutaneous and visceral forms of leishmaniasis in preclinical studies. I will discuss the advancement of the LmCen-/- live attenuated vaccine toward human trials, identiofication of biomarkers of immunity, and use of leishmanin skin test (LST) as a surrogate biomarker of protective cellular immunity.

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REVIVAL OF LEISHMANIN SKIN TEST (LST) FOR SURVEILLANCE AND ASSESSING VACCINE IMMUNOGENICITY

Abhay Satoskar[1,2]
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Over 12 million people currently suffer from leishmaniasis, and approximately 2 million new cases occur each year, making it a major global health problem and a WHO classified neglected tropical disease (NTD). Cutaneous leishmaniasis (CL) is the most common form of *Leishmania* infection which manifests as localized skin lesions that can become chronic, leading to significant tissue destruction and disfigurement. Other forms of infections are mucosal leishmaniasis (ML), or life-threatening visceral leishmaniasis (VL) caused by *L. donovani* and *L. infantum*. VL is the second most common fatal parasitic infection after malaria and is characterized by dissemination of the parasites to liver, spleen and bone marrow. The zoonotic potential of human leishmaniasis has also been escalating with increased incidence of canine infections, which is the main reservoir host for *L. infantum* worldwide including the United States. The leishmanin skin test (LST) was used for decades to determine exposure and immunity to *Leishmania* infection but the leishmanin antigen used in the LST is no longer available. There are compelling reasons to bring back the LST. Firstly, the LST could identify asymptomatic individuals with previous exposure to *Leishmania* in villages and districts to support surveillance and epidemiologic studies in endemic countries. Second, since promising vaccines are advancing, the LST would be an effective surrogate marker to help determine vaccine efficacy. This talk will provide overview of development of a new GMP scalable LST antigen formulation and its utility for assessing vaccine immunogenicity in pre-clinical animal models and for immune surveillance in humans.

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ACADEMIC-INDUSTRY PARTNERSHIPS: ADVANCING LEISHMANIA DIAGNOSIS, TREATMENT, VACCINE, AND SURVEILLANCE TO ERADICATE THE DISEASE

Sanjay Singh
Gennova Biopharmaceuticals Ltd., Pune, Maharashtra, India

Eliminating visceral leishmaniasis requires translating scientific discoveries into manufacturable, regulated, and deployable interventions. Academic–industry collaborations, including partnerships between Gennova and academic institutions, advance *Leishmania donovani*–based vaccines and diagnostics from laboratory research and preclinical validation to GLP manufacturing and field-relevant translational applications globally today. Academic research enabled the rational design of genetically defined, live attenuated *L. donovani* strains through targeted centrin gene deletion (*LmCen* $^{-/-}$), establishing immunological mechanisms and protective efficacy in experimental models of vector-transmitted visceral leishmaniasis[i] . These candidates were subsequently transitioned into an industrial development pathway, where Gennova led GLP-compliant manufacturing, quality control, and preclinical toxicology assessments, demonstrating a favorable safety profile and genetic stability, both essential for regulatory advancement[ii] . The partnership further extended to translational efficacy testing in naturally exposed canine populations, where the *LmCen* $^{-/-}$vaccine conferred durable protection following repeated sand-fly challenges under endemic field conditions in Tunisia, highlighting its relevance for zoonotic reservoir control[iii] . In parallel, collaborative manufacturing and process standardization enabled the production of leishmanin skin test (LST) antigen from *L. donovani*, reviving a critical immuno-epidemiological surveillance tool to support vaccine evaluation and disease mapping efforts[iv]. Collectively, these programs demonstrate how delineated academic and industrial roles—spanning discovery, scale-up manufacturing, regulatory science, and implementation—can accelerate solutions for leishmaniasis prevention, diagnosis, and monitoring. Key partnership models, translational decision points, and lessons learned applicable to parasitic disease elimination initiatives are discussed. **References:** [i] *Communications Biology* (2021) - https://www.nature.com/articles/s42003-021-02446-x [ii] *Scientific Reports* (2024) - https://www.nature.com/articles/s41598-024-64592-6 [iii] *NPJ Vaccines* (2025) - https://www.nature.com/articles/s41541-025-01070-8 [iv] *Nature Communications* (2023) - https://www.nature.com/articles/s41467-023-42732-2

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VALUE OF A SAND FLY-INITIATED CHIM IN ADVANCING LEISHMANIASIS VACCINES

Eva Iniguez, Tiago Serafim, Jesus Valenzuela, Joshua Lacsina, Shaden Kamhawi
Vector Molecular Biology Section, Laboratory of Malaria and Vector Research, NIAID, National Institutes of Health, Rockville, United States of America

Leishmaniasis is an infectious vector-borne disease transmitted by phlebotomine sand flies. Over 700,000 cutaneous leishmaniasis and 50,000 visceral leishmaniasis cases occur annually, with over a billion people at risk. To achieve global elimination of leishmaniasis, we need a safe and effective vaccine and better surveillance. Responding to this urgent need, we have established a large consortium dedicated to the development of a live attenuated *LmCen-/-* vaccine. As part of the consortium, we are in the process of establishing a vector-initiated controlled human infection model (vCHIM) to assess *LmCen-/-* vaccine efficacy. The unique experimental design of our vCHIM incorporates the use of a single double-fed infectious sand fly to challenge healthy volunteers, mimicking more closely a natural challenge while overcoming the anticipated fluctuation in transmission intensity of a field setting. Considering the lengthy duration and expense of conducting Phase 3 clinical trials, we project that having a vCHIM as part of phase 2 trials will reduce the timeline to regulatory approvals and licensure by half, contributing to a faster delivery of the *LmCen-/-* vaccine, the only leishmaniasis vaccine currently in clinical development.

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LEISHMANIA VACCINE CLINICAL TRIALS: CURRENT STATUS

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Background and Aims: *Leishmania sp*. cause significant morbidity and mortality, with nearly a billion people worldwide living in areas where they are at risk for infection, and 2 million new cases each year. The live attenuated candidate *Leishmania major* Centrin-/- vaccine has demonstrated safety and efficacy in preclinical models. These encouraging results allowed the progression of the vaccine to human clinical trials. Methods: Three Phase 1 trials have been planned to evaluate this vaccine: in a non-endemic country and two in endemic countries. Each trial will be a double-blinded, placebo-controlled study that will consist of two different dose levels (10^5, 10^6 promastigotes) of the *Lm Centrin*$^{-/-}$ vaccine administered intradermally using the Terumo Immucise device. 18 participants per dose cohort will be randomized at each site to receive either the vaccine or a placebo in a 2:1 ratio. Results: Phase 1 trial sites have been identified, Leishmania non-endemic sites in the US, at Johns Hopkins University and Cincinnati Children's Hospital Medical Center, an Old-World Leishmania endemic site in Kilifi, Kenya at HJF Medical Research International (HJFMRI)/KEMRI, and a New-World Leishmania endemic site in Belo Horizonte, Brazil at the Universidade Federal de Minas Gerais. The US FDA has cleared the Investigational New Drug Application to proceed. The study is undergoing regulatory review in Kenya and Brazil, with trials anticipated to begin later in 2026. Conclusion: The first clinical trials evaluating the safety and immunogenicity of the *Lm Centrin*$^{-/-}$ vaccine will start in 2026 in Kenya, Brazil and the US.

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TOPIC 3: RESISTANCE AND DEVELOPMENT OF DRUGS AND VACCINES
DEVELOPMENT OF A LIVE ATTENUATED VACCINE AGAINST LEISHMANIA: PROGRESS THROUGH HUMAN CLINICAL TRIALS
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Q&A

Greg Matlashewski
McGill University, Microbiology And Immunology, Montreal, Canada

Symposium Title: Development of a live attenuated Vaccine against *Leishmania*: progress through human clinical trials. Chair: Greg Matlashewski, McGill University. Symposium Presenters: Shinjiro Hamano, Nagasaki University; Hira Nakhasi, FDA USA: Sanjay Singh, Gennova Biopharma; Abhay Satoskar, Ohio State Univ; Shaden Kamhawi, NIH USA; Kawsar Talaat, John Hopkins University. People cured of leishmaniasis are typically immune against re-infection demonstrating that a live infection generates protective immunity and represents a viable vaccine strategy. Moreover, vaccination by live infection with wildtype *L. major* has been practiced with success for decades in several Middle Eastern Countries but is no longer performed due to long lasting skin lesions caused by the vaccine. Therefore, this consortium members have used CRISPR gene editing to develop a live attenuated *L. major* vaccine (*Lmcen*) that generates the same protective immune response as wildtype *L. major* infection in animal models (mice, rats, hamsters, dogs) but does not cause lesions. Preclinical progress in developing, manufacturing, testing and identifying surrogate immunological endpoints will be presented including a human challenge infection model and regulatory approved human clinical trials.

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PROTOZOAN EXTRACELLULAR VESICLES: MESSAGES OF INFECTION AND PROTECTION
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GENOTYPE-SPECIFIC EXTRACELLULAR VESICLES MODULATE METRONIDAZOLE RESPONSE IN GIARDIA LAMBLIA

Maria Touz[1], Gabriel Luna Pizarro[1], Jerónimo Laiolo[2], Nehuén Salas[1], Constanza Feliziani[1], Andrea Ropolo[1]
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Background and Aims *Giardia lamblia*, a eukaryotic intestinal parasite, releases small extracellular vesicles (sEVs) that mediate parasite–parasite communication under environmental stress. The growing incidence of treatment failure in giardiasis cannot be fully explained by classical genetic resistance mechanisms, highlighting the need to investigate alternative adaptive strategies. This study examined whether sEVs derived from metronidazole (MTZ)-resistant clones transmit drug-response traits in a genotype-dependent manner. **Methods** MTZ-resistant parasites from assemblages A and B were generated through prolonged in vitro exposure to MTZ. sEVs were isolated by differential ultracentrifugation and characterized by nanoparticle tracking analysis and transmission electron microscopy. Internalization was assessed by fluorescent labelling and flow cytometry. Drug susceptibility, reactive oxygen species (ROS) production, and expression of enzymes involved in MTZ metabolism were evaluated by quantitative PCR (qPCR). **Results** sEVs from resistant clones were efficiently internalized by wild-type trophozoites, although uptake efficiency varied among assemblages. Vesicle exposure induced contrasting phenotypes: assemblage A parasites became hypersensitive to MTZ, while assemblage B parasites exhibited reduced susceptibility. These divergent responses correlated with genotype-specific modulation of metabolic enzymes and altered ROS production, indicating functional reprogramming of recipient parasites. **Conclusions** sEVs serve as vectors for the transfer of non-genetic information that shape early adaptive responses to drug pressure in *Giardia*. By identifying extracellular vesicle–mediated communication as a driver of drug-response plasticity, this work establishes a novel conceptual framework for understanding the acquisition of resistance beyond classical genetic mechanisms, with potential implications for the management of refractory giardiasis.

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MOLECULAR INSIGHTS INTO GIARDIA DUODENALIS EXTRACELLULAR VESICLES: VIRULENCE CARGO AND IMMUNOPROTECTIVE POTENTIAL

Gerardo Corral-Ruiz[1], Raul Argüello-García[1], Dalia Ortiz-Lozano[1], Lizbeth Salazar-Villatoro Lizbethb[2], Edar Pech-Santiago[1], Martha Espinosa-Cantellano[2], Daniel Hernández-Cueto[3], Angélica Silva-Olivares[2], Abigail Betanzos[2], Guadalupe Ortega-Pierres[4]
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Extracellular vesicles (EVs) are key mediators of intercellular communication, enabling trans-kingdom molecular exchange through the transfer of proteins, nucleic acids, lipids, and metabolites. In *Giardia duodenalis*, EVs have been implicated in parasite survival, host–pathogen interactions, and modulation of host immune responses. In this study, EVs were isolated from trophozoites cultured in two serum-free media of distinct nutritional complexity (TYI-33 and DMEM) and characterized to elucidate their protein composition, pathogenic potential, and immunogenic properties. Nanoparticle tracking analyses revealed cup-shaped vesicles with predominant size ranges from 51 to 150 nm in diameter. Proteomic profiling identified 227 proteins in TYI-33-derived EVs (TYI33-EVs) and 97 in those obtained from DMEM, including molecules associated with cell cycle regulation, cytoskeletal organization, metabolism, antioxidant defense, and virulence. Among the latter, giardipains 1–3, enolase, fructose-bisphosphate aldolase, ornithine carbamoyltransferase, and arginine deiminase were detected. Exposure of an intestinal epithelial line, IEC-6, to TYI33-EVs resulted in disruption of epithelial junctions and apoptotic-like morphology, consistent with EV-mediated cytopathic effects. To evaluate their protective potential, gerbils were intranasally immunized with TYI33-EVs and subsequently challenged with *G. duodenalis* trophozoites. Immunized animals showed a significant reduction in intestinal trophozoite burden and fecal cyst shedding at 7, 14, and 21 days post-infection, along with elevated serum levels of IL-17F and IL-22, two cytokines associated with mucosal defense and epithelial repair. Collectively, these findings indicate that *Giardia* EVs, while carrying virulence-associated factors that may facilitate intestinal colonization, also possess strong immunogenic and protective properties, supporting their potential as a novel mucosal vaccine platform against giardiasis.

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EXPLORING THE ROLE OF LEISHMANIA AMAZONENSIS EXTRACELLULAR VESICLES IN INFECTION AND IMMUNE MODULATION

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Extracellular vesicles (EVs) released by *Leishmania* spp. contribute to host–pathogen communication, participating in the initial infection, parasite survival, immune modulation, and disease progression. These EVs deliver their cargo, interfering with innate and adaptive immune responses. *Leishmania* spp. EVs can exacerbate infection by favoring a regulatory or anti-inflammatory immune profile, depending on the parasite species and host context. Conversely, there is evidence that EVs also hold significant potential as immunogenic components when strategically exploited. Thus, using purified EVs obtained from *Leishmania* parasites is an interesting approach to explore their role in pathogenesis, protection, and therapeutics. Our group demonstrated that intraperitoneal immunization with these EVs provided partial protection in an experimental model of cutaneous leishmaniasis. Furthermore, our data showed that intranasal immunization with *L. amazonensis* EVs induced a protective response in experimentally infected animals, with a significant reduction in parasite load, decreased inflammation, and induction of a Th1 response. Exploring the immunomodulatory activity of EVs in a treatment model is also worth evaluating. Our group observed that treatment with EVs stabilized skin lesions and parasite load in a cutaneous leishmaniasis model. Proteomic characterization of these EVs revealed the presence of immunogenic parasite-derived molecules. Furthermore, comparing the protein content of EVs from parasites with distinct virulence profiles revealed insights into the virulence factors present in these EVs. Overall, understanding the role of *Leishmania* extracellular vesicles in disease pathogenesis, immunization, and treatment is essential for the rational design of innovative therapeutic and prophylactic approaches to control leishmaniasis.

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RNA REGULATION DURING PARASITIC INFECTION
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EXPANDING THE LANDSCAPE OF REGULATORY NONCODING RNAS IN LEISHMANIA

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Leishmania parasites undergo major environmental transitions between insect vectors and mammalian hosts, requiring rapid and tightly coordinated changes in gene expression. Unlike most eukaryotes, these parasites lack canonical promoters for individual genes and rely predominantly on post-transcriptional regulation. Increasing evidence suggests that noncoding RNAs (ncRNAs) are central players in this process. Starting from the unexpected discovery of polyadenylated transcripts derived from 3'UTRs in *Leishmania major*, we performed a comprehensive analysis of ncRNAs across the life cycle of *Leishmania braziliensis*, identifying ~3,600 putative ncRNAs and extensive stage-specific expression patterns. We functionally characterized distinct classes of regulatory ncRNAs, revealing unexpected mechanisms linking RNA structure, chromatin dynamics, and parasite gene regulation. Comparative genomic analyses identified conserved structured ncRNAs, including lncRNA45, whose function depends on RNA secondary structure and is required for optimal parasite growth. A single nucleotide substitution disrupting the structured region altered RNA–protein interactions and abolished functional rescue. We also characterized two 3'UTR-derived ncRNAs that exist both as independent transcripts and embedded within their cognate mRNAs, undergoing noncanonical processing pathways. Both interact with the chromatin remodeling factor SPT16, a component of the FACT complex, and co-localize with R-loops, suggesting roles in chromatin-associated regulation. Functional analyses demonstrated their impact on parasite gene expression networks. Together, our findings reveal that ncRNAs in *Leishmania* are active regulatory molecules rather than transcriptional byproducts, uncovering new layers of gene expression control in an early-diverging eukaryote and highlighting ncRNA-mediated regulation as an important mechanism in parasite adaptation.

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UNCOVERING THE ROLE OF NON-CODING RNAS DURING FASCIOLA HEPATICA INFECTION

Nham Tran[1], Dayna Sais[1], Sumaiya Chowdhury[1], John Dalton[2], Sheila Donnelly[2]
[1]University of Technology Sydney, Sydney, Australia, [2]University of Galway, School Of Biological And Chemical Sciences, Galway, Ireland

Mammalian hosts rarely mount fully protective immune responses capable of expelling helminths. Instead, these long-lived infections are typically tolerated, with immune responses favouring tissue repair and damage limitation over parasite elimination and protective immunity. Yet the molecular mechanisms underpinning this host-parasite relationship are poorly understood. Our work has identified parasite-derived microRNAs (miRNAs) within host macrophages that appear to mimic host sequences, thus enabling them to engage with host regulatory networks to reshape immune responses. In doing so, they reduce the inflammatory and anti-pathogen activity of macrophages, redirecting immune responses away from parasite clearance and toward phenotypes that support persistence and tissue homeostasis. Deeper analysis of these miRNA sequences reveals features distinct from those found in free-living worms, suggesting evolutionary adaptation linked specifically to parasitic lifestyles. Importantly, these molecules also appear to be selectively packaged for export and communication with host cells, supporting a specialised signalling role rather than functions confined to parasite development. Together, these findings suggest that helminths may actively engineer immune tolerance through molecular mimicry, using secreted miRNAs to co-opt host immune regulatory networks. This research positions parasite-derived miRNAs as key mediators of chronic infection, with important implications for immunology, parasite biology, and future therapeutic discovery.

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RNA EDITING IN TRYPANOSOMATID PATHOGENS: FROM REGULATION TO DRUG DISCOVERY

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Trypanosomatid parasites rely on a uniquely complex mitochondrial RNA metabolism system that is absent from their mammalian hosts, making it both biologically fascinating and therapeutically attractive. A central feature of this system is uridine insertion/deletion RNA editing, in which cryptic mitochondrial transcripts are remodeled into translatable mRNAs by coordinated RNA-protein complexes. I will discuss recent advances in understanding how RNA editing is regulated within a broader mitochondrial RNA-processing network that includes mRNA 5' and 3' end maturation, A/U tailing, RNA circularization, and life-stage-specific gene expression. Emerging evidence suggests that circular mitochondrial mRNAs form a distinct RNA population with altered tail features, raising new questions about how transcript architecture influences RNA stability, editing, and translation. The talk will also connect these regulatory mechanisms to drug discovery. Using biochemical RNA-editing assays, compounds have been screened for their ability to inhibit editing, revealing molecules that disrupt RNA-editosome interactions or inhibit RNA ligase activity. Together, these findings highlight mitochondrial RNA editing and associated RNA-processing steps as promising targets for understanding parasite development and developing new antiparasitic strategies.

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DIVERSITY OF 3'UTR-MEDIATED POST-TRANSCRIPTIONAL REGULATION THROUGHOUT LEISHMANIA DEVELOPMENT

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Leishmania relies on posttranscriptional regulation (PTR) to control gene expression throughout its development. Cis-acting elements within 3'UTRs are pivotal to PTR. We have identified several classes of 3'UTR elements contributing to either mRNA stability or translation, and a new paradigm of PTR driven by 3'UTR-located retroposon elements. The *Leishmania* genome harbors formerly active Short Interspersed DEgenerated Retroposons (SIDERs) that have evolved to fulfill critical regulatory functions. Genome-wide expression profiling studies showed that members of the SIDER2 subfamily are mostly transcribed as part of 3'UTRs accounting for 13% of the *Leishmania* transcriptome and are broadly associated with low mRNA expression. The remarkable link of SIDER2 retroposons with downregulation of gene expression supports their co-option as major cis regulators of mRNA abundance in *Leishmania*. SIDER2 sequences also add to the diversification of the *Leishmania* gene expression repertoire with ~35% of SIDER2-containing transcripts being developmentally regulated, and a few encoding key virulence factors. SIDER2 retroposons can also form various evolutionary divergent clusters, each harboring an intriguing chromosomal organization of homologous SIDER2 sequences, contributing to chromosome dynamics and co-regulation. In addition, using Nanopore direct RNA sequencing, we showed that 3'UTRs can be the site of multiple processing events, leading to the formation of long non-coding RNAs (lncRNAs). These lncRNAs exhibit generally distinct expression patterns from the 3'UTRs they derived, and several are developmentally regulated, representing 27% of the *L. infantum* stage-regulated transcriptome. Altogether, these findings provide new important insights into the transcriptome complexity and RNA regulation dynamics in both stages of *Leishmania* development.

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PALEOGENOMICS LINKS HELMINTH INFECTIONS TO FISHER-GATHERER SUBSISTENCE IN A BRAZILIAN SAMBAQUI

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The *sambaqui* (shellmounds) people are recognized as fisher-gatherers who inhabited the coastal regions of Brazil, with a subsistence on a diet composed of fish, mollusks, and plants. Paleoparasitological investigations in these open-air sites are challenged by the severe taphonomic processes affecting parasite vestige preservation. Previous paleogenetic work reported parasite helminth infection by *Ascaris* sp. in an individual from Jabuticabeira II (JABII) *sambaqui*, located in the Santa Catarina state, Brazil (2,890 ± 55 to 1,805 ± 65 years BP). To expand our knowledge about helminth infections in *sambaqui* people, the present study applied a paleogenomic approach in JABII. Sediments from pelvic and sacral regions of 3 JABII individuals and environmental samples underwent ancient DNA extraction and *cox*1 gene PCRs. High-throughput sequencing data were compared to a custom-built reference database. The JABII dataset revealed reads that are mapped against plathelminth and nematode parasites. Plathelminth observations are probably attributable to pseudo-parasitism and are associated with the animal hosts identified within the JABII faunal assemblages. Two JABII individuals exhibited *Anisakis simplex*, *Contracaecum osculatum,* and *Ascaris* sp. Anisakids are common parasites of marine fish, crustaceans, and mammals that infect humans by consuming fish. Most of the fish identified at the JABII site are currently documented as being infected by Anisakidae. *Ascaris* sp. is verified in different JABII individuals from the previous study, confirming the spread of this soil-transmitted helminthiasis. The novel data herein agree with the subsistence of the JABII fisher-gatherers, the sedentary lifestyle, and the faunal surroundings in the Southern coastal region of Brazil.

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LIVING WITH PARASITES: A ONE PALEOPATHOLOGY OF 19TH CENTURY EASTERN CANADA CITIES

Aida Barberà
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What can ancient waste tell us about modern public health? Paleoparasitology is increasingly revealing how infection risks, transmission pathways, and host-environment relationships shaped human health across time. Adopting a One Paleopathology framework, this paper examines the interconnections between household health, sanitation, environmental pollution, and parasite infection risk in the rapidly urbanizing cities of 19th-century Eastern Canada. The sites of the Ward (Toronto) and Lower Drury (Kingston) were densely populated working-class neighborhoods, home to immigrant and minority communities, characterized by poor sanitation and pervasive environmental pollution. Hedley Lodge (Québec City), in contrast, was a wealthy manor household located in a growing industrial neighborhood. A total of 32 sediment samples from six privies across were analyzed for parasite eggs and pollen grains using a combined archaeoparasitological and palynological protocol. Co-infection with *Ascaris lumbricoides* and *Trichuris trichiura* was consistently detected across both working-class and elite households, revealing that urban environmental conditions were the great equalizer in parasite transmission. Palynological evidence further points to reliance on repetitive staple foods and medicinal plants, illuminating the complex and often inadequate strategies communities used to cope with persistent infection. Privies aggregate fecal remains from multiple, underscoring that parasitism and health was never an individual condition but entangled in shared infrastructure, ecology, and daily lives of entire communities. Viewed through a One Paleopathology lens, paleoparasitology offers a long-term perspective on how disrupted human-animal-environment relationships drive infection and why tackling them demands holistic thinking as urgently today as it did in the 19th century.

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HELMINTH INFECTIONS IN LATE ANTIQUE (5TH–6TH C. CE) APHRODISIAS, TÜRKIYE

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This research aimed to investigate the parasitic diversity in Aphrodisias, Türkiye in Late Antiquity (5th-7th c. CE). We processed and analyzed three latrine and drain samples from recent excavations completed in Aphrodisias. A 0.2 g subsample from each sample was disaggregated, microsieved, and then viewed under a microscope, capturing images and measurements of preserved helminth eggs. Both *Ascaris* sp. and *Trichuris* sp. eggs were recovered from the samples. In the latrine sample, egg concentrations were very high for both *Ascaris* sp. [5,755 eggs per gram (epg)] and *Trichuris* sp. (5,420 epg), compared to drain samples, which contained *Trichuris* sp. (15 epg) and *Ascaris* sp. (115 epg). In addition, *Dicrocoelium dendriticum* was recovered from the latrine. The presence of *Ascaris* and *Trichuris* is most likely attributable to Roman sanitation practices, which may not have been effective in curbing transmission of these soil-transmitted helminths (STHs). A predictive model was also built to evaluate if the soil pH of different regions in the Roman Empire is predictive of the retention of secondary morphological characteristics of *Ascaris* sp. and *Trichuris* sp. eggs (mamillated coat and polar plugs). This model broadens understanding of the impact of pH on the preservation of key morphological characteristics used to identify common parasites in archaeological settings. Most parasites recovered from the site are STHs, further supporting evidence for the widespread presence of these parasites in the Roman Mediterranean. The particularly high egg concentration seen in the latrine suggests high worm burdens or infection amongst numerous individuals using the latrine.

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ADVANCES IN SEDIMENTARY ANCIENT DNA METHODS TO REVEAL NEW INSIGHTS INTO ANCIENT PARASITE DIVERSITY IN THE ROMAN EMPIRE

Marissa Ledger[1,2], Tyler Murchie[3], Melanie Kuch[2], Piers Mitchell[4], Hendrik Poinar[2]
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The recovery of parasites from archeological feces provides a record of gastrointestinal helminth infections throughout human history. Microscopy is the most common method used, allowing for direct visualization of preserved helminth eggs, while ELISA is used to detect preserved antigens from protozoa. The main limitations of these methods are the inability to identify many taxa to the species-level or to identify poorly preserved parasites. Increasingly, ancient DNA methods are being employed to improve reconstruction of ancient parasite diversity. All three methods were used to identify parasites in fecal samples collected from six Roman period archeological sites located in Belgium, Italy and Türkiye (2nd c. BCE to the 7th c. CE), to explore parasite diversity in the Roman period and compare results from the different methods. Microscopy recovered eggs from *Ascaris* sp., Capillariids, *Dicrocoelium dendriticum*, *Taenia* sp., and *Trichuris* sp. ELISA detected *Giardia duodenalis* in two samples from Italy. Parasite DNA was recovered from five samples. Four samples contained *Ascaris* sp. DNA and two samples contained *Dicrocoelium dendriticum* DNA alongside microscopically identified eggs. From as little as 0.2g of ancient sediment, microscopy detected the highest diversity of taxa. Ancient DNA recovered from samples containing poorly preserved eggs allows for a more confident exploration of the morphology of ancient eggs, but these methods could not recover DNA from all taxa and performed better for taxa present in higher egg concentrations. In combination with other studies done in the Roman period, the most common gastrointestinal parasites recovered are those spread by ineffective sanitation, especially *Ascaris* and *Trichuris*.

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CYSTIC ECHINOCOCCOSIS IN AL-ANDALUS CEMETERIES (SOUTHERN IBERIA): INSIGHTS INTO ZOONOTIC DISEASES IN MEDIEVAL ISLAMIC EUROPE

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Certain parasitosis can be identified from the osteoarchaeological record, such as the case of *Echinococcus granulosus*, a zoonotic parasite that allows us to relate the existing interactions between human and canids, as a result of the reproductive cycle of the parasite (from canids to herbivores/human as intermediate host), linked to agro-pastoral communities throughout history. In this communication, we present the finding of two fragmented calcified formations associated with two individuals from medieval Islamic cemeteries in the Kingdom of Granada in al-Andalus, dated between the 12th and 15th centuries A.D. For their correct attribution, macroscopy, scanning electron microscopy and energy dispersive X-ray spectroscopy studies were carried out. These results were used in the subsequent differential diagnosis, showing that the calcifications are hydatid cysts caused by *E. granulosus*. This finding allows us a bioarchaeological approach to these populations, evidencing human-animal interactions and agropastoral practices in medieval period of the Kingdom of Granada in al-Andalus.

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INTESTINAL PARASITES FROM THE BINIADRIS BRONZE-IRON AGE FUNERARY CAVE IN MENORCA, SPAIN (C.1200-500 BCE)

Piers Mitchell[1], Marta Diaz-Zorita Bonilla[2], Auxilio Moreno Onorato[2], Ramon Lopez-Gijon[3]
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Background and aims: to determine which parasites affected the population of the Talayotic and Post-Talayotic cultures and their animals in late Bronze Age and Iron Age Menorca. This is important as little past research has explored parasite infection in those living on Mediterranean islands in prehistory. Methods: 131 sediment samples were taken during excavation of the Biniadris funerary cave, which contained large numbers of disarticulated human and animal remains (sheep, cattle, pig and dog) as well as ceremonial areas. The sediment samples were examined with light microscopy. Results: 8 taxa of helminth eggs were identified. Roundworm (*Ascaris* sp.), whipworm (*Trichuris* sp.) and pinworm eggs (*Enterobius vermicularis*) indicate the faecal contamination of the food, drink or hands of those humans buried within the cave. Capillaria, Echinostoma, Dicrocoelium, Fasciola and Taenia/Echinococcus eggs may have originated from the animal remains thought to have been left as offerings for the dead, or false parasitism in humans consuming animals. Conclusions: a broad range of intestinal parasites were present in Talayotic society, with impacts upon health. Ineffective sanitation was the main driver for helminth infection in the human population. Pinworm is very rare in prehistoric sites as eggs are often destroyed by soil organisms as the centuries pass, but in this case the cave environment may have enabled their preservation.

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TOPIC 3: RESISTANCE AND DEVELOPMENT OF DRUGS AND VACCINES
DECODING AND DIRECTING IMMUNITY IN LEISHMANIASIS: FROM HOST DETERMINANTS TO VACCINE AND DRUG DEVELOPMENT.
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LEARNING FROM OTHER PARASITES: QUINOLINE AND BENZIMIDAZOLE-FERROCENE HYBRIDS AGAINST CUTANEOUS LEISHMANIASIS

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Cutaneous leishmaniasis, the most prevalent form of the parasitic vector-borne disease leishmaniasis, is predominantly caused by *Leishmania major* in many developing regions. Current treatments, including amphotericin B and miltefosine, are limited by drug resistance, host toxicity, and high treatment costs, underscoring the need for novel therapeutic strategies. Drawing on pharmacophores with established antiparasitic activity in other disease contexts, molecular hybridisation and metal incorporation strategies were applied to synthesise a series of compounds containing quinoline, benzimidazole, ferrocene, and triazole scaffolds. The compounds demonstrated adequate stability at 37 °C over 48 hours. Antileishmanial screening against *L. major* promastigotes identified one lead compound (4a) with potent activity (IC_{50} = 6.63 ± 0.66 µM), comparable to amphotericin B (IC_{50} = 4.96 ± 0.13 µM). Compound 4a also showed significant efficacy against intracellular *L. major* amastigotes in murine bone marrow-derived macrophages (BMDMs) (IC_{50} = 15.4 ± 0.5 µM), with minimal host cell toxicity and a favourable selectivity index. Mechanistic profiling revealed that compound 4a induced concentration-dependent increases in intracellular reactive oxygen species, mitochondrial membrane depolarisation, and plasma membrane impairment in *L. major* promastigotes, suggesting multimodal antiparasitic activity. Ongoing functional studies are further evaluating host-protective immune responses elicited by the lead compound in cellular and experimental models. Collectively, these findings highlight the therapeutic potential of this hybrid scaffold as a promising platform for the development of novel antileishmanial agents.

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NEW FRONTIERS IN LEISHMANIASIS VACCINE AND TREATMENT DEVELOPMENT

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Leishmaniasis remains a major neglected tropical disease with limited therapeutic options, significant toxicity of current drugs, and no licensed human vaccine. Recent advances in immunology and biotechnology have opened new avenues for disease control. In the field of cell therapy, adipose-derived mesenchymal stem cells (AD-MSCs) demonstrated therapeutic potential in experimental cutaneous leishmaniasis caused by *Leishmania amazonensis*. AD-MSC treatment partially controlled lesion development, and when combined with meglumine antimoniate significantly reduced both lesion size and parasite burden. Building upon these findings, extracellular vesicles derived from AD-MSCs (AD-MSC-EVs) emerged as a promising cell-free therapeutic strategy. AD-MSC-EVs reduced lesion severity, modulated humoral and cellular immune responses, and promoted IL-10-dependent immunoregulation, highlighting their potential as adjunctive therapies. Immunotherapeutic approaches targeting immune checkpoint pathways have also shown encouraging results. *L. amazonensis* infection induces PD-1 expression on T lymphocytes and PD-L1 on antigen-presenting cells. Treatment with anti-PD-1 or anti-PD-L1 monoclonal antibodies restored T-cell functionality, enhanced IFN-γ production by $CD4^+$ and $CD8^+$ T cells, and significantly reduced parasite loads, supporting checkpoint blockade as a novel strategy against chronic leishmaniasis. Vaccine development has progressed from first-generation whole-parasite formulations to defined antigen and next-generation platforms. More defined candidates have demonstrated strong protective efficacy associated with Th1 polarization and parasite control. Future efforts will focus on integrating reverse vaccinology/mRNA technologies to identify and deliver multiple protective antigens simultaneously, aiming to generate broader and more durable immunity. Together, these advances illustrate how vaccines, immunotherapy, cell therapy, and extracellular vesicle-based approaches are reshaping the future landscape of leishmaniasis prevention and treatment.

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PURINERGIC SIGNALLING IN THE CONTROL OF LEISHMANIA AMAZONENSIS INFECTION

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PURINERGIC SIGNALLING IN THE CONTROL OF *LEISHMANIA AMAZONENSIS* INFECTION Leishmaniasis is a neglected tropical disease with global distribution across five continents, yet it remains a particularly significant public health burden in developing countries. The etiological agent, *Leishmania* spp., preferentially infects phagocytic cells, eliciting a complex inflammatory response. During infection, macrophages release adenosine triphosphate (ATP) as a danger-associated molecular pattern (DAMP), which engages purinergic receptors, like P2X7 receptor, expressed on immune cells and thereby modulates host defense mechanisms. Extracellular ATP is subsequently hydrolyzed by ectonucleotidases located on both host and parasite membranes, generating adenosine. This metabolite activates adenosine receptors and promotes the synthesis of immunoregulatory mediators, including prostaglandin E2 and interleukin-10 (IL-10), which contribute to an anti-inflammatory milieu and parasite survival in macrophages. Within this context, purinergic signaling, particularly via the P2X7 receptor emerges as a critical determinant of host resistance to *Leishmania amazonensis*. Activation of P2X7 receptor initiates a multifaceted cascade involving reactive oxygen species (ROS) generation, secretion of pro-inflammatory cytokines such as IL-1β and IL-17, and also leukotriene B4 with consequent reduction of parasite burden. **Thus, delineating the role of purinergic receptors in shaping the immunological landscape of leishmaniasis not only advances our understanding of host–pathogen interactions but also highlights potential therapeutic targets for the development of novel immunomodulatory interventions.**

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SPATIALLY EXPLICIT INDIVIDUAL-BASED MODELS FOR UNDERSTANDING THE FOCALITY OF SCHISTOSOMIASIS TRANSMISSION AND ITS INTERVENTIONS

Melissa Iacovidou[1], Max Lang[1], Betty Nabatte[2], Narcis Kabatereine[2], Goylette Chami[1]
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Introduction: Schistosomiasis persists despite sustained control efforts, driven by reinfection and heterogeneous environmental exposure. Transmission is highly focal, yet existing models assume spatially homogeneous mixing, limiting their ability to inform targeted interventions. This study develops a spatially explicit individual-based model (IBM) to better understand focal transmission and inform targeted mass drug administration (MDA). **Methods:** We developed an IBM linking humans and water contact sites using a bipartite network approach, with explicit snail dynamics at the site level. Human water contact behaviour was informed by wearable GPS-derived movement data and was dependent on distance and water contact site availability. The model was parameterised and calibrated using epidemiological, environmental, and malacological data collected by the SchistoTrack study during six timepoints. Simulations were used to characterise spatial transmission patterns and inform focal MDA targeting high-risk areas. **Results:** The model captures key features of intestinal schistosome transmission, including spatial clustering and seasonal variation. Results indicate that transmission was concentrated around a subset of frequently visited water sites, shaped by human movement patterns. Simulations suggested that targeting these high-risk locations may improve the efficiency of praziquantel distribution, with preliminary work suggesting the models may also be used to target malacological interventions. **Conclusion:** Explicit spatial representation of human–site interactions provides improved insight into the focality of schistosome transmission. If validated in other settings, our models may help national programmes develop targeted interventions accounting for infection focality, environmental variability, and human behaviour, improving MDA efficiency and possibly reducing costs with lower-level implementation units.

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INVESTIGATION OF DEPENDENCE AND SHARED RISK IN MALARIA–SCHISTOSOMA MANSONI CO-INFECTIONS TO GUIDE INTEGRATED CONTROL

Max Lang[1], Betty Nabatte[2], Narcis Kabatereine[2], Christl Donnelly[3], Goylette Chami[1]
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Introduction: Despite calls for disease control integration, evidence on which risk factors are shared between malaria and schistosomiasis remains limited. **Methods:** We analysed data at the point of recruitment from 4290 individuals aged 5-90 within SchistoTrack. A novel bivariate copula regression model with disease-specific margins for *S. mansoni* (Kato-Katz) and *P. falciparum* (RDT) was used to separate shared risk from disease-specific associations. Risk differences, population attributable fractions (PAFs), and subgroup-specific effects were derived for exposures to assess real-world scenarios for decision-making within national programmes. **Results:** Prevalence of *S. mansoni* was 41.5%, *P. falciparum* was 41.2%, and co-infection was 18.5%. Distance to primary healthcare was the strongest shared risk factor, increasing risk of both infections and accounting for 20.8% of co-infection cases. Female sex and higher education were negatively associated with both infections. Proximity to vegetated water bodies was associated with lower schistosomiasis risk but higher malaria risk. Satellite-derived shoreline distance (Sentinel-2) reproduced the field-mapped distance associations with schistosomiasis. Fishing occupations were the main risk factor (PAF 5.6%) for schistosomiasis though uninformative for malaria, while behavioural actions to treat water were negatively correlated with co-infection. For a scenario of reduced water-contact and universal WASH provision, the model estimated a maximum 35% reduction in co-infection cases. Conditional on the marginal covariates, little residual dependence remained between infections. **Conclusion:** Most observed dependence between malaria and schistosomiasis was explained by easy-to-measure covariates. Shared upstream determinants, particularly health-centre accessibility and WASH infrastructure, appear to be the most plausible candidates for integrated control of both infections.

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BURDEN OF SCHISTOSOMIASIS-RELATED MULTIMORBIDITY, ITS RISK FACTORS, AND TIMELINES/TRANSITIONS OF PROGRESSION

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Introduction: *Schistosoma mansoni* infection is a major cause of hepatosplenic multimorbidity, but there is a limited understanding of the burden, its risk factors, condition interactions, and pathogenesis/progression. **Methods:** Current and long-term effects influencing schistosomiasis-related hepatosplenic conditions were investigated. We analysed 45 diverse mild to life-threatening conditions on over 3100 participants within SchistoTrack. Interpretable Bayesian machine learning was applied to identify current risk factors. Significant risk factors were further incorporated into a dynamic graph model to characterise transitions between conditions and timelines of disease progression. **Results:** Over 55% of individuals in SchistoTrack had hepatosplenic multimorbidity. Older age, lower haemoglobin concentration, male sex, being a fisherman, and living in Western Ugandan districts were associated with the highest burden of multimorbidity. Current schistosome infection was not a risk factor for current hepatosplenic multimorbidity. However, persistent infection was a strong predictor of future periportal fibrosis (Niamey protocol C-F) after as little as 1-2 years. Multimorbidity progressed most rapidly in young adults (aged 16-25). Praziquantel was found to reduce the risk associated with periportal fibrosis only when consumed yearly. Regarding disease pathways, the largest number of transitions/interactions were found originating from moderate periportal fibrosis (Niamey protocol pattern D), and spleen and portal vein enlargements. Only two years were required to transition from mild periportal fibrosis (Niamey protocol pattern C) to severe periportal fibrosis (Niamey protocol pattern E/F). **Conclusion:** The high burden of hepatosplenic multimorbidity and rapid development, starting in adolescents and young adults, indicates a need for improved morbidity monitoring and management in schistosomiasis-endemic areas.

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TOWARDS CLINICAL DECISION SUPPORT SYSTEMS FOR SCHISTOSOMIASIS-INDUCED LIVER MORBIDITY

Eloise Ockenden[1,2], Betty Nabatte[3], Narcis Kabatereine[3], Alison Noble[2], Goylette Chami[1]
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Introduction: Schistosomiasis causes diverse presentations of liver fibrosis prevalent in rural sub-Saharan Africa. Despite WHO goals to eliminate schistosomiasis as a public health problem, schistosomiasis-related morbidities are not actively monitored in national control programmes, limited by the lack of assistive diagnostic tools. **Methods:** Deep learning-based classifiers were developed, trained, and evaluated on liver ultrasound images and video collected from 2140 participants aged 5–87 over 2 years within SchistoTrack. Images portrayed curated views of fibrosis following a predefined protocol based on the WHO Niamey protocol. Video sweeps were designed with local sonographers using anatomical anchors that were nonspecific to schistosomiasis morbidity. Clinical utility was assessed by predicting patient outcomes in subsequent years. A focus group with expert sonographers and an interview with a doctor were conducted to assess applications for model outputs to clinical decision-making. **Results:** Our image-based classifier gained an accuracy of 82.2% for classifying five patterns of liver fibrosis and healthy video frames, performing comparably to a second sonographer reading ultrasound images. Despite being a much more challenging task, the video classification model performed comparably to the image classification model, gaining positive predictive values of up to 0.56 for low-prevalence liver patterns. Focus group discussions emphasised standardisation as a key theme for diagnostics, and that model outputs should focus on highlighting liver fibrosis patterns within an image and generation of text reports to aid clinical management. **Conclusion:** We present the first machine learning models for the diagnosis of schistosomiasis-related morbidities, which may improve large-scale morbidity surveillance and clinical decision-making.

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ULTRASTRUCTURE AND CHEMICAL COMPOSITION OF TRICHURIS EGGS: INSIGHTS INTO INFECTION AND PREVENTION

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Soil-transmitted helminth (STH) eggs represent highly specialized survival structures that ensure parasite persistence under adverse environmental conditions. Among them, eggs of *Trichuris* spp. display remarkable resistance associated with a multilayered eggshell and distinctive polar plugs, yet the chemical organization underlying these structures remains poorly understood. We combined high-pressure freezing/freeze substitution (HPF/FS), transmission electron microscopy (TEM), scanning electron microscopy (SEM), energy-dispersive X-ray spectroscopy (EDS), and nanoscale secondary ion mass spectrometry (NanoSIMS) to investigate the ultrastructure and *in situ* chemical architecture of *Trichuris muris* eggs. Correlative analyses revealed a highly stratified elemental organization directly associated with functional compartments of the egg. The chitinous layer and other ultrastructural components of the egg exhibited distinct chemical organizations associated with structural resistance, larval protection, permeability, and other functional properties, while the polar plugs displayed a unique nanochemical composition, suggesting specialized roles in hatching regulation. These findings redefine the *Trichuris* egg as a chemically dynamic and spatially organized system rather than a passive protective structure. By integrating ultrastructural and high-resolution chemical imaging, this study provides new insights into parasite environmental adaptation, host infection processes, and potential vulnerabilities that may be explored for future control and prevention strategies targeting trichuriasis.

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ASCARIS AND THE LUNG: IMMUNE REPROGRAMMING, CHRONIC DISEASE, AND GLOBAL HEALTH IMPLICATIONS

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Ascariasis affects an over 500 million people globally and is increasingly recognized as a contributor to chronic respiratory disease in low- and middle-income countries. While *Ascaris* larvae are cleared from the host within weeks, a single episode of pulmonary larval migration can precipitate lasting lung damage and dysfunction that persists for months after infection resolution. The mechanisms sustaining this chronic pathology have remained poorly understood. Our laboratory has discovered two potential mechanisms. First, using high-resolution immunopeptidomics on pulmonary macrophages, we identified a unique, non-mammalian peptide sequestered and presented via MHC class II and a distinct population of epitope-specific CD4+ T cells maintained in both the lung and spleen, providing evidence of persistent antigen-driven immune activation. Second, we identified a persistent population of MMP-12 secreting pulmonary macrophages in the lungs that may have chronic effector function on pulmonary architecture over time. These findings offer a molecular explanation for helminth-induced chronic pulmonary morbidity and identify novel target for therapeutic and vaccine development strategies aimed at decoupling larval exposure from lifelong pulmonary disease.

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DETERMINANTS OF MATERNAL-FETAL MALARIA ANTIBODY TRANSFER AND BIRTH OUTCOMES

Bernard Kanoi
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Background: Neonates in malaria-endemic regions depend critically on maternally transferred IgG for early-life protection, yet the mechanism by which placental malaria (PM) disrupts this transfer remains poorly resolved. Conventional cord-to-maternal transfer ratios cannot distinguish whether infection blocks antibody entry into placental transport pathways, impairs fetal-side release, or both. **Methods:** We performed tri-compartment antibody profiling in mother-infant pairs from western Kenya, simultaneously quantifying IgG against an 11-antigen panel (9 *P. falciparum* antigens; 2 mosquito salivary proteins) in matched maternal peripheral, placental intervillous and umbilical cord blood. Transfer was decomposed into maternal-to-placental uptake (PB/MB) and placental-to-cord release (CB/PB) using robust regression path models. **Results:** Transfer was antigen-class dependent: *P. falciparum*-specific antibodies transferred less efficiently than mosquito salivary antibodies ($P < 0.01$), and higher maternal antibody burden was inversely associated with transfer efficiency ($P < 0.01$), consistent with receptor saturation. Despite the absence of a crude global transfer deficit, path analysis localised the PM-associated signal towards maternal-to-placental uptake ($P = 0.06$) rather than placental-to-cord release ($P = 0.52$). A gravidity-conditioned PM×gravidity interaction leaned towards modifing the overall transfer phenotype (interaction $P = 0.05$), with qualitative convergence across 10 of 11 antigens, implicating shared transport machinery rather than antigen-isolated effects. **Conclusions:** The malaria-exposed placenta did not show a strong selective, gravidity-conditioned bottleneck for neonatal antibody acquisition. By anatomically localising a possible transfer deficit to the maternal-facing syncytiotrophoblast interface, these data provide a mechanistic framework for evaluating maternal vaccines and antenatal malaria prevention strategies in terms of their effect on passive immune endowment of the newborn.

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PLENARY SESSION
INTEGRATIVE OMICS STRATEGIES TO UNRAVEL HOST-PARASITE INTERACTIONS IN FILARIAL WORMS
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INTEGRATIVE OMICS STRATEGIES TO UNRAVEL HOST-PARASITE INTERACTIONS IN FILARIAL WORMS

Elodie Ghedin
National Institute of Allergy and Infectious Diseases, NIH, Laboratory Of Parasitic Diseases, Bethesda, United States of America

Elucidating the complex biology of filarial parasites benefits from approaches that integrate molecular, metabolic, and spatial perspectives on host-parasite interactions. In this talk, I will discuss how multi-omics strategies—including transcriptomics, proteomics, glycomics, and metabolomics—can be combined to uncover key mechanisms in filarial worm biology. In *Brugia malayi*, a causative agent of lymphatic filariasis, we identified significant sex-specific differences in protein glycosylation that influence immunomodulatory interactions at the host interface. We also discovered parasite-derived metabolites, including novel acetylcholine analogs, that affect both host lymphatic physiology and parasite reproduction, revealing new connections between metabolism and host manipulation. Finally, I will highlight spatial transcriptomics methods to resolve gene expression within the architecture of filarial and host tissues, providing new insights into the spatial relationships among the host, the parasite, and its *Wolbachia* endosymbiont. Together, these integrative omics frameworks offer a systems-level perspective on host-parasite interactions and reveal biological vulnerabilities that may guide future therapeutic strategies against filarial diseases.

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HELMINTH MICROBIOMES AS DRIVERS OF PARASITE PHENOTYPE, TRANSMISSION AND HOST MANIPULATION

Robert Poulin
University of Otago, Zoology Department, Dunedin, New Zealand

After shaking the foundations of medical research, the microbiome revolution is now sweeping through ecology and evolution. In this context, interactions between a host and a parasite can no longer be seen as simple two-player games: they need to be reframed to incorporate the influence of the microbial communities residing within the host and also within the parasite. Focusing on the microbiomes of parasites themselves, and using recent results of our studies on the microbiomes of helminths of New Zealand animals, I will address questions including: Do helminths with complex life cycles possess a ‘core’ microbiome, i.e. a set of vertically-transmitted bacteria that are not shared with any host and persist through the life cycle? How much geographic variation does this microbiome show among parasite populations? Are extensive microbial exchanges occurring between host and parasite, and also between different parasites sharing the same host? Does the composition of a helminth’s microbiome affect its phenotype, i.e. its development and life history strategies? Can a parasite’s microbiome contribute to host manipulation? Can a parasite’s microbiome be manipulated experimentally? Incorporating symbiotic microbes into the theoretical framework through which we study parasitism is necessary for a better understanding of host-parasite ecology and coevolution. The smallest players of all have been ignored for too long: it’s time to look at microbes within parasites to determine what strings they can pull.

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IMMUNITY AGAINST HUMAN PATHOGENS IN BED BUGS

Jose E. Pietri
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Bed bugs are blood feeding human ectoparasites and ubiquitous pests of the built environment worldwide. Intriguingly, despite exhibiting multiple physiological and ecological similarities to arthropods that are prolific vectors of human pathogens, bed bugs have never been linked to the transmission of any microbes that cause human disease in nature. The reasons for this apparent inability to transmit pathogens have remained enigmatic for over a century. One hypothesis is that bed bugs have evolved unique, robust immune responses that prevent them from sustaining high pathogen loads, thus limiting subsequent transmission. This presentation will discuss new insight into the mechanistic basis of pathogen resistance in bed bugs. Findings covered will include the characterization of infection-induced bed bug genes, the role of hemocyte extracellular DNA traps, and the antimicrobial effects of bed bug endosymbionts.

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THE EVOLUTION OF TRICHINELLA; ARE PARASITISM GENES FOR REAL?

Dante Zarlenga
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Background and Aimes Parasitism among nematodes evolved independently at least 18 times and likely resulted from free-living worms living in close association with would be hosts. However, searching for parasitism genes has been enigmatic. Though rare among metazoans, we believe that horizontal gene transfer played a role in the acquisition of genes linked to parasitism within the genus *Trichinella* and among other select parasite lineages.
Methods Upon sequencing the *Trichinella* genome, a cyanase gene was identified that is common among bacteria, fungi, and plants, but is only observed among other eukaryotes if acquired by horizontal gene transfer. The gene/protein was cloned and expressed, tested for functionality and evaluated for its presence in the *Trichinella* spp. and other genera.
Results Cyanase was observed in select nematodes that possess at least one tissue phase in their lifecycles: however, the *Trichinella* cyanase clusters with plant proteins whereas all other nematode cyanases cluster with bacterial proteins consistent with independent transfer events.
The *Trichinella* cyanase is bio functional (in vitro and in vivo) and is localized to the worm hypodermis and muscle.
Conclusions Cyanase has not been found in free-living nematodes nor in those more recently evolved. It is perplexing that *Trichinella* actively produces and secretes an enzyme that degrades a substrate (cyanate) not normally produced by its hosts. However, we propose mechanisms where cyanate can indeed be generated when *Trichinella* reprograms the muscle cell and where worm and cell survival and longevity depend on *Trichinella's* ability to metabolize the cyanate thus linking gene acquisition to parasitism.

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WHY DEVELOP RESISTANCE TO A PATHOGEN THAT CAUSES NO FITNESS LOSS? MOSQUITOES AND DENGUE.

Carl Lowenberger
Simon Fraser University, Biological Sciences, Burnaby, Canada

The presence of parasites and pathogens drives host immune responses to eliminate the parasites or reduce their negative effects. These may include behavioural or physiological responses. The general concept is that if there is a negative response of the parasites, then there should be some response. In Colombia there are sympatric populations of *Aedes aegypti* that ingest Dengue viruses (DEN); one strain (MIB) eliminates the pathogen while the other strain (S) transmits the pathogen. The factors used by the MIB to eliminate DEN are immune-based physiological responses. In the S strain, we cannot measure a negative effect of the presence of DEN. Infected or uninfected females have similar lifespans, fitness, and number of eggs produced over multiple gonotrophic cycles. The resistant MIB strain in contrast has a slightly shorter lifespan and produces fewer eggs per cycle. Why develop resistance mechanisms if the pathogen produces no obvious negative effects, and why is the resistant strain maintained even in the absence of the pathogen? These factors will be discussed.

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IMMUNITY TO HELMINTH INFECTIONS: MECHANISMS OF HOST DEFENSE IN BARRIER TISSUES
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THE FRENEMY WITHIN: ON HELMINTH REGULATION OF THE INTESTINAL EPITHELIUM

Kaitlin Olsen[1], Tamar Eliyahu[2], Ghislaine Fontes[1], Blanca Callejas[3], Simon Hirota[3], Irah King[1], Danielle Karo-Atar[2]
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Background and aims: Intestinal helminths persist across the animal kingdom by reprogramming host immune and tissue responses to their advantage. The intestinal epithelium functions both as a physical barrier and as an active immune sensor capable of detecting and responding to infection and tissue damage. Although tissue-invasive, helminths are uniquely well tolerated by their hosts, making them an ideal model system for uncovering mechanisms of tissue protection. Our overarching aim is to define how helminths regulate tissue-protective pathways in the gut. **Results:** We have previously demonstrated that the enteric parasitic nematode *Heligmosomoides polygyrus bakeri* (*Hpb*) directly targets the intestinal stem cell compartment, shifting the epithelium into a pro-regenerative state characterized by the expansion of *Clusterin*-expressing revival stem cells (revSCs). Here, we show that TGFβ receptor (TGFβR) signaling is essential for this helminth-driven epithelial reprogramming. *Hpb* induces potent epithelial TGFβR activation in both mouse and human intestinal tissues, and *Hpb*-mediated revSC expansion requires TGFβR engagement. We further identify TGM, a TGFβ mimic secreted by *Hpb*, as the factor responsible for inducing the pro-regenerative transcriptional program and revSC expansion. Finally, we show that the *Hpb*-induced regeneration is accompanied by increased expression of barrier-protective tight junctions, and that *Hpb*-secreted factors can restore barrier integrity in a model of epithelial permeability following bacterial breached. **Conclusions:** These findings reveal a direct mechanism through which *Hpb* exploits epithelial plasticity to promote its chronic persistence while enhancing host barrier resilience.

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INTERACTIONS BETWEEN BILE ACIDS AND HELMINTHS IN THE MAMMALIAN INTESTINAL TRACT

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The species that can live in the guts of mammals, including bacterial members of the microbiota and parasitic worms, have each evolved mechanisms to survive and exploit the mammalian intestinal environment. Bile acids are a group of potentially toxic, immunomodulatory molecules encountered in the intestinal tract, and the ability to chemically modify bile acids- which can change their functional properties- is widespread amongst the bacterial microbiota. Our lab has collected evidence that parasitic worms can also directly chemically modify bile acids (independently of the bacterial microbiota), which likely enhances the ability of gut-dwelling helminths to survive in the mammalian host. In this talk, I will show evidence that mice infected with a small intestine-dwelling helminth have a disrupted bile acid composition in the small intestinal lumen, including reduced concentrations of certain amino acid-conjugated primary bile acids. Changes to the bile acid pool during helminth infection occur both in mice with an intact microbiota (specific pathogen-free mice) and in mice devoid of a bacterial microbiota (germ-free mice), and, helminths cultured in sterile conditions ex vivo secrete an enzyme that can directly deconjugate primary bile acids. Given that different bile acids are known to influence many aspects of mammalian intestinal physiology, exploring the functional consequences of perturbations to the bile acid pool during helminth infection will deepen our understanding of the impact of helminth infections on host health.

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MAPPING THE GENETIC ARCHITECTURE OF HELMINTH MUCOSAL IMMUNITY

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Helminth infections affect more than 1.5 billion people worldwide, yet the host genetic determinants governing protective immunity and immunopathology remain poorly understood. *Ascaris* spp., the most prevalent human helminth, undergo a transient migration through the lungs, eliciting a robust eosinophil-rich type 2 immune response that closely resembles human Loeffler syndrome. The murine *Ascaris* model recapitulates this pulmonary phase and provides a powerful platform to investigate the genetic regulation of mucosal immunity. In this presentation, I will discuss our efforts to define the genetic architecture of host resistance to *Ascaris* infection using the Collaborative Cross (CC), a genetically diverse panel of recombinant inbred mouse strains that models human genetic variation. We hypothesize that naturally occurring genetic variation shapes distinct immune responses that determine parasite clearance and pulmonary inflammation. Our preliminary studies across the eight CC founder strains revealed striking differences in parasite burden and lung pathology, identifying the resistant wild-derived CAST/EiJ strain, which develops a unique SiglecF^{+} eosinophil-dominated inflammatory response associated with rapid parasite control. We are now extending these studies to 40 fully sequenced CC strains, integrating quantitative phenotyping of parasite burden and lung inflammation with immune profiling, transcriptomics, and quantitative trait locus (QTL) mapping to identify genetic loci controlling resistance and type 2 immunopathology. This systems genetics approach will identify novel genes and pathways regulating helminth mucosal immunity, providing mechanistic insights into host-parasite interactions and the genetic basis of immune heterogeneity. These findings may ultimately inform new strategies to prevent and treat helminth infections and other eosinophilic inflammatory diseases.

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GIARDIA-INDUCED TYPE 2 IMMUNITY ATTENUATES BYSTANDER MUCOSAL INFLAMMATION

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Diarrheal diseases are the second leading cause of death in children worldwide. Epidemiological studies indicate that co-infection with the protozoan parasite *Giardia intestinalis* is associated with reduced diarrheal severity. Here, we observed a high incidence of asymptomatic *Giardia* infection in school-aged children from Nigeria. To understand asymptomatic carriage, we developed a mouse model of giardiasis and show that *Giardia* induces a Type 2 mucosal immune response characterized by antigen-specific Th2 cells, IL-25, Type 2 cytokines, and goblet cell hyperplasia. Single-cell RNA sequencing and multiparameter flow cytometry revealed expansion of IL-10-producing Th2 cells, which promoted parasite persistence and protected against *Toxoplasma gondii*-induced ileitis and DSS-induced colitis. This protective effect was STAT6-dependent, as IL-4R blockade or STAT6 deficiency impaired IL-10$^+$ Th2 responses, leading to Th1/Th17-driven inflammation, tissue damage, and parasite clearance. Finally, we investigated whether *Giardia* infection in the gut can remotely shape immune responses in distal mucosal tissues such as the lung. To address this, *Giardia*-infected mice were orally challenged with *Ascaris suum* eggs. We found that prior *Giardia* infection conferred protection against *Ascaris*, as evidenced by a reduced pulmonary larval burden at day 8 post-infection, along with enhanced Th2 cell expansion in the lungs of co-infected mice. Together, our findings demonstrate that *Giardia* reprograms mucosal immunity toward a Type 2 profile, that facilitates parasite persistence while conferring local and distal protection against inflammatory and infectious challenges. These results, highlight a previous underappreciated role for gastrointestinal protists in regulating mucosal tissues immunity and host defense.

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ROLE OF HELMINTH EXTRACELLULAR VESICLES IN COMMUNICATION AND BEYOND

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Helminths release extracellular vesicles in their excretory-secretory products. These membranous nanosized structures contain lipids, proteins, nucleic acids as well as metabolites, and have been described as participating in intercellular and interspecies communications. Our group “rediscovered” extracellular vesicles in two species of trematodes in 2012, and since then, their study has expanded exponentially, with numerous descriptions in different helminth species. These investigations have confirmed active roles of extracellular vesicles in host-parasite communications. We here review major milestones on helminth extracellular vesicles, pointing out current knowledge gaps regarding their characterization and biological functions. Their usefulness in controlling helminthiases (showing diagnosis, vaccination and therapeutic applications) will be also discussed. Finally, current data will be presented on different helminth stage-specific extracellular vesicles, their composition, and their potential role in infection using in vitro models. Supported by Grant PID2023-146116NB-I00 funded by MICIU/AEI/10.13039 /501100011033 and “ERDF/EU”, and Conselleria de Educación, Cultura, Universidades y Empleo, Generalitat Valenciana, Valencia, Spain (Grant CIPROM/2023/54).

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HARNESSING CROSS-SPECIES EXTRACELLULAR VESICLE EXCHANGE FOR THERAPEUTIC DEVELOPMENT AGAINST INTESTINAL NEMATODES

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Background and aims Intestinal helminth infections impose a major global burden on humans and animals. Increasing drug resistance and limited efficacy of existing treatments call for therapeutic alternatives to control these parasites. This study aims to develop transgenic native gut *Escherichia coli* capable of producing and delivering RNA with anthelmintic properities via outer membrane vesicles (OMVs) directly to the site of infection in the gastrointestinal tract following re-engraftment. **Methods** Transgenic native gut *E. coli* strains were generated by stable genomic integration of sequences encoding nematode-specific RNA- and protein-based therapeutics, including self-circularising RNA sponges designed to sequester essential nematode microRNAs. Noncoding RNAs known to impair nematode viability and the nematocidal protein Cry5B were included as controls and proof-of-concept effectors. Transgenic bacteria were characterized and production and extracellular release of the anthelmintic molecules were quantified by RT-qPCR. The non-parasitic model organism *Caenorhabditis elegans* and the parasitic nematode *Heligmosomoides polygyrus* were exposed to the engineered bacteria, and phenotypic outcomes were assessed. **Results** Engineered bacteria produced and secreted the intended therapeutic molecules, with extracellular localization observed in *E. coli* OMVs. Exposure of *C. elegans* to bacteria expressing different therapeutic products induced measurable changes in larval development and chemosensory behaviour compared to wildtype controls. Additionally, the transgenic bacteria were able to re-engraft in mice and persist for several weeks. **Conclusion** We show that transgenic native *E. coli* can serve as a platform for the production and delivery of anthelmintics. While further validation is required, this approach shows promise as a novel strategy for nematode control.

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HELMINTH EVS IN PATHOGENESIS: THE CASE OF LIVER FLUKE

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Extracellular vesicles (EVs) have emerged as key mediators of host-parasite communication in helminth infections. Here, we examine the role of EVs in the pathogenesis caused by the carcinogenic liver fluke *Opisthorchis viverrini*. This parasite, a major risk factor for cholangiocarcinoma, secretes abundant EVs that can be fractioned into small and large vesicle populations with distinct biological properties. Proteomic analyses reveal that *O. viverrini* small EVs contain over 500 proteins, including canonical exosomal markers. These small EVs are actively internalized by human cholangiocytes, a process that can be blocked by antibodies against EV surface components. Functional studies demonstrate that small EVs, but not large EVs, induce proliferation of cholangiocytes, supporting a direct contribution to parasite-driven carcinogenesis. Furthermore, comparative omic approaches show that small and large EVs differ markedly in their protein and miRNA cargo, leading to distinct transcriptional responses in recipient host cells. Large EVs promoted the downregulation of host genes involved in carcinogenic pathways, whereas small EVs promoted downregulation of genes involved in focal adhesion and upregulation of genes associated with antiviral immune viral pathways. Intriguingly, small EVs are enriched in viral small RNAs and large EVs are enriched in viral mRNAs suggesting a potential mechanism for delivering viral signals to host cells and adding an additional layer of complexity to host-parasite interactions. Together, these findings reinforce the concept that helminth EVs reprogram host cells at the molecular level, highlight helminth EVs as central drivers of pathogenesis, and represent promising targets for novel interventions.

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LIFE STAGE-DEPENDENT GLYCOSYLATION OF EXTRACELLULAR VESICLES FROM SCHISTOSOMES CONTRIBUTES TO RECEPTOR TARGETING AND HOST IMMUNE RESPONSE

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Glycans play an essential role in pathogen-host interactions. *Schistosoma* express life-stage dependent and species-specific glycan and glycoconjugate antigens that give rise to innate and adaptive immune responses in the host. For instance, specific glycan elements such as the Lex trisaccharide Galβ1–4(Fucα1–3)GlcNAc and similar motifs on egg glycoproteins can mediate immunomodulatory responses whereas multifucosylated GalNAcβ1–4GlcNAc (LDN) glycan motifs of the cercarial glycocalyx, adult worm glycolipids or in egg secretions give rise to strong antibody responses. During glycomics-driven studies we have established that *Schistosoma* release distinct subsets of glycoconjugates as excretory/secretory (ES) products, including extracellular vesicles (EVs). EVs derived from *S. mansoni* contain N-glycosylated proteins and glycolipids exposed on their surface and inside, carrying different fucosylated and mannosylated glycans motifs, respectively. These EV, which show a wide size distribution profile by NTA and long protruding filaments by cryo EM, bind in a glycan-dependent manner to human dendritic cells (hDC) via the C-type lectin receptor (CLR) DC-SIGN. Functionally, this interaction leads to increased IL-10 and IL-12 release. In contrast, EVs released by *S. mansoni* adult worms, have a narrower size distribution than schistosomula EVs, lack the filaments and do not conntain glycolipids. LDN is the most abundant N-glycan motif on the surface of adult worm EVs, which confers specfic interaction with the macrophage galactose-type lectin (MGL), an LDN-binding CLR expressed on hDCs and macrophages. Together with recent comparative studies of *S. haematobium* glycosylation these data indicate that EV glycosylation and resulting parasite EV-host interactions are schistosome life-stage and species dependent.

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HSP90 INHIBITOR-BASED FORMULATION CURES EXPERIMENTAL CUTANEOUS LEISHMANIASIS BY DIRECTLY TARGETING AND KILLING THE PARASITE

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Current leishmaniasis chemotherapy is limited by invasive administration routes, severe side effects, treatment failure, and disease recurrence, highlighting the urgent need for new drugs and therapeutic strategies. We investigated the molecular chaperone Hsp83 of *Leishmania braziliensis* — a homolog of human Hsp90 involved in the folding and stabilization of client proteins critical for parasite survival, growth, and host-pathogen interactions — as a potential chemotherapeutic target. We evaluated 17-DMAG, a water-soluble Hsp90 inhibitor with improved pharmacokinetics relative to its analog 17-AAG, using complementary in vitro and in vivo approaches. Cytotoxicity was assessed in bone marrow-derived macrophages, and antiparasitic activity was evaluated against both promastigotes and intracellular amastigotes. In BALB/c mice infected with *L. braziliensis*, intraperitoneal 17-DMAG treatment significantly reduced lesion size and parasite load in ears and draining lymph nodes. The compound showed high selectivity toward intracellular amastigotes (SI = 432) with minimal host toxicity. To confirm Hsp83 as the molecular target, we expressed and purified recombinant *L. braziliensis* Hsp83 and demonstrated that 17-DMAG induced a significant thermal stabilization shift (+4°C for LbHsp83full; +8°C for LbHsp83N), indicating binding at the N-terminal domain. Dose-response and competition assays with AMP-PNP confirmed that 17-DMAG acts as a competitive ATP inhibitor. Additionally, 17-DMAG modulated the host immune response in treated mice, reducing pro-inflammatory cytokines (IL-6, IFN-γ, TNF) and the regulatory cytokine IL-10 in lymph node cells, suggesting it contributes to infection resolution beyond its direct leishmanicidal activity. These findings establish 17-DMAG as a potent antileishmanial agent targeting LbHsp83, supporting its further therapeutic development.

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BIOMARKERS FOR THE MANAGEMENT AND CONTROL OF VISCERAL LEISHMANIASIS

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Leishmaniasis is a parasitic disease with an extraordinary clinical and epidemiological complexity, whichs requires a comprehensive approach for its effective control. The development and validation of new biomarkers is essential for important aspect as early diagnosis of leishmaniasis, evaluation of the chemotherapy success, prevention of relapses, monitoring of immunocompromised patients, and prevention of the development of resistance. In recent studies, we have developed and applied new methodologies and biomarkers to address these needs. The combination of PCR and cytokine quantification in cell stimulation assays allows us to evaluate the success of pharmacological treatments, avoiding secondary prophylaxis, and preventing relapses in immunocompromised patients with VL. We have also identifie biochemical, hematological, and immunological variables associated with the progression of PKDL by using machine learning approaches. Furthermore, we analyzed the proteomic profiles of extracellular vesicles from immunocompetent patients with active VL or 1, 3, or 6 months after completing treatment, and observed differential expression of 132 proteins before and after treatment. Six of these proteins were analyzed by ELISA in whole plasma and proved useful for monitoring treatment response. Finally, we analyzed the genome of clinical Leishmania infantum strains isolated from both human patients and animal reservoirs, and we found biomarkers of drug resistance in most of them, which represent a challenge for effective treatment of leishmaniasis. These new methodological approaches and biomarkers identified in our studies are undoubtedly useful for decision-making in the clinical management of patients with VL and PKDL, preventing treatment failures and relapses, especially in immunocompromised patients..

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DECODING THE SILENT HOST: A PERSPECTIVE ON THE DYNAMICS OF ASYMPTOMATIC LEISHMANIA CARRIAGE TRYPANOSOMATID PARASITES

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Leishmaniasis, a neglected tropical disease caused by protozoan parasites of the genus *Leishmania* constitute a spectrum of diseases that entails from asymptomatic cases to symptomatic visceral and tegumentary leishmaniasis. Asymptomatic infections represent a major hidden reservoir present in endemic regions, that constitute a main challenge for the control and potential elimination of the disease. This review discusses current knowledge on the immune dynamics, diagnostics challenges and transcriptomic signatures associated with asymptomatic *Leishmania* infection and contrasts these with the signatures and immune dynamics of symptomatic cases concentrating on the visceral and cutaneous forms of the disease. Understanding the molecular and immune processes behind asymptomatic carriage is essential for the strategies to be taken at many levels- therapy, transmission, as well as control. We show further that transcriptomics, proteomics and other advanced approaches that have emerged represent powerful tools to identify and compare in symptomatic and asymptomatic cases differential gene expression, immune pathways, and metabolic changes.

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GOLD AND SELENIUM-BASED COMPLEXES AS ANTILEISHMANIAL METALLODRUGS

Alessandra Mara De Sousa[1], Katherine Bruno[2], Tulio Resende Freitas[1], Aline De Paula Costa[1], Paula Nogueira[3], Heveline Silva[2], Rubens Do Monte Neto[4]
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In the absence of a safe and effective vaccine for human use, the control of leishmaniasis relies primarily on chemotherapy. We identified novel gold-based selenium-containing imidazole compounds with promising antileishmanial activity against *Leishmania* species. Two of these compounds, AuMeSO and $AuCF_3Se$, are original molecules that exhibited potency, with IC_{50} values against amastigotes ranging from 0.2 to 4 µM and selectivity indices of 255 and 106, respectively. The antileishmanial activity of these compounds is not mediated by nitric oxide, as they did not stimulate NO release *in vitro* or *in vivo*. Furthermore, the tested gold-based complexes failed to induce a pro-inflammatory response *in vivo* following administration, as evidenced by unchanged serum TNF-α and IFN-γ levels determined by cytometric bead array (CBA). Acute toxicity studies followed by 15 days of oral treatment with 25 mg/kg/day of each gold-based compound revealed no signs of toxicity. Liver function markers, AST (aspartate aminotransferase) and ALT (alanine aminotransferase), remained within normal ranges. Similarly, kidney function was preserved, as demonstrated by normal serum creatinine and urea levels. The compounds are undergoing evaluation of their *in vivo* antileishmanial efficacy. In addition, attempts to generate drug-resistant mutants through stepwise selection of susceptible strains failed to produce viable resistant parasites to the gold-based compound AdtEt, providing evidence of a multitarget mechanism of action. To investigate the molecular pathways affected by these compounds, RNA-seq analyses have been performed and are currently under evaluation. Collectively, these findings support the translational potential of gold-based compounds as innovative metallodrugs for the treatment of leishmaniasis.

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HIGHLY SENSITIVE AND SPECIFIC ANTIGEN AND QPCR TESTS FOR VALIDATION OF ELIMINATION OF SCHISTOSOMIASIS.

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Leiden University Medical Center (LUMC), Leiden University Centre For Infectious Diseases - Research (lucid-r), Leiden, Netherlands

Successful schistosomiasis control efforts have led to an increasing demand for highly sensitive diagnostic methodologies in endemic settings. Novel assays must be capable of detecting low-burden infections that are frequently undetectable by conventional stool- or urine-based microscopy. This presentation will discuss recent advances in the detection of *Schistosoma* worm-associated circulating cathodic antigen (CCA) and anodic antigen (CAA) using lateral flow assays, as well as the detection of *Schistosoma*-specific DNA by real-time PCR (qPCR) as an alternative to classical microscopy. These methodologies have already been demonstrated to be highly sensitive and specific for the diagnosis of active *Schistosoma* infection. However, they present several context-specific challenges, including species-dependent limitations. We will present new data from multiple small-scale assay validation studies aimed at improving repeatability and reproducibility, as well as technical adaptations to facilitate in-country implementation, including approaches for enhanced quality assurance. Overall, the findings highlight species-specific differences in diagnostic performance of these antigen- and DNA-based assays, as well as the influence of sample type and volume, sample preparation procedures, and availability of laboratory infrastructure. The implications of these factors for validating schistosomiasis elimination using appropriate diagnostic strategies will be discussed.

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HIGH THROUGHPUT RECOMBINASE POLYMERASE AMPLIFICATION (RPA) ASSAYS FOR DIAGNOSIS OF SCHISTOSOMIASIS IN ELIMINATION SETTINGS.

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Urogenital schistosomiasis, caused by Schistosoma haematobium, remains a major public health problem in many endemic regions and can lead to chronic urinary tract pathology as well as female genital schistosomiasis (FGS), an associated gynaecological manifestation of high clinical relevance. As countries move toward elimination and infection intensities decline, highly sensitive diagnostics are increasingly needed to detect low-level infections that may be missed by urinary egg microscopy. This need is even greater for FGS, where diagnosis is complex and requires bespoke tools that support clinical assessments and management. Molecular diagnostics have high sensitivity and specificity. Standard PCR-based assays have proved highly sensitive and specific for detecting Schistosoma DNA in urine and gynaecological samples, but often require sophisticated laboratory infrastructure and trained personnel. In this context, isothermal amplification methods such as recombinase polymerase amplification (RPA) offer promising alternatives, as they maintain high sensitivity while being more suitable for near point-of-care or field-based diagnostics for both urogenital schistosomiasis and FGS. This talk will present the Sh-Dra1 RPA assay currently under evaluation for urine-based diagnosis of urogenital schistosomiasis in low-resource settings, highlighting both its utility and current limitations. It will also describe how the assay has been adapted for the detection of active FGS and is being evaluated as a promising screening tool in endemic countries where access to specialised gynaecological diagnostics remains limited. Further, we will discuss the feasibility and future direction of molecular diagnostics for schistosomiasis in field settings, highlighting the need for context-appropriate diagnostic approaches rather than a one-size-fits-all solution.

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TOPIC 5: DIAGNOSTICS, HOST-PARASITE INTERACTION AND EMERGING TECHNOLOGIES
IN-FIELD VALIDATION OF INNOVATIVE DIAGNOSTIC METHODS FOR SCHISTOSOMIASIS HAEMATOBIUM
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ARTIFICIAL INTELLIGENCE MICROSCOPY FOR DIAGNOSIS OF LIGHT-INTENSITY S. HAEMATOBIUM INFECTIONS.

Lindsay Broadfield, Peter Ward, John Bergelin
Enaiblers AB, Uppsala, Sweden

The WHO 2021-2030 Roadmap for NTDs identifies a target to eliminate schistosomiasis (SCH) as a public health problem globally by 2030. To achieve this, affected areas must have <1% of the population with high-intensity infections and be followed up with post-elimination surveillance and test-and-treat strategies. Thus, diagnostics with high sensitivity to identify and monitor low-intensity infections are crucial to support this goal. Manual single urine filtrate (UF)-microscopy is a standard diagnostic for SCH but has several limitations hindering its performance in low prevalence settings and in populations under surveillance following decreased or halted mass drug administration campaigns. Microscopy is highly error- and fatigue-prone and requires parasitology expertise. Artificial intelligence (AI) microscopy can address many key challenges faced by diagnostic microscopy but requires diagnostic performance validation against the reference method. Here, we describe an AI microscope scanner designed for use in resource-limited settings that identifies Schistosoma haematobium eggs in UF preparations. Initially developed to address the WHO target product profile for soil transmitted helminths diagnosis, the platform was adapted to address SCH in UF samples. In this session, we will explain the AI scanner functionality and design, the development process to generate performant AI models for SCH in UF, and methodology for human-in-the-loop verification. Finally, we present AI model results and performance during a first SCH diagnostic performance study in Pemba, Zanzibar. With promising diagnostic performance in its first in-field validation study, this AI microscopy tool presents a bright future for improved SCH diagnosis in light intensity infections.

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TOPIC 5: DIAGNOSTICS, HOST-PARASITE INTERACTION AND EMERGING TECHNOLOGIES
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DIAGNOSTIC TEST PERFORMANCE IN SCHISTOSOMIASIS ELIMINATIONS SETTINGS: LESSONS AND NEEDS FROM PEMBA, TANZANIA.

Naomi Ndum[1], Said M. Ali[2], Mohammed N. Ali[2], John Bergelin[3], Jan Hattendorf[1], Pytsje Hoekstra[4], Lisette Van Lieshout[4], Tom Pennance[5], Khamis R. Suleiman[2], Jürg Utzinger[1], Peter Ward[3], Bonnie Webster[5], Stefanie Knopp[1]
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As countries progress towards the schistosomiasis elimination goals set by World Health Organization for 2030, prevalences and infection intensities decrease, which brings about diagnostic challenges. In schistosomiasis elimination settings, accurate diagnostic tools are needed to determine prevalence thresholds for assigning or stopping interventions, guide pre- and post-elimination surveillance, and verify whether elimination has been reached. We conducted the first prospective, head-to-head accuracy evaluation of six diagnostic tests, ranging from standard urine filtration (UF)-microscopy and haematuria assessment to advanced molecular and antigen tests and a new artificial intelligence (AI)-scanner for egg microscopy, all evaluated using the same urine sample, for *S. haematobium* detection in the near-elimination setting Pemba Island, Tanzania. We showed that examining multiple-day urine samples from the same individual with standard UF-microscopy, substantially increased the number of cases, especially when infection intensities were light. With 5-day UF-microscopy as the reference test, among all investigated single-sample tests the AI-scanner and qPCR showed the highest accuracy. Sensitivity of all tests increased with increasing infection intensity. In near-elimination settings, multiple-day urine examination with standard UF-microscopy substantially improves case detection but is operationally challenging. For single-sample testing, the qPCR and AI-scanner proved to be the most accurate methods. While qPCR is a very valuable approach in well-equipped laboratories, the AI-scanner might offer a promising alternative for research, clinical and programme use in basic laboratories but requires further validation in other settings and cost-effectiveness analyses.

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EXPLOITING HOST GENETIC DIVERSITY TO DISSECT PARASITE-HOST INTERACTIONS
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GENETIC SCREENS FOR VACCINES TO TOXOPLASMA GONDII

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Toxoplasma gondii, the causative agent of human toxoplasmosis, is a globally spread intracellular parasite, with *Mus musculus* being an important intermediate host. Mouse strains differ widely in their ability to generate protective immunity to *T. gondii* following vaccination or natural infection, presenting an excellent model to explore mechanisms of immunological memory development and vaccine efficacy to high virulent challenge. To address a knowledge gap in vaccine development for *T. gondii*, we used the Collaborative Cross (CC), a large and diverse multi-parent recombinant inbred mouse panel that incorporates alleles from 3 subspecies of *Mus musculus*, in a secondary infection model for *T. gondii*. We identified a single significant chr11 QTL (51.5-53.3 Mbp; LOD=15) surrounding *Tcf7* that determines vaccine efficacy against virulent *T. gondii* strains. Survival correlates with enhanced CD8 T cell memory, Tfh development and high expression of *Tcf7*, which encodes the canonical Wnt-transcription factor Tcf1. The contribution of specific *Tcf7* alleles to immunity was further validated using chromosomal consomic and deficient mice generated from wild-derived genetic backgrounds. Collectively, these data demonstrate that allelic variation exerts a significant quantitative influence on vaccine-induced immune responses and underscore the utility of diversity outbred mouse panels as a translational model for capturing population-level genetic heterogeneity.

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GENETIC AND IMMUNOLOGICAL CONTROL FOR DEVELOPMENT OF ASYMPTOMATIC MALARIA

Tracey Lamb
University of Utah, Pathology, Salt Lake City, United States of America

A substantial proportion of the population in many malaria endemic areas (>60%) can carry asymptomatic *Plasmodium* infections, particularly in high transmission areas. Although a large body of work defines the immune mechanisms involved in the immunopathogenesis of malaria, there are major gaps in our understanding of the immune response that facilitates the asymptomatic carriage of *Plasmodium* parasites. Asymptomatic carriage of *P. falciparum* is often regarded as a result of acquired host immune responses that limit parasite multiplication to levels below the pyrogenic threshold ("anti-parasite immunity"). However, this is not supported by data in children infected with *P. falciparum* where asymptomatic malaria can occur at varying levels of circulating parasitemia and humoral response. Here, using an established colony of wild-derived *Mus musculus domesticus* that has been maintained in a specific pathogen free (SPF) facility for 23 generations, and several collaborative cross mouse lines, we have been able to demonstrate the genetic basis for asymptomatic carriage of *Plasmodium* parasites. Regulation of inflammation in response to *Plasmodium* parasites, often referred to as clinical immunity, is hypothesized to develop with age and could provide an immune environment which allows *Plasmodium* parasites to circulate without symptoms. Several studies shed some doubt on the hypothesis that adaptive immunity is a key component in the development of asymptomatic malaria, particularly in children. The data presented here explores the role of the innate immune system in dampening inflammatory pathology during *Plasmodium* infections.

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COLLABORATIVE CROSS MOUSE MODEL REVEALS GENETIC BASIS FOR SUSCEPTIBILITY AND RESISTANCE TO NAEGLERIA FOWLERI INFECTION

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Naegleria fowleri, also known as the brain-eating amoeba, is a free-living amoeba that is the causative agent of Primary Amoebic Meningoencephalitis (PAM). Infection occurs when amoebae enter the nasal passages in contaminated water and migrate to the brain, where they feed on neural tissue and induce PAM, a highly fatal disease. *N. fowleri* has a peculiar infection pattern; many infectious agents, such as COVID-19 or *Plasmodium* spp., have millions of new cases each year, with mortality rates averaging less than 1%. In comparison, only 5 PAM cases on average are documented in the U.S. each year, with a severe 97% mortality rate in infected individuals. Surveillance efforts suggest that *N. fowleri* lives ubiquitously in freshwater across the globe, putting millions of individuals at risk every year for PAM. How is it that *N. fowleri* has this highly unusual infection pattern with such dire disease outcomes in humans? We hypothesized that genetic differences in key host factors correlate with susceptibility and refractory responses to *N. fowleri* infection. To test our hypothesis, we infected 45 mouse lines from the Collaborative Cross (CC), a model of genetic diversity that represents over 90% of the common genetic variations found in *Mus musculus*. Infections in these mice revealed a gradient of diverse disease outcomes as measured by survival time and quantification of amoebae burden. Quantitative Trait Loci (QTL) mapping revealed three genomic regions associated with differential survival phenotypes, one of which on Chromosome 12 appears to mediate an increased susceptibility to *N. fowleri*.

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GENETIC AND ENVIRONMENTAL INTERACTIONS IN IMMUNE VARIATION

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The relative and synergistic contributions of genetics and environment to interindividual immune response variation remain unclear, despite implications in evolutionary biology and medicine. Here, we quantify the interactive effects of genotype and environment on immune traits by investigating C57BL/6, 129S1, and PWK/PhJ inbred mice, rewilded in an outdoor enclosure and infected with the parasite *Trichuris muris*. Whereas cellular composition was shaped by interactions between genotype and environment, heterogeneity in cytokine responses, including IFNγ concentrations, was primarily driven by genotype, with consequences for worm burden. In addition, we show that other traits, such as expression of CD44, were explained mostly by genetics on T cells, whereas expression of CD44 on B cells was explained more by environment across all strains. Notably, genetic differences under laboratory conditions were decreased following rewilding. These results indicate that nonheritable influences interact with genetic factors to shape immune variation and parasite burden. We have continued to use other inbred strains of mice to explore this idea in other tissue sites. to expersiy this hypothesis in detail.

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TOPIC 2: TRANSMISSION DYNAMICS, EPIDEMIOLOGY, AND GLOBAL HEALTH
DETECTION OF PARASITES IN FOOD, CLINICAL AND ENVIRONMENTAL SAMPLES: ADVANCEMENTS, INTERPRETATION OF MICROBIOLOGICAL TEST RESULTS AND PREVENTION OF OUTBREAKS
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FROM DETECTION TO PUBLIC HEALTH SIGNIFICANCE: INTERPRETING MOLECULAR RESULTS FOR INTESTINAL PARASITIC PROTOZOA IN FOOD AND ENVIRONMENTAL SAMPLES

The contamination of fresh produce and agricultural water with intestinal protozoan parasites — particularly *Cyclospora cayetanensis* and *Cryptosporidium* spp. — represents a persistent and significant food safety concern. Recurrent outbreaks associated with herbs, leafy greens, and environmental water sources underscore the urgent need for sensitive, validated, and reproducible detection methods. In response, non-culture-based molecular tools are increasingly employed in routine surveillance and regulatory testing; however, the accurate interpretation of results from these methods remains a complex challenge. This presentation will explore advances in molecular detection developed at FDA's Human Foods Program, including the adaptation and multi-laboratory validation of the BAM 19c dead-end ultrafiltration method for *C. cayetanensis* across diverse water matrices, the integration of digital PCR alongside validated real-time PCR platforms, and the development of sequencing-based confirmation strategies using mitochondrial gene targets. For *Cryptosporidium* spp., newly developed qPCR and dPCR assays enable sensitive detection and species-level differentiation of *C. parvum* and *C. hominis* from food and water samples. Critically, this presentation will address the nuances of interpreting molecular results for *Cyclospora cayetanensis* and *Cryptosporidium* spp. including the role of method validation in establishing performance characteristics such as sensitivity, specificity, and detection limits — and how well-validated testing results can inform risk management strategies and contribute to the prevention of foodborne outbreaks from a regulatory agency perspective.

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DETECTION OF PARASITES IN FOOD, CLINICAL AND ENVIRONMENTAL SAMPLES: ADVANCEMENTS, INTERPRETATION OF MICROBIOLOGICAL TEST RESULTS AND PREVENTION OF OUTBREAKS
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IMPORTANT ASPECTS TO CONSIDER WHEN DESIGNING STUDIES TO VALIDATE METHODS FOR DETECTION OF PARASITES IN FOODS AND ENVIRONMENTAL SAMPLES

Laura Lalonde
Canadian Food Inspection Agency, Centre For Food-borne And Animal Parasitology, Saskatoon, Canada

The validation of methods for detecting parasites in food and environmental matrices requires consideration of biological, methodological, and matrix-specific variables to ensure sensitivity, specificity, and fitness for purpose, and poses unique challenges compared with bacterial and viral detection. Chief among these is the typically low concentration of parasites in complex matrices, coupled with the absence of enrichment steps, which places critical importance on both analytical sensitivity and recovery efficiency during sample processing. Food and environmental samples contain substantial debris and diverse background, necessitating highly specific and selective detection systems to ensure accurate inclusivity and exclusivity, while minimizing interference and inhibition. The limited availability of ISO standards and reference materials complicates harmonization, although international guidance is emerging. Acquisition of suitable reference material remains challenging, as many parasites lack robust culture systems or animal models, and specificity assessments are constrained by limited availability of parasite species for inclusivity/exclusivity panels. Matrix variability can influence assay performance, underscoring the need for matrix-specific validation and appropriate controls. Parasite life stage variability and the inability of some methods to determine viability further complicate interpretation of results, particularly in risk assessment contexts. Validation is a systematic process that establishes an assay's fitness for an intended purpose, whether for surveillance, outbreak investigation, or regulatory use, while balancing technical feasibility, cost, and risk. Overall, rigorous validation, quality assurance, and standardized implementation ensure reliable detection and contribute to effective contamination prevention strategies. Collectively, these considerations support development of robust, fit-for-purpose methods for parasite detection in complex food and environmental samples.

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EXPLORING MICROBIOLOGICAL TESTING DATA TO UNDERSTAND AND PREVENT OUTBREAKS CAUSED BY PARASITES

Benjamin Rosenthal
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Foodborne outbreaks of parasitic infection, though somewhat seasonal and occasionally tied to particular food types, remain episodic and difficult to predict. Risk mitigation protects consumer health, and the reputation of producers, by lessening the risk and cost of outbreaks and product recalls. Such measure are especially warranted for fresh produce consumed raw, and especially challenging for parasites that endure conventional santizers. Here, I review a body of work employing Eimeria surrogates to advance the goal of mitigating the risk of foodborne Cyclospora, which has caused outbreaks via leafy greens, berries, and fresh herbs. I overview progress in detection, parasite viability assessment, inactiviation via UV light and ozonation, and prevention via low-cost irrigation water filtration. I will also emphasize new opportunities to convert biological insights to practical tools for surveilance and control.

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TOPIC 2: TRANSMISSION DYNAMICS, EPIDEMIOLOGY, AND GLOBAL HEALTH
WILDLIFE PARASITOLOGY IN THE SOUTHERN HEMISPHERE: UNDERSTANDING HOST–PARASITE CONNECTIONS THROUGH A ONE HEALTH LENS
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WILDLIFE PARASITOLOGY IN AOTEAROA NEW ZEALAND: OUR PIPELINE THAT MAKES THE MOST OF EVERY ANIMAL

Jerusha Bennett[1], Bronwen Pressswell[1], Kate Hutson[2], Robert Poulin[3]
[1]University of Otago, Dunedin, New Zealand, [2]Cawthron Institute, Nelson, New Zealand, [3]University of Otago, Zoology Department, Dunedin, New Zealand

Baseline data on parasite diversity and distribution is crucial for wildlife conservation efforts, especially in the face of environmental change. In Aotearoa New Zealand, our research group take a proactive approach to wildlife parasitology by opportunistically sampling hosts and inventorying their parasitic fauna all before disease outbreaks occur. This data allows us to discover new species, identify potential problematic parasites and assess changes over time and across host species. In this talk, I will introduce our multi-agency collaborative parasite investigation pipeline focused on native birds across New Zealand. Deceased native birds have been donated through a collaborative network of conservation agencies and wildlife hospitals. Parasites were obtained during workshops and wānanga during undergraduate labs with students, scientists and Māori communities, and identified using molecular and morphjological techniques. This pipeline has led to the discovery of multiple parasite species new to science, numerous new host and geographic records, increasing known parasites in NZ birds by over 30%. Our data contributes to understanding the ecology and evolution of parasite communities within New Zealand birds. Importantly, our dissection pipeline has impacts beyond scientific discovery, including repatriation of materials to indigenous communities and embedding within undergraduate teaching at Otago University. This proactive, multi-faceted pipeline maximizes the use of each deceased bird in NZ, contributing to baseline data for future assessments of environmental change.

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WILDLIFE PARASITOLOGY IN THE SOUTHERN HEMISPHERE: UNDERSTANDING HOST–PARASITE CONNECTIONS THROUGH A ONE HEALTH LENS
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FUNDING, FIELD AND FRONTIER: OVERCOMING LOGISTICAL AND FINANCIAL BARRIERS IN AMAZONIAN WILDLIFE PARASITE RESEARCH

Francisco Tiago Melo, Bianca Nandyara, Leandro Mauricio Silva
Federal University of Pará, Belem, Brazil

The Amazon region is considered a global biodiversity hotspot; however, it remains an unknown frontier for wildlife parasitology. Understanding this "hidden" diversity is vital within the One Health framework, which recognizes the links between ecosystem integrity, animal health, and human well-being. Parasites are sensitive indicators of ecosystem shifts, yet their study in Amazonian wildlife faces formidable logistical and financial hurdles, including high expedition costs, complex permitting, and large, remote sampling areas, making parasitological research in the region even more challenging. These barriers severely constrain our ability to baseline zoonotic risks, particularly those about foodborne diseases, which constitute a significant yet neglected public health threat among riverine and indigenous communities. This presentation will dissect these core challenges, drawing on field experiences to illustrate how limited funding restricts long-term surveillance. Furthermore, we will discuss strategic approaches to overcoming these obstacles through the formation of collaborative, interdisciplinary networks and capacity building for local and international institutions. Advancing this frontier is not merely an academic endeavor but a critical step toward proactive zoonotic disease management in one of the world's most dynamic environments.

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WILDLIFE PARASITOLOGY IN THE SOUTHERN HEMISPHERE: UNDERSTANDING HOST–PARASITE CONNECTIONS THROUGH A ONE HEALTH LENS
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PARASITES AS ENVIRONMENTAL CONNECTORS: INSIGHTS FROM SOUTHERN HEMISPHERE SYSTEMS

Shokoofeh Shamsi
Charles Sturt University, School Of Agricultural, Environmental And Veterinary Sciences, Wagga wagga, Australia

Australia provides a distinctive setting for wildlife parasitology, shaped by its long geographical isolation, which has given rise to highly endemic host–parasite assemblages. However, this evolutionary separation has been increasingly disrupted since colonisation, with the introduction of numerous parasites through livestock, companion animals, and human activities. As a result, Australian ecosystems now represent a unique convergence of endemic and introduced parasite systems, creating complex and dynamic transmission landscapes. This presentation draws on Australian case studies to explore the role of wildlife parasites as environmental connectors within a One Health framework. Focusing on parasites of relevance to food safety and biosecurity, it examines transmission pathways across wildlife–livestock–human interfaces and highlights how introduced parasites can establish, spread, and interact with native fauna. Examples from both aquatic and terrestrial systems will illustrate how environmental change, host ecology, and anthropogenic pressures shape parasite dynamics, with implications for livestock production, wildlife health, and human wellbeing. Case studies of parasites with significance for industry and public health will demonstrate the role of wildlife as reservoirs and sentinels, as well as the challenges associated with detecting and managing risks in these interconnected systems. By integrating ecological, molecular, and applied perspectives, this presentation provides a regionally grounded understanding of parasite transmission in Australia. These insights contribute to broader discussions on wildlife parasitology in the Southern Hemisphere and highlight the importance of considering both historical isolation and contemporary human influence in shaping parasite ecology within a One Health context.

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PLENARY SESSION
EXPERIMENTAL PARASITOLOGY TO SERVE PUBLIC HEALTH IN SUB-SAHARAN AFRICA
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EXPERIMENTAL PARASITOLOGY TO SERVE PUBLIC HEALTH IN SUB-SAHARAN AFRICA

Abdoulaye Djimde[1], Laurent Dembélé[2], Dinkorma Ouologuem[2], Mohamed Maiga[2], Koba Privat Agniwo[2], Safiatou Doumbo Niaré[2]
[1]University of Science, Techniques and Technologies of Bamako, Parasites And Microbes Research And Training Center, Bamako, Mali, [2]Université des Sciences, des Techniques et des Technologies de Bamako (USTTB), Parasites & Microbes Research And Training Center (pmrtc), Bamako, Mali

Parasitic diseases remain major public health problems in sub-Saharan Africa. WHO estimated 282 million new malaria cases worldwide in 2024 with >94% of cases and deaths occuring in sub-Saharan Africa (SSA). Schistosomiasis affects at least 250 million people worldwide with up to 90% of the total global disease burden in Sub-Saharan Africa. Malaria and Schistomiasis elimination programs are hampered by novel biological threats such as drug resistance, increase in non-falciparum species, development of new hybrids in schistosomes, etc. Effectively addressing these challenges will require novel tools to be developped in parasite endemic countries, as close as possible to the patients and the communities affected. Yet, scientific progress on these fields is hindered by the lack of field-deployable adequate in vitro assays. At the Parasites and Microbes Research and training Center, we are developping novel experimental parasitology to address these challenges in Mali (West Africa). We use in silico approaches to accelerate the clinical development of antimalarial drugs. We develop novel ex vivo/in vitro culture systems that allow drug screening on *Plasmodium malaria* and *P. ovale* asexual parasites and *P. falciparum* gametocytes directly from patients. We have successfully replicated the life cycle of *Schistosoma haematobium* in the laboratory and are testing compounds against different stages of schistosomes. These experiments are building the foundation for better understanding of the biology of the parasites and improving our capacity to assay novel compounds on parasites and developmental stages that are currently impossible to cultivate in vitro. Experimental parasitology is improving public health in sub-Saharan Africa.

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TOPIC 4: HOST IMMUNITY, MOLECULAR INTERACTIONS, AND MICROBIOME
NEW MOLECULAR ASPECTS AND IMMUNOPARASITOLOGY OF NEGLECTED TROPICAL DISEASES
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IRON SEQUESTRATION BY LEISHMANIA PARASITES ALTERS IMMUNITY

Fernanda Novais, Bridget Bowman
The Ohio State University, Microbial Infection & Immunity, Columbus, United States of America

Leishmania parasites infect, survive, and persist within innate immune cells, particularly macrophages. Many intracellular pathogens depend on host-derived iron for survival, yet how this competition for iron shapes host immune responses remains poorly understood. Since the host and the parasite require iron for survival, we investigated whether iron acquisition by *Leishmania* influences macrophage function. Utilizing iron-sensitive probes, we found that infected macrophages exhibit a marked reduction in cytoplasmic iron compared to uninfected bystander cells, consistent with parasite-driven sequestration of iron within the phagolysosome. To define how iron availability shapes macrophage responses broadly, we performed mRNA sequencing on uninfected and infected macrophages and compared these data with those from infected macrophages where cytoplasmic iron availability was restored by iron overloading. These studies identified Programmed Death-Ligand 1 (PD-L1) as a key gene that is induced by infection, however, this induction is ablated when the competition for iron is broken. Indeed, this pattern was observed in vitro and in vivo, where infection alone robustly induces PD-L1 protein expression, and when macrophages are overloaded with iron, PD-L1 induction is lost. Mechanistically, we found that infection stabilizes the transcription factor Hypoxia Inducible Factor-2α (HIF-2α) and that macrophage-specific HIF-2α deficiency impairs parasite-induced PD-L1 upregulation, linking iron deprivation to immune checkpoint regulation. Together, these findings suggest that *Leishmania* iron acquisition reprograms macrophages, creating a permissive environment for the parasite.

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CELL-INTRINSIC DEFENSES AGAINST MALARIA PARASITES IN THE LIVER.

Sam Kurup
University of Georgia, Cellular Biology/ctegd, Athens, United States of America

Plasmodium parasites undergo obligate development and replication within hepatocytes before initiating the erythrocytic stage responsible for clinical malaria. Although innate immune responses in the liver are known to limit infection, the mechanisms by which infected hepatocytes directly control intracellular parasites remain poorly understood. Using transcriptomic analyses, genetic approaches, and transgenic Plasmodium parasites that enable selective interrogation of infected hepatocytes, we demonstrate that direct type I interferon (IFN-I) signaling within infected hepatocytes is essential for parasite control. Hepatocytes actively mount IFN-I-dependent, ROS-mediated cell-intrinsic immune responses that target intracellular Plasmodium, resulting in parasite lysis and selective removal of infected hepatocytes, thereby limiting parasite survival and progression to the blood stage. These hepatocyte-intrinsic defense programs operate in both mouse and human systems, and disruption of these pathways results in markedly increased susceptibility to malaria in vivo. Together, these findings establish hepatocytes as active participants in innate immunity and reveal previously unrecognized cell-intrinsic defense mechanisms that control Plasmodium infection at its earliest stage, providing a mechanistic foundation for developing host-directed strategies to prevent malaria.

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NEW INSIGHTS INTO THE CONSEQUENCES OF ASCARIS PATHOGENESIS IN THE HOST STOMACH

Nina Tang, Yifan Wu, Mariel Burquez, Brian Keegan, Alexander Kneubehl, Maria Elena Bottazzi, Jill Weatherhead
Baylor College of Medicine, Pediatric Tropical Medicine, Houston, United States of America

Ascaris roundworms infect more than 700 million people worldwide and are a major source of morbidity in resource-limited settings. In endemic regions, infection typically begins in early childhood, with reinfection occurring throughout life. Our laboratory has previously demonstrated that *Ascaris* eggs can hatch within the host stomach, releasing larvae that translocate across the gastric mucosa. This larval migration damages gastric foveolar surface mucous cells and promotes the development of pyloric metaplasia upon repeated injury. Given that pyloric metaplasia is an established precancerous lesion associated with increased risk of gastric carcinogenesis, these findings suggest a previously unrecognized role for *Ascaris* infection in chronic gastric disease and gastric cancer pathogenesis. A scoping review from our laboratory has demonstrated a pattern of gastric injury in humans and non-human organisms with *Ascaris* infection, including gastritis, ulceration, and perforation. Our established murine models of single and repeated *Ascaris* infection have corroborated these findings by demonstrating increased gastric mucosal inflammation, transcriptomic alterations, and clinically evident upper gastrointestinal bleeding manifested by melena originating from the stomach. Ongoing translational studies will evaluate the effects of *Ascaris* infection on the gastric and downstream intestinal microbiomes and characterize the gastric immune responses following infection. Large-scale epidemiologic investigations are evaluating clinical associations between chronic gastric disease diagnoses and *Ascaris* exposure history. Together, these translational and epidemiologic studies explore the paradigm-shifting hypothesis that *Ascaris* larval translocation in the stomach serves as a trigger for chronic gastric disease, including gastric cancer, which remains the fifth leading cause of cancer mortality worldwide.

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NEW MOLECULAR ASPECTS AND IMMUNOPARASITOLOGY OF NEGLECTED TROPICAL DISEASES
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SPATIALLY RESOLVED CELL ATLAS OF THE BONE MARROW REVEALS MARKED HETEROGENEITY IN TISSUE REORGANIZATION IN FATAL P. VIVAX INFECTION

Joao Silva-Filho[1], Kevin Couper[2], Marcus Lacerda[3], Fabio Costa[4], Matthias Marti[5]
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P. vivax (Pv) infection can cause severe clinical outcomes despite low blood parasitaemia, with a “hidden” parasite population in organs such as the bone marrow (BM) and spleen. This study aims to characterize perturbations induced by Pv infection in BM and spleen and their role in disease pathogenesis by creating a spatially resolved cell atlas in fatal Pv. We analyzed BM (n=3) and spleen (n=5) samples from acute fatal Pv and non-malarial cases using IMC and spatial transcriptomics integrated to H&E. In both organs, we identified cell types and states including infected RBCs (iRBCs) and tracked their maturation. Tissue niches and alterations linked to specific host responses revealed unique features of BM and spleen architecture and marked heterogeneity in tissue reorganization across cases. In two patients, IMC showed iRBCs interacting with activated neutrophils and macrophages and correlating with reduced parasite biomass. CoSMx analysis revealed strong activation of innate immune responses, antigen (Ag) processing and presentation along with activation of cytokine signaling. These patients died from acute respiratory distress, which may be linked to the myeloid- and T lymphoid-driven antiparasitic response in BM. The third patient had high parasite biomass, with Pv-iRBCs mostly interacting with each other and uninfected RBCs. Transcriptomic analyses showed reduced Ag processing and presentation and suppressed T-cell responses. The higher parasite biomass in this BM sample may be due to parasite evasion, possibly related to a local immune deficient response. Thus, BM responses are associated with different disease trajectories leading to fatal outcome.

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ANTIMICROBIAL PEPTIDES IN HELMINTH EXCRETED-SECRETED PRODUCTS – A GOLD MINE FOR ANTHELMINTIC DISCOVERY?

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The global crisis of antimicrobial resistance and the mounting challenge of multidrug-resistant parasitic nematodes demand new approaches to infectious disease control. While traditional research has viewed gastrointestinal helminths and the host microbiome as separate entities, emerging evidence suggests they are inextricably linked in a complex "holobiont". Parasitic nematodes have evolved in microbe-rich environments for millions of years, developing sophisticated innate immune defenses. This evolutionary "arms race" has produced a diverse repertoire of antimicrobial peptides that can selectively target bacteria and other members of the gut microbiome. With over 15,000 putative AMP-like peptides already identified via computational mining, the bottleneck is no longer discovery, but validation. The integration of machine learning algorithms with advanced *in vitro* and *ex vivo* models and the possible development of a high-throughput pipelines allows to predict AMP mode of action, spectrum of activity and efficacy. This study characterises the biochemical features, structure and function of selcted AMPs within the secretome of the 'brown stomach worm', *Teladorsagia circumcincta*, revealing their potential as potent antimicrobial agents and their putative roles in parasite survival mechanisms within polymicrobial host environments. We argue that these molecules represent both a novel source for biotherapeutic discovery and a distinct class of biological interactions that may be exploited as targets for future anthelmintic development.

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CROSS-KINGDOM-MEDIATED DETECTION OF INTESTINAL PROTOZOA THROUGH THE INNATE IMMUNE RECEPTOR NLRP6

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Intestinal protists are detected by the host innate immune system through mechanisms that remain poorly understood. Here, we demonstrate that *Tritrichomonas* protozoa induces thickening of the colonic mucus in an NLRP6-, ASC- and Caspase-11-dependent manner, consistent with the activation of sentinel goblet cells. Mucus growth is recapitulated with cecal extracts from *Tritrichomonas*-infected mice but not purified protozoa, suggesting that NLRP6 may detect infection-induced microbial dysbiosis. In agreement, *Tritrichomonas* infection causes a shift in the microbiota with expansion of *Bacteroides* and *Prevotella*, and untargeted metabolomics revealed a dramatic increase in several classes of metabolites, including sphingolipids. Finally, using a combination of gnotobiotic mice and ex vivo mucus analysis, we demonstrate that wild-type but not sphingolipid-deficient *B. thetaiotaomicron* is sufficient to induce NLRP6-dependent sentinel goblet cell function, with the greatest effect observed in female mice. Thus, we propose that NLRP6 is a sensor of intestinal protozoa infection through monitoring microbial sphingolipids.

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HELMINTH-MICROBIOTA INTERACTIONS IMPACTING HOST HEALTH

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Helminths remain endemic in many Indigenous populations, yet their impact on gut microbiome function and viral composition is poorly characterized. We integrated metagenomic, metatranscriptomic, and immune profiling data from helminth-endemic Indigenous Orang Asli (OA) in Peninsular Malaysia and urban Kuala Lumpur (KL) residents to investigate how helminth infection and lifestyle shape gut microbiome function and host immunity. *Prevotella* showed higher transcriptional activity in OA whereas *Bacteroides* dominated in KL, corresponding to distinct immune signatures. Microbial GWAS identified helminth-associated variants in 14 species, including a stop-gained variant in the *trpB* gene of *Prevotellamassilia timonensis*, implicating tryptophan biosynthesis as a helminth-modulated microbial pathway. Abundance-independent transcriptional analysis revealed 543 microbial genes suppressed in helminth-infected individuals, enriched in amino acid biosynthesis, carbon metabolism, quorum sensing, and chemotaxis, demonstrating helminths remodel specific microbial function. Within the virome, helminth infection was associated with greater bacteriophage diversity, mirroring elevated bacterial diversity, suggesting helminths shapes gut ecosystem beyond bacteria. Enteric RNA viruses including *Norovirus* genogroup IV and *Echovirus B18* were detected exclusively in OA, with high seropositivity to the rare *Norovirus* GIV.1 observed in OA villagers. Assembly of Malaysian metagenome-assembled genomes uncovered 307 novel species, and 96.6% of 4,470 viral operational taxonomic units lacked genus-level classification, with 37.6% unique to Malaysian, revealing a largely uncharacterized microbial diversity. Together, these findings characterize helminth infection and lifestyle as a key ecological force reshaping gut microbiome function, and viral community structure, expanding our understanding of the host-helminth-microbiota interactions in endemic Indigenous communities.

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HARNESSING PARASITE REGULATION OF IMMUNITY FOR THERAPEUTIC DEVELOPMENT

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We have previously identified that *S. mansoni* infection induces a unique form of myeloid training that protects male but not female mice from high fat diet induced disease. Here we demonstrate that ovarian derived hormones account for this sex specific difference. Ovariectomy of females prior to infection permits metabolic reprogramming of the myeloid lineage, with BMDM exhibiting carbon source flexibility for cellular respiration, and mice protected from systemic metabolic disease. The innate training phenotype of infection can be replicated by in vivo injection of SEA, and by exposure of bone marrow to SEA in culture prior to macrophage differentiation (Day 0). This protective phenotype is linked to increased chromatin accessibility of lipid and mitochondrial pathways in BMDM including Nrf1 and Tfam, as well as mitochondrial biogenesis. This work provides evidence that *S. mansoni* antigens induce a unique form of innate training inhibited by ovarian-derived hormones in females.

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NOVEL INSIGHTS INTO GENOMIC HETEROGENEITY IN LEISHMANIA AND TRYPANOSOMA SPECIES THROUGH COMPARATIVE SINGLE CELL GENOMICS

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Leishmania spp. and *Trypanosoma spp.* are vector-borne protozoan parasites that cause neglected diseases of humans and mammals predominantly in tropical and subtropical regions. These parasites possess unique biological features, such as the presence of kinetoplastid DNA, polycistronic transcription and the absence of transcriptional regulation at the level of individual genes. Their genomes are generally diploid with small chromosomes of various lengths and are characterized by multi-gene family expansions and frequent copy number variations. Because of their dixenous life cycle, these trypanosomatids need to be able to adapt and survive very different the intra- or extra-cellular environments of the mammalian host and the insect vector. Genome instability is one of the mechanisms that can drive adaptation in these unicellular pathogens. To study genome instability and genomic heterogeneity, we developed and validated a novel single cell genomics workflow (scDNAseq), which exploits semi-permeable capsules for high-throughput cell encapsulation and combines it with primary template-directed amplification (PTA) for accurate whole-genome amplification. This high-throughput method allowed us to determine the karyotypes of hundreds of *Leishmania* parasites, detect distinct CNVs between different cell populations, and identify sub-populations of cells harboring distinct nucleotide variants. Next, we applied this scDNAseq protocol to study genome instability in the clonal populations of two *Leishmania (Viannia)* species, three *Trypanosoma cruzi* strains and *Trypanosoma brucei brucei, Trypanosoma brucei gambiense* and *Trypanosoma congolense*. The results of these single cell genomics analyses will be discussed in the context of potentially different levels of genome instability and adaptive strategies of these trypanosomatid parasites.

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GENETICS OF EMERGING LEISHMANIA LINEAGES ON THE INDIAN SUBCONTINENT

Patrick Łypaczewski
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Due to concerted international efforts, the cases of visceral leishmaniasis have been declining in recent years across the globe and particularly in India, Nepal and Bangladesh. However, recent research from multiple groups has shown that the traditionally visceral disease-causing *Leishmania donovani* has increasingly moved to a cutaneous disease phenotype in Indian Sub Continent. In the Sri Lankan region, we have observed three independent mechanisms that *L. donovani* has undertaken to modulate its pathology: genetic mutations in key genes, interspecies hybridization with *L. major,* and interspecies hybridization with *L. tropica*. Through whole genome sequencing and bioinformatic analysis of *L. donovani* from atypical leishmaniasis cases in the Himachal Pradesh region in India, an additional modulation mechanism (**intra**species *L. donovani*hybridization) as well as evidence for another instance of genetic mutations in key genes was found. In neighboring Nepal, a previously rare clade of parasites (ISC1, “Yeti”) has recently been shown to cause the traditional disease but more frequently also results in cutaneous infections. While it remains unclear what is the environmental pressure on these atypical *L. donovani* strains to modulate their pathologies, the mounting evidence of repeated independent changes in disease progression strongly suggests the parasites are responding to an external stimulus. These observations raise concerns in elimination programs as the parasites appear to readily modulate their tissue tropism from visceral to cutaneous and may readily be able to revert to a visceral phenotype, serving as a hidden reservoir primed to fuel a resurgence of visceral infections.

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GENOMIC INSTABILITY AND MONO-PARENTAL EXPRESSION MITIGATE GENOMIC SHOCK IN CROSS-SUBGENUS LEISHMANIA HYBRID

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Hybridization, the merging of distinct genomes, is increasingly recognized as a major evolutionary force among eukaryotic pathogens, including facultatively sexual protist parasites like *Leishmania* and *Trypanosoma*. Despite its importance in promoting pathogen virulence and drug resistance, hybridization remains poorly understood, particularly how genetic distance between parental cells influences genomic compatibility and which compensatory mechanisms ensure hybrid viability. Using *in vitro* crossing, we produced an unusual sexual hybrid between *Leishmania* species infecting mammals (*L. infantum*) and reptiles (the *Sauroleishmania L. tarentolae*), and used this unique genetic model system to address these open questions. Our data provide evidence of genomic compatibility between even highly divergent *Leishmania* species, offering new insights into the evolutionary potential of *Leishmania* and related pathogens. We demonstrate that the genomic shock caused by the fusion of distinct genomes can be mitigated by two key mechanisms: (i) at the genomic level, chromosome loss allows the establishment of mosaic aneuploidy in the newly formed hybrid, and (ii) at the post-transcriptional level, preferential mono-parental allelic expression acts as a secondary compensatory mechanism. Our findings establish genome instability and post-transcriptional regulation as central processes in *Leishmania* hybridization, which may be of broad relevance to other biological systems undergoing genetic exchange.

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MITOCHONDRIAL MINICIRCLE DNA DIVERSITY SHAPES VIRULENCE IN LEISHMANIA MAJOR

Tiago Ferreira[1,2], Zihao Chen[3], Ehud Inbar[1], Carol Catta Preta[1,4], Mariana Chaves[1], Alasdair Ivens[3], Nicholas Savill[3], Achim Schnaufer[3], David Sacks[1]
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Background and aims: Mitochondrial gene expression in *Leishmania* depends on guide RNA-mediated editing of maxicircle transcripts, yet the consequences of naturally occurring variation in the minicircle repertoire during mammalian infection remain poorly defined. We tested whether kinetoplast DNA minicircle diversity determines *Leishmania major* virulence. **Methods:** We generated experimental selfing hybrids by co-infecting sand flies with two *L. major* Fn clones displaying contrasting virulence in mice and assessed lesion development following mammalian infection. Minicircle repertoires and mitochondrial RNA editing were examined by DNA and RNA sequencing. To test a causal role for respiratory complex I, we disrupted a nuclear-encoded complex I subunit and evaluated an in-locus complemented line. **Results:** Hybrid progeny segregated into hypervirulent, virulent, and avirulent phenotypes despite their close nuclear similarity. Avirulent parasites showed contraction of the minicircle repertoire, including loss of guide RNA-encoding minicircles required for editing complex I transcripts. This loss was associated with impaired editing of complex I messenger RNAs, most prominently *ND8* and *ND9*, whereas parasites causing more severe lesions displayed higher levels of editing efficiency for those transcripts. Deletion of the complex I subunit attenuated infection, and genetic complementation partially restored lesion development in mice. **Conclusions:** These findings identify kinetoplast DNA minicircle repertoire diversity as a heritable determinant of *L. major* virulence and link variation in mitochondrial RNA editing to parasite fitness in the mammalian host. Mitochondrial complex I function therefore emerges as an important contributor to disease severity in cutaneous leishmaniasis.

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IMPROVING VACCINE EFFICACY BY ENHANCING THE IMMUNOGENICITY OF KEY VARIANTS OF THE VAR2CSA VACCINE CANDIDATE

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Placental malaria caused by *Plasmodium falciparum* (PM) remains a significant threat to maternal and child health in areas of stable malaria transmission. The pathogenesis of PM involves placental insufficiency, precipitated by the selective sequestration of infected erythrocytes (IE) in the placental intervillous space. This results in maternal anemia and pregnancy complications. The *P. falciparum* VAR2CSA protein, expressed on the surface of IEs, plays a key role in placental malaria. It is currently the leading candidate for a preventive vaccine against PM. However, the inherent antigenic polymorphism of VAR2CSA poses a challenge for vaccine development. Our team recently identified a rare variant of *P. falciparum* that particularly infects multigravid women living in Benin who already have high levels of anti-VAR2CSA antibodies. We established African parasite isolates expressing this particular type of VAR2CSA variant in *in vitro* culture and characterized the binding and antigenic properties of the native and recombinant proteins of these variants. The results show that these VAR2CSA-expressing parasite variants have epitopes in the CSA binding site that are distinct from those of common variants such as FCR3 and 3D7, and that they evade CSA binding inhibition by antibodies induced against these common variants. These observations are particularly relevant for guiding optimization efforts for the VAR2CSA-based vaccine currently under development. These data will be presented in detail at the congress.

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VAR2CSA-BASED PLACENTAL MALARIA VACCINE: CURRENT CLINICAL DEVELOPMENTS

Benoit Gamain[1], Sem Ezinmegnon[2], Clara-Eva Paquereau[1], Morten Nielsen[3], Issa Nébié[2], Anthony Bove[1], Sayit Erdogan[3], Flavia D'Alessio[4], Sodiomon Sirima[2], Benjamin Mordmüller[5], Alphonse Ouedraogo[2]
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Nulligravid and primigravid women are especially vulnerable to malaria in pregnancy. Two VAR2CSA-derived vaccines for use in nulligravid girls aiming to mimic naturally acquired immunity and confer protection to placental malaria are currently in clinical development: PAMVAC-cVLP and PRIMVAC. PAMVAC-cVLP is a re-engineered version of the PAMVAC antigen spanning the CSA-binding region. PAMVAC was previously tested in a Phase Ia/Ib clinical trial, demonstrating that the vaccine was safe, well tolerated, and induced a strong immune response in adult malaria-naive volunteers. The PRIMALVAC Phase Ia/Ib trial demonstrated that three doses of PRIMVAC, were safe and well tolerated, achieving 100% seroconversion, with 93% remaining seropositive after one year. However, both vaccines showed limited cross reactivity to other VAR2CSA variants. Following these clinical trials, the GHIT-funded VAC4PM project was launched in October 2021 to study long-term immunity and the boosting effects of PRIMVAC. Preliminary findings indicate that over 80% of vaccinated women still have antibodies seven years after vaccination. As part of VAC4PM, a Phase Ib trial will start soon in Burkina Faso to administer a single dose of PRIMVAC to nulligravid, primigravid, and multigravid women and evaluate its ability to boost naturally acquired VAR2CSA immunity. In parallel, the EU-funded ADVANCE-VAC4PM project seeks to improve and broaden the immune response induced by PAMVAC-cVLP and evaluate co-administration of PRIMVAC and PAMVAC-cVLP in a Phase Ia/Ib conducted in the Netherlands and Burkina Faso. Finally, in the context of a DGIS funded project, a PRIMVAC Phase II trial in African nulligravid women is under preparation.

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EPITOPE MAPPING OF HUMAN VAR2CSA-SPECIFIC MONOCLONAL ANTIBODIES

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Plasmodium falciparum placental malaria (PM) is a severe complication during pregnancy in areas with stable parasite transmission. It is caused by the adhesion of infected erythrocytes (IEs) to chondroitin sulphate A (CSA) exclusively expressed in the placenta, mediated by the parasite antigen VAR2CSA. PM mainly affects first-time mothers, as naturally acquired immunity (NAI) develops with subsequent pregnancies. This immunity has been associated with antibodies targeting VAR2CSA and inhibiting binding to CSA. However, it remains unclear whether protection is mediated by broadly cross-reactive monoclonal antibodies (mAbs) or by a repertoire of variant-specific mAbs. The VAR2CSA regions targeted by protective immunity are also not well defined. To address these gaps, seven full-length VAR2CSA variants were used to isolate antigen-specific B cells from Ghanaian pregnant women with NAI and were used to perform LIBRA-seq (linking B cell receptor to antigen specificity through sequencing). This enabled the sequence determination of 641 mAbs together with their antigen specificity and cross-reactivity. The majority (85%) of the identified mAbs were cross-reactive, and pan-reactivity was rare (0.5%). Forty-six mAbs were expressed as recombinant proteins, and ELISA and flow cytometry testing confirmed their VAR2CSA specificity. Epitope mapping established that most mAbs targeted the DBL3 and DBL5 domains, while regions critical for CSA binding (ID1-ID2, DBL4 and the NTS) were rarely recognized. Notably, none of the identified mAbs significantly blocked IE binding to CSA. This dataset reveals the antigenic landscape of VAR2CSA during natural infection and highlights key challenges for developing broadly protective vaccines or prophylactic antibodies against PM.

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EFFECTOR FUNCTIONS OF VAR2CSA-SPECIFIC IGG

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In pregnant women, parasites expressing VAR2CSA-type PfEMP1 bind to oncofetal chondroitin sulfate A (CSA), causing placental malaria (PM), a significant threat to mother-child health. Women exposed to *P. falciparum* during pregnancy acquire VAR2CSA-specific IgG, with high levels being associated with protection against adverse pregnancy outcomes, due to their ability to block infected erythrocyte adhesion to the placenta. However, this association is not always consistently observed, suggesting that other effector functions are involved. In recent years, we have consistently demonstrated that a high proportion of VAR2CSA-specific IgG acquired after malaria exposure during pregnancy selectively lacks fucose in the Fc region (Fc-afucosylation). The resulting afucosylated IgG has increased affinity for FcγRIIIa expressed on NK cells, enabling efficient antibody-dependent cellular cytotoxicity (ADCC) against IgG-opsonized infected erythrocytes. Fc-afucosylation increases with parity, as a proxy for exposure, and confers clinical protection against low birth weight and PM. In contrast, specific IgG induced by VAR2CSA vaccination is fully Fc-fucosylated and consequently unable to induce ADCC. Our findings provide new insights into protective immunity against PM and highlight the importance of NK cell-mediated destruction of IgG-opsonized infected erythrocytes, which requires Fc-afucosylated IgG. The evidence also calls for a revision of the current focus of PfEMP1-based vaccine development and suggests that alternative vaccination strategies are necessary.

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WEATHER DEPENDENT CHALLENGES OF SCHISTOSOMIASIS SURVEILLANCE AND CONTROL IN MALAWI.

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Both malaria and schistosomiasis remain highly endemic in southern Malawi and collectively give rise to a significant local public health burden. Of special note, each disease is firmly tied with the aquatic landscape, with a dominant physical feature being Lake Malawi and the Shire River Valley drainage basin. Contrary to commonly held assumptions, the ecological dynamics of this shoreline and riverine setting are surprisingly diverse, with both short- and long-term seasonality. For either natural or manmade reasons, this area is particularly vulnerable to acute flooding with a particularly notable cyclonic event of Cyclone Freddy. This struck southern Malawi in mid-March 2023, causing catastrophic damage and derailed several routine public health services. Cyclone Freddy delivered roughly six months' worth of rainfall in just six days, triggering intense flooding, with mudslides, displacing 600,000+ people. In this presentation, I discuss disease-surveillance activities set within the recently completed SHIRE_VEC and HUGS research projects to highlight the lesser known impacts of these now all too regular cyclonic events. The former project was funded by the UK's National Institute of Health Research (NIHR) and focused upon controlling vector borne diseases in emerging agricultural systems. The latter project was funded by the Wellcome Trust and addressed hybridisation in urogenital schistosomiasis, exploring several OneHealth dimensions, inclusive of environmental change and landscape use over the last decade.

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IMPACT OF CLIMATE CHANGE ON INTESTINAL PROTOZOA

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Background and aims Climate change is expected to increase the burden of waterborne diarrhoeal diseases by altering temperature, precipitation patterns, and pathogen dynamics. The Horizon Europe SPRINGS project (www.springsproject.eu) investigates these effects through an interdisciplinary framework integrating clinical and environmental surveillance with anthropological studies, hydrogeological modelling, and climate projections across four case study sites (Ghana, Italy, Romania, and Tanzania), including the city of Naples. **Methods** SPRINGS includes diarrhoeal surveillance through retrospective and prospective studies in vulnerable populations and water quality monitoring on drinking water sources and wastewater. Pathogen detection focuses on four index pathogens (*Campylobacter*, *Cryptosporidium*, *Giardia*, and rotavirus) through standardized procedures and molecular methods. Epidemiological findings will be integrated with climatic, anthropological, and hydrogeological data to identify climate-sensitive drivers of pathogen transmission and support risk assessment. **Results** Surveillance activities have been implemented across the four study sites, generating harmonized datasets on pathogen occurrence in human populations and water matrices. Protozoan pathogens were detected in vulnerable cohorts in the Italian case study site through both retrospective and prospective studies. Wastewater monitoring identified only *Giardia*, highlighting variability in its occurrence and concentration under varying temperature and precipitation conditions, although without statistically significant associations. **Conclusions** The project provides an integrated framework to investigate waterborne pathogen circulation under changing climatic conditions. The multidisciplinary approach will inform key climate, environmental, and health adaptation policies, in order to support and prepare citizens,

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communities and governments by better measuring the impact of future climate shocks on the burden of waterborne diarrheal diseases.

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TOPIC 1: PARASITE ECOLOGY, BIODIVERSITY, AND TAXONOMY
CLIMATE CHANGE, PARASITE ADAPTATION AND CONTROL
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ZOONOTIC MALARIA IN A CHANGING CLIMATE: LINKING VECTOR SURVEILLANCE, TRANSMISSION DYNAMICS, AND CONTROL STRATEGIES

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Zoonotic malaria has emerged as an important public health concern in Southeast Asia, particularly following increasing reports of zoonotic transmission involving *Plasmodium knowlesi* and other non-human primate–associated *Plasmodium* parasites. Environmental modifications, deforestation, and climate variability are increasingly recognized as key drivers influencing vector ecology, host interactions, and disease transmission dynamics. Changes in temperature, rainfall patterns, humidity, and land use may alter *Anopheles* vector distribution, abundance, biting behaviour, and transmission potential, posing challenges to malaria control efforts in the region. This study aims to investigate the relationship between climate change and zoonotic malaria transmission through integrated vector surveillance and intervention approaches. Over a two-year study period (currently 1.5 years completed), longitudinal *Anopheles* surveillance was conducted to characterize vector abundance, species composition, and temporal distribution patterns in relation to climate variables. Climate parameters including temperature, rainfall, humidity, and environmental factors were examined to determine their influence on vector population dynamics. Concurrently, malaria infection rates among vectors were assessed while entomological inoculation rates (EIR) were estimated to evaluate transmission intensity. In addition, a vector control intervention utilizing pyriproxyfen-based strategies was implemented to assess its effectiveness in suppressing mosquito populations and reducing transmission potential. By integrating ecological surveillance, parasite detection, transmission indicators, and targeted vector control measures, this study aims to provide evidence on how climate-driven changes may influence zoonotic malaria transmission and contribute toward sustainable vector management strategies for emerging zoonotic malaria in Southeast Asia.

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TOPIC 1: PARASITE ECOLOGY, BIODIVERSITY, AND TAXONOMY
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IMPACT OF CLIMATE CHANGE ON ANTHELMINTIC/ACARICIDE USAGE AND CLIMATE-FLEXIBLE APPROACHES TO PARASITE CONTROL

Hannah Vineer
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Climate change is one of the greatest challenges facing agriculture and conservation. Changes in the epidemiology of parasitic infections, and geographic ranges of parasites and vectors, have been documented over recent decades, challenging the ability of livestock keepers to uphold high standards of health and welfare. This presentation will explore, using examples, the role that mathematical and machine learning models play in supporting adaptation to climate change. For over a decade, a weather-dependent phenological model of *Nematodirus battus* spring hatching has supported UK farmers and veterinarians to target anthelmintic usage and control nematodirosis in lambs sustainably in the face of increasingly variable hatching patterns. In Norway, a similar approach has been used, since 2018, by veterinary services to target responses to climate-related outbreaks of *Elaphostrongylus rangiferi* (brainworm). More recently, machine learning and mathematical models are being developed to support responses to shifting geographic range of the tick, *Amblyomma variegatum*, which has been associated with tens of thousands of cattle deaths in Botswana since 2015. Lessons learned and implementation templates from these examples offer a route to accelerated development and uptake of additional climate-dependent models, for rapid response to emerging parasite and vector challenges.

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TOPIC 2: TRANSMISSION DYNAMICS, EPIDEMIOLOGY, AND GLOBAL HEALTH
FOOD AND AGRICULTURE ORGANIZATION OF THE UNITED NATIONS (FAO) EXPERT MEETING ON MICROBIOLOGICAL RISK ASSESSMENT OF PARASITES IN FOODS: PART I PROTOZOA
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OVERVIEW OF FAO EXPERT MEETING ON MICROBIOLOGICAL RISK ASSESSMENT OF FOODBORNE PROTOZOAN PARASITES

Laura Lalonde
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The FAO expert meeting on microbiological risk assessment of protozoan parasites in foods reviewed recent scientific developments and identified six parasites of highest foodborne concern: *Cryptosporidium* spp., *Cyclospora cayetanensis*, *Giardia duodenalis*, *Entamoeba histolytica*, *Toxoplasma gondii*, and *Trypanosoma cruzi*. These pathogens pose substantial health risks due to their environmentally resistant life stages, complex transmission routes, and challenges with detection and source attribution. Fresh produce emerged as the primary food vehicle of concern for intestinal protozoa, with contamination arising from irrigation water, soil amendments, or food handling. Other implicated foods include unpasteurized dairy and juices, and undercooked meats or bivalve shellfish. For extraintestinal parasites like *T. gondii*, foodborne transmission occurs mainly through undercooked meat and contaminated produce, and genotype virulence varies geographically. Food-borne *T. cruzi* infections are often linked to unpasteurized juices contaminated by triatomine vectors and are associated with severe illness, with climate-driven changes in vector ecology increasing risk. Although thermal treatments can inactivate protozoan parasites, they are not always applicable to raw foods, emphasizing the need for preventive measures such as good agricultural practices, clean water use, and improved hygiene. Detection methods remain limited, particularly for determining parasite viability, though molecular tools are advancing. The FAO meeting report highlights regional differences in parasite–food risks and identifies cross-cutting factors including globalization, climate change, and limited diagnostic capacity. Key knowledge gaps requiring attention to support quantitative risk assessment and improve control strategies include standardized detection methods, strengthened surveillance, molecular source-tracking tools, and enhanced education and training within a One Health framework.

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TOXOPLASMA FOODBORNE TRANSMISSION: CHALLENGES AND ALTERNATIVES FOR MONITORING

Jorge Gomez-Marin
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Foodborne transmission is the primary mode of *Toxoplasma gondii* transmission in humans. Infection may occur through the ingestion of oocysts present on fresh fruits or vegetables, uncooked shellfish, or bradyzoites in undercooked meat. Current climate change trends appear to enhance the occurrence, survival, distribution, and transmission of foodborne *T. gondii* infections. This is facilitated by increased runoff, leading to crop contamination, elevated levels of coastal contamination, and alterations in the geographic distribution, population density, and migration patterns of rodents, migratory birds, and arthropods. The risk of foodborne *T. gondii* infection varies significantly according to dietary habits and food preparation practices. Despite the low prevalence of infection in cattle, beef consumption is a significant source of meat-borne infections. Surveillance studies have reported the presence of *T. gondii* oocysts in fresh fruits and vegetables globally, indicating another source of foodborne infection. Current recommendations for *T. gondii* monitoring in food involve determining initial contamination levels using DNA-based tests. These methods tend to overestimate exposure to infective parasites by detecting all cyst populations (i.e., live and infectious, live and non-infectious, and dead), thus providing the maximum occurrence and contamination level for a given matrix. Comprehensive meat monitoring remains a distant goal; however, pre-harvest preventive measures should be implemented to ensure food safety. Cooking meat thoroughly and washing vegetables and fruits with boiled water can mitigate the risk of foodborne *T. gondii* infection at the individual level.

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SARCOCYSTIS IN FOOD ANIMALS: EMERGING RISKS, SILENT LOSSES, AND DIAGNOSTIC GAPS

Shokoofeh Shamsi
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Sarcocystids are among the most underestimated protozoan parasites affecting food animals globally. Despite their ubiquity, they receive only a fraction of the surveillance, diagnostic effort, and regulatory attention devoted to *Toxoplasma* or *Cryptosporidium*. In Australia, recent investigations have revealed high infection burdens across multiple hosts, including cattle, sheep, pigs, deer, dogs, and foxes yet most cases remain undetected or are reported generically at the genus level. This obscures important species-specific differences in zoonotic potential, pathogenicity, and epidemiology. This work combines new field data with a review of Australian sarcocystid records to clarify the true diversity and distribution of this group. Preliminary analyses point to the presence of previously unreported sarcocystids in food-producing animals, indicating that current surveillance frameworks underestimate their spread and public health relevance. Molecular diagnostics also present unique challenges: with more than 200 recognised species worldwide, sequence-based identification without accompanying morphological context can be misleading, resulting in diagnostic ambiguity. Our research highlights the emerging need for species-level surveillance, demonstrates how improved diagnostic literacy can prevent under-reporting, and outlines practical approaches for veterinarians and regulators to incorporate sarcocystids into existing food safety risk assessments. As global interest in foodborne protozoa expands, sarcocystids must be elevated to the same level of strategic concern as other tissue-cyst forming parasites.

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PERSISTENCE OF PROTOZOAN PARASITES IN ENVIRONMENTAL SAMPLES

Ynes Ortega
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Protozoan parasites can cause illness in humans and animals. Their mode of transmission can be person-to-person, through a reservoir, or via water or food. Two parasites are the most frequently associated with food and waterborne outbreaks: *Cryptosporidium* and *Cyclospora*. *Cryptosporidium* spp. can infect many animals, but the ones relevant to human health are *C. parvum*, which is primarily zoonotic and infects young animals, and *C. hominis*, which is considered anthroponotic. To date, *Cyclospora cayetanensis* infects only humans. Both parasites are highly resistant to environmental conditions and have been associated with the consumption of contaminated water or minimally processed raw vegetables. The source of *Cryptosporidium* oocysts is more likely to come from contaminated water or soil that came into contact with the product, or from contaminated food workers. In the case of *Cyclospora*, the oocysts require 7-15 days outside the human host to fully sporulate and become infectious. Our studies suggest that oocysts can persist viable in various types of agricultural soil, making preventive measures not just limited to water quality but also to the presence of feces in the soil. Molecular detection methods do not provide information on oocyst viability; thus, alternative methods are needed to assess the efficiency of oocyst inactivation on raw vegetables and fruits, as well as to determine the suitability of an agricultural field for crop production.

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TOPIC 5: DIAGNOSTICS, HOST-PARASITE INTERACTION AND EMERGING TECHNOLOGIES POPULATION GENETIC STUDIES TO INTERROGATE EPIDEMIOLOGICAL AND CLINICAL PHENOTYPES IN MALARIA
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GENOTYPIC AND SEROLOGICAL PERSPECTIVES ON HETEROGENEOUS MALARIA INFECTION RISK

Daniel Neafsey
Harvard TH Chan School of Public Health, Immunology And Infectious Diseases, Boston, United States of America

We have explored heterogeneity in malaria infection risk using Illumina-based amplicon sequencing of highly heterozygous antigens in longitudinal cohorts sampled cross-sectionally every 2-4 weeks. By noting the appearance and disappearance of parasite genotypic markers over time, we can measure strain-level infection dynamics in both observational cohort studies and clinical trial interventions in unprecedented detail. We note that infection risk by Plasmodium falciparum is highly heterogeneous in all surveyed African populations and age groups, with a small proportion of individuals exhibiting much higher rates of infection. In a pediatric cohort study conducted in Kalifabougou, Mali for which many participant covariates were measured, the majority of infection risk variation could not be explained. We hypothesize that infection risk variation is driven by invidivual-level biological and behavioral features that result in variable entomological innoculation rate. To measure individual-level exposure to Anopheles mosquitoes, we developed a phage-display library containing peptides representing the entire Anopheles spp. salivary proteome sialome, and measured the breadth of IgG response to the sialome in cohort participants. Breadth of sialome seropositivity was significantly associated with P. falciparum strain-level infection rate. As nearly study participants reported use of insecticide-treated bednets, we hypothesize that other features make some individuals intrinsically more attractive to mosquitoes.

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LEVERAGING THE NATURAL GENETIC CROSSES OF A HIGH TRANSMISSION SETTING TO UNDERSTAND HOW DRUG RESISTANCE SPREADS THROUGH POPULATIONS

Myo Naung[1], Dulcie Lautu-Gumal[2], Kirsty Mccann[1], Zahra Razook[1], Rebecca Narokobi[3], Tamarah Kolealea[4], Elma Nate[2], Coletah Kibai[3], Manuel Hetzel[5], Livingstone Tavul[6], Shazia Ruybal-Pesantez[7], Ivo Mueller[8], Leanne Robinson[4], Moses Laman[3], Maria Ome-Kaius[2], Alyssa Barry[1]
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Resistance of *Plasmodium falciparum* to antimalarial drugs remains a major threat to malaria control. Partial resistance to artemisinin first emerged in Cambodia in the early 2000s and spread across the Greater Mekong Subregion, driven largely by parasites carrying the *kelch13* C580Y mutation. This spread was facilitated by low host immunity and limited genetic outcrossing, enabling clonal expansion on a permissive genetic background. The recent emergence of *k13* mutations in high-transmission regions, including eastern Africa and Papua New Guinea (PNG), raises concern, although higher immunity and recombination may slow establishment. C580Y was first detected in PNG in 2016 on a hybrid PNG–Indonesian genetic background, confirming local transmission but leaving its origins unresolved. Nationwide surveillance subsequently showed a low prevalence of *k13* mutations until 2018, followed by rapid expansion of C580Y between 2019 and 2024. To investigate the drivers of this spread, we analysed whole-genome sequence data from parasites collected in 2019–2020 across C580Y hotspots and key sites. C580Y parasites are clonal in Morobe Province, recombining with wild-type parasites in Madang, and likely imported into East Sepik. The mutant population displays multiple selective sweeps, including loci previously associated with artemisinin resistance. In contrast, wild-type parasites show limited evidence of selection. This suggests an introduction of C580Y into mainland PNG, hybridisation with local parasites and westward spread along the north coast. The associated selective sweeps support a critical role for a permissive genetic background in facilitating resistance expansion. This work provides a genomic framework to predict resistance spread and inform targeted containment strategies.

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DISSECTING GENETIC FACTORS OF DRUG RESISTANCE AND OTHER CLINICAL MALARIA PHENOTYPES BY EXPRESSION QUANTITATIVE TRAIT LOCI (EQTL)

Zbynek Bozdech
Nanyang Technical University, Singapore, Singapore

The emergence and spread of *Plasmodium falciparum* lineages resistant to artemisinins is threatening the efficacy of Artemisinin Combination Therapies (ACTs), the global first-line treatment for *falciparum* malaria. Emerging first in Southeast Asia in the early 21st century, it has recently risen in Sub-Saharan Africa, posing a major challenge for global malaria control programs in the near future. Artemisinin resistance is a complex genetic trait in which nonsynonymous SNPs in *PfK13* cooperate with other, currently unknown, genetic variations to mediate the resistance phenotype. Population genetics and transcriptomics studies conducted over the last two decades have uncovered numerous genetic variants that contribute to or support the PfK13-dependent (and possibly independent) mechanism of artemisinin resistance, operating at several levels of malaria parasite pathophysiology. These include factors of *(i.)* hemoglobin degradation in the digestive vacuole (DV) affecting drug activation; *(ii.)* unfolded proteins /oxidative protein damage response (UP/ODR), mitigating the drug cytotoxic effect, and *(iii.)* senescence/quiescence signaling, alleviating the parasite's death pathways. In this presentation, we will discuss and summarize recent discoveries of genetic factors associated with artemisinin resistance, directly and/or indirectly, as mediators of parasite fitness and transmissibility, which are also known to be essential for the emergence and spread of P. falciparum lines with decreased drug sensitivity and, subsequently, ACT failures.

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PARASITE GENETIC BACKGROUNDS THAT SUSTAIN DRUG RESISTANCE VIA INCREASED TRANSMISSION

Matthias Marti
University of Zurich, Vetsuisse, Zurich, Switzerland

Plasmodium falciparum parasites are exposed to variable environmental conditions during infection. The switch from asexually replicating blood stage forms to transmission-competent gametocytes is sensitive to the host environment and assumed to be subject to natural selection. Data will be presented from a genome-wide association study with matched *P. falciparum* genomes and transcriptomes, using gametocyte marker expression as phenotypic readout. Geographic distribution of SNP associated with variation in gamateocyte expression segregated according to transmission settings (SNPs associated with high gametocyte expression in low transmission settings and vice versa). These data suggest that *P. falciparum* optimises the trade-off between within-host survival *versus* between-host transmission across malaria epidemiological settings based on genetic variation. Importantly, SNPs associated with artemisinin resistance and other drug resistance phenotypes were only present in the genetic background of parasites with alleles associated with high gametocyte marker expression, suggesting that increased transmission investment alleviates the fitness cost associated with the drug resistance trait. By linking natural selection for parasite investment in transmission with emergence and spread of drug resistance, these findings have major implications for containing drug resistant malaria. Potential translational applications are discussed.

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NOVEL STRATEGIES FOR HELMINTH ELIMINATION IN SE ASIA
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EFFICACY AND COST-EFFECTIVENESS OF THE BALATRINE IN INDONESIA

Darren Gray
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Background: Soil-transmitted helminths continue to pose a significant global health challenge, particularly in areas lacking improved sanitation. We evaluated the effectiveness of an integrated BALatrine intervention to prevent soil-transmitted helminth infections in Central Java, Indonesia. **Methods:** We conducted a cluster randomised controlled trial involving 2,155 participants in 8 village clusters between October 2016 and October 2017. Intervention villages received mass drug administration, the BALatrine, and community hygiene education, while the control group received only mass drug administration. We compared outcomes between study arms using a generalized linear mixed-effect model, with a p-value of less than 0.05 considered statistically significant. This trial is registered with the Australian and New Zealand Clinical Trials Registry (ACTRN12613000523707). **Results:** At baseline, the prevalence of any soil-transmitted helminth infection was 13.0% (95% CI: 10.7–15.7) in the control group versus 12.4% (95% CI: 9.9–15.3) in the intervention group. At the follow-up, the BALatrine intervention had a significant overall effect on any soil-transmitted helminth infection (adjusted OR: 0.22, 95% CI: 0.15–0.32). Stratifying by species also showed a significant effectiveness of 100% for *Ascaris lumbricoides* and 95% for hookworm (adjusted OR = 0.05, 95%CI: 0.01–0.21), but the reduction in *Trichuris trichiura* was not statistically significant (adjusted OR = 0.71, 95%CI: 0.48–1.11). **Conclusion:** The integrated BALatrine intervention was associated with a reduced incidence of overall soil-transmitted helminths infections, particularly *Ascaris lumbricoides*, and hookworm**.** This study highlights the potential interventions that focus on sanitation and hygiene, combined with health education and treatment to control and eliminate soil-transmitted helminths**.**

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THE MAGIC GLASSES PHILIPPINES: A SCALABLE HYGIENE EDUCATION TOOL

Mary Lorraine Mationg[1,2], Gail William[3], Veronica Tallo[4], Remigio Olveda[4], Portia Alday[4], Mark Donald Reñosa[4], Chona Mae Daga[4], Jhoys Landicho[4], Maria Paz Demonteverde[4], Eunice Dianne Santos[4], Eindra Aung[5], Franziska Beiri[6], Yuesheng Li[1], Archie C. A. Clements[7], Peter Steinmann[8], Kate Halton[9], Andrew Bedford[2], Donald Stewart[10], Donald Mcmanus[11], Darren Gray[1]
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Soil-transmitted Helminths (STHs) infect more than a billion people globally, causing up to 3.30 million DALYs, with the greatest burden among schoolchildren. Regular treatment through mass drug administration (MDA) of the at-risk population remains the main approach. However, MDA as a standalone intervention does not prevent reinfection. Improved provision of safe water and sanitation infrastructure, together with effective health education, is critical to interrupting the transmission and achieving sustainable control. The Magic Glasses Philippines (MGP) is a culturally tailored, video-based health education intervention package designed to prevent STH infection among schoolchildren. The MGP was evaluated in a cluster-randomized controlled trial involving >2000 schoolchildren in Laguna province, the Philippines. The trial demonstrated that MPG improved STH-related knowledge and behaviours, increased deworming uptake, and reduced STH infections in schools with a baseline prevalence of <15%. A cost analysis showed that the intervention is highly affordable, at just 30 cents USD per child (with most costs in kind, as it involves only teacher time to deliver the intervention). Based on this evidence, the Philippine Department of Education (DepEd) has endorsed MGP for integration into the WASH in Schools (WINS) program, including the school-based deworming program to control STH infections among learners. This presentation outlines the process from scientific findings to policy adoption and planned implementation, emphasizing the partnerships, evidence generation and stakeholder engagement that have facilitated the translation of this research into policy. The MGP program provides a practical model for implementing evidence-driven school-based health intervention in the Philippines and other STH-endemic countries.

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NOVEL STRATEGIES FOR HELMINTH ELIMINATION IN SE ASIA
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POC DIAGNOSTICS FOR REMOTE COMMUNITIES: THE PHILIPPINE EXPERIENCE

Emmanuel John Tabilin[1,2], Eleonor Cervantes[3], Mary Lorraine Mationg[4], Marianette Inobaya[5], Darren Gray[4], Megumi Sato[6], Marcello Sato[7], Hong You[8], Yasuhito Sako[9], Mario Jiz[3], Pengfei Cai[1], Catherine Gordon[1,2]
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Schistosomiasis caused by *Schistosoma japonicum* remains highly prevalent in the Philippines despite decades of preventive chemotherapy, partly due to the absence of accurate, field-deployable diagnostics. We recently developed two semi–point-of-care diagnostics (POC) tools: (1) an equipment-free cotton-syringe stool DNA extraction device paired with portable quantitative PCR, and (2) a latex microsphere-based lateral flow immunoassay detecting anti-SjSAP4 antibodies in serum. Laboratory validation using infected human samples showed diagnostic performance comparable to established methods—87.7%/92.1% sensitivity/specificity for the cotton-syringe system relative to Kato-Katz (KK) and commercial stool qPCR, and 80.6%/98.0% for the anti-SjSAP4 LFIA compared with KK. To evaluate field applicability, both POCs were deployed in six endemic Philippine villages, where community health workers were trained to perform the tests on residents (n=250 per village). Acceptability was assessed through questionnaires and focus group discussions. Preliminary findings highlight key challenges, including reluctance among health workers to handle stool samples and workflow bottlenecks during stool homogenization in the cotton-syringe method. Addressing these operational pain points will be essential for improving uptake and ensuring that these POCs can support more accurate, community-level schistosomiasis surveillance and monitoring.

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ARTIFICIAL INTELLIGENCE FOR RAPID, AUTOMATED HELMINTHAND SCHISTOSOMA EGG DETECTION IN FIELD SETTINGS

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Population-based surveys are essential for determining the presence and prevalence of soil-transmitted helminths (STH) and schistosomiasis (SCH), as outlined in the WHO 2030 NTD roadmap. Microscopy remains the diagnostic standard for STH and SCH, requiring manual reading of stool and urine filtrate slides. However, this approach is labor-intensive, prone to error and fatigue, and dependent on parasitology expertise that is increasingly limited worldwide. In programmatic settings, samples are collected in communities and analyzed either on-site or in centralized laboratories. In endemic countries, surveys are often conducted locally, with field teams traveling to remote communities across various environments, including archipelagos, remote villages, and other geographically challenging environments. Here, we describe a low-cost, portable digital pathology platform designed for both field and laboratory use to support monitoring and evaluation surveys low resource settings. The platform rapidly scans and digitizes sample slides and applies onboard artificial intelligence (AI) models for STH/SCH egg detection. Through a human-in-the-loop workflow, detected eggs can be verified locally or remotely, while results are automatically recorded and can be translated into prevalence intensity metrics, and integrated into study dashboards when internet connectivity is available. Clinical validation studies for STH and *Schistosoma mansoni* have demonstrated usability in mobile field settings, sensitivity in low-intensity prevalence areas, and potential for multiplexed parasite detection. Although further development is required for SCH species differentiation, this platform has strong potential to expand diagnostic access for remote communities with limited resources.

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TOPIC 2: TRANSMISSION DYNAMICS, EPIDEMIOLOGY, AND GLOBAL HEALTH
NOVEL STRATEGIES FOR HELMINTH ELIMINATION IN SE ASIA
08-20-2026 2:10 PM - 4:10 PM

ENVIRONMENTAL DNA FOR ONE HEALTH PARASITE SURVEILLANCE IN ENDEMIC REGIONS

Megumi Sato[1], Tiengkham Pongvongsa[2], Tippayarat Yoonuan[3], Phoyphaylinh Prasayasith[4], Mark June Revolteado[5], Joseph Valencia[6], Bunthea Chhor[1], Poom Adisakwattana[3], Somphou Sayasone[7], Marcello Sato[8]
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Using opisthorchiasis as a study model, this presentation will highlight our latest findings from field surveys and animal-host intervention studies, demonstrating the value of environmental DNA (eDNA)-based surveillance and the impact of a One Health approach for tackling opisthorchiasis in Laos. *Opisthorchis viverrini* remains highly prevalent in Lao PDR, where an estimated 46% of the population—approximately 2.5 million people—is infected, particularly in the central and southern provinces. Chronic *O. viverrini* infection is strongly associated with cholangiocarcinoma, leading to its classification as a Group 1 carcinogen. Therefore, prevention and control of *O. viverrini* infection are directly linked to reducing the burden of liver cancer in endemic communities. Control of the disease remains challenging because of the complex life cycle of the fluke, which involves humans and animals as definitive hosts, *Bithynia* snails as the first intermediate host, and cyprinid fish harboring the infective metacercarial stage. Consequently, control programs based solely on the treatment of human infections are insufficient to interrupt transmission. This presentation will discuss how integrated surveillance strategies combining eDNA technology, field epidemiology, and interventions targeting animal reservoirs can improve the detection of transmission hotspots and support more effective, sustainable control strategies within a One Health framework.

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TOPIC 2: TRANSMISSION DYNAMICS, EPIDEMIOLOGY, AND GLOBAL HEALTH
EMERGING PATTERNS AND DRIVERS OF ECHINOCOCCUS MULTILOCULARIS TRANSMISSION
08-21-2026 8:00 AM - 10:00 AM

HUMAN ALVEOLAR ECHINOCOCCOSIS IN EUROPE: EVIDENCE, SURVEILLANCE AND POLICY IN A CHANGING EPIDEMIOLOGICAL LANDSCAPE

Adriano Casulli
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Introduction: Human alveolar echinococcosis (AE) is a notifiable parasitic infectious disease in most European countries; however, in practice, it is under-reported by national health systems. This study aims to shed light on the unrecognized burden of AE in Europe, unveiling its epidemiological impact by providing a quantitative measure of number, incidence and trends of human cases during the period 1997–2023. **Materials and Methods:** The main inclusion criterion of this systematic review was primary data reporting probable or confirmed human AE cases in included European countries during 1997–2023. **Results:** This systematic review identified 4,207 human AE cases from 28 of the 40 European countries investigated. Historically endemic Austria, France, Germany, and Switzerland accounted for 2,864 (68.08%) of 4,207 cases documented in Europe, and Lithuania, Poland, and Slovakia represented an additional 887 (21.08%) cases. Based on incidence rates and trends detected in this study, two main epicentres were seen in countries in the Alpine and the Baltic areas. The mean annual incidence from 1997 to 2023 throughout Europe was 0.063 cases per 100,000 people and in EU member states was 0.060/100,000 people. **Conclusion:** Data suggest that AE is emerging in almost every country where this neglected parasitic infectious disease has been detected. Findings from this study should be used to support the planning of surveillance of *Echinococcus multilocularis* in animals and human in Europe in a One Health perspective. This study was funded by the KNOW-PATH project within the European Union's Horizon European Partnership on Animal Health and Welfare (GA 101136346).

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TOPIC 2: TRANSMISSION DYNAMICS, EPIDEMIOLOGY, AND GLOBAL HEALTH
EMERGING PATTERNS AND DRIVERS OF ECHINOCOCCUS MULTILOCULARIS TRANSMISSION
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TRANSMISSION OF ECHINOCOCCUS MULTILOCULARIS: EPIDEMIC INSIGHTS FROM CENTRAL ASIA AND WESTERN CHINA

Paul Torgerson
University of Zürich, Veterinary Epidemiology, Zürich, Switzerland

Human alveolar echinococcosis (AE) was first reported from the then Russian empire 1879. In Kyrgyzstan in central Asia, few cases had been reported before the start of the 21st century, although *Echinococcus multilocularis* had been isolated from wildlife in the 1950s. Over the last 25 years, the incidence has increased to over 200 cases per year. In China the first reported cases of AE was in Xingjiang in 1956. Subsequently large numbers of human AE cases have been described from western provinces of China. It is uncertain if this is a true epidemic or improved public health services that have uncovered large numbers of cases. In central Asia and western China, dogs are frequently infected with *E. multilocularis*. The close contact between domestic dogs and their owners may be driving the epidemic. This contrasts, with Europe, where canine infection is infrequently reported. In Kyrgyzstan the economic changes following independence from the USSR may have promoted the colonization of dogs by *E. multilocularis* leading to onward transmission to humans. However, many infections are not be attributable to dog or fox contact but may be associated with contamination of water supplies and/or widespread environmental contamination. Geographical and climatic variables are also associated with the distribution of AE which may be linked to conditions that optimize wildlife populations and environmental survival parasite eggs. The predominant *E. multilocularis* genotype is the Asian genotype. This may be more pathogenic to humans than the European or North American genotypes increasing the likelihood of clinical disease following exposure.

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TOPIC 2: TRANSMISSION DYNAMICS, EPIDEMIOLOGY, AND GLOBAL HEALTH
EMERGING PATTERNS AND DRIVERS OF ECHINOCOCCUS MULTILOCULARIS TRANSMISSION
08-21-2026 8:00 AM - 10:00 AM

TRACKING ECHINOCOCCUS MULTILOCULARIS IN EUROPE: INSIGHTS FROM MOLECULAR SURVEILLANCE AND GENOTYPING

Jenny Knapp
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Echinococcus multilocularis **is an endemic cestode in the Northern hemisphere, with an important focus in Europe. The parasite is the infectious agent of alveolar echinococcosis, and based on a prey-predator interaction, involves red fox as definitive host and small mammals as voles, occasionally humans or captive primates as intermediate hosts. This hermaphroditic parasite presents a low genetic diversity and its study was challenged during these last 35 years, with partial or full exploration of the mitochondrial DNA and few nuclear targets . Through the study of the tandemly repeated microsatellite EmsB, a core area in Europe was described in the north of the Alps (Switzerland and Jura Swab), with important parasite richness and diversity in EmsB profiles. Moreover, the transmission of the parasite into peripheral regions seems to be governed by a "mainland–island" system, suggesting a relatively recent colonization of the parasite in these peripheral regions. The presence of similar genetic profiles in both zones indicated a founder event. The origins of the parasite were studied and Asian backgrounds were hypothesized from mitochondrial and EmsB data. Moreover, outside of the European continent, the introduction of European *E. multilocularis* strains into North America was highlighted, without possible dating. With the advent of new technologies of sequencing, tracking *E. multilocularis* genetic diversity through the exploration of mitogenomes (13738 pb) is now possible and allow to described locally distributed haplotypes. The exploration of large DNA datasets could permit to better understand the circulation of the parasite in but also beyond the European continent**

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EMERGING EPIDEMIOLOGY OF ECHINOCOCCUS MULTILOCULARIS IN NORTH AMERICA: PARALLELS AND CONTRASTS WITH EUROPE

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Objectives We sought evidence that the zoonotic tapeworm *Echinococcus multilocularis* is emerging in North America and compared ecology, management, and challenges with the situation in central Europe, long considered highly endemic for this parasite. **Methods** We searched the peer-reviewed literature (PubMed, Web of Science, and Google Scholar) for reports of *E. multilocularis* infections in wildlife, dogs, and humans in North America published since 2015. Additional studies were identified through “snowballing” of cited references. **Results** The main sylvatic definitive hosts in North America are coyotes (*Canis latrans*) and red fox (*Vulpes vulpes*), with local prevalences from 0% to over 70%. Primary sylvatic intermediate hosts include arvicoline and neotomine rodents, and most recently, in a chipmunk (*Tamias striatus*). Previously, endemic regions of North America were divided into the Arctic (driven by arctic fox) and the prairie region of central North America (3 Canadian provinces and 13 US states). Now, new findings demonstrate *E. multilocularis* in animal hosts in 9 Canadian provinces and 24 US states, a major range expansion. This corresponds with unprecedented autochthonous cases of canine and human alveolar echinococcosis, especially in the Alberta province of Canada, all associated with European type strains of the parasite also found in sympatric wildlife. **Conclusions** There is increasing evidence that European type strains of *E. multilocularis* are emerging in North America, requiring a One Health approach to enhance surveillance in wildlife, humans, and dogs to increase awareness and tools for early detection and to increase access to existing and improved treatments for both human and animal patients.

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EMERGING PATTERNS AND DRIVERS OF ECHINOCOCCUS MULTILOCULARIS TRANSMISSION
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WILDLIFE TRANSMISSION OF ECHINOCOCCUS MULTILOCULARIS: EPIDEMIOLOGICAL INSIGHTS FROM SWITZERLAND AND EUROPE

Caroline F. Frey[1], Emily Bessell[1], Britta Lundström-Stadelmann[1], Antti Oksanen[2], Jussa-Pekka Virtanen[2], Emily Jenkins[3]
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Background and aims Alveolar echinococcosis (AE) caused by *Echinococcus multilocularis* is a rare but serious zoonotic disease. It is currently emerging. Wildlife can act as both definitive hosts, harboring the adult tapeworms in the intestine, as well as intermediate hosts, with metacestode growth mainly on the liver. This talk will cover the main wildlife hosts in Europe and highlight recent developments. **Methods** We searched the peer-reviewed literature for reports of *E. multilocularis* infections in wildlife in Europe published since 2015. Additional studies were identified through cited references. **Results** The main definitive host in Europe is the red fox (*Vulpes vulpes*) with local prevalences ranging from 0% to over 40%. Wolves (*Canis lupus*), golden jackals (*Canis aureus*), raccoon dogs (*Nyctereutes procyonoides),* and arctic foxes (*Vulpes lagopus*) are definitive hosts of local importance. Many wildlife species act as intermediate or aberrant hosts, developing liver lesions or AE. Besides small rodents, especially Arvicolinae, Eurasian beavers (*Castor fiber*) are particularly affected. The first detection of AE in a wild canid (golden jackal) and brown hare (*Lepus europaeus*) fell in the reviewed decade. Geographically, an expansion into two Balkan countries (Serbia and Bosnia & Herzegovina) was observed, as well as a slow range expansion in France, Italy, and Sweden. **Conclusions** Surveillance in the main definitive host, red fox in Europe, is key to determining local risk in endemic areas. National reporting of both animal and human cases would help to get a One Health overview and early alerts in newly affected countries.

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TOPIC 3: RESISTANCE AND DEVELOPMENT OF DRUGS AND VACCINES
NEW DRUGS IN DEVELOPMENT AGAINST FILARIASIS AND STH
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EFFICACY AND SAFETY OF OXFENDAZOLE IN ADULTS WITH TRICHURIS TRICHIURA INFECTION IN PEMBA ISLAND, TANZANIA: A DOUBLE-BLIND, PHASE 2A, RANDOMIZED CONTROLLED TRIAL

Annina Schnoz[1], Hannah Jeanguenat[1], Abrahaman Msellem[2], Said Mohammed Ali[2], Jennifer Keiser[1]
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Soil-transmitted helminths (STH), which include *Trichuris trichiura*, *Ascaris lumbricoides* and the hookworms *Necator americanus* and *Ancylostoma duodenale*.remain a major public health problem infecting over 1.5 billion people globally. The widely used benzimidazoles albendazole and mebendazole show low efficacy to treat *T. trichiura* infections compared to the other STH. Therefore, to reach the goal of the WHO to eliminate this neglected tropical disease as a public health problem by 2030, more effective medicines need to be developed. In this double-blind, phase 2a, randomized, placebo-controlled trial, we aimed to determine the safety and efficacy of oxfendazole in adult participants with *T. trichiura* infections on Pemba Island, Tanzania. In addition, we assessed the efficacy of oxfendazole in co-infections with *A. lumbricoides* and hookworm. A total of 127 participants were randomized to receive either placebo, 400 mg oxfendazole once , 800 mg oxfendazole once or 800 mg oxfendazole on three consecutive days. The highest efficacy was observed with the multiple dose of oxfendazole with a cure rate of 76% and a geometric mean egg reduction rate of 99%. The single doses of oxfendazole achieved cure rates of 31% (400 mg) and 35% (800 mg), respectively. Across all arms oxfendazole was well tolerated with few and minor adverse events. Abdominal discomfort was the most common adverse event reported. Overall, these findings suggest that multiple-dose oxfendazole is a safe and effective treatment for *T. trichiura* infections. Our data support further clinical development of oxfendazole for helminth infections.

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OXFENDAZOLE: A PAN-NEMATODE DRUG CANDIDATE

Karen Dequatre Cheeseman[1], Rella Manego[2], Glory Amambo[3], Sonja Zehetmayer[4], Benjamin Lenz[5], Annina Schnoz[6], Lidwine Badjina[7,8], Alexander Kazakov[5,9], Fabiana Barreira[1], Ivan Scandale[1], Said Mohammed Ali[10], Joel Tarning[11], Michael Ramharter[7,8,12], Achim Hoerauf[5,9], Martin Posch[4], Samuel Wanji[3], Dieudonne Ngoyi[13], Ghyslain Mombo-Ngoma[2], Jennifer Keiser[6], Sabine Specht[1], Marc Hübner[5,9]
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The WHO's goals to eliminate onchocerciasis are hampered by the lack of a safe, short-term macrofilaricidal, adult worm killing, drug. Similarly, safe and effective therapeutics are required for treating other filarial diseases, specifically loiasis and mansonellosis, as well as the intestinal helminth infection trichuriasis. Here, we present the clinical development of the repurposed, broad-spectrum veterinary anthelmintic oxfendazole to address these critical gaps. Within the EU-funded "HELP – Helminth Elimination Platform" consortium, a bioavailability study using a newly developed, field-applicable oxfendazole tablet formulation was conducted in Tanzania. Subsequent population pharmacokinetic analyses supported a 5-day dosing regimen of 400 mg or 800 mg oxfendazole daily for people with filarial infection. The efficacy and safety of oxfendazole are currently being evaluated by the EDCTP3- and SERI-funded eWHORM consortium using a "basket trial" design. This adaptive trial evaluates participants with onchocerciasis, loiasis, mansonellosis, and trichuriasis across four sub-Saharan African countries under a harmonized master protocol and includes an interim analysis to drop non-efficacious treatment arms and allocate additional study participants to efficacious or new treatment arms. The patient-centric approach allows the inclusion of co-infected individuals and the detection of country-specific differences. To date, over 200 people with filarial infections have been treated and the recruitment of the first 360 filariasis participants required for interim analysis is

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expected by Q2 this year; results of the trichuriasis sub-study are presented separately. Collectively, oxfendazole represents a highly promising pan-nematode macrofilaricidal candidate to support global elimination efforts.

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TOPIC 3: RESISTANCE AND DEVELOPMENT OF DRUGS AND VACCINES
NEW DRUGS IN DEVELOPMENT AGAINST FILARIASIS AND STH
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CORALLOPYRONIN A - A NOVEL ANTIWOLBACHIA ANTIBIOTIC

Kenneth Pfarr[1,2], Andrea Schiefer[1,2], Frederic Risch[1,2], Samuel Wanji[3], Jan Heitkötter[2,4], Miriam Grosse[5,6], Birthe Sandargo[5,6], Marc Stadler[5,6], Silke Alt[7], Thomas Hesterkamp[7], Rolf Müller[6,8], Karl Wagner[2,4], Marc Hübner[1,2], Achim Hoerauf[1,2]
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Onchocerciasis and lymphatic filariasis are two of the five diseases comprising 90% of the Neglected Tropical Disease burden in Africa. They are caused by filarial nematodes that are dependent on *Wolbachia* endobacteria for fertility, development and survival. Antibiotics targeting *Wolbachia* can be used to control/eliminate these diseases. However, doxycycline, the gold standard antibiotic, requires >4 weeks daily treatment and is contraindicated for children and pregnant/breastfeeding women. We are developing the RNA polymerase inhibitor corallopyronin A (CorA) to treat filarial infections. We have demonstrated oral drug efficacy (>2-log *Wolbachia* depletion) against *Litomosoides sigmodontis* in mice and Mongolian gerbils, *Onchocerca ochengi* in mice, and *Dirofilaria immitis* in dogs using human equivalent doses given ≤14 days. Pharmaceutical safety and toxicology studies have identified the major mechanisms of CorA metabolism and shown that CorA is nontoxic in rats and dogs, supporting advancement to clinical trials. We developed oral formulations with field-ready stability (>12 weeks at 40 °C) and bioavailability (mouse >59%, dog >53%). The drug substance and drug product manufacturing processes have been successfully transferred to the GMP facilities Phyton Biotech and Quotient Sciences, respectively. With funding from the German Center for Infection Research and the Global Health Innovative Technology Fund, in cooperation with Eisai Co., Ltd., we will produce clinical trial material and conduct a phase 1 first-in-human trial. Successful development will provide a novel treatment to help eliminate filarial infections as defined in the WHO road map for neglected tropical diseases 2021–2030.

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SCHISTOSOMA MEKONGI: PROGRESS ON AN ELIMINATION PROGRAM
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ESTABLISHING THE FOUNDATIONS FOR SCHISTOSOMA MEKONGI ELIMINATION IN CAMBODIA: PILOT AND BASELINE OUTCOMES

Molyden Vann[1], Virak Khieu[2], Catherine Gordon[3]
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Background: Cambodia has made substantial progress in reducing *Schistosoma mekongi* transmission through praziquantel-based control, but persistent focal risk remains in Mekong River communities. Elimination will require surveillance tools capable of detecting residual transmission, alongside locally acceptable interventions addressing water contact, sanitation, and community understanding of infection. We conducted a mixed-methods pilot study in historically endemic villages in Kratie Province to inform a larger One Health elimination program incorporating environmental DNA, animal reservoir surveillance, WASH assessment, and health education. **Methods:** The six villages: Achen, Kampong Krabei, Koh Dambang Kraom, Damre, Kampong Pnow, and Koh Cbar. Household questionnaires and knowledge, attitudes, and practices relating to *S. mekongi*. The animal faecal samples were collected from dogs, cats, cattle, and water buffalo following owner consent, with samples preserved for FECT and qPCR. Formative research included draw-and-write activities and focus group discussions with schoolchildren and parents,and interviews with teachers and health workers to guide development of a Cambodian *S. mekongi* Magic Glasses health education package. **Result:** A total of 563 household questionnaires were completed, representing 38–65% of households per village. River water use was widespread, including for drinking, cooking, and washing. Among households with latrines but inspection identified gaps in functionality, 92% heard of *S. mekongi*, but only 42% and 53%, respectively, correctly identified infected water contact as the transmission pathway. Water contact was frequent, with 72% in Achen and 91% in Kampong Krabei reporting freshwater contact, through bathing, washing clothes, wading, and collecting water. Open defecation was also reported. Two dogs were positive for S. mekongi by qPCR.

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SCHISTOSOMA MEKONGI: PROGRESS ON AN ELIMINATION PROGRAM
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LAO PDR'S PROGRESS TOWARDS SCHISTOSOMA MEKONGI ELIMINATION: INSIGHTS FROM PILOT AND BASELINE SURVEYS

Sysouphanh Many
Lao Tropical and Public Health Institute, International Program For Health In The Tropic, Vientiane Capital, Laos

Background: *Schistosoma mekongi* remains endemic in focal areas of Lao People's Democratic Republic (Lao PDR), where ongoing transmission is influenced by water contact, sanitation, animal reservoirs, and the ecology of the *Neotricula aperta* snail intermediate host. As prevalence declines following repeated mass drug administration, sensitive surveillance and locally tailored health education are needed to support elimination. We conducted a mixed-methods pilot study in Khong district to generate preliminary data for a multi-component elimination program integrating MDA, WASH, One Health surveillance, and school-based health education. **Methods:** A cross-sectional pilot study was conducted in two endemic villages, Tha Mak Hep and Som Ven Aok, with broader latrine mapping planned across six villages. Household questionnaires and latrine audits assessed water sources, sanitation infrastructure, open defecation, water contact, and knowledge, attitudes, and practices relating to *S. mekongi*. Formative research included draw-and-write activities and focus group discussions with schoolchildren, focus group discussions with parents, and key informant interviews with teachers and health officials to inform adaptation of the Magic Glasses health education package. Animal faecal samples were collected from potential reservoir hosts, preserved for microscopy and molecular testing, and assessed using FECT and qPCR. **Results:** A total of 187 households participated. Most respondents were farmers, and river water remained an important source for cooking and washing, although bottled water was commonly used for drinking. Latrine coverage was high, with >80% of households reporting latrine access and most latrines functional at inspection. However, untreated river water was commonly used for latrine purposes, and water contact remained frequent, with 78% of respondents in Tha Mak Hep and 82% in Som Ven Aok reporting freshwater contact in the previous month. Knowledge of *S. mekongi* was moderate to high, with >60% of respondents in both villages reporting prior awareness. Correct identification of infected water as the main transmission route was higher in Som Ven Aok than Tha Mak Hep, while uncertainty remained regarding animal reservoirs and whether treatment provides lasting cure. No animals tested positive for *S. mekongi*. **Conclusion:** This pilot identified strong community awareness but persistent behavioural and knowledge gaps relevant to transmission, particularly around water contact, untreated river water use, animal reservoirs, and treatment expectations. The findings support an

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integrated elimination strategy combining molecular One Health surveillance, WASH strengthening, and locally tailored school- and community-based health education.

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SCHISTOSOMA MEKONGI: PROGRESS ON AN ELIMINATION PROGRAM
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INFECTION RISK MAPPING AND ENVIRONMENTAL SURVEILLANCE FOR SCHISTOSOMIASIS MEKONGI: TRACKING EDNA ALONG THE MEKONG RIVER

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Schistosoma mekongi is a highly focal zoonotic trematode endemic to discrete areas of the lower Mekong River basin in Laos and Cambodia. First identified in 1957 and formally described in 1978 following the discovery of its unique intermediate snail host, *Neotricula aperta*, the parasite remains geographically restricted due to the snail's strict ecological requirements, including fast-flowing, oxygenated, rocky river habitats. Current reports indicate that the prevalence of *S. mekongi* has declined to low levels, generally below 1% in sentinel sites within endemic areas of Lao PDR and Cambodia, reflecting the impact of sustained mass drug administration and integrated control measures. However, transmission may persist in highly focal riverine hotspots associated with the distribution of *N. aperta*. These findings suggest that, although morbidity control has largely been achieved, the region remains in a pre-elimination phase that requires highly sensitive surveillance tools to detect residual infections. This presentation will highlight how a newly developed environmental DNA (eDNA)-based detection system can identify *S. mekongi* transmission hotspots and discuss its potential application for surveillance, supporting effective, sustainable control strategies and the possible future elimination of the disease.

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TOPIC 2: TRANSMISSION DYNAMICS, EPIDEMIOLOGY, AND GLOBAL HEALTH
SCHISTOSOMA MEKONGI: PROGRESS ON AN ELIMINATION PROGRAM
08-21-2026 8:00 AM - 10:00 AM

DEVELOPMENT OF THE MAGIC GLASSES SCHOOL-BASED HEALTH EDUCATION AND COMMUNITY PROGRAM FOR SCHISTOSOMIASIS IN LAO PDR AND CAMBODIA.

Mary Lorraine Mationg[1,2], Darren Gray[1], Gail William[3], Archie Clements[4], Kinley Wangdi[5], Malcolm Jones[6], Sysouphanh Many[7], Kanchana Thilakoun[7], Molyden Vann[8], Somphou Sayasone[9], Virak Khieu[8], Catherine Gordon[6]
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Mekong schistosomiasis is an infectious disease caused by the trematode *Schistosoma mekongi*, which is endemic in focal areas of Cambodia and Lao PDR. The disease is a zoonosis involving domestic animals, with dogs and pigs serving as key reservoir hosts that contribute to transmission. Transmission is confined to areas of the Mekong River where the freshwater snail *Neotricula aperta,* the intermediate host, is present. Infection prevalence in endemic communities of Cambodia and Lao PDR ranges from 0.6% to 33%. The cornerstone for *S. mekongi* control is mass drug administration (MDA) with praziquantel (PZQ). Although MDA and surveillance have reduced the disease burden, attaining sustainable control and progress toward elimination requires complementary measures, including the development and implementation of effective health education to improve knowledge and encourage behaviour change, reducing exposure to parasite-infected water in the community. As part of a trial evaluating a multidisciplinary, integrated elimination program for *S. mekongi* in Cambodia and Lao PDR, incorporating water, sanitation and hygiene (WASH), environmental monitoring, PZQ treatment, and health education, we have adapted the evidence-based Magic Glasses health education package to develop a culturally tailored, school-based intervention. We also developed a complementary community-based health education program for *S. mekongi* by adapting the helminth elimination in the Lower Mekong (HELM) program, which is designed to prevent soil-transmitted helminth and liver fluke infections. This presentation will describe the formative research, adaptation process and development of the health education program components to support the integrated schistosomiasis elimination program.

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TOPIC 2: TRANSMISSION DYNAMICS, EPIDEMIOLOGY, AND GLOBAL HEALTH
ONE GENUS, MANY STORIES: THE GENETIC, CLINICAL, AND ECOLOGICAL DIVERSITY OF STRONGYLOIDES
08-21-2026 8:00 AM - 10:00 AM

MOLECULAR EPIDEMIOLOGICAL STUDIES OF S FUELLEBORNI: FROM BABOONS TO VERVETS WITH HUMANS IN BETWEEN

Russell Stothard[1], Joel Barratt[2], Will Nevin[3], Lucas Cunnijgham[1], Ruth Cowlishaw[1], Alexandra Juhasz[1]
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Our interest in *Strongyloides fuelleborni* first started in 2021 with surveys of gastrointestinal parasites within semi-captive olive baboons at Knowsley Safari. There, from the safety inside their vehicles, the general public can have a close-up encounter with their baboon colony. From faecal surveys of these baboons, as well as by molecular diagnostic typing, the prevalence of strongyloidiasis typically ranged from 10% to 30%. An in-depth molecular characterisation of worms obtained from charcoal cultures was undertaken. Whole-genome sequencing data were generated with later phylogenetic analysis of mitochondrial (mt) cytochrome oxidase subunit 1 (*cox1*) and nuclear ribosomal 18S sequences against 300 published *Strongyloides* reference genotypes. The putative African origin of the UK *S. fuelleborni* was confirmed and full-length mt genome sequences were assembled to facilitate a more detailed phylogenetic analysis of 14 mt coding regions against all available *Strongyloides* species. Our analyses demonstrated that the UK isolate represented a novel African lineage not previously described. Using a recently revised clinical molecular diagnostic assay, we showed that around 20% of travel-acquired infections in Europe were due to *S. fuelleborni*, with co-infections with *S. stercoralis* also noted. From fieldwork in Malawi, zoonotic strongyloidiasis could be implicated in vervet monkeys, a non-human primate species becoming increasingly common in Southern Malawi. From a global perspective, infections with *S. fuelleborni* are often overlooked in terms of health policies and practices, however, it is now high time to reappraise the public health importance of this zoonotic threadworm.

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TOPIC 2: TRANSMISSION DYNAMICS, EPIDEMIOLOGY, AND GLOBAL HEALTH
ONE GENUS, MANY STORIES: THE GENETIC, CLINICAL, AND ECOLOGICAL DIVERSITY OF STRONGYLOIDES
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STRONGYLOIDES STERCORALIS IN HUMANS AND ANIMALS - ONE PATHOGEN OR MULTIPLE?

Adrian Streit
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There are more than 50 described species in the nematode genus *Strongyloides*. They are small-intestinal parasites of various vertebrates other than fish. Many questions around the taxonomy and the host specificity of *Strongyloides* spp. remain open. *S stercoralis* is the causing agent of the vast majority of human strongyloidiasis cases. Although most infections are associated with only minimal clinical symptoms, they may persist undetected for many years and eventually take a deadly turn, in particular in immuno compromised people. More than one hundred years ago, Fülleborn proposed that the *Strongyloides* worms in dogs belong to the same species as *S. stercoralis* in humans, but Brumpt disagreed and erected the species *S. canis*. Ever since, there exists an ongoing dispute if dogs and other animals might serve as reservoirs for zoonotic human strongyloidiasis. Over the last decades, epidemiological, cross-species experimental infection and morphological and molecular taxonomic studies attempted to address this question. I will review these studies and argue that most cases of human *S. stercoralis* infections are probably not zoonotic. However, non-human primates, dogs and, to a lesser extent, cats appear to be suitable hosts for at least some human infective *S. stercoralis*. Therefore, these animals might be the source for some human infections. The relative importance of zoonotic transmission for *S. stercoralis* epidemiology is unclear and likely to vary between different settings. Animals might, for example, serve as reservoir hosts, from which human infection re-starts after control interventions. Therefore, animals should be considered when devising *S. stercoralis* control strategies.

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ONE GENUS, MANY STORIES: THE GENETIC, CLINICAL, AND ECOLOGICAL DIVERSITY OF STRONGYLOIDES
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FRIEND OR FOE? EXPLORING THE GENETIC DIVERSITY OF S. FUELLEBORNI IN AFRICAN PRIMATES

Vladislav Ilík[1], Fréderic Niatou-Singa[2], Nelson Bukamba[3], Gaspard Nzayisenga[3], Václav Audrlický[4], Laurent Dumas[5], Rui Sá[6], Julius Nziza[3], Benard Ssebide[3], Klára Petrželková[1], Barbora Červená[1], Barbora Pafčo[1]
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Background: While *Strongyloides stercoralis* is a well-recognized zoonotic parasite with substantial pathogenic potential, the zoonotic role, transmission dynamics, and pathogenicity of *Strongyloides fuelleborni* remain poorly understood. In this study, we investigated *S. fuelleborni* infections in primates (including humans) and domestic dogs across multiple African localities, including these in the Central African Republic, Guinea Bissau, Uganda or Rwanda. **Methods:** Total DNA was screened for *Strongyloides* spp. using qPCR, and selected positive samples were further characterized using high-throughput sequencing (MGI and Illumina platforms). **Results:** *Strongyloides fuelleborni* predominated in non-human primates but also in humans and dogs. The presence of identical haplotypes across host species indicates ongoing cross-species transmission or parasite sharing among sympatric hosts. The high prevalence of *S. fuelleborni* and the occurrence of specific genetic variants in dogs suggest that dogs may play an important role in parasite dynamic. Notably, *Strongyloides* infection was detected in breastfed puppies only a few weeks old, consistent with direct maternal transmission. In contrast, *Strongyloides* prevalence was low in mountain gorillas and in dogs from Uganda and Rwanda, with no evidence of parasite sharing. Although gorillas were not infected with pathogenic *S. stercoralis*, a severe infection was observed in a gorilla infant, and high-throughput sequencing confirmed exclusive infection with *S. fuelleborni*, supporting its potential pathogenicity in this host. **Conclusions:** Overall, this study provides new insights into the genetic diversity, host range, and transmission dynamics of *S. fuelleborni*, with implications for strongyloidiasis surveillance and control strategies aligned with WHO 2030 targets.

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GLOBAL EPIDEMIOLOGY OF S. FUELLEBORNI AND S. STERCORALIS

Abhishek Mewara
Postgraduate Institute of Medical Education & Research, Medical Parasitology, Chandigarh, India

Human strongyloidiasis, once considered a poorly quantified infection of tropical foci, has now entered a new epidemiological era. S*trongyloides stercoralis* is estimated to infect over 600 million people worldwide, the highest burden concentrated in South-East Asia, Africa, the Western Pacific, and parts of Latin America. Yet, these estimates may be conservative because the prevalence varies with diagnostic method, urban-rural setting, surveillance intensity, and other factors; moreover, the data from endemic regions is sparse. When adequately mapped, the true global burden may plausibly be closer to one billion infections. *S. stercoralis* presents overlapping epidemiological facets of chronic human infection sustained by autoinfection and environmental transmission, and potential One Health infection involving dogs and cats. Molecular studies suggest diverse parasite populations, including human- and dog-associated lineages. Recent serological and hospital-based studies from various regions indicate substantial hidden infection in high-risk groups as well as general population. *Strongyloides fuelleborni* adds a second, less mapped story. Classically associated with Old World non-human primates, it causes human infections in Africa, Southeast Asia, and the Asia-Pacific, including infant disease historically described in Papua New Guinea. Recent molecular work has challenged older assumptions by demonstrating human *S. fuelleborni* infections in Asia-Pacific settings, possible cryptic diversity, and a broad African-Asian phylogeographic split. In Africa, evidence suggests both primate-associated spillover and possibly human-adapted transmission. In Asia, human infections appear more closely linked to primate contact. This talk will synthesize current global epidemiology of both species, highlighting diagnostic blind spots, regional data gaps, zoonotic interfaces, and priorities for integrated surveillance.

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TOPIC 5: DIAGNOSTICS, HOST-PARASITE INTERACTION AND EMERGING TECHNOLOGIES
HOW EXPANSION MICROSCOPY IS UNRAVELING THE HIDDEN BIOLOGY OF PARASITES
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MORPHOGENESIS IN TRYPANOSOMATIDS: HOW PARASITES GET THEIR SHAPE

Christopher De Graffenried, Laura Smithson
Brown University, Molecular Microbiology And Immunology, Providence, United States of America

Trypanosomatids undergo radical morphogenesis during their life cycles to assume shapes best suited to their host niches. Parasite shape is mediated by a sheet of closely packed microtubules, known as the subpellicular microtubule array, that underlies the plasma membrane. In *Trypanosoma brucei*, the causative agent of human African trypanosomiasis, the subpellicular array comprises ~120 microtubules spaced 18 nm apart. During cell division, new microtubules are added to the array and then partitioned to create two new arrays in the daughter cells, all while maintaining cell polarity. The tight spacing and helical twist of the microtubules in the array, along with the size of the parasite, have limited our ability to visualize the array in its entirety because no imaging modality offers sufficient resolution and capture size. Recently, expansion microscopy approaches have been used to resolve structures within parasites that previously could not be visualized without electron microscopy. We have now used ultrastructure expansion microscopy coupled with 3D super-resolution imaging to capture the full cohort of microtubules within subpellicular arrays for the first time, allowing us to better understand array composition and how the structure is inherited during division. We have also mapped the localization of microtubule-binding partners across different regions of the subpellicular array, showing that these proteins have distinct distributions along individual microtubules. Developing these imaging strategies enabled us to identify optimized epitope tags, fluorochromes, and methods for removing particulates from expansion gels, thereby greatly improving our imaging and interpretation.

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EXPANDING VIEWS OF TOXOPLASMA GONDII CELL DIVISION

Klemens Engelberg, Marc-Jan Gubbels
Boston College, Department Of Biology, Chestnut Hill, United States of America

Toxoplasma gondii is an obligate intracellular apicomplexan parasite that currently infects approximately one-third of the world's population. Although infection is typically asymptomatic in immunocompetent individuals, it can cause severe and potentially fatal disease in immunosuppressed patients and congenitally infected children. Clinical manifestations arise during the parasite's acute phase, which is characterized by rapid replication and host-cell lysis. During this lytic cycle, *T. gondii* proliferates through an internal budding mechanism that produces two daughter parasites within the mother cell. The parasite's specialized cytoskeleton, composed of the inner membrane complex (IMC) and subpellicular microtubules (SPMTs), plays essential roles throughout the lytic cycle and provides the scaffold for daughter bud formation. While many key components involved in daughter bud assembly have been identified, the molecular and structural mechanisms underlying bud initiation and the coordinated assembly of the IMC and SPMTs remain poorly understood. To address these questions, we implemented iterative ultrastructure expansion microscopy (pan-ExM), which, in our hands, achieves an approximately 13-fold physical expansion. This approach enables visualization of early budding events by light microscopy at a resolution previously inaccessible using conventional imaging techniques. Here, we present our recent findings on the earliest stages of daughter scaffold organization and examine the role of the γ-tubulin complex in scaffold formation. We further demonstrate how the daughter cytoskeleton contributes to the partitioning of the apicoplast during cell division. Together, these findings provide novel, high-resolution insights into *T. gondii* budding and reveal previously unappreciated aspects of daughter cytoskeleton organization and function.

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UEXM IN GIARDIA: OPPORTUNITIES AND CHALLENGES

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The development of ultrastructure expansion microscopy (UExM) has had a significant impact on the field of microscopy, enabling researchers to resolve complex structural details in biological samples. In recent years, a multitude of UExM-protocols have been published for a wide variety of cells, including the protist parasite *Giardia lamblia*. A distinctive characteristic of the parasite is its reduced subcellular compartment complexity, with only five endomembrane compartments described. One of these organelles is known as the peripheral endocytic compartments (PECs). The PECs mark the host-parasite interface for both endo- and exocytic trafficking and are localized in close proximity to the plasma membrane. The species is heavily dependent on nutrient uptake from the parasitized host through the fusion of PECs with the plasma membrane, therefore rendering them essential for parasite survival. A comprehensive understanding of the precise mechanisms responsible for the functions of PECs in both endo- and exocytic pathways remains uncertain, particularly with regard to the molecular mechanisms involved in membrane fusion events. One of the primary difficulties in providing a response to the given questions is the minimal distance between the plasma membrane and the PECs, which complicates the differentiation between the two compartments. The application of a UExM-protocol optimized for *Giardia* can facilitate the resolution of the issue and enable the distinction between the two structures. In order to capitalize on the numerous potential opportunities of UExM in *Giardia*, it is imperative to overcome the various challenges encountered.

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EXPLORING BIOGENESIS OF THE PLASMODIUM FALCIPARUM K13 RING BY EXPANSION MICROSCOPY

Stuart Ralph[1], Long Huynh[1], Sophie Collier[1], Haowen Deng[1], Niall Geoghegan[2]
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Introduction The primary determinant of artemisinin resistance, the K13 protein, forms a ring at the neck of the *Plasmodium* cytostome. This structure is required for cytostomal feeding in *Plasmodium* falciparum parasites. In artemisinin resistant parasites, K13 protein is less abundant, possibly due to decreased protein stability, and feeding is perturbed. As intra-erythrocytic parasites divide, new K13 rings form to provide merozoites with a K13 ring before egress. K13 rings are ~160nm in diameter - smaller than the diffraction limit of conventional light microscopy, so their biogenesis is challenging to monitor.
Methods To investigate biogenesis of K13 rings we employed expansion microscopy in asexual and sexual stages of P. falciparum. We used machine learning to automate image analysis to quantify K13 rings in artemisinin sensitive and resistant parasites. We also performed 48-hour 4D timecourses of intraerythrocytic forms to monitor the proliferation of K13 puncta.
Results K13 proliferates gradually throughout the asexual life cycle, then accelerating as nuclear division occurs in late stages. Several more K13 rings are produced than the number of daughter merozoites. These segregate so that each daughter parasite receives one K13 ring and several K13 puncta remain behind in the residual body of schizonts. A similar gradual proliferation of K13 rings occurs in gametocytes, but here, K13 rings do not segregate to the dividing microgametes. **Conclusions** K13 rings are segregated to daughter parasites, potentially to facilitate early blood cell feeding by intra-erythrocytic asexual and gametocyte forms. We present generalisable 3d automation tools that allow quantification of sub-cellular structures.

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PARASITIC NTDS IN SUB-SAHARAN AFRICA
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LEISHMANIASIS IN ZAMBIA AND MALAWI

Yasuyuki Goto
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Background and aims
Domestic dogs are the main reservoir of *Leishmania infantum*, a causative agent of visceral leishmaniasis in humans. In Zambia, the burden of leishmaniasis was poorly documented, potentially due to insufficiency of surveillance. Recently, three cases of autochthonous canine leishmaniasis were reported in Zambia intimating probable disease emergence. Therefore, we aimed at understanding the degree of *Leishmania* infection in domestic dogs in Zambia and the neighboring country, Malawi. **Methods**
Blood samples were collected from domestic dogs in Zambia and Malawi during 2022 - 2024. ELISA was performed for detection of antileishmanial antibodies against *L. donovani* soluble lysate antigen (LdSLA) and a recombinant protein rK39r4. For dogs in Malawi, DNA detection in blood was also performed by conventional PCR targeting the small subunit ribosomal RNA (SSU rRNA) gene. **Results**
A high seroprevalence was observed in dogs in both Zambia and Malawi, and seropositivity was up to ~17 % suggesting probable presence of *Leishmania* transmission hot spots in these countries. Moreover, on follow-up surveys of seropositive dogs, presence of antileishmanial antibodies was a risk factor for dog survival. In Malawi, PCR targeting SSU rRNA showed an overall detection rate of 7.9%. **Conclusions**
Our findings implies that *Leishmania* infection can be a health issue in domestic dogs in both Zambia and Malawi. Considering the risk of zoonotic transmission, the need for improved diagnosis and surveillance in both humans, dogs and sand fly vectors is highlighted in these countries.

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PARASITIC NTDS IN SUB-SAHARAN AFRICA
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UNDERSTANDING OF THE EPIDEMIOLOGY OF SLEEPING SICKNESS IN ZAMBIA FROM A ONE-HEALTH PERSPECTIVE

Kyoko Hayashida[1], Boniface Namangala[2], Lavel Moonga[2], Alex Kiarie[1], Tatsuki Sugi[1], Naoko Kawai[1], Elisha Chatanga[3], Janelisa Musaya[4], Mable Mutengo[5], Chihiro Sugimoto[1], Junya Yamagishi[1]
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Human and animal trypanosomiasis poses a serious burden in sub-Saharan Africa, threatening human and animal lives and hindering rural economic growth. In Zambia, diverse Trypanosoma species circulate within the Luangwa Valley and the Greater Kafue ecosystems, and the zoonotic *Trypanosoma brucei rhodesiense* continues to cause sporadic cases of human African trypanosomiasis (HAT). Controlling this disease requires a comprehensive One-Health perspective. To understand the current epidemiology, we collected tsetse flies and cattle blood from active and historical HAT-endemic foci in Zambia. Characterizing the trypanosome species composition using targeted NGS amplicon sequencing revealed an extremely rich genetic diversity. This highlights the highly complex nature of the parasite gene population within the area. We further investigated transmission dynamics by assessing tsetse fly host preferences by examining blood-meal sources using 12S rRNA metabarcoding. Alongside typical preferred hosts like Suidae and Bovidae, we identified unexpected opportunistic feeding sources, including domestic and fruit bats. This broader host preference implicates these atypical animals as potential disease reservoirs requiring further investigation. Finally, we developed a molecular tool to enable early diagnosis, which is critical for effective treatment. We modified the loop-mediated isothermal amplification (LAMP) assay for on-site HAT diagnosis in resource-limited settings. Reagents were dried and stabilized in a single tube using trehalose, thereby achieving an ambient shelf life of at least 6 months. Additionally, sample preparation was simplified, allowing direct amplification from detergent-lysed blood. Our developed LAMP diagnostic technique will contribute to improved diagnosis and a better understanding of the epidemiology of HAT in resource-limited endemic areas.

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PARASITIC NTDS IN SUB-SAHARAN AFRICA
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HYBRIDISATION IN UROGENITAL SCHISTOSOMIASIS IN MALAWI

Janelisa Musaya[1,2]
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Hybridisation between human and animal schistosomes is increasingly recognised across sub-Saharan Africa, yet remains largely invisible to control programmes. In Malawi, the Hybridisation in Urogenital Schistosomiasis (HUGS) project investigated its epidemiological and evolutionary significance in endemic settings. A cross-sectional study of 2,400 individuals in Mangochi and Nsanje integrated sampling from humans, livestock, snails and shared water bodies, with molecular diagnostics. Hybrid infections were identified in 7% of samples, predominantly involving Schistosoma haematobium × Schistosoma mattheei, demonstrating active multi-host transmission. These findings challenge the paradigm of urogenital schistosomiasis as a strictly human disease and reveal hybridisation as a potential mechanism of parasite adaptation under control pressure. By introducing zoonotic reservoirs and obscuring detection, hybrid schistosomes may sustain transmission and undermine elimination strategies, underscoring the urgent need for integrated molecular surveillance and One Health-informed control.

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ASIA-PACIFIC ASSOCIATION OF VETERINARY PARASITOLOGY: COLLABORATIVE EFFORTS TO ADDRESS MAJOR SOCIOECONOMICALLY IMPORTANT PARASITES IN THE REGION
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ADVANCING ONE HEALTH AND IMPROVING THE CONTROL OF SOCIO-ECONOMICALLY IMPORTANT PARASITES IN THE ASIA-PACIFIC

Reuben Sharma
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Across the Asia-Pacific region, many parasites and vector-borne pathogens continue to cause significant economic losses, reduced productivity, food insecurity, and pose a serious threat to public health. This comes in tandem with the changing dynamics of zoonotic parasite transmission due to climate change, environmental degradation, increased international trade, rapid urbanization, unsustainable livestock farming practices, and anthropogenic alterations to the landscape. The Asia Pacific Association of Veterinary Parasitology (APAVP) was established to play a pivotal role in advancing regional capacity and cooperation in veterinary parasitology and One Health. APAVP focuses on addressing the current challenges in surveillance, treatment, and control of parasitic diseases in the region by promoting excellence in research and education; facilitating the exchange of scientific knowledge and technological innovations; encouraging collaboration between veterinarians, public health professionals, conservationist, industry partners, and policymakers in the One Health framework; supporting the development of effective, sustainable, evidence-based parasite prevention and control strategies; and enhancing regional preparedness for emerging and re-emerging parasitic diseases. APAVP also seeks to enhance regional capacity, foster mentorship, and provide professional development opportunities through conferences, workshops, collaborative research networks, and outreach activities. It is envisaged that APAVP will contribute significantly towards reducing the burden of socio-economically important parasites, improving animal and human health, strengthening food systems, and promoting sustainable development across the diverse countries of the Asia-Pacific region.

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STRENGTHENING VETERINARY PARASITOLOGY EDUCATION TO ENHANCE KNOWLEDGE ON PARASITES IN THE ASIA-PACIFIC

Veterinary parasitology remains a core discipline in veterinary education as parasitic diseases affect animal health, productivity, public health, food security, animal welfare, and trade. In the Asia-Pacific region, the importance of veterinary parasitology is intensified by high biodiversity, diverse livestock production systems, wildlife-livestock interfaces, varying climates, uneven access to diagnostic infrastructure, and the continued importance of zoonotic and vector-borne parasitic diseases. However, veterinary education is changing. Curricula are increasingly compressed, students face heavy workloads, and veterinary graduates are expected to handle not only classical parasite identification but also diagnosis, treatment planning, herd-level control, surveillance, risk communication, antimicrobial and antiparasitic stewardship, one health collaboration, and emerging disease threats. This analysis was conducted to identify how veterinary parasitology is currently taught, how much emphasis is given to different subject areas, what teaching and assessment methods are used, what constraints are reported, and what improvements are recommended by respondents across the Asia-Pacific region. The analysis used a merged survey dataset consisting of 67 Google Forms responses and 18 Zoho responses. The two response sources were aligned using common survey variables, including country, respondent role, teaching experience, contact hours, topic coverage, teaching structure, teaching methods, resource adequacy, assessment methods, graduate preparedness, challenges, suggested improvements, and additional comments. Veterinary parasitology remains a well-established component of veterinary curricula among the responding Asia-Pacific institutions.

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IS277

TOPIC 1: PARASITE ECOLOGY, BIODIVERSITY, AND TAXONOMY
ASIA-PACIFIC ASSOCIATION OF VETERINARY PARASITOLOGY: COLLABORATIVE EFFORTS TO ADDRESS MAJOR SOCIOECONOMICALLY IMPORTANT PARASITES IN THE REGION
08-21-2026 8:00 AM - 10:00 AM

ADVANCED TECHNOLOGIES FOR THE CONTROL OF ENDEMIC AND EMERGING INTESTINAL PARASITES IN THE ASIA-PACIFIC

Vito Colella
The University of Melbourne, Melbourne Veterinary School, Melbourne, Australia

Background and aims Parasitic nematodes have substantial impacts on human and animal health, causing considerable morbidity and production losses that perpetuate cycles of poverty. This study aimed to develop and apply long-read nanopore metabarcoding approaches for the simultaneous characterisation of parasitic nematode communities across human and animal hosts. **Methods** We used a comparative molecular-target assessment to identify suitable barcoding regions for an assay capable of detecting a broad range of parasitic nematodes. We subsequently applied nanopore metabarcoding to investigate the diversity and transmission dynamics of intestinal nematodes among children and adults, dogs, and livestock in endemic areas of the Asia-Pacific region. **Results** The novel nemabiome assay detected a greater diversity of parasitic nematodes than conventional molecular approaches. Sequencing of mitochondrial and nuclear target regions enabled the characterisation of soil-transmitted helminth populations across human and animal hosts in Cambodia, Vietnam, and Vanuatu. Comparative sequence analyses identified settings in which transmission appeared to be predominantly human-to-human, as well as settings showing strong evidence of zoonotic transmission from animal reservoirs to humans. **Conclusions** Our nemabiome assay provides a comprehensive approach for characterising parasitic nematode communities affecting human and animal health. The combined nanopore sequencing strategy can uncover previously undetected parasites, including zoonotic and cryptic species, while helping to resolve inter-host transmission pathways and potential animal reservoirs. By enabling the simultaneous identification of multiple infections and generating high-resolution epidemiological data, this approach can support the design of more targeted and effective control interventions against (re)-emerging parasitic threats across the rapidly changing Asia-Pacific region.

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TOPIC 1: PARASITE ECOLOGY, BIODIVERSITY, AND TAXONOMY
ASIA-PACIFIC ASSOCIATION OF VETERINARY PARASITOLOGY: COLLABORATIVE EFFORTS TO ADDRESS MAJOR SOCIOECONOMICALLY IMPORTANT PARASITES IN THE REGION
08-21-2026 8:00 AM - 10:00 AM

UNDERSTANDING TICKS AND TICK-BORNE DISEASES IN A CHANGING ASIA-PACIFIC

Frederic Beugnet
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With a human population exceeding 695 million in 2024 across 11 countries, Southeast Asia (SEA) is one of the most densely inhabited regions globally. With its unique mix of landscapes, ranging from dense rainforests and mountainous zones to dynamic peri-urban and agricultural landscapes, SEA presents an exceptional diversity of tick species and host–vector–pathogen networks. At least 97 tick species have been recorded, and many are recognized as vectors of pathogens (TBPs). The identification of pathogens has increased in the past years, it is most probably due to the increased capacities of diagnostic, and due to the high biodiversity, rapid urbanization, climate variability, and human-animal-environment interactions. Most data being generated from Thailand and Malaysia, more data are needed from other countries. Progress in TBP detection confirmed the circulation of bacteria, i.e. *Anaplasma*, *Borrelia*, *Coxiella*, *Ehrlichia*, and *Rickettsia*, and of protozoans, i.e. *Babesia Hepatozoon*, and *Theileria*. Some pathogens (e.g. *Theileria orientalis)* impact livestock economies, while others (e.g. *Babesia vogeli*, *B. gibsoni*, *Hepatozoon canis*, *Ehrlichia canis*, and *Anaplasma platys*) impact companion animals. Canine vector-borne pathogens are increasingly prevalent in SEA, particularly among free-roaming dog populations, which serve as key reservoirs for transmission to companion animals and potentially to humans. Regarding human health, the expansion of the Bandavirus dabieense, agent of the Severe Fever with Thrombocytopenia Syndrome, is now confirmed in SEA with reports from Cambodia, Thailand and East Malaysia. The increased prevalence of pathogens and epidemiological changes in ticks and TBPs in SEA highlight the need for a multidisciplinary approach and regional surveillance programs.

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TOPIC 5: DIAGNOSTICS, HOST-PARASITE INTERACTION AND EMERGING TECHNOLOGIES
HUMAN LICE: BIOLOGY, EPIDEMIOLOGY, CLINICAL IMPACT, AND CONTROL
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HUMAN HEAD LICE IN ARCHAEOLOGICAL REMAINS

Kosta Mumcuoglu
The Hebrew University, Microbiology And Molecular Genetics, Jerusalem, Israel

In archaeological contexts, head lice have been documented in mummies, human remains, and louse combs. The aim of this presentation is to summarize our work on this subject and present unpublished findings.

Organic and inorganic debris trapped between the louse teeth were removed using a needle and subsequently examined under a stereomicroscope.

Six out of seven hair samples collected from mummies from Camarones, Chile (4000 BC) were positive for head louse eggs and/or nits. The oldest nit specimens reported to date derive from 10,000-year-old human hairs recovered in Brazil, while 9,000-year-old nits were identified in a hair sample obtained from an individual who lived in the Nahal Hemar Cave, Israel. In Israel, head lice and/or their eggs or nits have been recorded in louse combs recovered from archaeological excavations at Masada, Qumran, Wadi Farah, Nahal Ze'elim, Ein Rachel, the Ein Gedi Oasis, and Wadi Kedron.

The frequent archaeological association of lice with specialized louse combs clearly demonstrates intentional parasite control strategies, reflecting cultural awareness of pediculosis and routine delousing practices across historical periods. Comb morphology, raw material selection, and wear traces further enhance our understanding of technological responses to ectoparasitism in daily life. Moreover, ancient DNA analyses of lice and their eggs enable reconstruction of louse population structure, genetic diversity, and evolutionary history. Such data provide independent proxies for human mobility, population contact, and migration, given the close co-evolutionary relationship between lice and their human hosts.

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TOPIC 5: DIAGNOSTICS, HOST-PARASITE INTERACTION AND EMERGING TECHNOLOGIES
HUMAN LICE: BIOLOGY, EPIDEMIOLOGY, CLINICAL IMPACT, AND CONTROL
08-21-2026 8:00 AM - 10:00 AM

MICROBIOTA OF THE HUMAN HEAD LOUSE, PEDICULUS HUMANUS CAPITIS, AND THE HUMAN BODY LOUSE, PEDICULUS HUMANUS HUMANUS (ANOPLURA: PEDICULIDAE)

Aysegul Taylan-Ozkan[1], Kosta Mumcuoglu[1,2]
[1]Cyprus International University, Faculty of Medicine, Medical Microbiology, Nicosia, Cyprus, [2]The Hebrew University, Microbiology And Molecular Genetics, Jerusalem, Israel

Head lice (Pediculus humanus capitis) and body lice (Pediculus humanus corporis) are common ectoparasites of humans that inhabit distinct ecological niches, despite sharing similar morphological characteristics. These differences are expected to be reflected in their microbiota profiles. In this study, we aimed to comparatively evaluate the bacterial microbiota of head lice and body lice.

Head lice and body lice were collected from Israel and identified to the species level by morphological methods. Samples were washed to reduce external contamination, then total genomic DNA was isolated. For bacterial microbiota analysis, target regions of the 16S rRNA gene were amplified, and next-generation sequencing was applied. Sequencing data were quality controlled, operational taxonomic units were created, and taxonomic assignments were made at the genus level. Microbiota compositions were evaluated based on relative abundances.

The microbiota of body lice exhibited a more structured profile, dominated by specific bacterial genera, including Enterobacter and Acinetobacter. In contrast, the head louse microbiota showed a fragmented, heterogeneous structure, with a high proportion of low-abundance bacterial genera. There was no clear dominant genus in head lice, whereas the relative abundance of certain bacteria was higher in body lice.

The findings suggest that the microbiota of head lice and body lice are structurally distinct. This difference is thought to be related to the habitats and ecological characteristics of the two lice species.

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TOPIC 5: DIAGNOSTICS, HOST-PARASITE INTERACTION AND EMERGING TECHNOLOGIES
HUMAN LICE: BIOLOGY, EPIDEMIOLOGY, CLINICAL IMPACT, AND CONTROL
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FACING THE TRUTH: LICE ARE BECOMING INCREASINGLY RESISTANT TO PEDICULICIDES

Ariel Toloza[1], Kosta Mumcuoglu[2]
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The human head louse, Pediculus humanus capitis De Geer (Phthiraptera: Pediculidae), is an obligate ectoparasite responsible for pediculosis capitis, a condition that has affected humans since ancient times. This infestation is widespread worldwide, particularly among school-aged children, regardless of socioeconomic or hygienic background. Common treatments include topical formulations with pyrethroids, organophosphates, silicones, and ivermectin. However, the intensive and continuous use of these insecticides, especially permethrin, has led to resistance development, resulting in increased infestations and control failures across many countries.

This review discusses the occurrence, resistance mechanisms, and consequences of resistance in regions such as Africa, Asia, Oceania, Europe, and the Americas.

In this study, we examined a global population of head lice to detect the presence of genetic markers of resistance and the population genetic signatures of undergoing strong selection pressure exerted by the use of pediculicides by their host.

Currently, it is estimated that up to three-quarters of head lice globally are resistant to permethrin, a trend that has risen sharply over the past two decades. A reduction in the genetic diversity within louse individuals that possess the kdr mutations was detected. Additionally, new genetic evidence regarding the permethrin target—the voltage-sensitive sodium channel—has revealed a unique origin for kdr-type mutations.

For the establishment of an adequate resistance management program, it is imperative to detect early levels of insecticide resistance, promote preventive measures to avoid its spread, and inform public health authorities to treat pediculosis.

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TOPIC 5: DIAGNOSTICS, HOST-PARASITE INTERACTION AND EMERGING TECHNOLOGIES
HUMAN LICE: BIOLOGY, EPIDEMIOLOGY, CLINICAL IMPACT, AND CONTROL
08-21-2026 8:00 AM - 10:00 AM

UPDATES ON GLOBAL PREVALENCE OF HEAD LOUSE PEDICULOSIS - SYSTEMATIC ANALYSIS

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Background and Aims. Head louse pediculosis (HLP) due to infestation with *Pediculosis humanus capitis* is a global problem impacting economic development. In North America, UK and Europe head lice are the most common human parasitic agent, while in other regions they coexist with other ectoparasites. Our chief objective was to evaluate the worldwide prevalence of HLP in the 21st century with particular attention to epidemic and pre and post COVID-19 periods through systematic review of articles published in English.
Methods. One hundred and thirty articles were retrieved predominantly focused on HLP in school-aged children, although its impact on other populations was also examined.
Results. Prior to 2020 the highest HLP prevalence was reported from South and Central America (up to 61.4%), and lowest was from Europe (0.48%). Recently, circulation of pediculicide resistant lice was identified as a growing public health concern. COVID-19 lockdown resulted in a huge drop in infestations-estimated up to 66% to 72% based on school screening and corroborated by 40-44% decrease of pediculicide sales and 60.6% decline in clinical diagnosis in 2020. However, household crowding sustained circulation of lice and diagnosed cases were more severe due to delays in seeking professional help. Immediate post COVID prevalence of HLP varied by region but current trends indicate variable return to pre-pandemic levels.
Conclusions. In conclusion, further understanding region-specific social and biological variables that define the occurrence and circulation of head lice is critical to maintaining effective public health programs which target control of this parasitosis.

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KEYNOTE
FROM NEGLECT TO SHARED GLOBAL RESPONSIBILITY: INNOVATION, PARTNERSHIP, AND HOPE FOR THE NEXT ERA OF PARASITIC DISEASE CONTROL
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FROM NEGLECT TO SHARED GLOBAL RESPONSIBILITY: INNOVATION, PARTNERSHIP, AND HOPE FOR THE NEXT ERA OF PARASITIC DISEASE CONTROL

Osamu Kunii
Global Health Innovative Technology (GHIT) Fund, Tokyo, Japan

The GHIT Fund, a pioneering public-private partnership from Japan, drives R&D for malaria, tuberculosis, and neglected tropical diseases (NTDs). Amidst shrinking global health budgets, shifting from neglect to shared global responsibility is essential to combat parasitic diseases. To accelerate R&D and ensure equitable Access and Delivery (A&D) of life-saving medical tools, we must harness multi-dimensional innovations. This requires various innovations, especially cutting-edge digital technologies and AI, not only for R&D but also for A&D, including supply chain and financial innovations, such as pooled procurement and innovative financing models. Furthermore, sustainable progress depends on strengthening robust, diverse collaborations. We must expand partnerships beyond traditional actors to include startups, civil society, and affected communities. Crucially, fostering synergistic, equitable partnerships between the Global North and the Global South is imperative. While the global community has set ambitious targets like the "100-Day Mission" for pandemic preparedness, we must be equally bold in addressing endemic parasitic diseases. We propose a strategic, innovative, and synergistic "100-Month Plan" to enhance partnerships, maximize synergy, and bring lasting hope to the next era of global health.

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Oral Presentation Abstracts

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OP001

PARALLEL ORAL ABSTRACT SESSION
PARALLEL ORAL ABSTRACT 01: PARASITE DIVERSITY AND ECOLOGY IN WILDLIFE:FROM MOLECULAR TAXONOMY TO CONSERVATION IMPLICATIONS
08-17-2026 4:40 PM - 6:40 PM

GASTROINTESTINAL PARASITES OF THE WILD UNGULATES (MAMMALIA: CETARTIODACTYLA) IN THE HEMIS NATIONAL PARK, LADAKH, INDIA

Jigmet Zazay
Government Model Degree College Zanskar, Zoology, Kharshah, India

Background and Aims: Gastrointestinal (GI) parasites strongly affect the health and survival of wild ungulates, yet information from Hemis National Park (HNP), Ladakh, is scarce; therefore, this study aimed to assess the prevalence, diversity, seasonal variation, and intensity of GI parasites in wild ungulates of HNP.

Methods: A total of 327 faecal samples were collected from blue sheep (n = 127), Ladakh urial (n = 110), and Himalayan ibex (n = 90) between June 2021 and May 2022. Parasites were identified using standard flotation and sedimentation techniques, infection intensity was estimated using the McMaster egg-count method, and seasonal and interspecific differences were analysed using chi-square and Kruskal–Wallis tests.

Results: Out of 327 faecal samples examined, 165 were infected with seven different parasite genera, including one protozoan species, five nematodes, and one cestode. The overall prevalence of gastrointestinal (GI) parasites was 50.45%. *Eimeria* showed the highest prevalence across all three wild ungulate hosts, with an overall prevalence of 18.65%. Nematodes were more prevalent (16.20%) than cestodes (4.58%). Blue sheep exhibited the highest overall prevalence (55.11%), followed by Ladakh urial (49.09%) and Himalayan ibex (45.55%). Mixed infections were detected in 36 samples (11%). Parasite prevalence was highest during summer (64.42%), followed by autumn (51.13%), spring (44.77%), and winter (33.82%).https://doi.org/10.1007/s12639-024-01652-8

Conclusions: Although parasite intensity was low (<500 EPG), increasing livestock density may elevate transmission risks. Higher prevalence in blue sheep highlights the role of habitat sharing with livestock. Regulated grazing, periodic anthelmintic treatment, and habitat management are recommended to minimize parasitic risks to wild ungulate populations.

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OP002

PARALLEL ORAL ABSTRACT SESSION
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08-17-2026 4:40 PM - 6:40 PM

CHROMOSOME-CONTIGUOUS NUCLEAR GENOME OF THE ZOONOTIC FILARIAL PARASITE DIROFILARIA ASIATICA (SPIRURIDA: ONCHOCERCIDAE)

Neil Young[1], Vito Colella[2], Yuanting Zheng[1], Anson Koehler[1], Tao Wang[3], Sunita Sumanam[1], Ushani Atapattu[1], Bill Chang[1], Robin Gasser[3]
[1]The University of Melbourne, Melbourne Veterinary School, Melbourne, Australia, [2]The University of Melbourne, Melbourne, Australia, [3]Melbourne University, Veterinary Biosciences, Parkville, Australia

Background and Aims: Parasitic nematodes of the family Onchocercidae have evolved alongside vertebrate hosts for millions of years. While morphological methods have played a central role in identifying species, many taxa remain cryptic and are challenging to differentiate, complicating diagnosis. Among the clinically relevant genera are *Dirofilaria*, responsible for illnesses such as heartworm disease in canines and rare zoonotic infections in humans. Substantial gaps remain regarding other Dirofilaria spp. and genotypes, and their impact on both animal and human health. The aim of this study was to provide a comprehensive morphological and molecular resource for a new species of *Dirofilaria*.

Methods: Long-read PacBio and short-read Hi-C (high-throughput chromosome conformation capture) sequence data were used to characterise the mitochondrial (mt) and nuclear genomes. Comparative genomic analysis was then conducted to explore the specialised immunomodulatory proteins inferred to play a role in host adaption.

Results: The nuclear genome of *D. asiatica* comprises four autosomes and one sex-linked scaffold and encodes 9,658 genes. Comparative genomic analyses with *Dirofilaria immitis*, *Brugia malayi* and *Onchocerca volvulus* revealed both conserved chromosomal synteny and lineage-specific rearrangements. Predicted excretory/secretory (ES) proteins (n=881), were enriched for immune-relevant pathways, including proteolysis, lysosomal activity and antigen presentation. Notably, 229 (~26%) of these ES proteins were unique to *D. asiatica*, many of which are implicated in host–parasite interactions, immune evasion and metabolic adaptation in the mammalian host.

Conclusions: The genome of *D. asiatica* fills a significant gap in filarial genomics and provides a resource for studies into host adaptation, molecular epidemiology, and the development of improved diagnostics.

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OP003

PARALLEL ORAL ABSTRACT SESSION
PARALLEL ORAL ABSTRACT 01: PARASITE DIVERSITY AND ECOLOGY IN WILDLIFE:FROM MOLECULAR TAXONOMY TO CONSERVATION IMPLICATIONS
08-17-2026 4:40 PM - 6:40 PM

OCCURRENCE OF TOXOPLASMA GONDII AND OTHER APICOMPLEXAN PARASITES IN MUSTELIDS FROM DENMARK

Rebecca Berg[1], Rosalina Molberg Rotovnik[2], Christen Rune Stensvold[1], Morten Elmeros[3], Pikka Jokelainen[4], Henrik Vedel Nielsen[1]
[1]Statens Serum Institut, Laboratory Of Parasitology, Copenhagen S, Denmark, [2]University of Copenhagen, Veterinary And Animal Sciences, Parasitology And Pathobiology, Frederiksberg, Denmark, [3]University of Aarhus, Ecoscience, Aarhus, Denmark, [4]Statens Serum Institut, One Health And International Collaborations, Copenhagen, Denmark

Background and Aims: Denmark is home to seven native species of mustelids (family Mustelidae). Phylum Apicomplexa comprises multiple parasitic genera, with some causing disease in mustelids and some having zoonotic potential. This study aims to screen faecal, heart, and serum samples, collected post-mortem from mustelids, for selected Apicomplexa-specific DNA and antibodies against *Toxoplasma gondii*.

Methods: Blood samples were analysed for antibodies against *T. gondii* using a commercial ELISA. Heart and faecal samples were subjected to DNA extraction. All heart-DNAs were analysed by real-time PCR for *T. gondii* DNA. The faecal- and heart-DNAs are being analysed by metabarcoding targeting the 18S rRNA gene using Illumina sequencing, and additional confirmatory methods as needed to identify the parasites to species level.

Results: Antibodies against *T. gondii* were detected in 122/172 of the blood samples. *Toxoplasma gondii* DNA was detected in 48/201 heart samples. Positive cases were found in all regions in Denmark and in all mustelid species. Until now, 22/203 faecal DNAs and 23/201 heart DNAs have been analysed by metabarcoding. Preliminary results showed *Sarcocystis* in almost one third of faecal and heart samples, while *Babesia, Cryptosporidium, Cystoisospora, Eimeria,* and *Hepatozoon* were detected in at least one sample each.

Conclusions: Our findings support the role of mustelids as wildlife hosts of *T. gondii* in Denmark. Furthermore, while differences in tissue predilection may have limited detection of some apicomplexan species, our results suggest that the mustelids may serve as hosts or carriers of other apicomplexan parasites, some of which have potential relevance for zoonotic transmission.

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OP004

PARALLEL ORAL ABSTRACT SESSION
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SMALL BUT MIGHTY: PARASITES SHAPE HOST MIGRATORY BEHAVIOUR

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Background and Aims: Parasites can not only drive changes in individual host movement at a local scale, but also are increasingly recognized for their role in larger-scale patterns of host movement like migration. However, studying infection dynamics in migrating animals empirically is challenging. Luckily we can use theory to derive testable predictions for how infection and migratory behaviours might interact, and new technologies to test these predictions in natural systems.

Methods: We develop mathematical models to predict how parasites drive different types of host movement. Our models are guided by conceptual frameworks including migratory culling (infected individuals are more likely to die during migration), migratory escape (migrating animals escape further infection), and migratory recovery (migrating animals recover from infection).

Results: By considering multiple frameworks, we generate patterns that parallel those seen in empirical systems. Namely that the presence of parasites can favor hosts to have full migration (all individuals migrate), partial migration (only some individuals migrate), and differential migration (individuals migrate different distances). We also find that either migrants or non-migrants can have higher infection than the other, and that infection intensity can vary within migratory animals by the distance traveled.

Conclusions: Theory is currently outpacing empirical results. However, technological advances (e.g. drones, biologgers, molecular techniques) make studying large-scale animal movement and infection together more tractable than ever. We encourage researchers interested in wildlife disease to think about the impacts of infection on host behaviour beyond daily movement to better allow us to predict how these critical ecological processes will change in the future.

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OP005

PARALLEL ORAL ABSTRACT SESSION
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08-17-2026 4:40 PM - 6:40 PM

MOLECULAR TAXONOMIC CHARACTERISATION OF ANCYLOSTOMA SPP. AND STRONGYLOIDES SPP. IN THREE PROTECTED AREAS IN GHANA.

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[1]Sunyani Technical University, Pharmaceutical Sciences, Sunyani, Ghana, [2]Max Planck Institute for Biology, Tuebingen, Germany, [3]Kwame Nkrumah University of Science and Technology, Biology, Kumasi, Ghana

Background and Aims: Soil-transmitted helminths (STHs) continue to affect millions of people worldwide, but the role of animals in sustaining transmission remains poorly understood. This study examined the diversity and zoonotic potential of hookworms and *Strongyloides* across humans, dogs, and nonhuman primates in the three different study areas located in three ecological zones in Ghana, where humans live in close contact with domestic dogs and free-ranging nonhuman primates, creating opportunities for cross-species parasite exchange

Methods: Stool samples from humans, dogs, and nonhuman primates were analysed using the Baermann technique after faecal culture. Single larvae and young adult worms were genotyped at the nuclear small subunit, Hypervariable Regions (HVR) I and IV, ITS-2 and the mitochondrial *cox*-1 markers. Species identities and genetic relationships were explored using Neighbour-joining and Maximum-likelihood phylogenies in MEGA 12 and a Median-joining haplotype network in POpART.

Results: We detected several hookworm species, including *Ancylostoma braziliense, A. caninum, A. ceylanicum,* and *A. tubaeforme*. Several haplotypes were shared between humans and dogs, indicating active zoonotic transmission. An unexpected finding was the recovery of *A. braziliense* larvae from cultured human stool, indicating that its ability to infect humans may be broader than previously recognised. For *Strongyloides*, humans carried *S. stercoralis*, monkeys carried *S. fuelleborni*, and in dogs, we found a *Strongyloides* that appeared closely related to *S. papillosus*.

Conclusions: Our findings reveal substantial hookworm diversity and strong evidence of zoonotic transmission at the human-dog interface and reinforce the need for One Health approaches in STH studies in communities where humans and animals live in close proximity.

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OP006

PARALLEL ORAL ABSTRACT SESSION
PARALLEL ORAL ABSTRACT 01: PARASITE DIVERSITY AND ECOLOGY IN WILDLIFE:FROM MOLECULAR TAXONOMY TO CONSERVATION IMPLICATIONS
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CIRCULATION OF TOXOPLASMA GONDII IN THE GALAPAGOS ARCHIPELAGO

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Background and Aims: *Toxoplasma gondii* is a zoonotic protozoan parasite that represents a major concern for wildlife conservation due to its ability to infect virtually all homeotherms and cause severe disease in naïve species. In the Galapagos archipelago, introduced domestic cats are the only definitive hosts of *T. gondii*, yet the pathways linking terrestrial contamination to exposure of marine species remain poorly understood. This study aimed to integrate epidemiological and environmental evidence to describe *T. gondii* circulation in the Galapagos.

Methods: Three complementary studies were conducted. First, exposure was assessed in Galapagos land birds and seabirds through serology, with species grouped by trophic preferences. Second, coastal water contamination was evaluated using wild bivalves as sentinels, detecting *T. gondii* DNA and analyzing associations with environmental factors. Third, exposure and infection in Galapagos sea lions (*Zalophus wollebaeki*) were investigated using serology, molecular detection, and histopathology.

Results: Both land birds and seabirds were seropositive to *T. gondii*, including species on cat-free islands. Prevalence differed by diet and was highest in occasional carnivores. *Toxoplasma gondii* DNA was detected in oysters from all sampled islands, including sites located up to 20 km from cat populations, indicating the dispersal of oocysts via marine currents. In sea lions, seropositivity was detected in individuals from breeding colonies, and molecular and histological findings were consistent with infection.

Conclusions: These findings demonstrate the circulation of *T. gondii* across terrestrial and marine environments in the Galapagos and identify coastal waters as a critical interface for parasite dissemination and exposure of wildlife.

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OP007

PARALLEL ORAL ABSTRACT SESSION
PARALLEL ORAL ABSTRACT 01: PARASITE DIVERSITY AND ECOLOGY IN WILDLIFE:FROM MOLECULAR TAXONOMY TO CONSERVATION IMPLICATIONS
08-17-2026 4:40 PM - 6:40 PM

TOXOPLASMA GONDII IN WILDLIFE IN THE ARCTIC - WHO NEEDS CATS ANYWAY?

Emily Jenkins[1], Adrian Hernandez Ortiz[2], Emilie Bouchard[3]
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Background and Aims: *Toxoplasma gondii* is the most successful parasite in the world, infecting most vertebrates as intermediate hosts. This coccidian undergoes sexual reproduction in the intestines of felids, the only known definitive hosts. Despite the general absence of felids in the Canadian Arctic, human residents in some regions show levels of exposure to *T. gondii* 2-4 times higher than the general population. We explore multiple hypotheses about the sources and transmission of *T. gondii* in the Arctic.

Methods: We use validated molecular and serological methods for detection of *T. gondii* in samples from harvested Arctic wildlife.

Results: 1) Canadian lynx in boreal regions are a potential source of environmental contamination with *T. gondii*; 2) migratory geese seasonally introduce *T. gondii* acquired in their overwintering habitat into the Canadian Arctic; 3) terrestrial carnivores (foxes) are good sentinels for *T. gondii*, with higher exposure in the eastern vs western Canada Arctic, paralleling observed trends in human seroprevalence; 4) foxes in the Canadian Arctic consuming migratory geese and aquatic prey have higher exposure to *T. gondii* ; and 5) polar bears in the western Canadian Arctic are increasingly exposed to *T. gondii* concomitant with warmer and wetter summers.

Conclusions: This work suggests that aquatic transmission of oocysts from felids in the south (“parasite pollution”), south to north transport of tissue cysts in migratory wildlife. and trophic transmission through arctic food webs drive high wildlife and human exposure to *T. gondii* in regions of the Arctic undergoing unprecedented environmental change and increased global connectivity and importance.

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OP008

PARALLEL ORAL ABSTRACT SESSION
PARALLEL ORAL ABSTRACT 01: PARASITE DIVERSITY AND ECOLOGY IN WILDLIFE:FROM MOLECULAR TAXONOMY TO CONSERVATION IMPLICATIONS
08-17-2026 4:40 PM - 6:40 PM

NOVEL MANSONELLA OZZARDI-LIKE NEMATODE FOUND IN BLACK BEARS AND RACCOONS OF EAST TENNESSEE

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Background and Aims: *Mansonella ozzardi*-like nematodes have been reported sporadically in black bears in Japan and the United States (U.S.), and, most recently, have been reported in raccoons in the U.S. *Mansonella ozzardi* is a human filariid that can cause mansonelliasis in humans. Little is known about the *M. ozzardi*-like parasite that infects bears and raccoons, including its range and prevalence in different areas of the U.S.

Methods: We analyzed blood samples from 91 American black bears (Ursus americanus) and 47 raccoons (Procyon lotor) in eastern Tennessee from 2022 to 2025. A modified Knott's test was performed on all samples.Conventional PCR using filarial ITS F (5'- TTCCGTAGGTGAACCTGC-3') and filarial ITS R (5'-ATATGCTTAAGTTCAGCGGG-3') primers was performed on a select number of positive samples.

Results: Microfilaria was found on a total of 63 (63/91, 69%) bears and 15 (15/47, 32%) raccoons. Microfilaria sizes ranged from 175 to 300µm long and 2.5 to 6.25µm wide, with most being between 225 and 275µm long and 2.5 to 5µm wide. PCR samples sent to sequencing came back as 85.6% to 92.6% similar to Mansonella ozzardi.

Conclusions: These results demonstrate the relatively high prevalence of this parasite in bear and raccoon populations of this region. Given this prevalence, as well as the many other knowledge gaps surrounding this parasite including its life cycle, anatomical location of the adult nematodes, how the parasites affect bears and raccoons, and its zoonotic potential, further investigation into this novel filarial nematode is warranted.

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PARALLEL ORAL ABSTRACT 02 : INNOVATIONS IN SCHISTOSOMIASIS CONTROL: VACCINES, TRANSMISSION DYNAMICS, AND ZOONOTIC INTERFACES
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COMBATING SCHISTOSOMIASIS JAPONICA THROUGH TRANSMISSION BLOCKING VACCINES

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Background and Aims: *Schistosoma japonicum*, the Asian schistosome, is a zoonotic parasite that infects humans and more than 40 species of domestic animals. It is a major causative agent of schistosomiasis, a neglected tropical disease affecting approximately 240 million people globally. In endemic areas, bovines contribute 75–90% of environmental egg contamination, making them key reservoirs sustaining transmission. Current control strategies rely predominantly on mass drug administration (MDA) with Praziquantel, which, while effective at reducing worm burdens, does not prevent reinfection and may contribute to drug resistance if used as a sole intervention. These limitations underscore the need for integrated and sustainable control approaches. Mathematical modelling suggests that combining chemotherapy with an effective transmission-blocking vaccine (TBV) could achieve elimination of *S. japonicum* within a decade. This study aims to evaluate the immunogenicity and protective efficacy of an mRNA-based TBV candidates.

Methods: Murine models were immunized with lipid nanoparticle-encapsulated mRNA vaccines encoding the open reading frame (ORF) of parasite-derived proteins with demonstrated immunogenicity and protective potential. Vaccine-induced immune responses and protective efficacy were assessed following vaccination and parasite challenge.

Results: Preliminary findings demonstrate robust vaccine-induced immune responses, significant reductions in adult worm and egg burdens, and marked alleviation of schistosomiasis-associated liver pathology in vaccinated mice.

Conclusions: These results support the feasibility of subsequent development of a multivalent mRNA vaccine incorporating multiple parasite antigens to enhance protective efficacy and durability. Integration of such transmission-blocking vaccines with existing MDA programs represents a promising strategy for sustainable schistosomiasis control and improved global public health outcomes.

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HYBRIDISATION IN UROGENITAL SCHISTOSOMIASIS IN MALAWI: HIGHLIGHTING THE ZOONOTIC IMPORTANCE OF SCHISTOSOMA MATTHEEI

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Background and Aims: In southern Malawi there are significant changes in the epidemiology of urogenital schistosomiasis. To shed light on the transmission biology of hybrid schistosomes, a four year project coined as HUGS - *hybridisation in urogenital schistosomiasis*, was proposed and came to a successfull conclusion in December 2025.

Methods: The HUGS project conducted a longitudinal cohort study of multi-species infections in two sentinel commuities, Samama and Mthawira. Participants (approx. 2000) were sampled at baseline (BL), a 12-month follow-up (FU1) and a 24-month follow-up (FU2). Preventive chemotherapy (PC) was provided annually (BL-FU1) and biannually (FU1–FU2) and urine samples were analysed with molecular methods. Schistosome infections were also tracked in local snail and livestock populations.

Results: Urine egg-patent infections were higher in Samama than Mthawira (±0.0563, p-value = 1.3 × 10−11), as was mixed infections with *Schistosoma mattheei* (± 0.17, p-value = 3.84 × 10−10). By FU2, PC reduced the prevalence of *S. haematobium* and *Schistosoma mansoni*, but that of *S. mattheei* remained relatively stable, rising by 0.98% at Samama (± 0.19, p-value = 0.41) and decreasing by 0.43% at Mthawira (±0.39, p-value = 0.33). It appears that cattle are major local reservoirs of infection(s) for this hybrid variant which is able to use both *Bulinus africanus* and *Bulinus globosus* as intermediate snail hosts.

Conclusions: From HUGS observations on schistosomes in snails, people and livestock, we conclude that treatment alone will not be sufficient for control of zoonotic *S. mattheei*, but additional interventions will be required for progress towards elimination as a public health problem.

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MAPPING FIBROTIC MICROENVIRONMENTS: SINGLE-CELL RESOLUTION SPATIAL PROFILING OF SCHISTOSOMA MANSONI-INDUCED TISSUE FIBROSIS

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Background and Aims: Schistosomiasis-induced fibrosis, driven by immune response to trapped eggs, is the principal cause of pathology and schistosomiasis-related morbidity. Despite effective adult parasite clearance by praziquantel, no therapies reverse established fibrosis. This study maps the spatial organization of immune and stromal cells in granulomas/fibrotic regions and identifies localized molecular pathways and ligand–receptor (LR) interactions as therapeutic targets.

Methods: Mice were infected with 25 *Schistosoma mansoni* cercariae, and livers and intestines were collected 8 weeks post-infection. High-resolution spatial transcriptomics was performed using Xenium (10X Genomics). Regions of interest included parasite eggs, granulomas/fibrotic tissue, and adjacent healthy areas. Cells were clustered and annotated using Scanpy and standard marker genes. Cell–cell interactions (CCI) were inferred from spatial proximity with LR co-expression, highlighting communication between immune and collagen-producing cells. Findings were validated using qPCR and immunohistochemistry.

Results: Spatial profiling revealed 11 liver and 15 intestinal cell populations associated with schistosome-induced pathology. Hepatic granulomas/fibrotic regions displayed a structured organisation, with macrophages, eosinophils, and activated hepatic stellate cells (aHSCs) forming the inflammatory-fibrotic core, and T and B cells forming an outer layer. aHSCs were the principal source of collagen. In intestine, these regions showed similar architecture; however, collagen was predominantly produced by activated fibroblasts. Liver CCI analysis predicted strong communication between aHSCs and macrophages. This approach is being extended to the intestine to identify fibrosis-associated interactions and shared LR pairs; preliminary findings suggest shared targets.

Conclusions: Spatial transcriptomics enabled visualization of the fibrotic microenvironment and localized immune-stromal interactions, and ongoing work aims to identify ligand–receptor signals as potential therapeutic targets.

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HOW COMMON IS HYBRIDIZATION BETWEEN SCHISTOSOMA HAEMATOBIUM AND THE LIVESTOCK PARASITES S. BOVIS AND S. CURASSONI?

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Background and Aims: Evidence for hybridization between the human parasite S. haematobium and the livestock parasites S. bovis and S. curassoni has major implications for “One Health” strategies and parasite control. The dominant method to assign parasites to a hybrid class is by genotyping the mitochondrial cox1 and ribosomal ITS. The consensus from studies using these markers is that zoonotic spillover and contemporary hybridization between S. haematobium and livestock parasites is common. However, two markers have limited power to detect hybridization.

Methods: We directly compared hybrid classifications by genotyping 169 Nigerian parasites with the cox1/ITS and whole-genome sequencing to evaluate the predictive power of the two-marker approach.

Results: Two-marker genotyping suggested extensive hybridization and spillover in the Nigerian samples. Nearly half (43.7%) were classified as either livestock spillover in humans (20%), early generation human-livestock hybrids (10%), or a complex hybrid containing ancestry from all three species (13.7%). However, we found no evidence of recent hybridization or spillover infection using whole-genome SNVs. Instead, we observed regionally varying levels of S. bovis alleles at rates consistent with historical introgression.

Conclusions: Our results demonstrate that: (i) 2-marker genotyping is uninformative for detecting recent hybridization or spillover events, (ii) prior studies could have overestimated hybridization levels so (iii) previous data generated using this approach requires reinterpretation, and (iv) the terminology used to describe schistosome hybrids should have greater nuance.

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ANIMAL CONTRIBUTION TO SCHISTOSOMIASIS (ACTS) TRANSMISSION IN BENIN: FROM EPIDEMIOLOGICAL DATA TO GENOMICS

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Background and Aims: Schistosomiasis remains a major human and veterinary health burden in sub-Saharan Africa, and its control is challenged by the potential contribution of animal reservoir to transmission. In line with the WHO One Health roadmap for 2030, the ACTS project aims to determine the respective roles of humans, animals and the environment in the local transmission of urogenital schistosomiasis in southern Benin (Kessounou), where *Schistosoma haematobium*, *S. bovis*, and their hybrids appear to circulate between humans, livestock and rodents. ACTS combines epidemiological surveys, population genomics, experimental infections, and mathematical modelling to assess parasite zoonotic potential and reservoir contributions.

Methods: Field sampling was conducted in Kessounou in June 2025. Infection prevalence was estimated using standard parasitological methods in humans, cattle, and small mammals. Parasite larvae DNA were preserved on Whatman FTA Classic cards for RADseq analyses. In parallel, experimental infections of snails, rodents, and cattle have been initiated to evaluate host-specific transmission competence and parasite strains development success.

Results: Preliminary results revealed infection prevalences of 23.9% in humans and of 38.6% in cattle, while no infections were detected in rodents. RAD sequencing from human and livestock samples has been completed, and genomic analyses are currently underway. A second field mission

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scheduled for February 2026 will capture seasonal variation in host populations and transmission patterns, and may allow completion of RAD data from the rodent hosts.

Conclusions: By integrating epidemiological, genomic, experimental, and modelling approaches, ACTS will provide critical insights into the zoonotic nature of schistosomiasis and inform control strategies within a One Health framework.

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EXPANDED MOLECULAR EVIDENCE OF SOIL-TRANSMITTED HELMINTH AND SCHISTOSOMA MEKONGI INFECTIONS IN MYANMAR SCHOOLCHILDREN: A QPCR UPDATE

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Background and Aims: Schistosomiasis is a disease caused by blood flukes of the genus *Schistosoma* which are transmitted by freshwater snails. There are three identified species that cause human infections in Asia. Morphologically and genetically these species are very similar; it is difficult to differentiate species based on morphology alone. This study builds on earlier work that identified a high prevalence of soil-transmitted helminths in school students in Myanmar. We re-examined stored stool DNA samples from the same children to look for additional parasite infections.

Methods: A total of 264 stool samples that had previously undergone DNA extraction and qPCR for STH in 2017 were revisited to test additionally for schistosomiasis using two different assays capable of detecting both *S. japonicum* and *S. mekongi*, and additionally ran qPCR assays to detect *Strongyloides* spp., *Opisthorchis viverrini*, and the duplex qPCR for *A. duodenale* and *A. ceylanicum*.

Results: Two samples were positive for *Schistosoma* using one of the assays, one sample was positive for *Strongyloides*, and eleven samples were positive for *A. ceylanicum*. No samples were positive for *O. viverrini* or *A. duodenale*. We attempted Big Dye sequencing of the *Schistosoma* amplicons, however our attempts failed (no visible bands on gel), highlighting limitations in template quality or primer-target mismatch.

Conclusions: These molecular findings, though limited in number, reinforce evidence that schistosomiasis may be more widespread in Myanmar than previously acknowledged. The inability to sequence the amplicon prevents species identification; clarifying whether infections are due to *S. japonicum*, *S. mekongi*, or other species is essential for guiding control strategies.

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REGULATION OF SCHISTOSOMA INFECTIONS IN SNAIL POPULATIONS: A MODELLING FRAMEWORK WITH INHERITABLE RESISTANCE IN SNAILS

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Background and Aims: Freshwater snails play an indispensable role in the transmission of human *Schistosoma* species. In endemic regions, prevalences of patent *Schistosoma* infections in snails are usually low and apparently unassociated with schistosomiasis prevalences in humans. Through mathematical modelling, we investigated whether inheritable resistance in snails to *Schistosoma* infection can facilitate these infection patterns observed in nature.

Methods: We developed a deterministic model of *Schistosoma* transmission in snails (Figure 1), where snail resistance to infection was modelled as a monogenic, overdominant trait that was associated with a reproductive fitness cost. We evaluated the impact of resistance on the prevalence of patently infected snails in endemic equilibrium. By coupling the snail model with a simple model of *Schistosoma* worms in humans, we studied infection dynamics in snails after *Schistosoma* introduction and following implementation of control

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interventions.

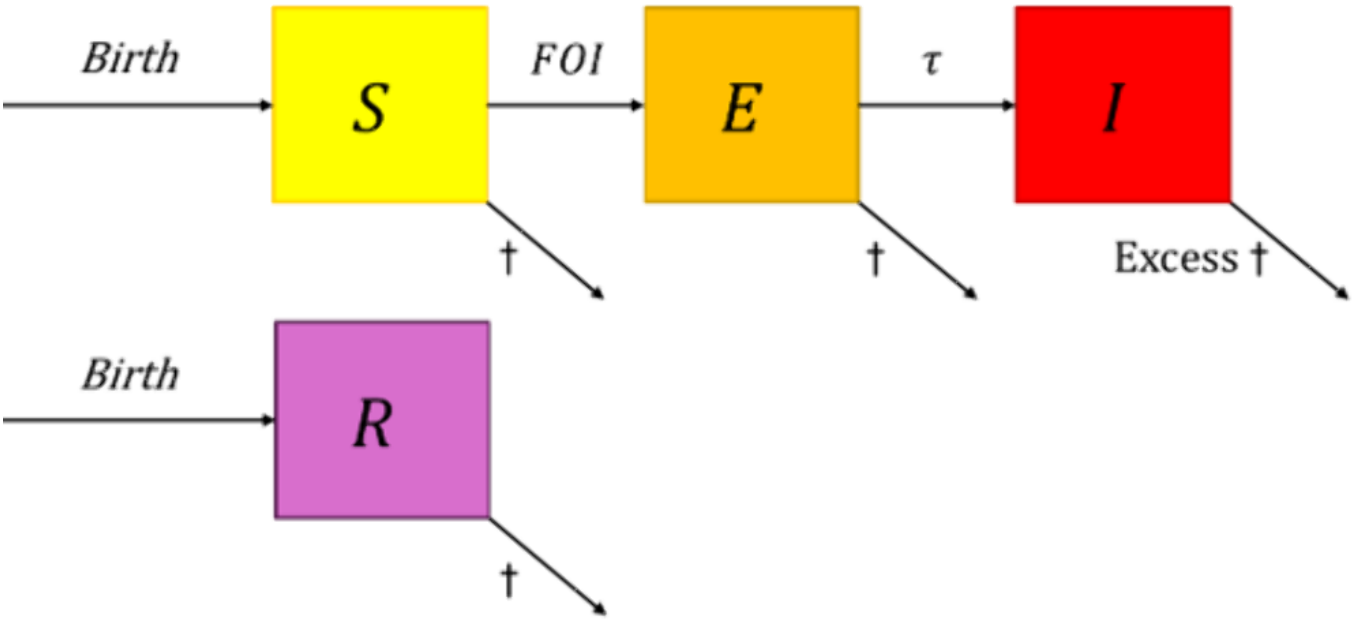


Figure 1: Schematic overview of the compartmental model of* Schistosoma *transmission dynamics in snails, with inheritable resistance. *The model simulates births, deaths (†),* Schistosoma *infections, and resistance inheritance in a snail population, which is divided in susceptible (S), prepatently infected (E), shedding (I), and resistant snails (R). Susceptible snails become prepatently infected through a force of infection (FOI). These infections develop to patency at a rate τ. Resistant snails are fully protected against* Schistosoma *infection.*

Results: Inheritable resistance in snails limited the equilibrium prevalence of patent infections to 11.5%, even under high parasite exposure. A first introduction of *Schistosoma* led to a transient peak in infection prevalence in snails, as also observed in field studies. Importantly, prevalence of shedding snails was highly resilient to decreases in human infection levels due to repeated anthelmintic treatment (Figure 2). Concurrent molluscicide helped to temporarily lower human reinfection rates. However, after termination of molluscicide, a rapid recovery of susceptible snails could cause rebounds in both snail and human

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infections.

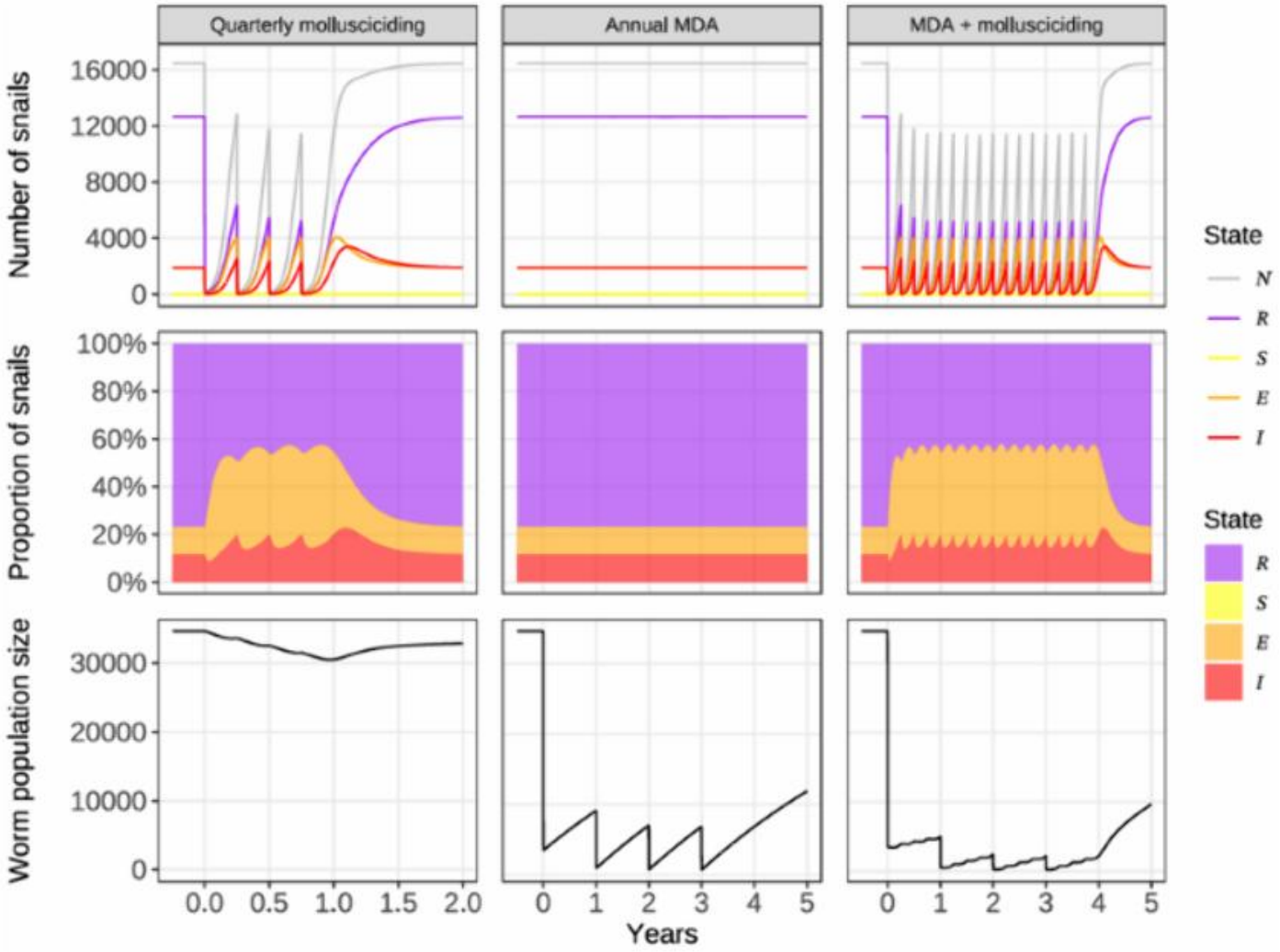


Figure 2: Impact of schistosomiasis interventions on infection dynamics in snails and worm population size in humans in a setting with high transmission potential. *The snail model simulates a snail population of size N, with susceptible (S), prepatently infected (E), shedding (I), and resistant snails (R). Quarterly molluscicide was simulated for one year (left panels) and four years (right panels), annual MDA was implemented for four consecutive years. Note how the time scale of the left panels is two years, compared to five years for the centre and right panels. The proportion of S snails is infinitely small and therefore not visible in any of the snail proportion plots.*

Conclusions: Inheritable resistance in snails can explain why *Schistosoma* transmission often rebounds or persists despite repeated interventions. This regulating mechanism will have significant implications for the feasibility and sustainability of control targets.

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ADVANCING SCHISTOSOMIASIS MONITORING THROUGH OPTIMISED ENVIRONMENTAL DNA DETECTION

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Background and Aims: Schistosomiasis remains a major public health burden in sub-Saharan Africa. Effective control relies on large-scale surveillance, yet conventional methods such as malacological surveys and stool or urine microscopy often lack sensitivity and scalability. This study aimed to evaluate and optimise environmental DNA (eDNA)-based detection of *Schistosoma mansoni* in freshwater systems, and to assess how sampling, preservation, and extraction approaches influence detection performance.

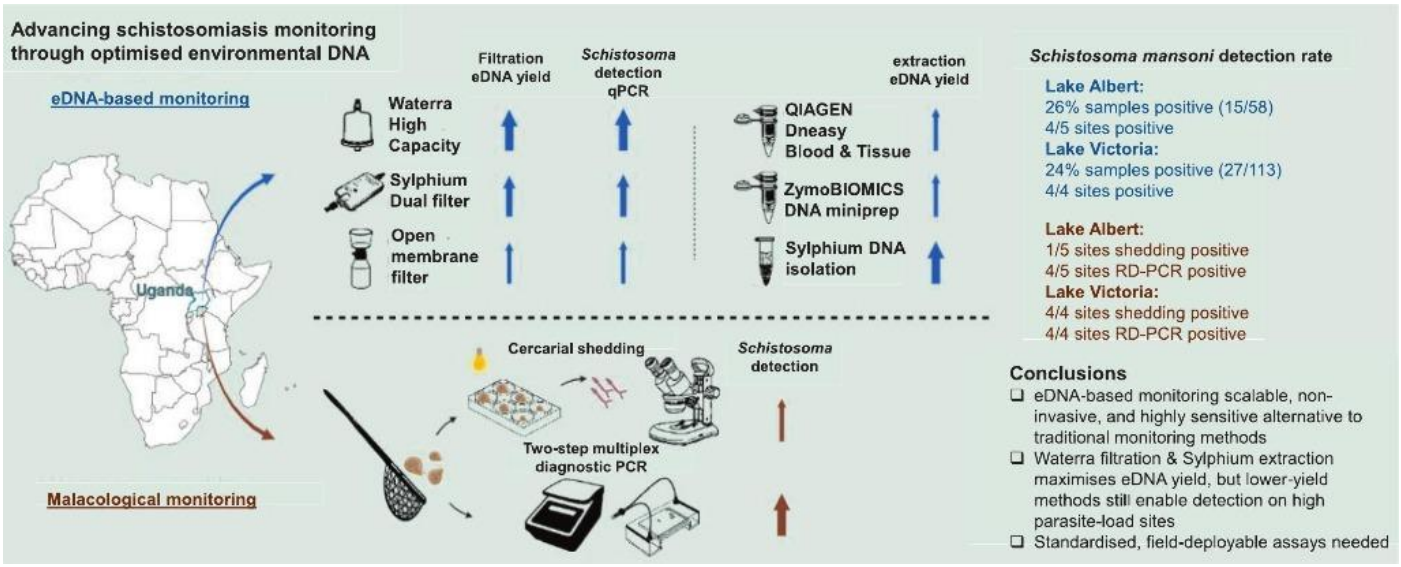
Methods: Water samples were collected from Lake Albert and Lake Victoria, Uganda. Three filtration techniques, open membrane filters, Waterra eDNA high-capacity capsules, and Sylphium eDNA dual filter capsules, were compared for eDNA yield and detection sensitivity. *Schistosoma mansoni* eDNA was quantified using a species-specific qPCR assay targeting the mitochondrial COI gene. Conventional malacological surveys were conducted in parallel. Statistical analyses examined associations between filter type, eDNA yield and detection probability.

Results: *Schistosoma mansoni* eDNA was detected in 26% (15/58) of samples from Lake Albert and 24% (27/113) from Lake Victoria. Waterra filters yielded the highest eDNA concentrations, while Sylphium purification significantly outperformed column-based extraction. Filter type and eDNA yield significantly influenced detection, with Waterra and Sylphium-single filters showing the highest amplification probabilities (~40%). Detection odds increased by ~0.8% per additional nanogram of eDNA. Open membrane filters showed low performance (~3%) and higher susceptibility to field contamination.

Conclusions: eDNA is a sensitive and scalable tool for schistosomiasis surveillance. While high-capacity filters and precipitation-based extraction methods maximise performance, lower-yield approaches can still enable detection in high-transmission settings. These findings support the adaptability of eDNA across resource contexts and highlight the need for protocol

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standardisation.



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TRACKING DRUG RESISTANCE AND GENETIC DIVERSITY IN PLASMODIUM FALCIPARUM MALARIA ACROSS NIGERIA USING TARGETED NANOPORE SEQUENCING

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Background and Aims: Background Genomic epidemiology is crucial in malaria control and elimination, providing insight into *Plasmodium falciparum* genetic diversity and drug resistance mutations. However, limited sequencing infrastructure in many malaria-endemic regions hinders the timely generation of genomic data. **Aim** This study employed targeted nanopore sequencing, implemented in-country, to analyse nine *P.falciparum* genes in febrile children under five years old across Nigeria's six geopolitical zones.

Methods: Methods A total of 108 samples confirmed positive for malaria parasites by 18SrRNA and VarATS PCR genes underwent multiplex PCR using 9 primer pairs targeting drug-resistance genes(*pfcrt, pfmdr1, pfdhfr, pfdhps* and *pfkelch13*), diagnostic test antigens(*pfhrp2 and pfhrp3*), polymorphic markers(*pfama1*), and vaccine target *pfcsp*. The resulting PCR products were sequenced using Oxford Nanopore Technology.

Results: The study identified sextuple mutations in *pfdhfr* (C59R: 96.3%, N51I: 94.4%, S108N: 97.2%) and *pfdhps*(A581G: 67.6%, A613S: 66.7%, S436A: 69.4%) across all six geopolitical zones of Nigeria. In the *pfcrt* gene, the K76T mutation was found in 77.8% of samples across six zones. Mutation N86Y and N1042D in *pfmdr1* was present in 63.0% and 50.9% of samples from 4 zones, while Y184F was found in 82.4% across 6 zones. Mutations A578S in the *pfkelch13* gene were detected in 26% of samples from the South-South and South-West, while V487I (novel mutation) was detected in 0.9% of the samples from the South-West. The *csp* gene harboured S301N(67.6%) and K317E (50%) mutations across all six zones.

Conclusions: The high prevalence of sextuple *pfdhfr/pfdhps* mutations across Nigeria indicates widespread resistance to Sulfadoxine-Pyrimethamine(SP), threatening the effectiveness of SP-based intermittent preventive treatment.

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USING BUMPED-KINASE INHIBITORS TO EXPLORE NOVEL KINASE TARGETS IN PLASMODIUM FALCIPARUM

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Background and Aims: *Plasmodium falciparum*, the deadliest human malaria species, continues to develop resistance to current therapeutics, driving the need to discover new drug targets. Its complex lifecycle within the human host relies heavily on kinases to regulate essential processes such as cell cycle progression, host cell invasion and erythrocyte egress. Kinases are a thoroughly explored drug target, and many *Plasmodium* kinases are known to lack human orthologues, making them potentially attractive targets.

Methods: To identify potential new kinase drug targets in *P. falciparum*, we utilised the bumped-kinase strategy. This approach requires “bumped’-kinase inhibitors (BKIs) that will only bind to a kinases’ ATP binding site after the replacement of a large-side group gatekeeper amino acid with a smaller-side-group alternative; these inhibitors are otherwise inactive against the erythrocytic stage of parasite growth.

Results: Eight kinases were chosen for CRISPR editing of the gatekeeper residue, with mutant parasite lines generated for four kinases, where the gatekeeper was edited to either an alanine or serine: TLK1, PK2, PK4, and ARK2. Using a library of 53 BKIs, a total of 265 inhibitor and parasite combinations were tested, with only four BKIs found to selectively inhibit parasites with an edited TLK1. TLK1, an uncharacterised putative serine/threonine kinase (PF3D7_1148000), was confirmed as essential using conditional knockdown. Using the SAR gleaned from the identified hits, ‘bumpless’ inhibitors were also developed, and their on-target capacity was explored.

Conclusions: Overall, we were able to identify a novel kinase drug target within *P. falciparum* using the highly selective BKI system.

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UNDERSTANDING ARTEMISININ RESISTANCE IN PLASMODIUM FALCIPARUM THROUGH HIGH RESOLUTION IMAGING

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Background and Aims: Resistance to the frontline antimalarial drug artemisinin is primarily mediated by mutations in a *Plasmodium falciparum* protein of unknown function called Kelch13 (K13). Drug resistant parasites with mutations in K13 (K13) ingest red blood cell haemoglobin more slowly. Artemisinin requires activation by haem released during parasite feeding to generate a toxic species, and decreased haemoglobin uptake likely reduces intracellular concentrations of toxic artemisinin species. How wild type or mutant K13 is involved in this feeding process is unknown and this study investigated the cell biological basis of this involvement.

Methods: We used a combination of light microscopy, expansion microscopy, super-resolution microscopy, and electron microscopy and tomography to investigate K13 in wild type and K13-pertubed parasites, imaging parasite feeding apparatus and K13 itself

Results: We show that K13 localises to the collar that maintains the cytostome, a stable invagination of the parasite used to take up host cytosol. 3D-electron microscopic analysis reveals that mislocalising K13 ablates formation of the cytostomal collar, and disrupts the formation of the cytostome itself. Moreover, haemoglobin degradation products haem and haemozoin are decreased when K13 is inactivated. We show that K13 collars proliferate to provide a new K13 collar to each daughter cell before division, but that this biogenesis is altered in mutant parasites.

Conclusions: We demonstrate that K13 is required for normal parasite uptake of haemoglobin via its function in cytostome formation. This suggests that artemisinin resistance arises through perturbation of K13 function through aberrant formation of cytostomes, subsequent decreased endocytosis, and thereby reduced activation of artemisinin.

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OP020

PARALLEL ORAL ABSTRACT SESSION
PARALLEL ORAL ABSTRACT 03 : MALARIA DRUG RESISTANCE AND ANTIMALARIAL DISCOVERY: FROM MECHANISMS TO MOLECULES
08-17-2026 4:40 PM - 6:40 PM

HIJACKING TRNA CHARGING: A NEW STRATEGY FOR DEVELOPMENT OF ANTI-MALARIALS

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Background and Aims: In an effort to overcome the increasing levels of resistance to antimalarial drugs, we explored nucleoside sulfamates from the Takeda Pharmaceuticals library and an Open Source Malaria Project.

Methods: We identified nucleoside sulfamate compounds that exhibit high potency, high selectivity and long half-lives *in vivo*. An exemplar compound demonstrates single dose oral efficacy in the *P. falciparum* SCID mouse model. We identified a second class of reaction hijacking inhibitors with drug-like physical parameters and a synthetically accessible scaffold. An exemplar, OSM-S-106, shows potent activity against parasite cultures and low mammalian cell toxicity.

Results: We identified the nucleoside sulfamate target as *P. falciparum* tyrosyl-tRNA synthetase (*Pf*Tyr-RS). *Pf*Tyr-RS catalyses the formation of a highly stable inhibitory sulfamate conjugate, via a mechanism we call reaction-hijacking (Xie et al. *Science* 376, 1074-1079 (2022)). Enzyme kinetics and X-ray crystallographic studies of plasmodium and human Tyr-RS reveal differential flexibility of a loop over the catalytic site determing differential susceptibility to hijacking by nucleoside sulfamates. We identified the OSM-S-106 target as the *P. falciparum* cytoplasmic asparagine RS (*Pf*AsnRS). Targeted mass spectrometry revealed that *Pf*AsnRS, but not *Hs*AsnRS, transforms the pro-inhibitor into an inhibitory conjugate. X-ray crystallographic and molecular modelling studies of *P. falciparum* and human AsnRSs in complex with Asn-OSM-S-106 adduct reveals that features that underpin the potency. Work with broad-specificity reagents reveals the opportunity to target a range of aminoacyl RSs.

Conclusions: Together the work points to the potential for the design of bespoke nucleoside sulfamates, with tuneable specificity and potency for applications in a broad range of infectious diseases.

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OP021

PARALLEL ORAL ABSTRACT SESSION
PARALLEL ORAL ABSTRACT 03 : MALARIA DRUG RESISTANCE AND ANTIMALARIAL DISCOVERY: FROM MECHANISMS TO MOLECULES
08-17-2026 4:40 PM - 6:40 PM

EVALUATION OF GENETIC VARIABILITY AND ITS POTENTIAL IMPACT ON THE ANTIGENICITY OF B-CELL EPITOPES OF THE PV41 AND PVGAMA PROTEIN IN BRAZILIAN ISOLATES OF PLASMODIUM VIVAX

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Background and Aims: Malaria parasites exhibit an extensive repertoire of genetic polymorphisms. In this context, Pv41
(Plasmodium vivax protein 41) is a surface protein expressed during the asexual (merozoite) stage, while PvGAMA (P. vivax GPI-anchored micronemal antigen) is a GPI-anchored protein involved in parasite cell adhesion and has been described as both antigenic and immunogenic.

Methods: To investigate the genetic variability of these proteins and their potential impact on immune recognition, genes encoding Pv41 and PvGAMA were PCR-amplified from 72 P. vivax field isolates collected across different endemic regions of the Brazilian Amazon. Amplicons were sequenced, and B-cell linear epitopes were predicted using multiple immunoinformatics tools (BepiPred, BCPREDS, and VaxiJen), followed by an analysis of the potential effects of antigenic variation on the most promising epitopes.

Results: Analysis of the pv41 gene revealed four synonymous and fifteen non-synonymous mutations. Notably, a non-synonymous substitution within the predicted PEKQIDEHL epitope at position 170 (H170L), as well as the A509T mutation, altered the antigenicity scores of this epitope, suggesting a possible impact on immune recognition.In contrast, analysis of pvGAMA identified only two non-synonymous mutations in the field isolates, T269C and G875A, with frequencies of 95.8% and 8.6%, respectively. Despite the high prevalence of the T269C mutation, neither polymorphism was located within predicted linear B- cell epitope regions.

Conclusions: Overall, these results indicate that PvGAMA exhibits a lower degree of polymorphism compared to Pv41, and the identified variants do not appear to affect predicted antigenic regions, reinforcing the potential of PvGAMA as a stable target for immune-based interventions.

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OP022

PARALLEL ORAL ABSTRACT SESSION
PARALLEL ORAL ABSTRACT 03 : MALARIA DRUG RESISTANCE AND ANTIMALARIAL DISCOVERY: FROM MECHANISMS TO MOLECULES
08-17-2026 4:40 PM - 6:40 PM

IMPACT OF SEASONAL MALARIA CHEMOPREVENTION ON PLASMODIUM FALCIPARUM RESISTANCE IN SENEGAL: EVIDENCE FROM FOUR MOLECULAR SURVEYS IN 2015

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Background and Aims: Seasonal malaria chemoprevention (SMC) with sulfadoxine–pyrimethamine plus amodiaquine has been widely implemented in Senegal to reduce malaria morbidity among children under 10 years. Although SMC has proven highly effective, concerns remain regarding its potential impact on the selection of *Plasmodium falciparum* drug resistance. This study aimed to assess the effect of SMC on the prevalence and temporal dynamics of molecular resistance markers in Senegal.

Methods: Four molecular surveys were conducted in 2015, including cross-sectional surveys in January, July, and December, as well as outpatient malaria cases collected between August and November. *P. falciparum* isolates were sequenced by the method of Sanger for polymorphisms in the **pfcrt, pfmdr1, pfdhfr,** and **pfdhps** genes. Allelic and haplotype frequencies were estimated overall and stratified by age (<10 years and ≥10 years). Temporal trends were assessed using 95% confidence intervals.

Results: High prevalences of **pfdhfr 51I, 59R, and 108N** mutations were observed across all surveys. Analysis of *pfdhfr* haplotypes showed a marked predominance of IRN-related haplotypes, while additional mutations such as **164L** or **140L** remained rare and showed no evidence of temporal expansion. In contrast, ***pfdhps*haplotypes associated with high-level sulfadoxine–pyrimethamine resistance,** including **540E** and **581G**, were rare or absent, with wild-type or low-level resistance haplotypes predominating. The ***pfmdr1* NF haplotype** was most prevalent overall, although an increase in the **NY haplotype** was observed after the SMC period, particularly among individuals ≥10 years.

Conclusions: SMC implementation in Senegal in 2013 did not critically compromise sulfadoxine–pyrimethamine efficacy, despite sustained selection of mutant *pfdhfr* haplotypes. Continuous molecular surveillance remains essential

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OP023

PARALLEL ORAL ABSTRACT SESSION
PARALLEL ORAL ABSTRACT 03 : MALARIA DRUG RESISTANCE AND ANTIMALARIAL DISCOVERY: FROM MECHANISMS TO MOLECULES
08-17-2026 4:40 PM - 6:40 PM

TARGETED COVALENT INHIBITORS OF PLASMODIUM PHOSPHATIDYLINOSITOL 4-KINASE AS POTENTIAL TREATMENTS FOR MALARIA

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Background and Aims: Malaria remains a critical public health issue, with drug-resistance undermining treatment effectiveness. This work aimed to synthesise and characterise a range of novel compounds designed as targeted covalent inhibitors (TCIs) against the clinically-validated target *Plasmodium* phosphatidylinositol 4-kinase (PI4K).

Methods: A series of putative TCIs were synthesised based on the scaffolds of known noncovalent kinase inhibitors MMV390048 and VE-821, bearing an array of warheads designed for covalent modification of *Pf*PI4K cysteine 1361. Warhead stability was evaluated using mass spectrometry analysis of the derivatives under aqueous or glutathione-containing conditions. Whole cell washout assays at 6 or 24 hours post incubation were applied to screen for covalent modification behaviour by the putative TCIs. Mass spectrometry was used to determine covalent binding of the best derivatives to *Pv*PI4K protein through detection of peptide fragments containing modifications at the target cysteine.

Results: Most putative TCIs had potent whole cell anti-parasitic activity and *in vitro Pv*PI4K inhibition, although there was no correlation between these values. Three of the tested putative TCI derivatives demonstrated prolonged activity against *P. falciparum* following compound washout, an indicator of covalent action. Mass spectrometry confirmed 10% target covalent modification by one of the three derivatives.

Conclusions: Exploiting a covalent binding mechanism to *Plasmodium* drug targets should improve selectivity and reduce resistance risk. This work demonstrates that cysteine 1361 of *Plasmodium* PI4K is covalently modifiable using rationally-designed TCIs. The demonstrated prolonged duration of action in *P. falciparum* exemplifies potential benefits to TCI use in a clinical setting.

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OP024

PARALLEL ORAL ABSTRACT SESSION
PARALLEL ORAL ABSTRACT 03 : MALARIA DRUG RESISTANCE AND ANTIMALARIAL DISCOVERY: FROM MECHANISMS TO MOLECULES
08-17-2026 4:40 PM - 6:40 PM

MULTI-OMIC PROFILING REVEALS GENETIC MARKERS ASSOCIATED WITH ARTEMISININ TOLERANCE IN PLASMODIUM FALCIPARUM KELCH13 C580Y MUTANT PARASITES WITH DIFFERENT SURVIVAL PHENOTYPES

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Background and Aims: Artemisinin-based therapies are essential for malaria control, but reduced susceptibility in *Plasmodium falciparum* remains a major challenge. Mutations in the β-propeller domain of Kelch13 (K13), including the prevalent K13-C580Y allele in Southeast Asia, are strongly associated with diminished artemisinin sensitivity. We sought to identify molecular markers beyond K13 contributing to artemisinin tolerance.

Methods: We conducted a comparative analysis of a K13-C580Y isolate at three time points: immediately after collection (INITIAL), after 8 months of continuous culture (DERIVED), and after 13 months of culture with intermittent dihydroartemisinin exposure from months 8–13 (PRESSURED). Ring survival assays showed increased artemisinin susceptibility in DERIVED parasites and a partial restoration of tolerance in PRESSURED parasites. The K13-C580Y mutation was retained at all time points, indicating that artemisinin tolerance is mediated by factors beyond K13. To define transcriptional signatures underlying these variable ring survival phenotypes, we performed single-cell transcriptomic analyses.

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Results: We identified transcriptomic signatures unique to INITIAL and PRESSURED parasites, shedding light on conserved expression programs linked to artemisinin tolerance. We also compared transcriptional responses at each collection point after 6-hour dihydroartemisinin treatment. A minor population of parasites exhibited a distinct transcriptional profile, potentially representing forms capable of entering a dormancy-like state to mitigate artemisinin-induced stress. Whole-genome analysis linked transcriptomic differences to specific genomic alterations.

Conclusions: Integrating scRNA-seq with long-read DNA sequencing deepens our understanding of artemisinin tolerance and highlights loci associated with variable drug response in *P. falciparum* K13-C580Y mutants. These findings reveal additional genetic factors that contribute to artemisinin tolerance alongside K13 mutations.

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OP025

PARALLEL ORAL ABSTRACT SESSION
PARALLEL ORAL ABSTRACT 04 : HOST IMMUNE RESPONSES AND MODULATION IN PARASITIC INFECTIONS
08-17-2026 4:40 PM - 6:40 PM

INFLAMMATORY PD-L1^{+}PD-L2^{+} MONOCYTE-DERIVED MACROPHAGES CREATE AN IMMUNE-EVASIVE PATHOGEN NICHE THAT ABROGATES PRE-EXISTING TH1 IMMUNITY

Matheus Carneiro[1], Santiago Soares[2], Chris Tiessen[1], Camila Gaio[1], Ben Perks[1], Leah Hohman[1], Bruna David[1], Paul Kubes[1], Matthias Mack[3], Ehud Inbar[4], Nathan Peters[1]
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Background and Aims: Humans develop immunity against many infectious diseases following natural exposure, called natural acquired immunity (NAI), which provides a blueprint for vaccination. However, many vaccination strategies have failed against these infections. “Leishmanization”, the inoculation of viable *Leishmania major* (*L.m.)* parasites into the skin, remains the only ‘immunization’ strategy known to generate protection against subsequent natural vector transmission in mice and humans. In the parasitic *Leishmania major (L.m)* model of self-healing cutaneous disease, protection against secondary infection relies on pre-existing T helper (Th)1-dependent activation of skin-infiltrating monocytes for elimination of intracellular parasites. To better understand immune-evasion of pre-existing Th1 immunity by pathogens, we investigated whether NAI protects against non-healing cutaneous disease caused by the *L. amazonensis (L.a)* parasite.

Methods: C57BL/6, STAT6$^{-/-}$, IL-10$^{-/-}$, and STAT6$^{-/-}$IL-10$^{-/-}$ (dKO) mice were infected in the footpad with 10^4 *L.m* parasites. After healing, 12 weeks post-primary infection, Leishmanized mice were challenged with *L.m*. (homologous) or *L.a*. (heterologous) in the skin and lesion development and parasite load was followed up to 20 weeks post-secondary challenge. Flow cytometry analysis of skin was performed for characterization of the innate and adaptive inflammatory responses.

Results: Following secondary heterologous challenge, pre-existing Th1 NAI initially controlled infection but protection ultimately failed. Loss of protection was not overtly STAT6- or IL-10-mediated. Rather, monocyte-lineage tracing revealed infected monocyte-derived macrophages, which acquired a highly inflammatory PD-L1^{+}PD-L2^{+} niche-adapted phenotype, facilitated evasion of pre-existing immunity and CCR2^{+} monocyte-depletion post-challenge improved parasite control

Conclusions: This unique immune-evasive cellular niche represents a target for successful vaccination and treatment strategies against phagosomal infections.

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OP026

PARALLEL ORAL ABSTRACT SESSION
PARALLEL ORAL ABSTRACT 04 : HOST IMMUNE RESPONSES AND MODULATION IN PARASITIC INFECTIONS
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CD47 IS REQUIRED FOR PROTECTIVE CD4 T CELL RESPONSE TO L. MAJOR INFECTION

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Background and Aims: CD47 is a ubiquitously expressed cell surface protein that regulates immune homeostasis by engaging signal regulatory protein alpha (SIRPα) on phagocytes to inhibit activation. While the CD47-SIRPa axis is well characterized to regulate innate and adaptive responses in cancer and autoimmunity, its role in infectious disease remains poorly defined. *Leishmania major* (*L. major*) is an intracellular protozoan parasite that infects phagocytes. Because phagocytes and CD4+ T cells are critical for immunity to *L. major* infection, we interrogated the role that CD47-SIRPα plays in immunity to *L. major*.

Methods: We infected *Cd47*$^{-/-}$ & *Sirpα*$^{-/-}$ mice on a resistant C57BL/6 background via subcutaneous footpad injection. We harvested draining lymph nodes and spleens at critical time points for flow cytometry. T cells were assessed further by intracellular cytokine and transcription factor staining. Parasite burden was assessed using limiting dilution assay.

Results: Disease in *Cd47*$^{-/-}$ and *Sirpα*$^{-/-}$ mice is severe. They present increased lesions, swelling, and parasite burden. Overall decrease in T cells and increase in antigen experienced T cells is observed. Cytokine analysis reveals reduced IFNγ and TNFα producers, likely liable for disease severity. Both of these cytokines promote parasite killing. In contrast, T cell activation was increased at acute time points (day 15pi) when disease severity is similar.

Conclusions: Further studies are required to characterize differences, and why CD47- and SIRPα-KO mice don't control infection despite adequate T cell responses early on. These studies support the novel finding that CD47-SIRPα majorly contributes to the induction and maintenance of protective T cell responses to infectious disease.

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OP027

PARALLEL ORAL ABSTRACT SESSION
PARALLEL ORAL ABSTRACT 04 : HOST IMMUNE RESPONSES AND MODULATION IN PARASITIC INFECTIONS
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ENTERIC HELMINTH INFECTION IMPAIRS THE MEMORY T CELL RESPONSE TO A RECOMBINANT VESICULAR STOMATITIS VIRUS VECTOR VACCINE

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Background and Aims: Vaccination relies on the induction of effective antibody responses, together with the priming and maintenance of long-lived antigen (Ag)-specific memory T lymphocytes. Gastro-intestinal helminths have been associated with reduced vaccine efficacy, through various immunoregulatory mechanisms. However, how helminth infection would impact the dynamics and response of memory T cells remains elusive.

Methods: We used a recombinant vesicular stomatitis virus (rVSV) vector to investigate the memory T cell responses to the chicken ovalbumin (Ova) model Ag during enteric infection with the parasite nematode *Heligmosomoides polygyrus*. Naïve or *H. polygyrus* -infected mice were vaccinated at day 14 after infection with rVSV-Ova intramuscularly.

Results: We observed a significant reduction of total, Ag-specific $CD8^+$ T cells and adoptively transferred Ova-specific OT-I cells in blood and spleen when vaccination occurred during helminth infection. To go further, splenocytes at day 3 or 7 after rVSV-Ova administration were depleted of B cells, NK cells and neutrophils before single-cell (sc)RNA sequencing, including T cells, monocyte/macrophages and dendritic cells. While a cluster of *Cd4*-expressing T cells upregulated *Gata3, Cxcr6, Il4* and *Il1rl1* (T1/ST2) after *H. polygyrus* infection, a cluster of *Cd8a/Cd8b1*-expressing T cells upregulated effector/memory signature genes (*Gzmb, Cx3cr1, Klrg1, Itga4*) and was strongly enriched from day 3 to day 7 after vaccination. Strikingly, effector/memory $CD8^+$ T cells following vaccination of *H. polygyrus*-infected mice showed significantly lower enrichment, with reduced cytotoxic (*Gzma, Gzmb, Gzmk*) and activation (*Ccl5, Klrk1, Cx3cr1*) gene signatures.

Conclusions: These results demonstrate that helminth infection impacts memory T cell responses and opens new avenues for mechanistic insights.

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OP028

PARALLEL ORAL ABSTRACT SESSION
PARALLEL ORAL ABSTRACT 04 : HOST IMMUNE RESPONSES AND MODULATION IN PARASITIC INFECTIONS
08-17-2026 4:40 PM - 6:40 PM

ASSESSING ANTIGEN-SPECIFIC CD8 T CELL IMMUNITY AND THE PROTECTIVE POTENTIAL OF HIGHLY-CONSERVED, MULTI-STAGE PLASMODIUM IMMUNOGENS IN A NON-HUMAN PRIMATE MODEL OF PLASMODIUM INFECTION

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Background and Aims: Next-generation malaria vaccines will likely require induction of parasite-specific CD8+ T cells to improve efficacy and durability. Immunopeptidomics of *Plasmodium vivax (Pv)*-infected reticulocytes identified >400 parasite peptides presented on classical MHC-I and non-classical MHC-E molecules [Barbosa 2025, preprint]. Most were ribosomal proteins with high sequence conservation, expression, and immunogenicity across *Plasmodium* species in blood and liver stages. We aim to utilize these antigens and a non-human primate (NHP) model of *Pv* to define the phenotype and protective role of *Plasmodium*-specific CD8 T cells under classical and MHC-E-restricted presentation.

Methods: Antigen-specificity during infection and vaccination was assessed by *ex vivo* stimulation and phenotyping of immune cells from NHPs infected with *Plasmodium* or vaccinated with live-attenuated parasites, protein-in-adjuvant or, cytomegalovirus vectors inducing MHC-Ia or MHC-E-restricted responses. We additionally assessed infected red blood cell (**iRBC**) killing by CD8 T cells using an *ex vivo* CD8:iRBC co-culture assay with and without MHC-E blocking.

Results: Antigen-specific CD8 T cell responses were induced and varied by infection and vaccine platform. CD8:iRBC co-culture under MHC-E blockade reduced, but did not eliminate, iRBC killing, indicating a partial role of MHC-E presentation to CD8 T cells. Vaccinated NHP challenge is underway and will be presented with detailed T cell phenotyping.

Conclusions: Using the first unbiased and validated set of MHCI-presented *Plasmodium* antigens, we characterized the phenotypic and functional role of CD8 T cells across parasite life stages. These results will be critical in the design of next-generation vaccines that include the induction of CD8 T cell immunity.

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OP029

PARALLEL ORAL ABSTRACT SESSION
PARALLEL ORAL ABSTRACT 04 : HOST IMMUNE RESPONSES AND MODULATION IN PARASITIC INFECTIONS
08-17-2026 4:40 PM - 6:40 PM

MULTISTAGE DISRUPTION OF B CELL DEVELOPMENT DURING TRYPANOSOMA BRUCEI INFECTION

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Background and Aims: African trypanosomosis is a chronic parasitic disease affecting humans and animals across sub-Saharan Africa. The causative extracellular protozoan parasite, *Trypanosoma brucei*, establishes persistent infection through multiple immune evasion strategies. While antigenic variation of the variant surface glycoprotein (VSG) coat enables escape from antibody-mediated clearance, accumulating evidence indicates that *T. brucei* also profoundly impairs host B cell immunity. In experimental mouse models, infection disrupts humoral immune responses by suppressing B cell lymphopoiesis in the bone marrow and progressively depleting splenic B cell subsets, including marginal zone and follicular B cells. This is accompanied by polyclonal B cell activation, clonal exhaustion, and destruction of the B cell memory compartment, ultimately preventing the generation of long-term protective antibody responses.

Methods: To investigate how *T. brucei* infection interferes with B cell development prior to maturation, we analyzed a C57BL/6 mouse model infected with the *T. brucei* AnTat 1.1 strain using single-cell transcriptomic profiling.

Results: In the bone marrow, infection induces marked defects in B cell lymphopoiesis, characterized by sequential reductions in pre–pro-B, pro-B, pre-B, and immature B cell populations. This is followed by a decline in transitional B cells in the spleen. Notably, *T. brucei* subsequently triggers extramedullary B cell lymphopoiesis, generating precursor populations that fail to mature into transitional B cells, leading to sustained loss of marginal zone and follicular B cells.

Conclusions: Together, these findings demonstrate that *Trypanosoma* infection disrupts B cell lymphopoiesis at multiple developmental stages over time, rendering the host unable to maintain effective antibody responses during recurrent parasitemia.

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OP030

PARALLEL ORAL ABSTRACT SESSION
PARALLEL ORAL ABSTRACT 04 : HOST IMMUNE RESPONSES AND MODULATION IN PARASITIC INFECTIONS
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ABNORMAL CD8 T CELL DIFFERENTIATION IN RHESUS MACAQUES INFECTED WITH LEISHMANIA INFANTUM

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Background and Aims: In visceral leishmaniasis, co-infection with human immunodeficiency virus (HIV) worsens disease outcomes. Nonhuman primates (NHPs) have contributed substantially to advances infectious disease research in human owing to their close anatomical and immunological systems. Our research has underlying the reprogramming of innate immune cells associated with microbial persistence. Consequently, persistence may potentially weaken CD8 T-cell immunity and increasing susceptibility to CD8 T-cell–dependent viral infections. By using NHPs, we aimed at identifying CD8 T cell alterations in tissues in which *Leishmania infantum* (*L.i*) persist.

Methods: In this study, we examined CD8 T cell differentiation by flow cytometry and cell distribution by confocal microscopy in a rhesus macaque (RM) model infected with *L.i*. Gene expression from sorted CD8 T cells was quantified using quantitative RT-PCR (qRT-PCR).

Results: Our results revealed that the expansion and differentiation of the splenic CD8 T cell pool was associated with the induction of T-bet and EOMES that regulate distinct effector functions. While T-bet was strongly associated with the expression of granzyme B and IFNG, EOMES levels persisted elevated even after loss of expression of these effector molecules. Interestingly, this profile of CD8 T cells was associated with the upregulation of several inhibitory T cell receptors. Our study provides also new insights into the orchestration and distribution of CD8 T cell dynamic in secondary lymphoid organs.

Conclusions: This model provides a support for understanding in whole body the immune alterations associated with *L.i* infection. Reprogramming of CD8 T cells associated with exhaustion may alter immune defense contributing in the exacerbation of HIV infection.

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OP031

PARALLEL ORAL ABSTRACT SESSION
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08-17-2026 4:40 PM - 6:40 PM

ADJUVANT-SPECIFIC PROGRAMMING OF CELLULAR AND HUMORAL IMMUNITY IN NA-GST-1 VACCINATION AGAINST HOOKWORM INFECTION

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Background and Aims: Current hookworm control relies on anthelmintic drugs that clear infection but do not prevent reinfection, underscoring the need for an effective vaccine. *Necator americanus* Glutathione-S-Transferase-1 (Na-GST-1) is an advanced vaccine candidate, yet the optimal adjuvant remains undefined. We aimed to compare the immune responses induced by Na-GST-1 formulated with Alhydrogel alone or in combination with CpG.

Methods: Samples were obtained from vaccinated participants enrolled in a Phase 1 Clinical Trial following a three-dose vaccination schedule. Peripheral blood mononuclear cells were analyzed using multiparameter flow cytometry and unbiased clustering to characterize antigen-specific $CD4^+$ T-cell subsets. Cytokine secretion profiles were assessed by Luminex following in vitro re-stimulation with GST-1. Circulating antibody levels in plasma were accessed by ELISA.

Results: CpG-formulated vaccine induced higher GST-1-specific IgG levels, peaking after second dose and remaining sustained throughout the study, but showed limited activation of the T-cell compartment. Alhydrogel formulation elicited inconsistent IgG responses that peaked after each dose but weren't sustained, while promoting expansion of $CD4^+$ T-cell subsets and increased cytokine secretion. GST-1/Alhydrogel-induced $CD4^+$ T cells corresponded to effector memory Th1 subsets defined by CXCR3/CD69/IFN-γ and CD69/CD154/IFN-γ/TNF expression. Luminex analysis demonstrated robust induction of both type 1 (IFN-γ, TNF-α) and type 2 (IL-4/5/13, IL-10) cytokines upon antigen stimulation, particularly after the third dose. Ongoing single-cell and B-cell analyses aim to further understand these responses.

Conclusions: These data suggest that Alhydrogel may drive functional remodeling of $CD4^+$ T-cells toward polyfunctional responses, whereas CpG preferentially enhances humoral immunity, highlighting distinct immune compartments engaged by each adjuvant.

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OP032

PARALLEL ORAL ABSTRACT SESSION
PARALLEL ORAL ABSTRACT 04 : HOST IMMUNE RESPONSES AND MODULATION IN PARASITIC INFECTIONS
08-17-2026 4:40 PM - 6:40 PM

SINGLE-CELL TRANSCRIPTOMICS REVEALS DYNAMIC IMMUNE CELL INFILTRATION DURING EXPERIMENTAL CEREBRAL MALARIA PATHOGENESIS

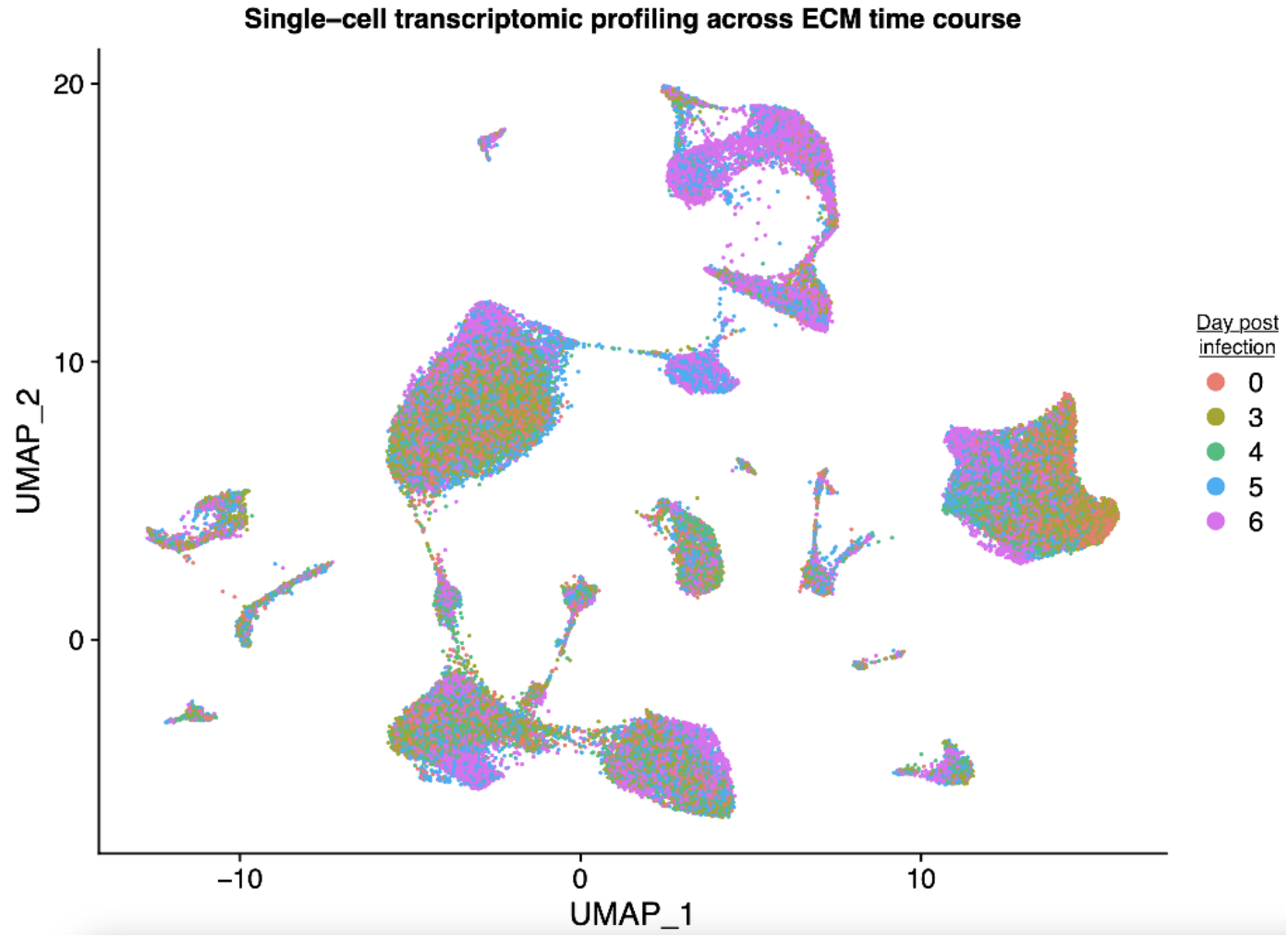
Rebecca Bellworthy, Julia Luo, Albena Pramatarova, David Langlais
Victor Phillip Dahdaleh Institute of Genomic Medicine, Human Genetics, Montreal, Canada

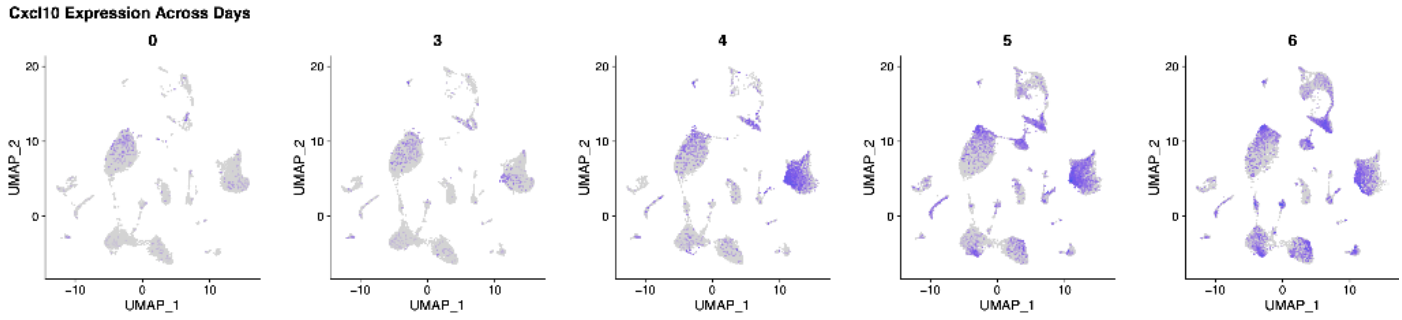
Background and Aims: Experimental cerebral malaria (ECM) caused by *Plasmodium berghei* ANKA results in severe neuroinflammation and immune cell infiltration into the murine brain, resembling the human pathology. However, the temporal dynamics and heterogeneity of infiltrating immune populations during disease progression remain incompletely characterized. We applied single-cell RNA sequencing (scRNA-seq) to comprehensively profile cellular composition and gene expression changes across the ECM disease time course.

Methods: C57BL/6 mice were infected with *P. berghei* ANKA and brain tissue collected at days 0, 3, 4, 5, and 6 post-infection (n=4 per timepoint, balanced for sex). Brain cell suspensions were enriched for immune and vascular populations by O4+ oligodendrocyte depletion and processed using Parse Biosciences scRNA-seq. We obtained 84,000 high-quality cell transcriptomes and performed unsupervised clustering, differential expression analysis, and cell type identification.

Results: Transcriptomic profiling revealed dramatic cellular shifts during ECM progression, identifying distinct populations including brain-resident microglia, infiltrating T lymphocytes, neutrophils, monocytes/macrophages, endothelial cells, and brain parenchymal populations. T lymphocytes showed striking increase [DL1] with 16-fold increased Ifng expression from day zero to day six, across all cells. CXCR3-ligand chemokines Cxcl9 (20-fold) and Cxcl10 (8-fold), along with Ccl5 (17-fold), drove robust T cell recruitment. Endothelial E-selectin upregulation (5-fold) indicated BBB activation. Peak expression at days 5-6 coincided with severe disease, emphasizing coordinated multi-cellular pathogenesis underlying ECM. Ongoing cluster-specific transcriptional analysis will delineate pathogenic populations and temporal dynamics.

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Conclusions: This single-cell atlas provides unprecedented cellular resolution of immune dynamics across the timeline of ECM and establishes a framework for identifying pathogenic populations and therapeutic targets in cerebral malaria.

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OP033

PARALLEL ORAL ABSTRACT SESSION
PARALLEL ORAL ABSTRACT 05 : EMERGING TECHNOLOGIES IN HOST–PARASITE INTERACTIONS AND MOLECULAR DIAGNOSTICS
08-17-2026 4:40 PM - 6:40 PM

INTEGRATIVE SINGLE-CELL ATLAS OF MALARIA TRANSMISSION STAGES REVEALS CONSERVED AND VIVAX-SPECIFIC SIGNATURES OF SEXUAL DEVELOPMENT

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Background and Aims: Malaria transmission is driven by the differentiation of asexual blood-stage parasites into male and female gametocytes. In contrast to the prolonged gametocytogenesis of *P. falciparum*, vivax-type parasites initiate sexual development early and efficiently transmit prior to symptom onset, suggesting fundamental mechanistic differences in transmission biology. To explore these differences, we constructed an integrative single-cell atlas of sexual development for *P. vivax* and *P. cynomolgi*, a closely related simian malaria parasite species.

Methods: *P. vivax* and *P. cynomolgi* gametocytes were purified from blood from experimentally infected humans (n=3) and macaques (n=3), respectively, by fluorescence-activated cell sorting, and processed using the CEL-Seq2 single-cell RNA-sequencing protocol. Datasets were integrated with published *P. falciparum* and *P. berghei* data enabling cross-genus comparisons.

Results: Our atlas comprises 9,604 vivax-type parasites, including 2,825 gametocytes. Cluster analyses across four *Plasmodium* species resolved distinct male and female populations arranged along progressive developmental trajectories. Notably, mature gametocytes accounted for the majority (*P. cynomolgi* – 85%, *P. vivax* – 57%) of vivax-type parasites captured. Differential expression analysis identified 540 genes conserved among mature gametocytes across all species, highlighting biological processes essential to transmission, such as cell motility and host cell remodelling. In contrast, 532 genes were specifically present in vivax-type gametocytes, highlighting lineage-specific features of gametocytogenesis.

Conclusions: This single-cell atlas defines conserved and vivax-specific signatures of sexual development, providing a framework for understanding how divergent transmission biology is controlled at the molecular level. The apparent scarcity of early gametocyte progenitors in peripheral blood suggests that vivax-type gametocytes mature outside circulation – a central hypothesis of ongoing work.

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OP034

PARALLEL ORAL ABSTRACT SESSION
PARALLEL ORAL ABSTRACT 05 : EMERGING TECHNOLOGIES IN HOST–PARASITE INTERACTIONS AND MOLECULAR DIAGNOSTICS
08-17-2026 4:40 PM - 6:40 PM

HIGH-RESOLUTION, SINGLE-TUBE PCR–NGS TARGETED AMPLICON SEQUENCING AND HYBRID CAPTURE METHODS FOR RAPID, SENSITIVE GENOTYPING OF CYCLOSPORA CAYETANENSIS

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Background and Aims: *Cyclospora cayetanensis* is a significant cause of foodborne outbreaks associated with fresh produce and water, where outbreak detection and source tracking are critical. WGS is expensive, low-throughput, labor- and time-intensive, and requires advanced bioinformatics expertise. The aim of this study was to develop rapid, high-resolution genotyping methods to support surveillance, outbreak detection, and source tracking while overcoming the limitations of WGS.

Methods: Two single-tube PCR–NGS assays based on targeted amplicon sequencing (TAS) were developed. The first method enables high-resolution genotyping, and the second method combines TAS with hybrid capture for high sensitivity. Assay design was based on all 40 publicly available *C. cayetanensis* genome assemblies available on NCBI. The assay targets 63 amplicons and enables clustering of *C. cayetanensis*. Performance was evaluated using mixed salad, basil, blackberry, and fecal samples. The assay is supported by data analysis software that requires no bioinformatics expertise.

Results: The standard TAS assay demonstrated excellent genotyping performance and robust clustering, supporting outbreak detection. The TAS plus hybrid capture method showed remarkable sensitivity, detecting targets down to a few copies. Both methods performed reliably across food and clinical matrices and generated results within 12–48 hours depending on Illumina sequencing platform used.

Conclusions: The *Cyclospora* genotyping assay provides a high-resolution, rapid, user-friendly, and cost-efficient alternative to WGS, supporting routine surveillance, outbreak detection, and source tracking in food safety and public health investigations.

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OP035

PARALLEL ORAL ABSTRACT SESSION
PARALLEL ORAL ABSTRACT 05 : EMERGING TECHNOLOGIES IN HOST–PARASITE INTERACTIONS AND MOLECULAR DIAGNOSTICS
08-17-2026 4:40 PM - 6:40 PM

SPATIAL TRANSCRIPTOMICS REVEALS TISSUE-LEVEL IMMUNE ARCHITECTURE GOVERNING SCHISTOSOME–SNAIL COMPATIBILITY

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Background and Aims: Compatibility between *Schistosoma mansoni* and its snail host *Biomphalaria glabrata* depends on immune processes embedded within complex tissue environments. We aimed to spatially resolve host–parasite interfaces and identify tissue-specific immune architectures associated with both resistance and susceptibility.

Methods: Resistant (BS-90) and susceptible (M-line) snails were challenged with fluorescently labelled *S. mansoni* and fixed at 0, 12, 24, and 48 hours post challenge. Spatial transcriptomics were performed using a custom 140-plex MERSCOPE panel targeting key parasite transcripts as well as host pattern recognition receptors, opsonin and effector molecules, antimicrobials, mucosal and neuro-immune factors, molecules related to stress and detoxification, and genome-defence components. We then compared spatial co-expression and tissue-level differential expression patterns between strains.

Results: While certain historically important immune transcripts are found to localize to proliferating and aggregating hemocyte populations, we also see contributions in barrier epithelia, and hematopoietic regions in the kidney and heart, forming discrete transcriptional hotspots. Resistant snails rapidly deploy a coordinated signalling network at invasion sites where expression of genes such toll-like receptors and progranulin by multiple cell types and tissues is critical; a view that has traditionally been missed in studies of isolated hemocytes. We also see that susceptible snails show delayed or less localized responses and that transcriptional profiles for parasite virulence factors are altered in resistant snails over time.

Conclusions: MERSCOPE spatial transcriptomics has allowed us to create the first immune atlas of transcription for a molluscan host, linking tissue-level gene organization to compatibility phenotypes and revealing microenvironments that promote or restrict schistosome development.

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OP036

PARALLEL ORAL ABSTRACT SESSION
PARALLEL ORAL ABSTRACT 05 : EMERGING TECHNOLOGIES IN HOST–PARASITE INTERACTIONS AND MOLECULAR DIAGNOSTICS
08-17-2026 4:40 PM - 6:40 PM

TRANSGENIC SCHISTOSOMES SECRETE SARS-COV-2 NEUTRALIZING ANTIBODY

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Background and Aims: Platyhelminths have proven difficult to genetically edit. We sought to establish a tractable procedure for generating transgenic lines of shistosomes, to provide the helminth field with a new tool to decipher the biology and pathophysiology of these pathogens.

Methods: Using CRISPR/Cas, we generated heritably transgenic *Schistosoma mansoni* that secrete a C5 nanobody–IgG1-Fc fusion targeting the SARS-CoV-2 spike protein. The C5-Fc transgene was inserted into the schistosome genome, and parental lines were derived from snails infected with single edited miracidia.

Results: Through successive selection in snail and mouse hosts, we established an F2 generation of the C5-Fc–transgenic line in which all schistosomes carried the transgene and secreted C5-Fc into the venous circulation of infected mice. Sera from mice infected with C5-Fc transgenic parasites potently neutralized SARS-CoV-2 *in vitro*.

Conclusions: This report provides the first demonstration of a helminth delivering a therapeutic protein *in vivo* and the first derivation of a stable, heritable transgenic flatworm line. With controlled human infection models available, these findings support developing transgenic schistosomes as single-dose delivery vehicles that continuously produce and deliver therapeutic proteins. Moreover, they provide the helminth field with a new approaches to investigate biology and pathophysiology of these pathogens.

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OP037

PARALLEL ORAL ABSTRACT SESSION
PARALLEL ORAL ABSTRACT 05 : EMERGING TECHNOLOGIES IN HOST–PARASITE INTERACTIONS AND MOLECULAR DIAGNOSTICS
08-17-2026 4:40 PM - 6:40 PM

STRUCTURAL PREDICTION AND MOLECULAR VALIDATION OF IMMUNOMODULATORS FROM HELIGMOSOMOIDES BAKERI

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[1]Cornell University, Department Of Molecular Biology And Genetics, Ithaca, United States of America, [2]University of Calgary, Department Of Biological Sciences, Calgary, Canada, [3]University of Dundee, Division Of Cell Signalling And Immunology, Dundee, United Kingdom

Background and Aims: Mammalian-parasitic nematodes have excreted/secreted proteins (ESPs) that are thought to modulate their host's immune system and thus enable infection. Many of these ESPs are encoded by multigene families that are transcriptionally upregulated during infection, are specific to parasites, or both. The profusion of ESP genes in parasitic nematodes has made it difficult to experimentally test their functions. The mouse-parasitic nematode *Heligmosomoides bakeri* has become a key model for host-parasite interactions. We are thus using large-scale structural predictions and *in vitro* assays to screen *H. bakeri* ESPs for new immunomodulatory functions.

Methods: We mapped cDNAs encoding ESPs to genes of a *H. bakeri* genome assembly. We used RNA-seq to identify which ESP genes were most strongly expressed during early infection (4 days post-infection). We used AlphaFold3 to predict binding between *H. bakeri* ESPs and mouse immune system proteins. We are now using AVEXIS to experimentally test which predicted binding events occur *in vitro*.

Results: We individually tested 163 *H. bakeri* ESPs with AlphaFold3 for binding to mouse IL-25, and predicted one ESP to bind (ipTM = 0.81, 0.00 being worst and 1.00 being best). To increase throughput, we then tested these 163 ESPs against eight mouse immune proteins at once, individually retesting any predicted bindings; this predicted two ESPs to bind IL-4 (ipTM = 0.81) or IL-33 (ipTM = 0.76). AVEXIS assays to test these predicted interactions are ongoing.

Conclusions: We intend to identify new immunomodulatory proteins of *H. bakeri* that suppress defined targets in the mammalian immune system.

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OP038

PARALLEL ORAL ABSTRACT SESSION
PARALLEL ORAL ABSTRACT 05 : EMERGING TECHNOLOGIES IN HOST–PARASITE INTERACTIONS AND MOLECULAR DIAGNOSTICS
08-17-2026 4:40 PM - 6:40 PM

A WORLD-FIRST CLINICAL TRIAL OF A GENE DRIVE MALARIA PARASITE TO CONTROL DISEASE

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Background and Aims: Malaria is on the rise. Existing tools are failing to control disease. Gene drives offer the capacity to repress, perhaps even eradicate, problem organisms like the parasite that causes malaria[1]. But do gene drives work, and are they safe?

Methods: We are building and testing a variety of gene drives in *Plasmodium* spp. Our gene drives aim to reverse drug resistance, ameliorate malaria-in-pregnancy, prevent spillover of *P. knowlesi* into humans, restore the efficacy of rapid diagnostic tests, and collapse *falciparum* malaria transmission.

Results: We have tested gene drives in human and rodent malaria parasites. Our drives have targeted six different genes and have been inserted into four different loci (chromosomes) in the parasite genomes. We have built homing drives, repression drives, and modifying drives—all of which work extremely efficiently.

Conclusions: Deploying gene drive parasites entails considerable safety, ethical, social, and compliance considerations. We have been funded for in-human safety and efficacy trials of a homing, sex biasing *falciparum* gene drive. Exploration of ethics, economics, social licence, and regulatory frameworks for a field trial of our gene drive in a malaria endemic region are also built into the project. Our team thus has the first chance to assess the utility and acceptability of a new malaria control tool. We will present our progress-to-date in building and testing malaria parasite gene drives. We will also weigh the pros and cons of these powerful and innovative tools. 1. Wilde, M-L *et al.* (2025) *Novel techniques for disrupting malaria transmission*. Trends in Parasitology **41**: 657-9

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OP039

PARALLEL ORAL ABSTRACT SESSION
PARALLEL ORAL ABSTRACT 05 : EMERGING TECHNOLOGIES IN HOST–PARASITE INTERACTIONS AND MOLECULAR DIAGNOSTICS
08-17-2026 4:40 PM - 6:40 PM

ACETYLEISH: ACETYLATION-DRIVEN CONTROL OF STAGE DIFFERENTIATION AND VIRULENCE IN LEISHMANIA MEXICANA

Suellen Rodrigues Maran[1], Anelise Gonçalves Marino[1], Ariely Barbosa Leite[1], Bruno Souza Bonifácio[1], Gabriela Gomes Alves[1], Artur Honorato Reis[1], Miguel Nascimento Garcia[1], Myrna Zanchetta Costa[1], Ana Caroline De Castro Nascimento Sousa[1], Paulo Otávio Lourenço Moreira[2], Alessandra Mara De Sousa[2], Paula Monalisa Nogueira[2], Angela Moreira Bezerra[3], Danielle Nascimento Moura[3], Naiwen Zhang[4], Clara Barbiéri[1], Sergio Schenkman[1], Antonio Mauro Rezende[2], Marcelo Santos Da Silva[5], Tiago Ferreira[6], David Sacks[7], Rubens Lima Do Monte Neto[2], Qijun Chen[4], Nilmar Moretti[1]
[1]Federal University of Sao Paulo, Sao Paulo, Brazil, [2]René Rachou Institute - Oswaldo Cruz Foundation, Belo Horizonte, Brazil, [3]Aggeu Magalhaes Institute - Oswaldo Cruz Foundation, Recife, Brazil, [4]Shenyang Agricultural University, Shenyang, China, [5]Sao Paulo University, Sao Paulo, Brazil, [6]NIAID, NIH, Laboratory Of Parasitic Diseases, Bethesda, United States of America, [7]NIAID, Bethesda, United States of America

Background and Aims: Protein acetylation regulates essential processes across eukaryotes. In trypanosomatids, stage-specic acetylation suggests roles in parasite differentiation. The levels of protein acetylation is balanced by the activity of lysine acetyltransferases and lysine deacetylases, which adds and removes the acetyl group from proteins. Here, we functionally characterized zinc-dependent lysine deacetylases (DAC1, DAC3, DAC4, and DAC5) in *Leishmania mexicana*.

Methods: To characterize the DACs we used gene editing, transcriptomics, proteomics and several in vitro and in vivo phenotypic approaches.

Results: CRISPR-Cas9-mediated disruption revealed that DAC1 and DAC3 are essential for procyclics, while DAC4 and DAC5 are dispensable. DAC1 and DAC5 are localized in the cytoplasm, and DAC3 and DAC4 in the nucleus. Functional analysis implicates DAC1, DAC3, and DAC5 in procyclic proliferation, whereas DAC1 and DAC5 drive promastigote-to-metacyclic differentiation. DAC5 was required for metacyclogenesis in the sand flies, the promastigote–amastigote transition, and amastigote intracellular replication. Notably, DAC5-null parasites failed to induce lesions in mice, displaying an attenuated phenotype. Proteomic proling uncovered altered acetylation patterns in DAC mutants, linking DAC5 to cytoskeleton regulation and cell cycle control.

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Conclusions: These findings identify acetylation as a central regulator of *Leishmania* stage differentiation and highlight DAC5 as a key factor in parasite virulence, oppening the opportunity to explore these enzymes as potential drug targets in the future.

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OP040

PARALLEL ORAL ABSTRACT SESSION
PARALLEL ORAL ABSTRACT 05 : EMERGING TECHNOLOGIES IN HOST–PARASITE INTERACTIONS AND MOLECULAR DIAGNOSTICS
08-17-2026 4:40 PM - 6:40 PM

GAMIFIED HEALTH EDUCATION TO ADDRESS INTESTINAL PARASITIC INFECTIONS IN URBAN POOR COMMUNITIES: A ONE HEALTH PILOT STUDY.

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Background and Aims: Intestinal parasitic infections (IPIs) represent a significant yet often overlooked public health challenge in rapidly urbanising regions. In Malaysia, existing research and intervention efforts have predominantly focused on Indigenous communities, resulting in a limited understanding of IPI risks among the urban poor residing in public housing projects (PPRs), characterised by overcrowding, shared sanitation, and inadequate waste management. This pilot study aimed to assess the effectiveness of a community-based, gamification-driven health education intervention in enhancing knowledge, attitudes, and preventive practices (KAP) related to IPIs among residents of urban public housing.

Methods: A pre- and post-intervention outreach program was implemented at one PPR in the Klang Valley, involving ninety participants across three age groups: children (7–12 years), adolescents (13–17 years), and adults (>18 years). Interactive games, including charades, Pictionary, and Bingo, were utilised to deliver crucial messages regarding parasite prevention and hygiene. KAP related to IPIs was evaluated using standardised questionnaires administered before and after the intervention.

Results: indicated varying levels of IPI awareness across age demographics, with adolescents demonstrating higher initial knowledge. Post-intervention assessments revealed significant improvements in all KAP domains, along with high engagement levels and positive participant feedback. The gamified approach fostered a safe learning environment, particularly benefiting adults with limited prior exposure to IPI education.

Conclusions: Gamified, culturally relevant health education shows promise for strengthening parasite-related health literacy in underserved urban populations. By bridging scientific knowledge with community realities, this low-cost and scalable approach aligns with One Health principles and complements existing public health initiatives.

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OP041

PARALLEL ORAL ABSTRACT SESSION
PARALLEL ORAL ABSTRACT 06 : PARASITES IN WILDLIFE ECOSYSTEMS: DIVERSITY, HOST–PARASITE INTERACTIONS, AND CONSERVATION CHALLENGES
08-17-2026 4:40 PM - 6:40 PM

HOST-PARASITE INTERACTIONS IN QUEBEC FISHES: STATE OF KNOWLEDGE AND EMERGING THREATS

Ariane Côté[1], Juliane Vigneault[2], Melissa Pimentel[3], Sandra Binning[1]
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Background and Aims: Despite their vital role in ecosystem health and biodiversity, parasites are often overlooked or misunderstood by the public, scientists, and managers. Thus, our understanding of many parasite species, including their impacts on hosts and ecosystems, is limited, making it difficult to predict how infection dynamics may shift in a warming world. This lack of knowledge is particularly concerning in Canadian aquatic systems, where fishes, are increasingly threatened. Synthesizing knowledge of host-parasite interactions in Canadian fishes can help identify areas that require further research and establish a baseline for monitoring disease dynamics over time and space.

Methods: We conducted a narrative literature search for papers with information on the parasite fauna of fishes from Quebec, Canada on June 25th 2024 and on February 19th 2025 based on PRISMA guidelines.

Results: One hundred and twelve publications across 52 journals published before December 31th 2024 were screened. Although information from sixty-four fish species and 145 parasite genera across 132 water bodies were found in the literature, there was no record of the parasitic fauna from 65 known freshwater fishes, including from the 5 endangered species, in the province. Our synthesis also identified geographic biases in terms of regions studied which was unrelated to population density.

Conclusions: Focusing on freshwater fishes in Quebec, this perspective assesses the current state knowledge of metazoan fish parasites and highlights species and geographic locations in need of further research. We also explore how emerging threats may impact host-parasite relationships. This work offers a foundation for similar studies across Canada and beyond.

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OP042

PARALLEL ORAL ABSTRACT SESSION
PARALLEL ORAL ABSTRACT 06 : PARASITES IN WILDLIFE ECOSYSTEMS: DIVERSITY, HOST–PARASITE INTERACTIONS, AND CONSERVATION CHALLENGES
08-17-2026 4:40 PM - 6:40 PM

DOES A SEMI-NATURAL HELMINTH INFECTION IMPACT THE THERMAL TOLERANCE OF A FRESHWATER FISH?

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Université de Montréal, Sciences Biologiques, Montréal, Canada

Background and Aims: Freshwater ectotherms, such as fishes, are often exposed to multiple stressors, including warming waters and parasite infections. However, few studies consider the potential influence of parasites on the physiological and behavioural responses of fishes to thermal stress. Fewer still use experimental or semi-natural infections to establish causal links between parasites and host thermal tolerance metrics. Therefore, we investigated the thermal limits, agitation behaviour and the acclimatization capacities of parasite-naïve pumpkinseed sunfish (*Lepomis gibbosus*) following a semi-natural helminth infection.

Methods: Using aquatic cages, we exposed fish to helminth (cestodes and trematodes) infections over one summer month in a lake with naturally high levels of parasites (infected fish). The Critical Thermal Method (CTM) was then used to measure an individual's lower (CT_{min}) and upper (CT_{max}) thermal limits and agitation behaviour (T_{ag}). These result were compared to a group of fish from the same population also following a month-long exposure in cages placed in a naturally uninfected lake (uninfected fish).

Results: The thermal tolerance windows ($CT_{max} – CT_{min}$) and the acclimatization capacities of infected and uninfected fish did not differ. Although parasite abundances in our infected fish were relatively low (median of 22 trematodes and 6 cestodes per fish), infection with trematode parasites reduced T_{ag} in infected fish, prolonging their agitation period during CT_{max} trials.

Conclusions: Trematode infections induced greater thermal agitation in pumpkinseeds but did not alter their thermal limits, indicating that parasites, even at low levels of infections, may have different impacts on the behavioural and physiological responses of their hosts to thermal stress.

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OP043

PARALLEL ORAL ABSTRACT SESSION
PARALLEL ORAL ABSTRACT 06 : PARASITES IN WILDLIFE ECOSYSTEMS: DIVERSITY, HOST–PARASITE INTERACTIONS, AND CONSERVATION CHALLENGES
08-17-2026 4:40 PM - 6:40 PM

APPLIED OUTCOMES LEAD TO FUNDAMENTAL INSIGHTS: OBSERVATIONS FROM A STUDY ON CARDICOLA SPP. (APOROCOTYLIDAE) INFECTING RANCHED SOUTHERN BLUEFIN TUNA OFF PORT LINCOLN, SOUTH AUSTRALIA.

Nathan Bott[1], Cecilia Power[1], Maree Widdicombe[1], Melissa Carabott[1], Jemma Hudson[1], Rebecca Van Gelderen[1], Paul Ramsland[1], Neil Young[2], Sho Shirakashi[3], Barbara Nowak[4]
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Background and Aims: Aporocotylid blood flukes, *Cardicola forsteri and Cardicola orientalis,* are economically significant parasites of Southern bluefin tuna (SBT), *Thunnus maccoyii.* SBT are ranched off the coast of Port Lincoln, South Australia.

Methods: A multi-year collaboration between the Australian Southern Bluefin Tuna industry and researchers has developed important applied outcomes for the industry. This has been achieved by providing insights into husbandry methods and the use of the anthelminthic praziquantel for the ongoing management of aporocotylids.

Results: A novel, rapid, in-field diagnostic molecular test (Recombinase Polymerase Amplification), targeting the 2nd internal transcribed spacer of ribosomal DNA (ITS-2 rDNA) was developed for both *C. forsteri* and *C. orientalis* in-field detection. Importantly this industry-researcher collaboration has allowed important studies on the biology of aporocotylids to be undertaken. Developments in aporocotylid glycomics, genomics and transcriptomics will be discussed, as well as important insights into host-parasite immune interactions.

Conclusions: This research not only has importance for aporocotylid management in bluefin tuna, but also provided important information about the Aporocotylidae and the Trematoda.

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OP044

PARALLEL ORAL ABSTRACT SESSION
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DIVING DEEP; MITOCHONDRIAL GENOMICS SHEDS LIGHT ON EVOLUTIONARY ADAPTATIONS OF MARINE PARASITES IN WILD CETACEAN POPULATIONS.

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Background and Aims: The diversity, evolutionary history, and host-associated adaptations of cetacean parasites remain poorly understood. This study investigates the mechanisms that enable trematodes of the family brachycladiidae to parasitise cetacean hosts with markedly different ecological traits, with implications for the conservation and health assessment of wild cetacean populations.

Methods: Genomic, population genetic and phylogeographic analysis are combined to investigate population biology, diversity and adaptation of the brachycladiidae family. A holistic approach was taken to characterising highly-specific brachycladiid mitochondrial adaptations in response to the marine environment that cetaceans inhabit, drawing on well-established methodologies used to investigate mitochondrial adaptations to extreme environmental conditions in a variety of other organisms.

Results: Well-supported divergence between brachycladiid species is attributed to affinities for different infective sites within cetacean hosts (Figure 1). Furthermore, brachycladiid speciation and divergence mirrors patterns of host speciation and divergence, in response to exploiting emerging ecological niches as environmental conditions changed (Figure 2). Finally, specific adaptations are identified that allow brachycladiid parasites to infect hosts across diverse environmental

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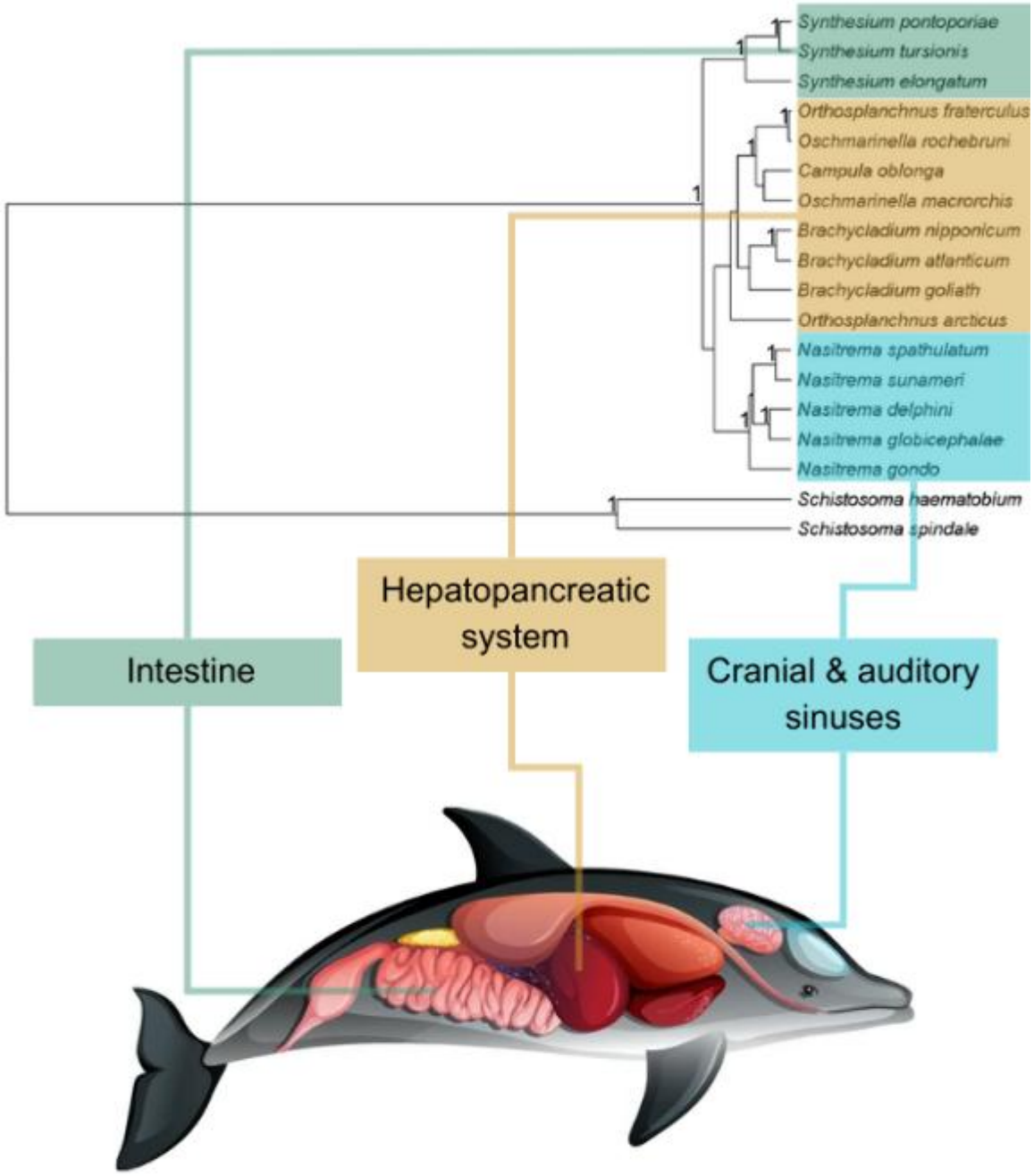


Figure 1. Bayesian phylogeny of parasites within the brachycladiidae family, showing phylogenetic topology corresponding to target organ within cetacean definitive hosts.

gradients.

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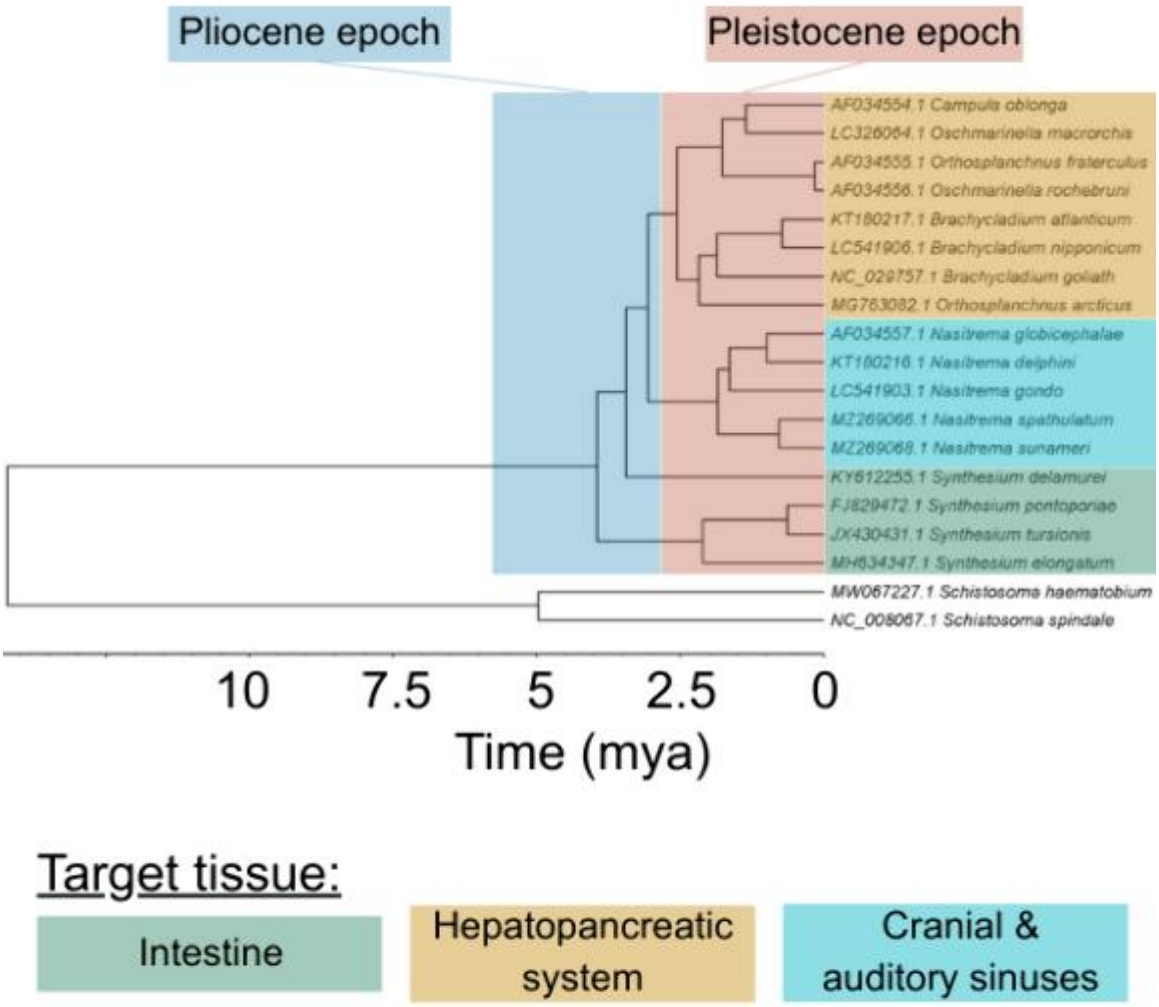


Figure 2. Molecular clock illustrating divergence times of parasites within the brachycladiidae family, labelled with geological time periods.

Conclusions: Congruence in speciation and divergence patterns between cetaceans and the brachycladiidae family highlights the role of marine mammal hosts in shaping the evolutionary history of their parasites, signalling potential for use of parasite genotype as a proxy for host genealogy and ecology. Parasite divergence timings show responses of hosts to changing environmental conditions.

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Specific mitochondrial adaptations show the process of brachycladiid evolution in greater detail. Specifically, changes in response to increased metabolic demands are shown, indicating adaptations required by the brachycladiidae family to parasitise cetaceans so successfully.

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IDENTIFIYING RESEARCH GAPS IN HELMINTH PARASITES OF EUROPEAN AMPHIBIANS EXAMPLIFIED BY SPECIES OF THE GENUS SALAMANDRA IN GERMANY

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Background and Aims: Amphibians are among the most threatened vertebrates, affected by habitat alteration and emerging pathogens. While this has driven research on amphibian pathogens, macroparasites (i.e., helminths) remain relatively understudied, particularly in Europe. Therefore, our goal was to assess the current state of European amphibian helminthology and to compare historical records with contemporary parasite communities, highlighting research gaps on parasite diversity, their life history, and the availability of molecular data.

Methods: To achieve this goal, we compiled literature data from public databases, museum collections and relevant grey literature. In parallel, we examined helminth communities in selected German populations of fire and alpine salamanders using morphology and DNA barcoding, as a contemporary case study.

Results: We found records of 146 helminth parasites infecting 86 amphibian species in Europe. The majority of available data is over 50 years old, with the majority of species lacking any sequencing data, pointing towards yet undiscovered parasite diversity. Consequently, by examining 162 salamanders, we found four helminth species, including two previously undetected taxa (*Cosmocerca longicauda*, *Amphibiocapillaria* sp.), also observing scarcity of available life history and molecular data.

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Conclusions: By performing an extensive database review combined with contemporary analyses of helminths in *Salamandra* species, we illustrate that European amphibian helminthology lags behind other parasitological fields. By providing a comprehensive list of helminth species and their amphibian hosts, including information on available DNA sequences, we offer a foundation for future studies. Finally, we outline major challenges in amphibian helminthology and propose strategies to address them to stimulate renewed research interest.

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AT A SNAIL'S PACE: TREMATODE RECOLONIZATION IN RESTORED FRESHWATER SYSTEMS

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Background and Aims: While ecological succession of free-living organisms in restored freshwater ecosystems is well studied, parasite recolonization and development remain largely unexplored. Digenean trematodes are particularly well suited to examine parasite recolonization in freshwater systems, as their heteroxenous life cycles are typically water-bound, with aquatic gastropods serving as first intermediate hosts. Here, we assess trematode recolonization and succession following large-scale stream restoration.

Methods: We investigated trematode occurrence in gastropods in the Emscher catchment (Germany), formerly among Europe's most heavily polluted and degraded freshwater systems. Following a large-scale restoration project exceeding €5 billion over the past three decades, the catchment now includes the newly restored Emscher main stem alongside tributaries restored during the last 20 years. Over two years, we conducted spatially and temporally fine-scaled sampling in the catchment. Trematode host specificity, species richness, and community composition were analysed to assess successional trajectories of trematodes and their free-living hosts.

Results: We examined 7,811 snails and identified 27 trematode species from 11 families. Initial trematode recolonization was patchy in prevalence and sampling localities and dominated by host-generalist species dependent on allogenic definitive hosts. Trematode component communities were broadly similar across restoration stages, indicating similar definitive host assemblages across the catchment. In sites restored for over 5 years, restoration history became less influential, while land use and definitive host availability emerged as primary drivers of trematode prevalence and richness.

Conclusions: Trematodes and their gastropod hosts can rapidly recolonize restored streams, with generalist trematodes using mobile definitive hosts establishing first, while landscape context drives trematode dynamics in later stages.

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HOW DOES DIGENETIC TREMATODE HOST SPECIFICITY IMPACT THE DIVERSITY-DISEASE RELATIONSHIP?

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Background and Aims: How biodiversity influences disease remains widely debated, particularly for parasites with complex, multi-host life cycles such as digenetic trematodes. The relationship between host and parasite diversity may depend not only on host community composition, but also on whether trematode communities are dominated by host specialists or host generalists. While host specialization has typically been defined based on relationships with definitive hosts, we examined how trematode infection prevalence relates to the diversity of first intermediate snail hosts.

Methods: From 2019-2022, freshwater snails were collected biweekly between June and August at eight wetland sites. Trematode infections were identified from patently infected snails using cytochrome oxidase subunit I (*COI*) or adenine dinucleotide dehydrogenase subunit 1 (*nad1*) sequencing. Trematode species were classified as specialists or generalists based on observed snail-trematode interactions. Generalized linear mixed-effects models were used to examine the relationships between 1) snail richness and overall, generalist, and specialist infection prevalence, 2) snail richness and trematode richness, and 3) the impact of sampling year on infection prevalence.

Results: Throughout the study, 22 397 snails were collected, with 1 981 infected (8.84%), yielding 74 trematode species from five snail species. Increasing snail richness was associated with higher overall and generalist infection prevalence, but not trematode richness. Infection prevalence declined across all trematode groups in 2022 relative to 2019.

Conclusions: Increased snail richness was associated with higher trematode infection prevalence, driven primarily by generalist trematodes. These results highlight the importance of incorporating first intermediate host diversity and trematode-host dynamics when evaluating disease dynamics.

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FISH RETURN, PARASITES RESPOND: ESTABLISHMENT OF COMPLEX LIFE CYCLES DURING ECOSYSTEM RECOVERY

Maya Kays[1], Florian Scharff[1], Milen Nachev[1], Daniel Grabner[1], Bernd Sures[1,2]
[1]University of Duisburg-Essen, Aquatic Ecology, Essen, Germany, [2]Research Alliance Ruhr, Research Center One Health Ruhr, Essen, Germany

Background and Aims: The Emscher River in the Ruhr Area (North Rhine-Westphalia, Germany) represents a unique case of large-scale urban river restoration. After serving as an open sewage system for over a century, it has been sewage-free since early 2022. Restoration measures worth over €5 billion, including removal of concrete channels and partial re-naturalisation, have initiated a gradual recovery of aquatic biodiversity. Alongside free-living organisms, parasite biodiversity has begun to re-establish. Parasites closely mirror host diversity and ecosystem complexity, so their reappearance indicates the reconstitution of functional life cycles in a formerly parasite-free system. In parallel, the bioindicator properties of helminths allow assessment of long-term ecosystem degradation through quantification of toxic metal burdens in selected parasite taxa and their hosts.

Methods: Fish were sampled by electrofishing and subjected to complete parasitological examination using morphological and molecular identification. Parasite prevalence, abundance, and community composition were analysed to track recolonisation dynamics. Selected parasite taxa and host tissues were analysed for metal concentrations using inductively coupled plasma mass spectrometry (ICP-MS) to assess contamination patterns in both parasites and hosts.

Results: This project identifies temporal patterns of fish remigration and parasite community assembly, allowing inferences about additional, often cryptic, host species required for parasite life cycles. Metal analyses revealed differences in accumulation between hosts and parasites, providing insights into ongoing chemical stressors.

Conclusions: By integrating parasite biodiversity, fish recolonisation, and metal contamination data, this long-term study (starting in 2025) assesses ecosystem recovery in the Emscher River and demonstrates the value of parasites as sensitive indicators of restoration success.

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LEVERAGING MACHINE LEARNING FOR ENHANCED SPATIOTEMPORAL FORECASTING OF MALARIA MORTALITY IN CHILDREN UNDER FIVE IN THE DEMOCRATIC REPUBLIC OF CONGO

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Medical research circle, Department Of Research, Goma, Congo, Republic of

Background and Aims: Malaria remains a major public health challenge in the Democratic Republic of Congo (DRC), particularly affecting children under five years of age. Accurate and timely spatiotemporal forecasts of malaria mortality are critical for optimizing resource allocation and implementing effective control strategies.

Methods: This study developed and evaluated two machine learning (ML) models, Random Forest and XGBoost, to forecast monthly provincial-level malaria mortality in children under five across the DRC. Monthly mortality data from 2020 to 2023 were used for extensive spatio-temporal feature engineering, incorporating lagged mortality values, rolling means, and cyclical seasonal components. Model performance was assessed using Root Mean Squared Error (RMSE) and Mean Absolute Error (MAE).

Results: Both ML models demonstrated strong predictive capabilities on the training data, with XGBoost slightly outperforming Random Forest (RMSE: 8.97 vs. 10.25; MAE: 5.92 vs. 6.88). Feature importance analysis identified lagged mortality, provincial identifiers, and seasonal indicators as the most influential predictors. Forecasts for 2024 revealed persistently high-burden provinces, such as Kongo-Central and Maniema, with strong seasonal peaks between October and December. Mann-Kendall trend tests indicated significant increasing mortality trends in provinces like Tshuapa and Lualaba, whereas Haut-Katanga exhibited a decreasing trend.

Conclusions: These findings highlight the potential of ML models to provide accurate and granular spatio-temporal forecasts of malaria mortality. Such predictive insights are invaluable for optimizing resource allocation, strengthening early warning systems, and guiding targeted public health interventions in the DRC. The proposed framework also offers a transferable methodology for improving disease surveillance and response in other high-burden settings.

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HIGH GENETIC DIVERSITY AND CLONAL ACTIVATION OF HYPNOZOITES OF PLASMODIUM VIVAX ISOLATES IN LOW TRANSMISSION SETTING OF ETHIOPIA

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Background and Aims: *Plasmodium vivax* causes recurrent infections. The recurrent infection can be due to recrudescence, reinfection, or relapse. In malaria endemic area to distinguish between relapse and reinfection are required

Methods: *P. vivax* DNA was extracted from 370 blood samples collected from 215 individuals visiting health facilities within sugarcane plantations and surrounding areas in Jimma-Arjo and Dabo-Hanna districts of Oromia Region, southwestern Ethiopia. All samples were subjected to amplicon deep sequencing of the *pvmsp1* gene.

Results: This study revealed high population genetic diversity—generating 67 unique haplotypes, with haplotype diversity (Hd = 0.799), nucleotide diversity (𝜋 = 0.044), and expected heterozygosity (HE) = 0.826. However, low multiplicity of infection (MOI) = 1.4 and 34.6% polyclonal infections were observed. In paired analysis of primary and recurrent episodes high genetic homology (81.3%) were observed in between the recurrent and the preceding episode, with 55.6% of the homologous pairs sharing identical alleles. Furthermore, relapse episodes that occurred between days 29-60 after the primary attack showed 100% homology in children under the age of five.

Conclusions: This study demonstrated high population level genetic diversity however, low diversity at individual level, likely driven by migrant workers introducing diverse parasite genotypes into a low-transmission setting. Most recurrent infections were relapses, as evidenced by identical shared alleles, occurrence beyond day 29 after the primary attack, and homologous relapses in children under five. The finding highlights the need for strengthening malaria surveillance and tailored intervention particularly for mobile population.

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EXPANSION MICROSCOPY AS AN EMERGING TOOL TO INVESTIGATE THE BIOLOGY OF MOSQUITOES AND THEIR MALARIA PARASITES.

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Background and Aims: Light microscopy is the most widely used technique in the field of cell biology, however many of the organelles of malaria parasites are smaller than the resoltuion of a conventional light microscope; making studying their cell biology difficult or impossible. Since its first application to malaria parasites in 2021, a technique called ultrastructure-expansion microscopy (U-ExM), which takes parasites and makes them ~5-fold larger, has revolutionised our understanding of parasite cell biology. While U-ExM has already been used to make significant progress in our understanding of parasite cell biology, to date it has almost exclusively been used on parasites grown *in vitro*.

Methods: To investigate the formation of oocyst sporozoites in the mosquito midgut, and their subsequent invasion into the mosquito salivary gland, we developed expansion microscopy for isolated mosquito tissues (MoTissU-ExM). More recently, we have gone a step further and developed expansion microscopy for whole infected mosquitoes.

Results: Using MoTissU-ExM, we constructed a timeline for rhoptry biogenesis, visualised salivary gland invasion by sporozoites, and characterised the essential role of RON11 in these two processes. With our newly developed whole-mosquito expansion microscopy protocol, we have been able to visualise sporozoite development in the mosquito midgut and invasion into the salivary, all within the same mosquito

Conclusions: Collectively, these newly developed techniques enable insight into the biology of mosquito-stage parasites at previously impossible detail and enable ultrastructural analysis of both malaria parasites and their mosquito hosts at the same time. Ongoing work will establish whether these techniques can applied to other arthropod vectors of parasites.

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TOWARDS AN EARLY WARNING SYSTEM (EWS) FOR CLIMATE-DRIVEN MALARIA DYNAMICS IN KEUR SOCÉ, SENEGAL

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Background and Aims: Despite advances in interventions (SMC, LLINs, IRS), malaria transmission continues to show strong spatial and temporal heterogeneity, particularly in rural areas of Senegal where climatic and ecological conditions strongly modulate vector abundance. This study proposes a mechanistic climate–vector–host model to understand malaria transmission in the Sahelian zone.

Methods: The model combines a SEIR (Susceptible-Exposed-Infectious-Recovered) model for humans and a SEI (Susceptible-Exposed-Infectious) model with a larval stage for mosquitoes, explicitly integrating the effects of temperature, humidity, and rainfall on vector survival, the extrinsic incubation period (EIP), and larval habitats. A three-dimensional bioclimatic window S (T, RH, RR) continuously modulates the transmission rates and allows for the expression of an instantaneous reproduction number $R_0(t)$.

Results: Simulations show that the seasonality of malaria results from climate fluctuations and that transmission persists only when $R_0(t) > 1$. Extreme scenarios (high rainfall, drought, warming) confirm the system's strong sensitivity to climate. The simulated dynamics align with vectors bionomic (HLC, PSC) data collected in the municipality of Keur Socé, located in the Ndoffane health district.

Conclusions: This model identifies critical transmission periods and provides a solid foundation for an early warning system and the optimal planning of vector control interventions.

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EMERGENCE OF HRP2/HRP3 GENE DELETIONS IN PLASMODIUM FALCIPARUM AND THE ROLE OF UNDERRECOGNIZED HUMAN MALARIA PARASITES IN BANGLADESH'S ELIMINATION EFFORTS

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Background and Aims: Bangladesh has made substantial progress toward malaria elimination by 2030 through the use of rapid diagnostic tests (RDT), artemisinin-based combination therapy, and vector control. Although five human malaria parasite species circulate nationally, surveillance and case management focus primarily on *Plasmodium falciparum* and *P. vivax*. The emergence of *hrp2/hrp3* gene deletions and infections caused by underrecognized malaria species threatens the reliability of current diagnostic strategies.

Methods: This study was conducted from January to December 2025 in Alikadam Upazila, a high-transmission area of Bandarban District, Bangladesh. Patients presenting to the Upazila Health Complex with clinical symptoms suggestive of malaria but testing negative by RDT were enrolled. Repeat RDTs and microscopy were performed locally. Residual blood samples were analyzed at the Parasitology Laboratory, icddr,b, using molecular methods for parasite species identification and detection of *hrp2/hrp3* gene deletions. Routine malaria case data were retrieved from hospital records.

Results: In 2025, a total of 1,496 malaria cases were reported from Alikadam Upazila, of which 133 originated from Alikadam UHC. Molecular analysis revealed nine *P. falciparum* infections that were discordant with RDT results due to ***hrp2* gene deletions**, seven of which also lacked ***hrp3***. Furthermore, five *P. ovale* infections (*P. ovale curtisi* and *P. ovale wallikeri*) and three *P. malariae* infections were identified exclusively through molecular diagnostics.

Conclusions: The presence of *hrp2/hrp3* deletions and unrecognized malaria parasite species in endemic areas undermines malaria diagnosis and surveillance in Bangladesh. Strengthening molecular surveillance and transitioning away from HRP2-based RDTs are critical to sustaining progress toward malaria elimination.

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TOWARDS MALARIA ELIMINATION IN NORTHERN MEXICO: SOCIODEMOGRAPHIC DESCRIPTION OF REMANENT FOCI IN CHIHUAHUA

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Background and Aims: The sociodemographic conditions and natural characteristics of the state of Chihuahua in northern Mexico have historically posed challenges for the implementation and monitoring of malaria control programs. The objective of this study was to identify and characterize transmission patterns in malaria-endemic localities in the state and analyze sociodemographic variables associated with transmission in these communities over the last twenty years (2000–2022). Also, review the joint activities made through the state's two public universities regarding vector surveillance.

Methods: Retrospective analysis using surveillance data from malaria databases and entomological control activities by locality in the state of Chihuahua, along with other open-access databases. The analysis was conducted to determine the prevalence of malaria transmission in all foci evaluated. Additionally, semi-structured interviews were conducted with operational staff of the malaria control program.

Results: Based on observations of the epidemiological trend of active outbreaks, it was determined that annual case variation was associated with population changes across localities, predominantly due to transit limitations associated with local economic activities. Social, environmental, and clinical factors, such as migration, geographical isolation, and ethnic beliefs and traditions, were identified as associated with changes in malaria transmission. On-site studies on the bionomics of *Anopheles pseudopunctipennis* (Diptera: Culicidae) have been conducted to support entomological activities of the malaria control group.

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Conclusions: This is the first study to integrate the operational experience of the control and surveillance program in the area with a comprehensive approach to the region's social context, including research groups, thereby supporting the ongoing elimination program in the country.

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NOVEL APPROACHES TOWARDS MOLECULAR DIAGNOSIS OF SINGLE NUCLEOTIDE POLYMORPHISMS IN CHLOROQUINE RESISTANT GENES OF PLASMODIUM VIVAX AND PLASMODIUM FALCIPARUM CLINICAL ISOLATES

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Background and Aims: Antimalarial drug-resistance is major hurdle for eliminating malaria. So routine surveillance of markers of resistance is useful tool for tracking resistance. Different molecular methods are used for detecting markers of drug-resistance, but these are, time-consuming and require well-equipped labs . So the primary aim of this study is to test LAMP as a, reliable tool for detecting SNPs in Chloroquine resistance genes in field settings.

Methods: In 88-P.vivax and 27-*P.falciparum clinical samples Pvmdr 1*, *Pfcrt* genes of CQ resistance were amplified respectively. Amplified PCR products were sent for Sanger sequencing and analyzed using Clustal W. LAMP primers were designed to amplify mutants over the wild types and conditions for LAMP were optimized. Limit of detection for the assay was detected by calculating copy number of cloned DNA and preparation of serial dilutions. Assay was also tested on other strains including *P.falciparum, L.major, T.gondii* for detecting cross-reactivity

Results: For *Pfcrt* gene 29.6% isolates were wild type , 7.4% were single mutant ($C_{72}V_{73}M_{74}N_{75}T_{76}$) and 62.96% were double mutant ($S_{72}V_{73}M_{74}N_{75}T_{76}$). 100% of the *Pvmdr1 gene* with haplotype M958 Y976 Y1028 L1076 showe double mutation. The assay was 100% sensitive and 100% specific for SNPs detection in Pvmdr1. For *Pfcrt* gene assay was 100% sensitive and 83 % specific. The limit of detection was 0.9 copies/µl for *Pvmdr1* gene and 1 copy/ µl for *Pfcrt* gene.

Conclusions: Observed prevalence of polymorphisms indicates trend towards *P.vivax and P.falciparum* antimalarial drug resistance. Study optimized LAMP for detecting SNPs in *P.vivax* and *P.falciparum* cases in field settings, thus can detect significant hubs of Chloroquine resistance and ultimately helping in management of suitable antimalarial drug policy.

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PARALLEL ORAL ABSTRACT 07 : INTEGRATED APPROACHES TO MALARIA CONTROL: EPIDEMIOLOGY, CO-INFECTION, AND EMERGING TECHNOLOGIES
08-17-2026 4:40 PM - 6:40 PM

SPATIAL REPELLENTS DO NOT SHRINK THE ASYMPTOMATIC PLASMODIUM FALCIPARUM RESERVOIR IN WESTERN KENYA

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Background and Aims: Spatial repellents, which prevent mosquitos from entering houses, were conditionally recommended by the WHO in 2025 after they were shown to reduce the number of infections in children. They are the first new vector control intervention recommended in several decades. Asymptomatic *Plasmodium falciparum* infections are the source of >90% of malaria transmission. We aimed to determine the ability of spatial repellents to reduce the asymptomatic reservoir, and to understand sources of heterogeneity in transmission intensity.

Methods: We conducted a cluster randomized trial in western Kenya. We collected 9187 blood samples in repeated cross-sectional surveys in 10 intervention and 10 placebo clusters and screened them by qPCR and microscopy. The study was powered to detect a 30% reduction in prevalence.

Results: At baseline, prevalence by qPCR was 49.8% and ranging from 26-70% per cluster. Prevalence did not decrease over two years after spatial repellents were implemented in intervention compared to placebo clusters. We observed significant associations between entomological indices of transmission intensity and prevalence. An increase of 10 mosquitos per household per night in the last 3 months resulted in 7.3% higher odds of infection (q=0.032), and a 1% increase in the proportion of fed mosquitos in the last one month resulted in 1.1% higher odds of infection (q=0.002). A random forest's model including maximum precipitation, wetland tree cover, cropland lost to trees, minimum wet-bulb temperature, and maximum precipitation explained 84% of the variation in positivity across clusters.

Conclusions: Entomological and remote-sensing data can explain pronounced small-scale heterogeneity in parasite prevalence.

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OP057

PARALLEL ORAL ABSTRACT SESSION
PARALLEL ORAL ABSTRACT 08 : ADVANCING ANTHELMINTIC DRUG DEVELOPMENT: FROM TARGETS TO THERAPEUTICS
08-17-2026 4:40 PM - 6:40 PM

EVALUATING TRICLABENDAZOLE AND ALBENDAZOLE COMBINATIONS FOR ALVEOLAR ECHINOCOCCOSIS: IN VITRO SYNERGY AND IN VIVO PHARMACOKINETIC BARRIERS

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Background and Aims: *Echinococcus multilocularis*, the causative agent of alveolar echinococcosis (AE), is amongst the most lethal zoonotic helminth infections in the Northern Hemisphere. Current therapy relies on the parasitostatic benzimidazole albendazole, which requires prolonged, often lifelong, administration. This study aimed to identify benzimidazole-based alternatives with improved anti-parasitic activity and to assess their pharmacokinetic behavior *in vitro* and *in vivo*.

Methods: An extended *in vitro* drug-screening platform was developed to accommodate the slow-acting nature of benzimidazoles. Twenty-seven compounds (40µM) were tested against cultured *E. multilocularis* metacestodes by damage-marker release assay and against isolated germinal layer cells by viability assays. Selected candidates and combinations were evaluated in a murine AE model. Mice received 10-week treatments (5d/w) with albendazole (200mg/kg), triclabendazole (100mg/kg), alternating albendazole-triclabendazole regimens, or vehicle control. Drug and metabolite concentrations were quantified in serum and parasite tissue.

Results: Triclabendazole and its metabolites exhibited superior *in vitro* activity against metacestodes (>50% damage after 15d) and markedly reduced germinal layer cell viability (<15% after 5d), whereas ABZ was inactive. Combination treatment suggested synergy *in vitro* based on a Zero Interaction Potency model. *In vivo,* however, no synergy was observed and triclabendazole monotherapy did not

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reduce parasite burden despite high systemic exposure. Triclabendazole-sulphoxide accumulated at lower concentrations in parasite tissue (1h: 0.94μM) than albendazole-sulphoxide (1h: 2.56μM), while displaying higher and more sustained serum concentrations (triclabendazole-sulphoxide, 1h:48.6μM; 7h: 62.4μM; albendazole-sulphoxide, 1h: 26.5μM; 7h: 8.8μM).

Conclusions: Triclabendazole represents a promising candidate for AE therapy, particularly in combination with albendazole, and underscores the importance of host-parasite pharmacokinetics in optimizing benzimidazole-based treatment strategies.

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OP058

PARALLEL ORAL ABSTRACT SESSION
PARALLEL ORAL ABSTRACT 08 : ADVANCING ANTHELMINTIC DRUG DEVELOPMENT: FROM TARGETS TO THERAPEUTICS
08-17-2026 4:40 PM - 6:40 PM

ADVANCING AR102, A NOVEL COMPOUND TARGETING JUVENILE AND ADULT STAGES OF SCHISTOSOMIASIS, TOWARDS CLINICAL CANDIDATE

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Background and Aims: Schistosomiasis affects over 200 million people and remains a major burden in low-resource regions. Praziquantel, the current treatment, kills only adult parasites, allowing juvenile stages to sustain transmission. Mass drug administration mainly reaches children, leaving many infected adults untreated. There is an urgent need for a therapy efficacious against all parasite stages. A new compound class, including the lead candidate AR102, shows single-dose efficacy against juvenile and adult parasites, offering a promising pathway toward more effective and sustainable disease control.

Methods: Ro 13-3978, an aryl hydantoin originally developed by Roche, showed potent anti-schistosomal activity but also antiandrogenic effects. A lead-optimisation programme reduced androgen-receptor interactions while maintaining favourable ADME properties, yielding AR102 as the lead candidate and AR89 as backup. The safety profile was assessed through in vitro and in vivo exploratory toxicology studies. Additional investigations explored the mechanism of action and the disconnect observed between in vitro and in vivo efficacy.

Results: AR102 shows no cytotoxicity, no genotoxicity or phototoxicity, and minimal off-target activity. It demonstrates potent activity against juvenile and adult *Schistosoma* species in mouse models, supported by a long half-life and strong PK/PD profile. PBPK modelling predicts that single-dose human treatments could be effective. Mechanistic studies show that this compound class inhibits parasite evasion of neutrophil extracellular traps (NETosis) and granulocyte responses, enabling effective immune-mediated parasite clearance.

Conclusions: The AR compounds appear to counteract the schistosome elastase inhibitor SmKI-1, enhancing innate immune-mediated parasite clearance. These findings support progression of AR102 toward GLP safety studies and subsequent clinical development.

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OP059

PARALLEL ORAL ABSTRACT SESSION
PARALLEL ORAL ABSTRACT 08 : ADVANCING ANTHELMINTIC DRUG DEVELOPMENT: FROM TARGETS TO THERAPEUTICS
08-17-2026 4:40 PM - 6:40 PM

GROWTH DYNAMICS IMPACT FLUKICIDE SUSCEPTIBILITY IN THE LIVER FLUKE FASCIOLA HEPATICA

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Background and Aims: Migratory juvenile liver fluke cause acute disease yet most flukicides target the adult with only triclabendazole (TCBZ) effective against early juveniles and adults. TCBZ resistance is common and new control options need to target juveniles to counter acute disease. Here we examine the impact of parasite growth dynamics on drug susceptibility and resistance.

Methods: We use an *in vitro* parasite culture and drug screening platform to investigate the growth dynamics of drug resistant and drug suscpetible liver fluke isolates. Using irradiation and gene silencing-based functional genomics we alter parasite growth and expose the profound impacts this has on drug susceptibility. Further studies validate the potential of genes involved in parasite growth as new flukicide targets.

Results: *In vitro* drug-responses in TCBZ-susceptible and TCBZ-resistant field isolates revealed that *in vivo* resistance in juveniles correlated most closely with parasite growth dynamics. The *in vitro* growth of resistant and susceptible fluke field isolates, exposed a strong correlation between growth and drug susceptibility. *In vitro* manipulation of growth using irradiation or RNAi revealed that reducing growth rate in resistant fluke increased drug susceptibility and enhancing growth in susceptible fluke reduced drug susceptibility. The interrogation of genes hypothesised to regulate growth exposed growth dysregulation as a common phenotypic readout, but also revealed lethal phenotypes, including uncontrolled apoptosis encompassing dramatic stem cell dysregulation.

Conclusions: The data support the hypothesis that growth dynamics play a key role in drug susceptibility, and potentially inform resistance phenotypes. They also encourage the dysregulation of parasite growth as an appealing control strategy.

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OP060

PARALLEL ORAL ABSTRACT SESSION
PARALLEL ORAL ABSTRACT 08 : ADVANCING ANTHELMINTIC DRUG DEVELOPMENT: FROM TARGETS TO THERAPEUTICS
08-17-2026 4:40 PM - 6:40 PM

DISCOVERY OF THE ANTHELMINTIC ACTIVITY OF THE CYCLOTIDES FROM THE AFRICAN PLANTS ALLEXIS BATANGAE AND ALLEXIS OBANENSIS (DAACAPABAO)

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Background and Aims: Cyclotides are a family of plant peptides containing a cyclized and knotted cystine framework that exhibit anthelmintic activity against the gastrointestinal parasites Haemonchus contortus and Trichostrongylus colubriformis, two important nematodes in sheep. In this study, we examined the in vitro effects of newly discovered natural cyclotides on the viability of larval and adult stages of these pests.

Methods: In the current study, the presence of cyclotides in *Allexis batangae* and *Allexis obanensis* from the Allexis genus in the Violaceae family was investigated. Five bracelet cyclotides, including three novel cyclotides, Alba A-C, were isolated and characterized using MS-MS analysis and NMR spectroscopy and the potential anti-Alzheimer's activity was evaluated. Cyclotides were isolated and characterized using MS-MS analysis and NMR spectroscopy and the potential Anthelmintic Activity was evaluated.

Results: Five bracelet cyclotides, including three novel cyclotides, Alba A-C, were isolated and characterized. The naturally occurring cyclotides have demonstrated activity up to 18 times greater than that of the prototype cyclotide kalata B1 in nematode larval development assays. In motility tests on adult *H. contortus*, Alba A and C showed significantly greater potency than kalata B1.

Conclusions: Natural cyclotides have been tested for their anthelmintic activity against nematode larvae, and it has been observed that the number of charged residues in their sequence correlates with these activities. These findings are promising and could lead to a new class of agents for combating diseases caused by nematodes.

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OP061

PARALLEL ORAL ABSTRACT SESSION
PARALLEL ORAL ABSTRACT 08 : ADVANCING ANTHELMINTIC DRUG DEVELOPMENT: FROM TARGETS TO THERAPEUTICS
08-17-2026 4:40 PM - 6:40 PM

DEVELOPMENT OF TARGETING STRATEGY FOR THE TREATMENT OF ALVEOLAR ECHINOCOCCOSIS

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Background and Aims: Alveolar echinococcosis (AE) is a hepatic parasitic disease caused by the larval stage of *Echinococcus multilocularis*. The larval stage consists of vesicles with an inner germinal layer containing proliferative cells, enabling its continuous growth, making them the main target for treatment. Current treatment options are limited, relying on invasive surgery followed by parasitostatic benzimidazoles, leaving many patients without curative solutions. This highlights the need for more effective therapeutic strategies. This study aimed at developing a nanoparticle-assisted drug delivery strategy for niclosamide, an *in vitro* active drug against *E. multilocularis*.

Methods: We developed a formulation of niclosamide in lipid nanoparticles (niclo-NP) that exhibited stable physiochemical properties with preserved drug content. Vesicle damage induced by this treatment was evaluated in vitro using an assay quantifying an enzyme within larval vesicles. To assess parasiticidal activity and hepatocyte toxicity, we evaluated viability by ATP quantification after drug exposition on parasite germinal layer cells and HepaRG cell line respectively.

Results: Niclo-NP showed anti-echinococcal activity comparable to free niclosamide, with LC_{50} (concentration with 50 % dead cells) of 0.15 ± 0.04 µM and 0.18 ± 0.01 µM, respectively, indicating that the formulation does not impair drug efficacy. Free niclosamide had an LC_{50} of 0.10 ± 0.02 µM on germinal layer cells, while niclo-NP showed 0.09 ± 0.01 µM. Hepatocyte toxicity yielded an LC_{50} of 3.16 ± 0.33 µM for niclosamide and 3.04 ± 0.43 µM for niclo-NP.

Conclusions: Data obtained show that Niclo-NP represents a promising formulation for improved drug delivery in AE that must be evaluated in vivo.

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OP062

PARALLEL ORAL ABSTRACT SESSION
PARALLEL ORAL ABSTRACT 08 : ADVANCING ANTHELMINTIC DRUG DEVELOPMENT: FROM TARGETS TO THERAPEUTICS
08-17-2026 4:40 PM - 6:40 PM

IDENTIFICATION AND FUNCTIONAL VALIDATION OF A CYTOSOLIC MALATE DEHYDROGENASE AS A DRUG TARGET IN ALVEOLAR ECHINOCOCCOSIS

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Background and Aims: Alveolar echinococcosis (AE), caused by the larval stage (metacestode) of *Echinococcus multilocularis*, is a life-threatening zoonosis with limited drug treatment options. The parasite strongly depends on anaerobic energy metabolism, including the malate dismutation pathway. This study aimed to identify and characterize cytosolic malate dehydrogenase (cMDH) as a potential metabolic drug target in *E. multilocularis*.

Methods: Expression levels of putative *E. multilocularis* cMDH homologs were analyzed by quantitative PCR. The main candidate gene, EmuJ_000417100, was recombinantly expressed and purified, and enzyme kinetics were determined. Functional relevance was assessed through RNA interference in parasite primary cell cultures. Additionally, inhibition studies were performed using the oxidoreductase inhibitor gossypol.

Results: EmuJ_000417100 showed the highest transcriptional expression among predicted cMDH homologs. The recombinant protein exhibited strong catalytic activity, favoring oxaloacetate reduction with low Michaelis-Menten constants (K_m(oxaloacetate) = 16.4 ± 3.2 µM; K_m(NADH) = 11.5 ± 3.9 µM) and high catalytic efficiency ($K_{cat}/K_m = 6.6 \times 10^6\ M^{-1}\ s^{-1}$). Knock-down of cMDH significantly impaired parasite development and proliferation *in vitro*. Gossypol inhibited recombinant cMDH activity in a dose-dependent manner (IC_{50} = 0.28 ± 0.09 µM) and reduced parasite viability (IC_{50} = 6.18 ± 0.24 µM).

Conclusions: This study identifies cMDH as an essential metabolic enzyme in *E. multilocularis* and highlights it as a promising target for novel anti-parasitic drug development.

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OP063

PARALLEL ORAL ABSTRACT SESSION
PARALLEL ORAL ABSTRACT 08 : ADVANCING ANTHELMINTIC DRUG DEVELOPMENT: FROM TARGETS TO THERAPEUTICS
08-17-2026 4:40 PM - 6:40 PM

INTEGRATED INNOVATIVE INTERVENTION STRATEGIES TO ENHANCE ANTI-FILARIAL EFFICACY AND SUPPORT THE ELIMINATION OF RIVER BLINDNESS

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Background and Aims: Mass drug administration (MDA) with ivermectin (IVM) is designed to interrupt onchocerciasis transmission, yet after >20 years of MDA only one country in Africa was verified as free of onchocerciasis, making it unlikely that the WHO 2030 elimination of transmission target will be met with IVM alone. The absence of macrofilaricides or vaccines underscore the need for new integrated treatment strategies that target different parasite stages. Modeling shows that pairing IVM with a macrofilaricide (~60% efficacy) or a prophylactic vaccine greatly increases the probability of elimination compared with monotherapy. We present preclinical evidence supporting integrated, multi-stage treatment strategies.

Methods: Strategies evaluated include: (1) repurposing adulticidal drugs as larvicides; (2) combining adulticidal candidates using one- or two-dosing regimens; (3) pairing larvicidal and adulticidal treatments; and (4) testing vaccine-linked chemotherapy using filaricidal drugs plus a vaccine targeting key filarial antigens expressed across all parasite stages.

Results: Emodepside and oxfendazole, currently in clinical development as macrofilaricides, also displayed strong larvicidal activity. In *B. pahangi*–infected gerbils, early emodepside treatment reduced adult worm burden by ~92% and suppressed fecundity and microfilariae production. We will also show that two treatment schedules with emodepside and oxfendazole-like drug improved efficacy over single-dosing regimens. A fusion vaccine antigen containing Bm-103 and Bm-RAL-2 significantly reduced parasite establishment by 60%. Protection induced by Ov-FUS-1 vaccine is through antibody-, neutrophil-, and complement-mediated mechanisms.

Conclusions: Integrated larvicidal, macrofilaricidal, and vaccine-linked chemotherapy strategies combined with existing MDA programs could more effectively reduce parasite burden, enhance immune-mediated clearance, and accelerate progress toward sustained elimination of onchocerciasis.

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OP064

PARALLEL ORAL ABSTRACT SESSION
PARALLEL ORAL ABSTRACT 08 : ADVANCING ANTHELMINTIC DRUG DEVELOPMENT: FROM TARGETS TO THERAPEUTICS
08-17-2026 4:40 PM - 6:40 PM

BACILLUS THURINGIENSIS CRYSTAL PROTEINS: DEVELOPING AN ARSENAL AGAINST GASTROINTESTINAL NEMATODES

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Background and Aims: Gastrointestinal nematodes (GINs) are the most common parasites of humans and animals and cause significant morbidity among livestock and impoverished humans worldwide. Nematode resistance to the few existing drugs is spreading. New drugs and new ways of thinking are needed to deal with this growing threat. *Bacillus thuringiensis* (Bt) is a natural soil bacterium that has been used successfully for more than 60 years in the biological control of insect pests and is by far the most commonly used biopesticide. We have been pioneering research on nematicidal Cry proteins and continue to identify increasing numbers of anthelmintic Cry proteins that can safely cure GIN infections in animals.

Methods: Here we present ongoing studies to reveal synergistic bioactivity against GINs between nematicidal Cry proteins. We are screening *Ancylostoma ceylanicum* hookworms, *Trichuris muris* whipworms, and *Ascaris suum* roundworms with Cry - Cry protein combinations and Cry - small molecule anthelmintic combinations to study how they interact. In order to generate further novelty, we are investigating the impact of domain swapping on anthelmintic Cry protein activity to speed up the evolutionary processes that occur naturally for Bt to increase efficacy and spectrum of activity.

Results: Here we will present the results of these studies to date, identifying which Cry - Cry and Cry - small molecule interactions show the most promise in control of gastrointestinal nematodes and how our studies to engineer novel anthelmintic Cry proteins is progressing.

Conclusions: Cry proteins alone and in combination show excellent potential for long-term, potent, and durable control of gastrointestinal nematode parasites.

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OP065

TOPIC 2: TRANSMISSION DYNAMICS, EPIDEMIOLOGY, AND GLOBAL HEALTH
EMERGING ISSUES IN CLINICAL GIARDIASIS: FROM PARASITE RESISTANCE TO CHRONIC DISEASE
08-18-2026 10:00 AM - 12:00 PM

WHAT CAN WE LEARN FROM NATURALLY RESISTANT GIARDIA PARASITES ABOUT METRONIDAZOLE RESISTANCE?

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Background and Aims: Giardiasis, a cosmopolitan disease caused by the protozoan parasite *Giardia intestinalis*, is treatable with metronidazole (MTZ). However, MTZ-refractory giardiasis is frequent. It remains unclear, however, whether treatment failure is caused by naturally MTZ-resistant parasites and what is the molecular basis of this resistance.

Methods: We developed a simple 24 h viability assay to assess the *in vitro* MTZ sensitivity of *Giardia* parasites. Followingly, whole genome differences between selected *Giardia* isolates were evaluated, and gene and protein expression was followed upon MTZ exposure. The interaction model was used to separate resistance-associated changes from the resistance-independent response.

Results: We tested 18 *Giardia* clinical isolates. Based on the minimum lethal concentration (MLC), the isolates clustered as sensitive, intermediate resistant, and resistant to MTZ. Interestingly, resistance was only manifested under microaerobic conditions. Two highly resistant isolates (MLC of 400 µg/mL MTZ) originated from patients with MTZ-refractory giardiasis, suggesting that natural parasite resistance likely contributes to treatment failure. However, two additional isolates from patients with MTZ-refractory giardiasis showed in vitro susceptibility. This indicates that treatment failure in giardiasis is multifactorial. Using multiomics characterization of selected isolates, we identified resistance-associated changes (mainly in reduced MTZ activation and enhanced detoxification).

Conclusions: Our study highlights the importance of oxygen concentration during assessment of *Giardia* resistance to MTZ. It also indicates that MTZ-refractory giardiasis may result from reasons other than parasite resistance. Despite limitations in the study design, the multiomics characterization of clinical isolates brought unique insights into the natural resistance to metronidazole.

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OP066

TOPIC 2: TRANSMISSION DYNAMICS, EPIDEMIOLOGY, AND GLOBAL HEALTH
EMERGING ISSUES IN CLINICAL GIARDIASIS: FROM PARASITE RESISTANCE TO CHRONIC DISEASE
08-18-2026 10:00 AM - 12:00 PM

INTERACTIONS BETWEEN DIET AND INFECTION PROMOTE WEIGHT LOSS IN GIARDIASIS

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Background and Aims: Giardiasis is a widespread protozoan infection leading to diarrhea and growth failure, especially in children in low- and middle-income countries. While mice fed standard diets do not exhibit growth failure when infected with *Giardia*, mice fed a diet with reduced calories derived from protein (and replaced with carbohydrate) display infection-dependent growth failure along with intestinal barrier defects. Since signaling through the aryl hydrocarbon receptor (AhR) has been shown to promote intestinal barrier function, we hypothesized that low levels of metabolites activate AhR in mice fed a low protein diet (LPD) would promote growth failure and that supplementation of LPD with tryptophan (Trp) would promote growth.

Methods: Groups of mice were placed on custom diets for 1 week and then infected with Giardia strain GS or a luciferase tagged strain developed in our lab. Weights and infection burden were monitored for 3 weeks.

Results: Our data show that mice fed LPD had reduced plasma levels of three indole compounds known to activate AhR and that supplementation with Trp increased these levels. Supplementation with Trp also eliminated infection-dependent growth failure and increased mRNA levels of the AhR target gene Cyp1a1. While mice fed LPD had higher parasite burdens than mice fed a standard diet, Trp supplementation did not impact parasite clearance, suggesting that effects on barrier function rather than immune responses are mediating growth failure.

Conclusions: These data suggest that diets rich in Trp may benefit children with giardiasis and potentially other enteric infections associated with growth failure.

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OP067

TOPIC 2: TRANSMISSION DYNAMICS, EPIDEMIOLOGY, AND GLOBAL HEALTH
EMERGING ISSUES IN CLINICAL GIARDIASIS: FROM PARASITE RESISTANCE TO CHRONIC DISEASE
08-18-2026 10:00 AM - 12:00 PM

MOLECULAR EPIDEMIOLOGY OF DOMESTICALLY CIRCULATING GIARDIA AND CRYPTOSPORIDIUM IN JAPAN

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Background and Aims: While amebiasis is recognized to spread domestically as a sexually transmitted infection in Japan, the domestic transmission of the other protozoa remains unclear. Here, we sought to characterize domestically transmitting *Giardia* and *Cryptosporidium* using phylogenetic analysis and subtyping.

Methods: We reviewed 624 stool specimens submitted for suspected infectious gastroenteritis (2022–2024). After fluorescent antibody microscopy screening, *Giardia duodenalis* was typed by nested PCR of the gdh gene; *Cryptosporidium* spp. were identified by nested PCR of 18S rRNA and subtyped by nested PCR of gp60, followed by Sanger sequencing and phylogenetic analysis.

Results: Among domestic giardiasis cases (n=10), 9 clustered tightly within Assemblage B and 1 was Assemblage A. Among imported cases (n=13), most were Assemblage B and 2 were Assemblage A. All domestic *Cryptosporidium* infections (n=11) were *C. hominis*, subtyped as IaA14R1 (n=7) or IaA16R5 (n=4). Imported Cryptosporidium cases comprised *C. hominis* IaA25R2 and IeA11G3Tc, *C. parvum* IImA7G1, and *C. meleagridis*, forming lineages distinct from domestic cases.

Conclusions: In Japan, domestic protozoal transmission appears dominated by MSM-associated, person-to-person spread of *Giardia* Assemblage B and *C. hominis* clusters, whereas imported cases reflect different exposure sources. Combining targeted screening with molecular typing enables surveillance of both domestic transmission networks and external introductions.

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OP068

PARALLEL ORAL ABSTRACT SESSION
PARALLEL ORAL ABSTRACT 09 : PARASITE EXTRACELLULAR VESICLES: FROM VIRULENCE FACTORS TO VACCINE DEVELOPMENT
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CIRCULATING EXTRACELLULAR VESICLES AS BIOMARKERS AND IMMUNOMODULATORS OF MURINE MALARIA PROGRESSION

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Background and Aims: Malaria remains a major global health burden, with 40% of the world's population at risk. Disease pathology is shaped by complex host–parasite interactions that regulate immune responses. Extracellular vesicles (EVs) are nanosized cellular carriers of biomolecules (proteins, lipids, DNA/RNA) released into circulation and serving in host-parasite communication. With rising resistance to antimalarial drugs and the absence of an effective vaccine, alternative intervention strategies are needed. EVs have therefore been proposed as novel platforms in vaccine and therapeutic research. This study aimed to characterize plasma-derived EVs during blood-stage malaria in mice and to assess their potential as vaccine and immunotherapeutic agents.

Methods: Plasma-EVs were isolated from C57BL/6 mice infected with *Plasmodium chabaudi* at distinct stages and characterized by Flow cytometry and LC-MS/MS. In vaccination studies, mice received two Intraperitoneal doses of plasma-EVs prior homologous infection. For immunotherapy experiments, infected mice were treated with a single intravenous dose of EVs three days post-infection. Parasitemia was followed by thin blood smear.

Results: EVs cellular origin was determined, and active infection was associated with immune and metabolic pathways, whereas homeostatic immune functions characterized naïve-EVs. Vaccination with log- and naïve-EVs reduced parasitemia. Therapeutic EV administration reduced parasitemia, with the strongest effects observed following treatment with naïve-EVs.

Conclusions: Plasma-derived EVs dynamically reflect the host immune and metabolic state during malaria. In both vaccination and immunotherapy settings, the use of circulating EVs significantly reduced parasite burden in a murine model. These findings highlight circulating EVs as potential biomarkers during infection and as emerging agents in malaria infection control.

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PLASMA EXTRACELLULAR VESICLE PROTEOMIC SIGNATURES REVEAL METABOLIC AND IMMUNE DYSREGULATION ASSOCIATED WITH CEREBRAL MALARIA IN CHILDREN

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Background and Aims: Cerebral malaria (CM) is a severe and frequently fatal complication of *Plasmodium falciparum* infection. Increasing evidence suggests that extracellular vesicles (EVs) contribute to CM pathogenesis; however, the molecular composition of EV cargo associated with neurovascular dysfunction in CM remains poorly characterized. This study aimed to identify EV-associated proteins linked to CM that could serve as prognostic biomarkers or therapeutic targets.

Methods: We performed an in-depth LC–MS/MS proteomic analysis of plasma-derived, purified, and characterized EVs obtained from children with CM, severe-non-cerebral-malaria (SNCM), and uncomplicated malaria (UM). Differential protein expression and disease-specific pathways were analyzed using KEGG pathway enrichment.

Results: EV size, morphology, and total protein concentration did not differ significantly among clinical groups. In contrast, EV protein cargo showed marked qualitative and quantitative differences associated with disease severity, with CM-derived EVs exhibiting the highest number of differentially expressed proteins compared with UM. Functional enrichment analyses highlighted metabolic and

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immune-related pathways. Notably, valine, leucine, and isoleucine degradation was the only KEGG pathway uniquely enriched in CM compared with SNCM, driven by differential expression of enzymes including HSD17B10, ALDH2, ACAA1, and AGXT2. CXCR4 and RPL13 were uniquely detected in CM-derived EVs, whereas AGXT2 was specific to SNCM. Parasite-derived proteins were present at lower abundance, with parasitophorous-vacuolar-protein-1 consistently detected in CM-associated comparisons.

Conclusions: CM is characterized by distinct EV-mediated metabolic and immune signatures rather than changes in EV abundance. Disruption of branched-chain amino acid metabolism and enhanced immune signaling within EV-cargo support a role for EV-driven metabolic–immune crosstalk in CM pathogenesis.

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EXTRACELLULAR VESICLE-MEDIATED DELIVERY OF TOG-CONTAINING TRNA FRAGMENTS BY TRICHOMONAS VAGINALIS HIJACKS HOST TRANSLATIONAL CONTROL

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Background and Aims: Terminal oligoguanine (TOG)–containing 5' tRNA fragments (5'tRFs or tiRNAs) are potent regulators of protein translation in eukaryotic cells, acting through higher-order RNA structures that disrupt the eIF4F initiation complex and induce stress granules. While well characterised in mammalian stress responses, it is unknown whether pathogens exploit this mechanism to manipulate host cell translation. Here, we test the hypothesis that the protozoan parasite *Trichomonas vaginalis* (Tvag) delivers TOG-containing 5'tRFs via extracellular vesicles (EVs) to hijack host translational control.

Methods: Comparative genomics and bioinformatic were used to characterise TOG-containing tRNAs across *T. vaginalis*, other trichomonads, and reference eukaryotes. Small RNA sequencing datasets from Tvag cells and EVs were analysed to identify and quantify TOG-5'tRFs, with selected species quantified by RT-ddPCR under multiple biological conditions. RNA structure formation and functional effects on host cells were assessed using translation reporter assays, eIF4F complex pull-downs, stress granule imaging, and immune-response readouts in human epithelial cells.

Results: Tvag exhibits a marked expansion of TOG-containing tRNAs, a feature conserved among trichomonads. These tRNAs are processed into abundant TOG-5'tRFs, and enriched in parasite-derived EVs. TOG-5'tRFs derived from few tRNAs dominate both cellular and EV RNA pools and display condition-dependent regulation. Functionally, parasite TOG-5'tRFs form tetramolecular RNA complexes, inhibit cap-dependent translation, disrupt the eIF4F complex, promote stress granule formation, and modulate host immune responses.

Conclusions: Our findings reveal a previously unrecognised mechanism by which *T. vaginalis* exploits conserved tRNA-fragment biology to modulate host translation via EV-mediated RNA delivery, uncovering a novel layer of host-parasite interaction with implications for mucosal immunity and disease.

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INTEGRATED EXTRACELLULAR VESICLE PROTEOMICS LINKS CURE AND TREATMENT FAILURE IN IMMUNOCOMPETENT AND IMMUNOSUPPRESSED VISCERAL LEISHMANIASIS

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Background and Aims: Visceral leishmaniasis (VL) is a systemic parasitic disease whose clinical outcome and therapeutic response are shaped by host immune status. Immunocompetent patients usually respond well to therapy, whereas immunosuppressed individuals—particularly those receiving tumor necrosis factor (TNF) antagonists—frequently develop more severe disease and poorer treatment responses. This study aimed to identify immune status–dependent biomarkers of disease activity and treatment response through extracellular vesicle (EV) analysis in immunocompetent humans and anti-TNF-immunosuppressed mice.

Methods: Plasma-derived EVs from immunocompetent VL patients were analyzed during active disease and at 1, 3, and 6 months after successful liposomal amphotericin B treatment using TMT-based proteomics. Serum-derived EVs from *Leishmania infantum*–infected BALB/c mice, treated or not with anti-TNF antibodies, were examined before and after antimonial therapy by label-free quantitative proteomics. Pathway analyses compared EV-associated processes across immune contexts, and selected biomarkers were validated by ELISA in plasma.

Results: In immunocompetent patients, EV proteomes during active VL were enriched in inflammatory, acute-phase, complement, and leukocyte migration pathways, which declined after treatment. Serum amyloid A showed an early marked decrease, emerging as a response biomarker. Parasite-derived *Leishmania* proteins were detected in human EVs and modulated by therapy. In mice, anti-TNF immunosuppression caused persistent dysregulation of EV proteins related to T-cell activation, interferon signaling, liver regeneration, and iron metabolism before and after treatment, consistent with impaired parasite clearance and reduced efficacy.

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Conclusions: EVs capture immune status–dependent molecular signatures that discriminate effective cure from treatment failure in VL. These data support EV proteomics as a translational platform for biomarker discovery and immune-adapted patient monitoring.

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FUNCTIONAL ANALYSIS OF THE TOXOPLASMA GONDII EXOSOME PROTEIN SRS20A WHICH IS MAYBE INVOLVED IN THE INTERACTION WITH HOST CELL PROTEINS

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Background and Aims: Parasite-derived exosomes contain abundant proteins that play critical roles in parasite invasion and host immune evasion. In our previous study, we identified surface antigen 1-related sequence (SRS) proteins within *Toxoplasma gondii* exosomes. These SRS superfamily proteins mediate attachment to host cells, stimulate immune responses, and regulate parasite virulence processes that collectively promote tissue cyst formation in intermediate hosts. Therefore, we aim to identify and characterize additional SRS family members to elucidate their functional mechanisms.

Methods: A novel *T. gondii* SRS protein (designated TgSRS20A) was identified and characterized. Recombinant TgSRS20A (rTgSRS20A) was expressed in *Escherichia coli* and subsequently purified. Antisera against rTgSRS20A were generated. The characterization and functional analysis of SRS20A were performed using indirect immunofluorescence assays (IFA), enzyme-linked immunosorbent assay (ELISA), and gene knockout technology.

Results: Antisera raised against rTgSRS20A specifically recognized the native SRS20A protein in *T. gondii* and its exosomes. IFA revealed that TgSRS20A localizes to both the cell surface and cytoplasm of *T. gondii* tachyzoites. ELISA results demonstrated that SRS20A serves as a viable diagnostic antigen. Protective efficacy evaluation indicated its potential as a vaccine candidate. The SRS20A gene knockout strain exhibited significantly reduced plaque formation on host cells and impaired invasion capacity. Additionally, interaction analysis between SRS20A and host cell proteins identified one cell lysis protein as being highly expressed.

Conclusions: This study demonstrates that TgSRS20A plays a critical role in *T. gondii* invasion of host cells. Further investigations are warranted to elucidate additional potential functions of TgSRS20A in parasite-host interactions.

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EXTRACELLULAR VESICLES FROM HYMENOLEPIS DIMINUTA INDUCE A PHENOTYPE IN MURINE MACROPHAGES THAT PROTECTS AGAINST COLITIS

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Background and Aims: Helminth-derived extracellular vesicles (EVs) can modulate host immunity, including regulation of macrophage function. Infection with *Hymenolepis diminuta* protects mice from DNBS-induced colitis and recent evidence indicates that *H. diminuta*-EVs (Hd-EVs) elicit IL-10, TGF-β, and CCL5 output from murine macrophages. We therefore proposed that macrophages treated with Hd-EVs (i.e. M(Hd-EVs) could inhibit murine colitis.

Methods: Adult *H. diminuta* were incubated in serum-free RPMI media for 6h and EVs isolated from media using the Exoeasy Isolation kit. C57Bl/6 or BALB/c mouse bone marrow-derived macrophages were treated with 5 µg/mL protein Hd-EVs for 24h. One million M(Hd-EV)s were injected interperitoneally into male strain-matched mice 2 days before colitis was induced via intrarectal di-nitrobenzene sulphonic acid (DNBS: 3 mg). Three days later, mice were euthanized and macroscopic disease activity score (DAS) calculated, colon length measured and samples of mid-colon collected for qPCR and histological assessment on H&E stained sections. Spleen cells were isolated and treated with concanavalin-A (5 µg/ml per $5X10^6$ cells) for 48h and IL-10 production was measured by ELISA.

Results: C57Bl/6 or BALB/c mice (n=4-5) treated with M(Hd-EV)s displayed substantially reduced severity of DNBS-colitis assessed by colon length and lower DAS and histopathology scores. qPCR analysis of colon extracts suggested increased *TGF-β* mRNA expression in M(Hd-EV)-treated mice. Spleen cells from M(Hd-EVs)-treated mice showed increased IL-10 expression.

Conclusions: Hd-EVs induce a regulatory phenotype in murine macrophages that exert an anti-colitic effect. We speculate that Hd-EVs are one aspect of the *H. diminuta*-suppression of DNBS-colitis via regulation of macrophages function.

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VAR2CSA-POSITIVE PLASMODIUM FALCIPARUM EXTRACELLULAR VESICLES IMPAIR PLACENTAL B-HCG SECRETION VIA A CSA-INDEPENDENT MECHANISM

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Background and Aims: Background/aims: Placental malaria, caused by *Plasmodium falciparum*, leads to adverse pregnancy outcomes. The disease pathology results from sequestration of infected red blood cells in the placenta via VAR2CSA binding to chondroitin sulfate A (CSA) on Syncytiotrophoblasts. While this adhesion mechanism is well characterized, the impact of *P. falciparum*-derived extracellular vesicles (PfEVs) on placental function remains unclear. This study investigated whether PfEVs from VAR2CSA^{+} *P. falciparum* impair placental β-hCG secretion and whether this effect is mediated by CSA-dependent interactions.

Methods: Methods: Extracellular vesicles (EVs) were isolated from VAR2CSA^{+} (CS2), VAR2CSA^{-} (E8B), and uninfected red blood cells (uRBCs). EVs were characterized by Nanoparticle Tracking Analysis, Transmission Electron microscopy, Western and dot blotting. Syncytiotrophoblast-like cells (STB) were treated with EVs, and β-hCG secretion was measured by ELISA, while cell viability was assessed via Lactate Dehydrogenase release. CSA dependency was evaluated by chondroitinase treatment prior to EV exposure.

Results: Results: PfEVs showed a broad size distribution (146–321 nm) with concentrations of 1.01×10^{10} to 4.2×10^{10} particles/mL. Western blot analysis confirmed the presence of EV-specific markers (Band 3/Stomatin/RESA/Flotillin-1) in the isolated EVs. Treatment of STB with EVs from iRBC or uRBC did not affect cell viability. Conversely, CS2-derived EVs significantly reduced β-hCG secretion ($p<0.0001$), whereas E8B- and uRBC-derived EVs had no effect. Removal of CSA did not modify the inhibitory effect of CS2 EVs on β-hCG secretion, indicating a CSA-independent mechanism suggesting the engagement of an alternative host receptor.

Conclusions: Conclusions: VAR2CSA^{+} *P. falciparum*–derived-EVs impair placental β-hCG secretion through a CSA-independent mechanism, indicating a novel contributor to placental dysfunction.

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EXTRACELLULAR VESICLE MIRNAS IN PLASMA AS BIOMARKERS FOR DISEASE SEVERITY IN CHILDREN WITH SEVERE MALARIA

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Background and Aims: Cerebral malaria (CM) and severe malarial anaemia (SMA) are two major complications of *Plasmodium falciparum* severe malaria. Severe malaria results in hundreds of thousands of deaths in children under age five, with survivors at risk of developing significant motor and neurocognitive impairment (NCI). Pathogenesis of severe malaria is not entirely understood, though, among other cellular and soluble factors, increased extracellular vesicle (EV) levels are associated with disease severity in individuals with CM. As reliable markers of disease severity are not available the aim of this study was to discover new biomarkers of disease severity through the analysis of the microRNA cargo carried by malaria patient EVs.

Methods: EVs contained in the plasma of in Ugandan children with severe malaria (n=4/group: CM with full recovery (FR), CM with fatal outcome (FO), CM with NCI, SMA with FR, and SMA with NCI) and asymptomatic community control children (CC) were extracted and their complete microRNA profile was analysed by next-generation sequencing (NGS). An Benjamini Hochberg (FDR) multiple testing correction of <0.05 was considered significant.

Results: This unbiased approach identified 5 differentially expressed microRNAs, hsa-miR-146a-5p, hsa-miR-21-5p, hsa-miR-152-3p, hsa-miR-30a-3p and hsa-miR-3195. hsa-miR-146a-5p and hsa-miR-21-5p with lower in expression in CM FO than CM NCI children, and were linked to the Malaria KEGG pathways along with several other pathogenic disease pathways.

Conclusions: Despite not identifying candidates discriminating all patient sub-groups, these results highlight EVs and miRNAs as involved in important pathways and further tests on a larger cohort are necessary to expand our findings and ensure reliable and reproducible diagnostic predictions.

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PARASITE COMMUNITIES: PART OF INTEGRATIVE SOLUTIONS TO STOCK IDENTIFICATION

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Background and Aims: Many marine fish species undertake migrations across different life-history stages, which can span thousands of km. These include the passive movement of eggs and larvae to nursery grounds and annual migrations to the feeding/spawning grounds. Fish from different populations or stocks might not overlap spatially or temporally during their respective migrations. As they migrate, they will acquire parasites endemic to the areas they travel through during their life cycle. Fish harbouring similar parasite communities are likely to have migrated to or through the same geographical areas. Therefore, parasites can be of potential use for stock identification. Several commercial species, important to Icelandic fisheries, are found around Iceland with little suspected movement between waters to the south (warm) and north (old), with some evidence suggesting the presence of multiple stocks. However, most species in Icelandic waters are managed as single stocks.

Methods: Here, we apply linear discriminant analyses on parasite community data for haddock and plaice sampled in Icelandic waters, and blue whiting sampled across the northeast Atlantic (from Greenland to the Barents Sea and from Svalbard to the Iberian Peninsula).

Results: The data demonstrate the potential of parasites as biological tags to identify multiple stocks in these three scenarios. Furthermore, the efficacy of parasites is comparable to that using otoliths collected from these same fish (shape and otolith microchemistry).

Conclusions: The high discriminatory power of parasite communities suggests that these should be considered as part of integrative approaches to stock identification for management of marine resources in Icelandic waters and beyond.

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COMPARATIVE ANALYSES OF 18S RRNA GENE FRAGMENTS AND REFERENCE DATABASES FOR ASSESSING BOVINE EIMERIA DIVERSITY USING METABARCODING

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Background and Aims: This study assessed the effects of different 18S rRNA gene fragments and reference databases on bovine *Eimeria* species profiling using metabarcoding.

Methods: A total of 32 diarrheic fecal samples were collected from cattle in the Republic of Korea and were confirmed to be *Eimeria*-positive by light microscopy and conventional PCR. Genomic DNA extracted from each sample was pooled and subjected to amplicon sequencing on the Illumina MiSeq platform (Illumina, San Diego, USA), targeting the V4 and V9 regions of the 18S rRNA gene as well as an *Eimeria* genus-specific 18S rRNA fragment (Pan-Eim-18S). Taxonomic assignment was compared using two reference databases, NCBI and SILVA.

Results: The results showed that Pan-Eim-18S analysis recovered more *Eimeria* species than V4 and V9 regions. In addition, the NCBI database provided higher species-level resolution and identified more *Eimeria* taxa than the SILVA database. To validate the metabarcoding results, conventional PCR followed by cloning was conducted using the same pooled DNA, and phylogenetic analysis was constructed. When compared with metabarcoding results, the species observed in Pan-Eim-18S analysis were most similar to those identified in the phylogenetic analysis.

Conclusions: These findings demonstrate that both target regions and database selection markedly affect *Eimeria* species identification. Further studies are needed to expand reference databases, thereby improving taxonomic resolution.

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BACTERIAL DIVERSITY ASSOCIATED WITH STREBLIDAE FLIES PARASITIZING CAVE-DWELLING BATS FROM NORTHEASTERN BRAZIL

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Background and Aims: Bats (Order Chiroptera) comprise one of the most diverse groups of mammals and play key ecological roles, in addition to serving as reservoirs for a wide range of microorganisms. Among their ectoparasites, bat flies of the family Streblidae (Diptera Hippoboscoidea) are highly specialized, yet their associated bacterial communities remain poorly characterized. This study aimed to characterize the bacterial microbiome of *Trichobius parasiticus* (n = 35) and *T. johnsonae* (n = 33) collected from cave-dwelling bats in northeastern Brazil.

Methods: Bacterial communities were profiled by Illumina NovaSeq 6000 platform sequencing the V3–V4 region of the bacterial 16S rRNA gene, followed by sequence processing using the DADA2 pipeline, OTU clustering with DECIPHER, and taxonomic assignment against the SILVA database (v138.1).

Results: The bacterial microbiota of both species was dominated by Gammaproteobacteria and Alphaproteobacteria, with recurrent endosymbiont-associated genera, such as *Arsenophonus*, *Wolbachia*, and *Bartonella*. Alpha and beta diversity analyses indicated that microbial community structure is shaped by the combined effects of ectoparasite species and sex, with a significant species × sex interaction, whereas species alone did not explain variation in alpha diversity. OTU intersection analyses further revealed higher OTU richness and a greater proportion of exclusive taxa in *T. johnsonae*, as well as greater microbial overlap between males and females of the same species than between species.

Conclusions: These results highlight the importance of host-related biological factors, particularly the interaction between ectoparasite species and sex, in structuring bacterial communities associated with bat flies, contributing to a broader understanding of ectoparasite microbiome host interactions.

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AVIAN THRICHOMONOSIS AS A CONSERVATION THREAT TO ENDANGERED RAPTORS IN THE LEVANT

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Background and Aims: Avian trichomonosis is a worldwide parasitic disease caused by the flagellate protozoan *Trichomonas gallinae*. The parasite colonises the upper respiratory and digestive tracts of birds, where it induces granulomatous lesions that occlude the oesophageal lumen. Often, infection leads to bird's mortality due to severe starvation or asphyxiation. In recent years, nestling mortality due to trichomonosis has been documented in several raptor species in Israel, including Golden Eagles, Bonelli's Eagles, Lanner Falcons, Barbary Falcons, and Long-legged Buzzards. These species have declining populations, are considered regionally threatened, and are the subject of ongoing conservation efforts.

Methods: As part of these efforts, 326 nestlings from 158 nests were sampled for *Trichomonas* diagnosis and molecular characterisation during the 2022-2025 breeding seasons.

Results: Overall, 69% of sampled raptors tested positive for *Trichomonas spp. T. gallinae* was the dominant species (80% of *Trichomonas*-positive samples). Two *T. gallinae* strains (Tag 1 and Tag 2) constituted a persistent and stable population across all raptor species, years and regions sampled. However, other *Trichomonas* taxa (*T. foetus-like* and *T. vaginalis-like*) showed significant temporal, host-specific, and spatial variation, correlating with fluctuations in raptors' morbidity and mortality. In addition, *T. gallinae* Tag 1 pseudocysts were detected predominantly in samples collected from Falcon nests in arid habitats.

Conclusions: Whether the observed spatiotemporal variation in *Trichomonas* strains reflects differences in the raptors' diet across habitats, environmental conditions within breeding territories, the developing immune systems of nestlings, or the opportunistic colonisation capacity of some *Trichomonas* strains remains to be determined.

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METAL ACCUMULATION IN METAZOAN PARASITES AND THEIR HOSTS: OVERARCHING PATTERNS AND LESSONS FROM 30 YEARS OF RESEARCH

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Background and Aims: In the early 1990s, it was first demonstrated that certain parasites, particularly acanthocephalans in fish, can accumulate metals to concentrations several hundred times higher than those in their hosts. This discovery triggered numerous studies on metal accumulation patterns in parasites and their hosts. After roughly 30 years of research in this field, this talk aims to synthesise and highlight overarching trends in metal accumulation across metazoan parasite–host associations.

Methods: Drawing on both original data and published studies, metal accumulation patterns were compared for the main metazoan parasite groups infecting the most species-rich vertebrate classes. The talk will address the suitability of parasites as bioindicators of metal contamination, their potential to reflect the biological availability of pollutants, and their function as pollutant sinks within host-parasite systems.

Results: Across studies, metal accumulation is consistently higher in parasites than in their hosts, and higher in endoparasites than in ectoparasites. Parasites of aquatic hosts, particularly freshwater fish, showed the greatest metal loads, while in mammals the highest levels occurred in terrestrial systems. Across parasite taxa, acanthocephalans and cestodes consistently exhibited the highest absolute metal concentrations.

Conclusions: Historical and contemporary research reviewed indicate that several parasite taxa show distinct metal accumulation patterns that can be explored to improve our understanding of environmental contamination. In particular, the extraordinary metal accumulation capacity of acanthocephalans can substantially reduce metal burdens in their hosts, revealing an unexpected positive effect of parasites that challenges the traditional view of them as purely detrimental organisms.

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OP081

PARALLEL ORAL ABSTRACT SESSION
PARALLEL ORAL ABSTRACT 10 : PARASITES ACROSS SCALES: BIODIVERSITY, TRANSMISSION DYNAMICS, AND GLOBAL HEALTH
08-18-2026 4:40 PM - 6:40 PM

THE ROLE OF SPATIAL SCALE IN MODULATING BIODIVERSITY-DISEASE DYNAMICS FOR THE MOST BURDENSOME INFECTIOUS DISEASES OF HUMANS

Dakeishla (Daki) Díaz-Morales[1,2], Kevin Lafferty[3,4], Armando Cruz-Laufer[5], Soha Ali[6], Alex Mcinturff[4,7], Vivian Hawkinson[2], Chelsea Wood[2]
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Background and Aims: Multiple drivers have been proposed to explain changes in global infectious disease burden, including biodiversity loss. Previous work has found that countries with high biodiversity tend to suffer greater disease impacts. An alternative hypothesis is that negative biodiversity-disease relationships are small-scale phenomena obscured in analyses conducted at the country scale. Understanding how spatial scale affects biodiversity-disease relationships can help to identify effective intervention strategies at the appropriate administrative levels. New data made it possible for us to evaluate associations between diseases and their putative drivers at national and subnational administrative units.

Methods: Thus, we analyzed the relationship between disability-adjusted life years (DALYs) and biodiversity changes as well as other potential drivers, such as population density, percentage of people living in urban areas, wealth, forest land use, transitioned pristine land, latitude, and mean temperature and precipitation at both subnational and national scales using piecewise structural equation models.

Results: Temperature and latitude effects on DALYs were seen only at the national scale, probably because among-nation variation in these variables is much higher than within-nation variation. Counter to expectation, the effects of other drivers were scale-independent, including the effect of biodiversity, which in general tended to be positive.

Conclusions: These analyses suggest that although administrative scales matter for temperature and latitude, a positive effect of biodiversity on disease occurs at both national and first-level administrative scales.

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OP082

PARALLEL ORAL ABSTRACT SESSION
PARALLEL ORAL ABSTRACT 10 : PARASITES ACROSS SCALES: BIODIVERSITY, TRANSMISSION DYNAMICS, AND GLOBAL HEALTH
08-18-2026 4:40 PM - 6:40 PM

HOUSEHOLD-LEVEL PATTERNS OF OPISTHORCHIS VIVERRINI AND SCHISTOSOMA MEKONGI DETECTED USING MULTIPLEX QPCR IN SOUTHERN LAO PDR

Mark June Revolteado[1], Parita Hansana[2], Joseph Valencia[1], Bunthea Chhor[1], Phoyphaylinh Prasayasith[1], Virasack Banouvong[3], Philippe Buchy[2], Moritoshi Iwagami[2], Marcello Sato[4], Megumi Sato[1]
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Background and Aims: *Opisthorchis viverrini* (Ov) and *Schistosoma mekongi* (Sm) remain endemic in Laos, but routine Kato–Katz microscopy misestimates prevalence due to misidentification of Ov eggs as minute intestinal flukes (MIFs) and poor sensitivity for low-intensity Sm. We evaluated a multiplex quantitative PCR (qPCR) assay for simultaneous detection of both trematodiases and assessed household infection patterns.

Methods: A cross-sectional survey was conducted in four endemic villages in Champasack Province in 2024. Stool samples from 1,037 participants, including 343 guardian–child pairs, were examined using Kato-Katz and multiplex qPCR targeting mitochondrial markers for Ov and Sm. Prevalence, co-infections, spatial clustering, and guardian–child concordance were analyzed.

Results: Multiplex qPCR substantially outperformed microscopy, identifying infections missed or misclassified by Kato-Katz. Microscopy failed to detect 22% of Ov infections and misclassified 11% of Ov cases as MIFs, while also missing all Sm infections. qPCR-estimated prevalence reached 38.7% for Ov and 3.3% for Sm. Ov transmission was widespread across all villages and disproportionately affected adults, whereas Sm infections were highly focal, concentrated in a single village, and more common in children. Despite shared living environments, guardian infection status was a poor predictor of child infection, showing only moderate concordance for Ov and low concordance for Sm.

Conclusions: These findings reveal fine-scale heterogeneity in transmission that conventional diagnostics overlook. The contrasting age patterns and weak household concordance indicate that child-centered health education alone is insufficient. Instead, interventions should target adult food-consumption behaviors for Ov and localized water-contact risks for Sm, with household-level strategies guided by molecular surveillance to focus control where transmission is actively sustained.

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OP083

PARALLEL ORAL ABSTRACT SESSION
PARALLEL ORAL ABSTRACT 10 : PARASITES ACROSS SCALES: BIODIVERSITY, TRANSMISSION DYNAMICS, AND GLOBAL HEALTH
08-18-2026 4:40 PM - 6:40 PM

TOXOPLASMOSIS IN SOUTH AMERICA: A HYBRIDIZATION-DRIVEN DISEASE BURDEN?

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Background and Aims: *Toxoplasma gondii*, the causative agent of toxoplasmosis, infects approximately 30% of the world's population and is transmitted to humans by domestic cats and other felids. Although generally considered benign, toxoplasmosis can cause severe disease, particularly in South America. In this region, severe cases also occur in immunocompetent individuals, with ocular manifestations that may lead to blindness, or disseminated forms that can be life-threatening.

Methods: We analyzed a global dataset of 156 *T. gondii* genomes, the largest assembled to date.

Results: We show that, despite the ancient presence of *T. gondii* in South America, modern domestic parasite populations on the continent result from extensive hybridization between domestic lineages of European and African origin, introduced during European colonization, and native South American wild strains. Strikingly, these hybrid strains share a unique, strongly selected haplotype spanning only 0.16% of the *T. gondii* genome, suggesting a key role in adaptation to the domestic environment.

Conclusions: These hybridization events appear to have shifted toxoplasmosis from a zoonosis primarily associated with wildlife exposure to a highly endemic domestic zoonosis, now infecting tens of millions of people in South America. In addition to inherited adaptation to domestic transmission, we hypothesize that these hybrids may also retain key pathogenicity determinants from their wild South American ancestors. The convergence of these two phenotypes (adaptation to domestic environment and augmented virulence) may underlie the disproportionate burden of toxoplasmosis in this region. **Galal L. et al. (2022).** *Nature Communications* **13**, 5778. **Galal L. et al. (2025).** *Trends in Parasitology* **41**:1086–1089.

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OP084

PARALLEL ORAL ABSTRACT SESSION
PARALLEL ORAL ABSTRACT 11 : GLOBAL PARASITES, GLOBAL IMPACT: TACKLING THE WORLD'S MOST BURDENSOME DISEASES
08-18-2026 4:40 PM - 6:40 PM

BUILDING A ONE HEALTH BLASTOCYSTIS NETWORK: OUTCOMES FROM COST ACTION CA21105 (2022–2026)

Anastasios Tsaousis
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Background and Aims: *Blastocystis* is the most prevalent intestinal microbial eukaryote, yet its clinical relevance, transmission routes, and subtype ecology remain contested. COST Action CA21105 (*Blastocystis* under One Health; 2022–2026) aimed to connect medical, veterinary and environmental parasitology with microbiome science to accelerate comparable evidence and practical guidance.

Methods: We coordinated a multidisciplinary network through working groups spanning epidemiology and diagnostics, biology and omics, and stakeholder engagement. Delivery was via funded short-term scientific missions, training schools, workshops, cross-laboratory protocol development, and coordinated communication to researchers, clinicians, and the public.

Results: The Action established an active community of >240 members and prioritised early-career leadership and inclusiveness. We delivered capacity building (10 STSMs, 3 training schools, plus multiple workshops) and produced reusable resources, including protocols, training videos, and outreach materials. Network-led outputs include peer-reviewed papers on prevalence, subtype distribution and One Health transmission interfaces, and a developing plan for a community database to harmonise subtype, host, sampling, and contextual metadata and enable cross-study synthesis.

Conclusions: A structured One Health network can rapidly turn fragmented Blastocystis research into shared methods, comparable data, and fundable collaborative agendas. The Action's legacy is a trained cohort, practical tools, and a roadmap focused on longitudinal multi-omics, curated live isolate collections, minimum requirements for FMT donor screening, and open data infrastructure to resolve Blastocystis' role in health and disease.

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OP085

PARALLEL ORAL ABSTRACT SESSION
PARALLEL ORAL ABSTRACT 11 : GLOBAL PARASITES, GLOBAL IMPACT: TACKLING THE WORLD'S MOST BURDENSOME DISEASES
08-18-2026 4:40 PM - 6:40 PM

MUSSELS TO MOANA: A ONE HEALTH APPROACH TO UNDERSTANDING ENVIRONMENTAL TRANSMISSION OF TOXOPLASMA GONDII IN AOTEAROA/NEW ZEALAND

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Background and Aims: *Toxoplasma gondii* is a zoonotic protozoan parasite found in all ecosystems globally. Infection with the parasite may cause toxoplasmosis in warm-blooded animals, including humans, making it a paradigmatic One Health disease threat. In Aotearoa/New Zealand, Critically Endangered Māui dolphins are known to be particularly susceptible to fatal toxoplasmosis. The leading hypothesis suggests land-to-sea transmission of *Toxoplasma* oocysts is the main source of infection risk to Māui dolphins, with freshwater playing a key transport role in this pathway. In our study, we aimed to use kākahi (freshwater mussels) as *in situ* bioindicators for *Toxoplasma* in rivers to better understand the environmental transmission dynamics of the parasite.

Methods: Over a two-year period, we non-lethally sampled haemolymph (blood) from kākahi every month at three sites within a key freshwater catchment flowing into Māui dolphin habitat. We then analysed haemolymph samples using three different PCR methods to detect *Toxoplasma* oocyst DNA and measure changes in prevalence from month to month. Furthermore, we compared each molecular technique to determine the most effective method for detecting *Toxoplasma* in field-collected haemolymph samples.

Results: We found kākahi were effective bioindicators for *Toxoplasma* oocysts and our sampling method allowed us to track changes in *Toxoplasma* prevalence over time in freshwater environments. We also highlight various benefits and limitations of different PCR methods for detecting *Toxoplasma* oocysts in haemolymph.

Conclusions: Using the One Health approach, our study recognises the interconnections between species and ecosystems to improve our understanding of parasitic disease transmission and further inform conservation efforts for endemic species in Aotearoa.

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OP086

PARALLEL ORAL ABSTRACT SESSION
PARALLEL ORAL ABSTRACT 11 : GLOBAL PARASITES, GLOBAL IMPACT: TACKLING THE WORLD'S MOST BURDENSOME DISEASES
08-18-2026 4:40 PM - 6:40 PM

IMPORTANCE AND EFFECTIVENESS OF IMPROVING TREATMENT BEYOND SCHOOL-AGED CHILDREN IN HIGH ENDEMICITY SCHISTOSOMA MANSONI COMMUNITIES

Christina Faust[1], Alon Atuhaire[2], Andrina Nankasi[2], Moses Arinaitwe[1], Lucy Henshall[1], Olivia Ericsson[1], Candia Rowel[2], Diana Ajambo[2], Andrew Rugigana[2], Moses Adriko[3], Edridah Tukahebwa[2], Poppy Lamberton[1]
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Background and Aims: Hotspots of schistosomiasis persist in Uganda and other sub-Saharan African countries despite nearly two decades of preventive chemotherapy through mass drug administration (MDA). Although praziquantel is donated for free and recommended for community-wide treatment in high endemic areas, coverage remains low and global supplies are limited. A key question is how to optimize drug distribution to maximise success, reduce transmission and minimize heavy infection intensities.

Methods: We conducted a longitudinal survey of 640 individuals aged nine months to 80 years old residing in Bugoto, Mayuge District, Uganda, a high endemic *Schistosoma mansoni* community. Stool samples were collected for Kato-Katz and isolation of miracidia for population genetic analyses. Reinfection and parasite genetic diversity was re-evaluated at five and 18 months after treatment and compared between age groups, alongside travel survey data.

Results: Following national MDA, infection intensities were higher in preschool-aged children (PSAC) and young adults compared to SAC. High infection intensities were associated with water contact frequency and duration, ineligibility for MDA, and young adults systematically not offered treatment. Following observed treatment, SAC had the highest reinfection rates. Examination of parasite genetic structure revealed significant differentiation between age groups but not across timepoints. While SAC are often the focus of control programs, we provide epidemiological and genetic evidence that these target groups can be rapidly re-infected by an untreated reservoir of high-risk PSAC and mobile adults importing parasites.

Conclusions: Improving praziquantel treatment coverage in age groups beyond SAC will help high endemic regions reach targets for elimination of schistosomiasis as a public-health burden.

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OP087

PARALLEL ORAL ABSTRACT SESSION
PARALLEL ORAL ABSTRACT 11 : GLOBAL PARASITES, GLOBAL IMPACT: TACKLING THE WORLD'S MOST BURDENSOME DISEASES
08-18-2026 4:40 PM - 6:40 PM

ONE HEALTH DETERMINANTS OF TICK INFESTATION IN COMMUNAL CATTLE PRODUCTION SYSTEMS OF SOUTH AFRICA

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Background and Aims: Background and Aim: Tick infestation constrains livestock productivity in Africa. This study examined management, socio-economic, and the human and environmental determinants of tick infestation in communal cattle production systems in rural South Africa

Methods: Methods: A cross-sectional study was conducted among 168 cattle-owning households across 17 villages using structured questionnaires to collect data on socio-demographics, tick control practices, and knowledge of tick-borne diseases. Data were analysed using descriptive statistics and mixed-effects models

Results: Results: Most households (82.7%) relied on communal grazing and had small herd sizes (median = 7 cattle). Although 95.8% of respondents recognized ticks as disease vectors, only 54.8% practiced regular tick control. Common tick control methods included pour-on acaricides (61.3%) and hand spraying (51.2%). Acaricide rotation was limited (27.4%). Dipping infrastructure was largely inadequate, with 94.6% of the respondents saying that their dip tanks were either non-functional or partially functional. 58.3% of households were located more than 5 km from a functional facility. Knowledge of tick-borne diseases (3.6%) and zoonotic risks (8.9%) was low, despite frequent human–tick contact (78.0%). Dysfunctional dipping infrastructure ($p < 0.001$), low household income ($p < 0.001$), communal grazing ($p = 0.002$), and irregular acaricide use ($p < 0.001$) were identified as significant predictors of high herd-level tick burden. Economically, 61.9% of households spent over ZAR 1,000 (≈USD 62) annually on tick control.

Conclusions: Conclusion: Persistent tick infestation in communal cattle systems is driven by socioeconomic constraints, dysfunctional dipping infrastructure, communal grazing, and irregular acaricide use

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OP088

PARALLEL ORAL ABSTRACT SESSION
PARALLEL ORAL ABSTRACT 11 : GLOBAL PARASITES, GLOBAL IMPACT: TACKLING THE WORLD'S MOST BURDENSOME DISEASES
08-18-2026 4:40 PM - 6:40 PM

ADVANCING TOWARDS A MULTIGENIC DNA VACCINE BY EVALUATING NEWLY DISCOVERED IMMUNOGENIC PLASMODIUM FALCIPARUM ANTIGENS AGAINST SEVERE MALARIA IN CHILDREN

Pedro José Alcolea[1], Paloma Abad[2], Azam Ghazi[1,2,3], Amalia Buitrago[2], África Vincelle[2], Isabel González-Azcárate[4], Susana Pérez Benavente[2], Amalia Díez[2,3], Sara Pérez-Luz[2,3], Ana Pérez De Ayala[3], Vicente Larraga[1], Julius Fobil[5], André Kazadi[6], Ana Alonso[1], José Manuel Bautista[2,3]
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Background and Aims: Malaria remains a major global health threat, with *Plasmodium falciparum* causing over 600,000 deaths annually, primarily among children in sub-Saharan Africa. Although the RTS/S vaccine is now available, its complex four-dose regimen poses significant logistical challenges for immunization campaigns in endemic regions. To address the need for a more practical and effective solution, we aimed to select antigen candidates to be included in a DNA vaccine based on the antibiotic resistance-free pPAL platform, already endorsed by the EMA for veterinary use (EMA/CVMP/858971/2022).

Methods: IgM-immunodominant epitopes on parasite membrane proteins were identified by screening with high-density peptide microarrays using patients' sera from Ghana and the DRC. Chimeras were obtained by computer-aided design specifically removing A/T-rich sequences involved in immune system evasion. These candidates were cloned into pPAL and their immunogenicity was tested in C57BL/6J mice, including circulating antibody titers, *in vitro* neutralization of *P. falciparum* (growth inhibition assays, GIA), and T cell response in spleen (spectral FACS).

Results: Six antigens exhibiting high-affinity IgM and IgG reactivity were selected. Engineered chimeric sequences were cloned into pPAL. PfAg3 has showed high potency, inducing strong IgM and IgG humoral responses along with the activation of specific Th2 (IL10) and cytotoxic (CD107a) T cell

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responses. GIA assays have confirmed that this candidate significantly inhibits *P. falciparum* replication.

Conclusions: The PfAg3 induces a Th2 and T cytotoxic response and inhbits parasite growth *in vitro* and will be included in the pPAL-based multigenic DNA vaccine. Funding: Thanks to the Spanish Research Agency (AEI) for funding the FALCIVAC P. falciparum malaria project PID2023-149637OB-I00.

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OP089

PARALLEL ORAL ABSTRACT SESSION
PARALLEL ORAL ABSTRACT 11 : GLOBAL PARASITES, GLOBAL IMPACT: TACKLING THE WORLD'S MOST BURDENSOME DISEASES
08-18-2026 4:40 PM - 6:40 PM

EFFECT OF BEHAVIORAL INTERVENTIONS ON SCHISTOSOMIASIS-RELATED KNOWLEDGE, ATTITUDES, AND PRACTICES OF SCHOOLCHILDREN IN PEMBA, TANZANIA: A 4-YEAR REPEATED CROSS-SECTIONAL STUDY

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Background and Aims: Behavior change communication ('BCC'), combined with other interventions is considered essential for reducing *Schistosoma* transmission and achieving elimination. This study evaluated the effect of BCC interventions on schistosomiasis-related knowledge, attitudes, and practices (KAP) among schoolchildren exposed to different frequencies of BCC within the 4-year SchistoBreak project in Pemba Island, Zanzibar, Tanzania.

Methods: From 2020 to 2024, repeated annual cross-sectional surveys were conducted among schoolchildren in grades 3–5 from 18 primary schools. BCC exposure varied by school: one period (4 schools), two consecutive periods (3 schools), two periods with a one-year gap (1 school), or no exposure (10 schools). In 2024, associations between BCC exposure and knowledge, attitude scores, or unsafe washing practices were analyzed using linear and logistic mixed models.

Results: A total of 4,196 children participated. Knowledge and attitude scores improved with increased and continuous BCC exposure. In 2024, knowledge scores were significantly higher among children exposed once (difference = 1.2; 95% CI: 0.6–1.9) or twice (difference = 2.1; 95% CI: 1.4–2.7) compared with unexposed children. Attitude scores were significantly higher only among children exposed twice (difference = 1.2; 95% CI: 0.8–1.6). Washing practices improved overall, independent of BCC exposure. Although up to half of BCC-exposed children were aware of installed washing platforms, usage remained low (7.5–43.1%).

Conclusions: BCC interventions effectively improved knowledge and attitudes among schoolchildren, particularly with repeated exposure. However, gains diminished after intervention gaps or cessation. Sustainable, long-term BCC, integrated with supportive infrastructure, is required to maintain KAP improvements and contribute to schistosomiasis elimination.

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OP090

PARALLEL ORAL ABSTRACT SESSION
PARALLEL ORAL ABSTRACT 11 : GLOBAL PARASITES, GLOBAL IMPACT: TACKLING THE WORLD'S MOST BURDENSOME DISEASES
08-18-2026 4:40 PM - 6:40 PM

URINARY SCHISTOSOMIASIS IN IRAN: A HISTORICAL OVERVIEW

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Background and Aims: Schistosomiasis is a waterborne parasitic disease. The causative agent, Schistosoma haematobium, has been endemic in Khuzestan Province, southern Iran, for many years. Studies conducted in Iran since 1949 have reported between 25,000 and 35,000 cases, although no positive cases were identified in 2000 and 2001

Methods: This study was conducted based on a review of epidemiological data, historical reports, and environmental conditions associated with schistosomiasis in Khuzestan Province. Factors such as the presence of host snails, local residents' behavioral habits, the condition of irrigation systems, and the region's geographical features were examined. The role of the Iran-Iraq border and population movement between the two countries was also considered.

Results: The findings suggest that, despite the absence of new cases over the past two decades, the conditions for the reemergence of schistosomiasis in Khuzestan remain in place. The presence of host snails, shared rivers between Iran and Iraq, constant population movement, and the prevalence of urinary schistosomiasis in some areas of Iraq all increase the risk of reintroduction of the parasite's life cycle. In addition, some areas of the province lack adequate surveillance systems to detect new cases.

Conclusions: Given the environmental and epidemiological conditions that favor the reemergence of schistosomiasis in southern Iran, especially in Khuzestan Province, it is essential for health authorities to implement a strict monitoring and surveillance system. Strengthening the monitoring of irrigation systems, controlling the host snail population, and monitoring suspected human cases can play an effective role in preventing the reemergence of this disease and its health consequences.

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OP091

PARALLEL ORAL ABSTRACT SESSION
PARALLEL ORAL ABSTRACT 11 : GLOBAL PARASITES, GLOBAL IMPACT: TACKLING THE WORLD'S MOST BURDENSOME DISEASES
08-18-2026 4:40 PM - 6:40 PM

TOXOPLASMA GONDII SEROPOSITIVITY AMONG PATIENTS WITH SICKLE CELL DISEASE: PREVALENCE AND ASSOCIATION WITH BLOOD TRANSFUSION HISTORY

Verner Orish
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Background and Aims: Patient with Sickle cell disease (SCD), due to frequent transfusion, might be at higher risk *Toxoplasma (T) gondii* infection. This study aimed at finding the prevalence of *T. gondii* infection and the association of blood transfusion transmission *among* patients with SCD.

Methods: A cross-sectional study involving SCD patients attending clinic at the Ho Teaching Hospital in the Volta Region of Ghana. Rapid Diagnostic Test (RDT), and Enzyme-linked Immunosorbent Assay (ELISA) were used to detect anti-*T. gondii* IgG and IgM. Chi-square and Logistic regression analyses were performed to find any significant association and odds of *T. gondii* infection with blood transfusion respectively.

Results: A total of 156 SCD patients participated in this study of which 124 (79.5%) and 32(20.5%) were HbSS and HbSC respectively. Among the study participants, 105 (67.3%) had a history of blood transfusion. A total of 60 (38.5%) and 83 (53.2%) patients were positive for RDT and ELISA respectively. Blood transfusion had a significant association with *T.gondii* diagnosis (RDT, p= 0.003; ELISA, p= 0.001). A total of 66 (62.9%) of SCD patients who had history of blood transfusion tested positive for ELISA and they had 3 times the odds of testing positive for ELISA (adjusted OR 3.14[95% CI 1.50-6.58]; p =0.002)

Conclusions: Blood tranfused SCD patents showed higher odds of infection in this study. This cross-sectional design allowed estimation of prevalence and associations, but not causality or temporality. More robust longitudinal studies are needed to help elucidate the true contribution of blood transfusion transmission of *T. gondii*.

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OP092

PARALLEL ORAL ABSTRACT SESSION
PARALLEL ORAL ABSTRACT 12 : CELLULAR AND MOLECULAR REGULATION OF PARASITE INVASION AND SURVIVAL
08-18-2026 4:40 PM - 6:40 PM

CHROMATIN HIJACKING IN THEILERIA ANNULATA INFECTION

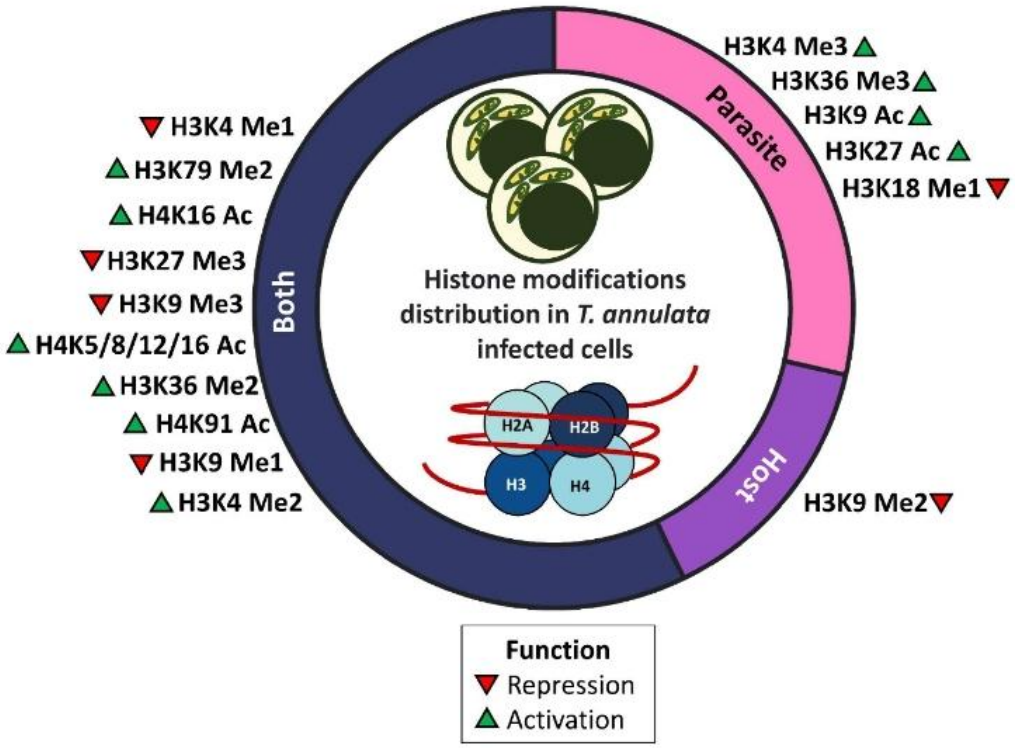
Sakshi Singh
National Institute of Animal Biotechnology, Host Microbe Interactions And Epigenetics Laboratory, Hyderabad, India

Background and Aims: *Theileria annulata*, an apicomplexan parasite of cattle, transforms infected leukocytes into a cancer-like state characterized by uncontrolled proliferation, immortalization, and dissemination. Despite extensive study, the mechanisms by which the parasite suppresses host defense pathways to maintain this transformed phenotype remain poorly defined. This work aimed to investigate how *T. annulata* manipulates the host epigenetic machinery to silence immune and apoptotic responses.

Methods: Infected lymphocytes were treated with a range of epigenetic and p53-modulating compounds to assess changes in histone modifications and the expression of genes governing chromatin regulation.

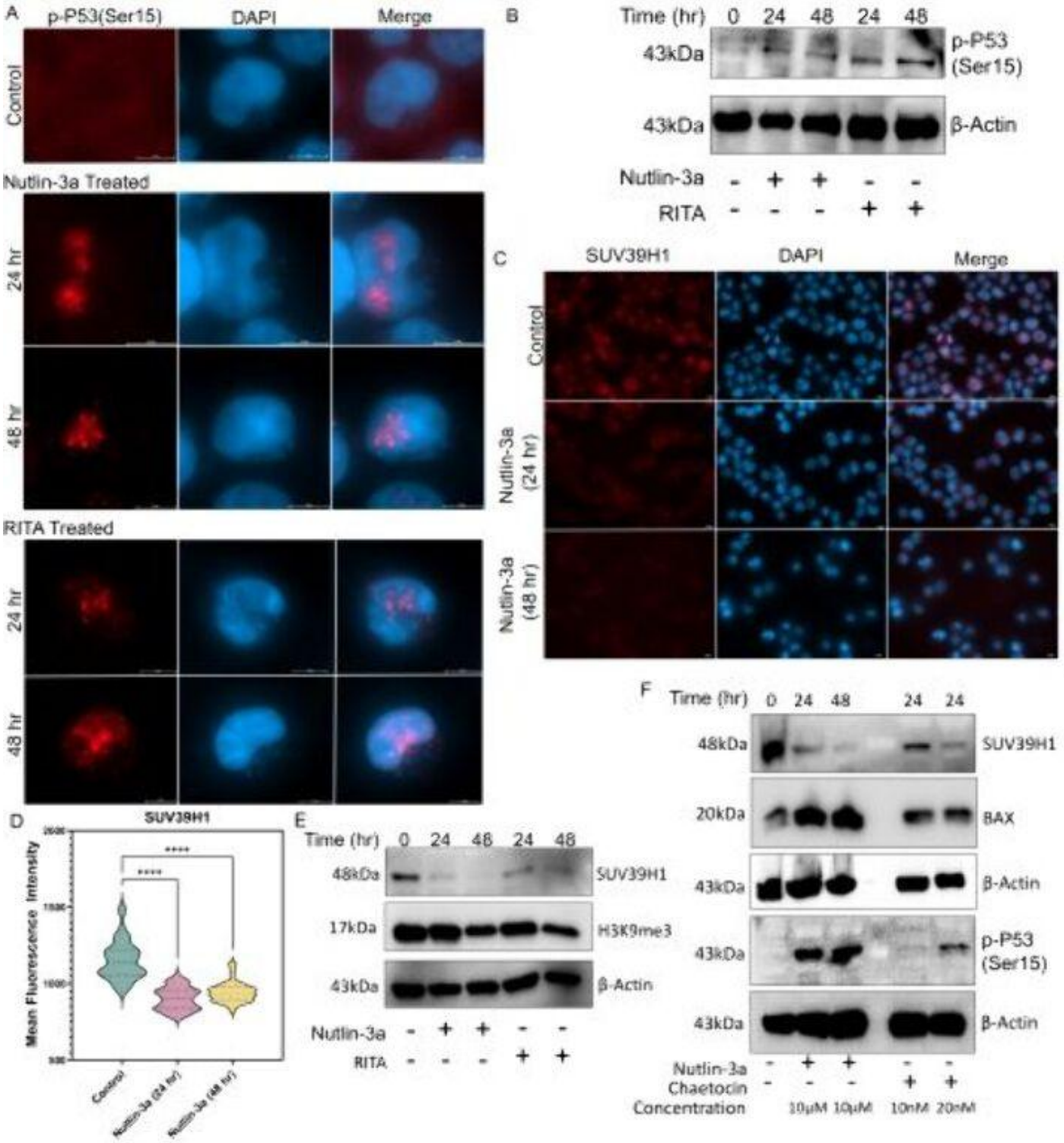
Results: Our findings show that *T. annulata* profoundly remodels the host chromatin landscape, leading to repression of innate immune signaling and p53-dependent apoptotic pathways. Profiling 18 histone modifications revealed strong enrichment of histone marks—H3K4me3, H3K36me3, H3K27ac, H3K9ac, and H3K18me1—predominantly in
parasites.

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Strikingly, parasite clearance rapidly reversed these epigenetic alterations, triggering robust p53 activation and apoptosis, underscoring the parasite's tight epigenetic control over host cell fate.We also identified a potential role for the H3K9me3–SUV39H1–USP7 axis in maintaining p53 suppression.

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Inhibition of SUV39H1 (Chaetocin) or USP7 (P005091) restored pro-apoptotic responses, revealing a chromatin-based mechanism exploited to evade host surveillance. Furthermore, p53-activating compounds such as Nutlin-3 and RITA amplified p53 signaling, including apoptotic, DNA damage response, and cell-cycle regulatory pathways.

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Conclusions: This study uncovers an epigenetic strategy through which *T. annulata* overrides host immune and apoptotic defenses to sustain leukocyte transformation. These insights highlight chromatin modifiers as promising therapeutic targets for restoring host immunity against intracellular pathogens.

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PARALLEL ORAL ABSTRACT SESSION
PARALLEL ORAL ABSTRACT 12 : CELLULAR AND MOLECULAR REGULATION OF PARASITE INVASION AND SURVIVAL
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THE MALARIA PARASITE PLASMODIUM FALCIPARUM VPS15 INTERACTS WITH PHOSPHATIDYLINOSITOL 3-KINASE AND IS CRITICAL FOR HOST-CELL CYTOSOL DELIVERY TO THE FOOD VACUOLE AND APICOPLAST BIOGENESIS.

Alexandra Bourgeois[1], Louis-David Martin[1], Harpreet Kaur[2], Dominic Gagnon[1], Joel Dacks[2], Dave Richard[1]
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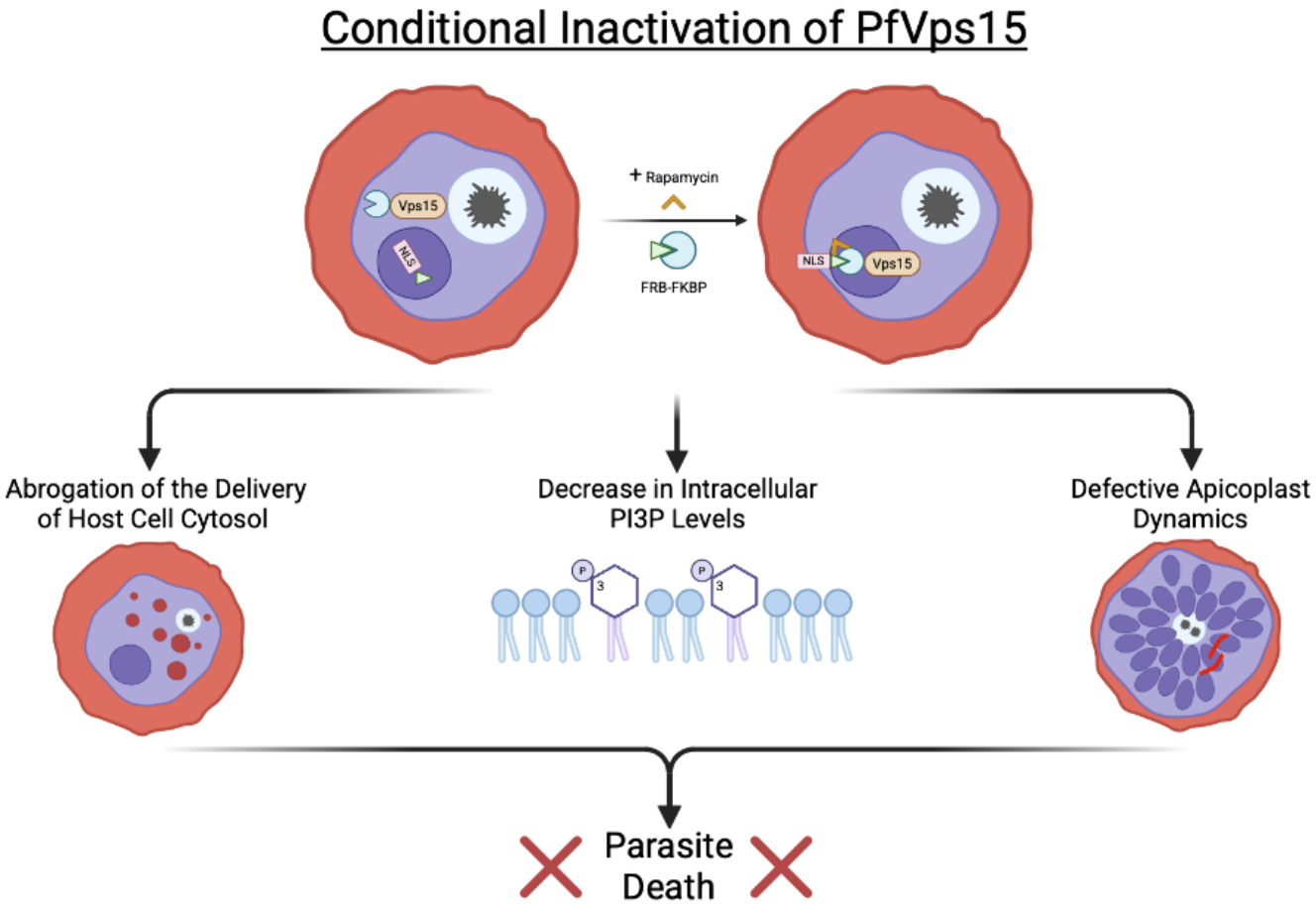
Background and Aims: The malaria parasite *Plasmodium falciparum* (*Pf*) spends an important part of its lifecycle within human erythrocytes. The endocytosis of host-cell cytosol (HCC) and its delivery to the food vacuole (FV) are critical for *Pf*'s survival. Evidence suggests that phosphatidylinositol-3-phosphate (PI3P), found at the FV and the apicoplast, plays a central role. In yeast, PI3P is generated by a single PI3-kinase, whose activity is regulated by the pseudokinase Vps15. *Pf* also possesses a single PI3K and an uncharacterized putative orthologue of Vps15. Our aim is to characterize the role of *Pf*Vps15 in the erythrocytic cycle of *Pf* in order to investigate the role that PI3P plays in *Pf*'s biology.

Methods: Using selection-linked integration, we were able to endogenously tag *Pf*Vps15 allowing the characterization of protein expression, localization and interaction partners. Conditional inactivation of PfVps15 using Knock Sideways was used to further characterize the resulting phenotype.

Results: Immunoprecipitation of PfVps15 showed that it forms a complex with only PfPI3K, contrary to the heterotetrameric complexes found in eukaryotes, suggesting *Plasmodium*-specific adaptations. We determined PfVPS15 to be expressed throughout the erythrocytic cycle and localize near the FV, branching apicoplasts, and some endosomal structures.Conditional inactivation of PfVps15 leads to rapid parasite death, characterized by a decrease in PI3P levels, the abrogation of delivery of HCC to the FV and defective apicoplast biogenesis.

Conclusions: Collectively, our data has identified a protein crucial for the biogenesis of PI3P and provides a direct demonstration for the critical role of this lipid in the HCC uptake pathway and in apicoplast
dynamics.

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AN EXPORTED PROTEIN OF PLASMODIUM KNOWLESI MEDIATES ROSETTE FORMATION

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Background and Aims: In Southeast Asia, the zoonotic malaria parasite *Plasmodium knowlesi* is a growing public health threat. A critical pathogenic mechanism of malaria is rosetting which is the binding of infected red blood cells (iRBCs) to uninfected ones (uRBCs). This causes microvascular obstruction and severe systemic complications in *falciparum* malaria. While rosetting ligands in *P. falciparum* (e.g., PfEMP1) are potential therapeutic targets, those in *P. knowlesi* remain uncharacterized. This study aims to identify the parasite-derived molecular factors responsible for *P. knowlesi* rosetting to provide knowledge on severe disease development and inform future therapeutic development.

Methods: To identify *P. knowlesi* rosetting factors, we enriched rosettes from a single *P. knowlesi* clone using density gradient and performed bulk transcriptomics. We validated candidate rosetting genes via overexpression and also developed a high-throughput Convolutional Neural Network (CNN)-based imaging cytometry method to assess rosetting rates.

Results: A single gene, we designated "*P. knowlesi rosetting-related protein 1*" (*PkRRP1*), exhibited the most significant upregulation based on False Discovery Rate (FDR) correction ($Log_2FC = 13.61$, $q < 0.01$). Overexpression of PkRRP1 resulted in a significant increase in the rosette rate compared to other lines (mean = 9.00%; 95% CI: 1.92–16.07; $p < 0.0001$). Immunofluorescence assays and western blotting for Myc-tagged PkRPP1 revealed export to the iRBC cytosol.

Conclusions: Further identification and characterization of additional molecules involved in *P. knowlesi* rosetting will enhance our understanding of the function, evolution, and diversity of *Plasmodium* virulent factors and their role in pathology of malaria, providing novel targets for therapeutic intervention aimed at mitigating severe disease outcomes.

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HOST-PARASITE INTERACTION: PLASMODIUM VIVAX TRYPTOPHAN-RICH ANTIGEN PVTRAG36.6 OF PV-FAM-A FAMILY INTERACTS WITH RETICULOCYTE RECEPTOR CD71 FOR INVASION PROCESS.

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All India Institute of Medical Sciences, Biotechnology, New Delhi, India

Background and Aims: The limited information on the interaction mechanisms between host and parasite molecules, during the red cell invasion, is an impediment to the development of immuno-therapeutics against the disease caused by the most common human malaria parasite, *Plasmodium vivax*.

Methods: Using tools like Immuno pulldown, Label transfer assay and vitro invasion assays we describe here the identification of the red cell's receptor for one of the *P. vivax* tryptophan-rich antigens called PvTRAg36.6.

Results: We show here that PvTRAg36.6 binds to the human reticulocytes, and the naturally acquired human anti-PvTRAg36.6 antibodies produced during Plasmodium vivax infection, or antibodies raised in rabbit against this antigen, were able to inhibit this receptor-ligand interaction and thus affected the parasite growth in-vitro. This trypsin and chymotrypsin sensitive reticulocyte receptor for PvTRAg36.6 has been identified here as Transferrin Receptor Protein 1, also known as CD71, by two different assays followed by LC-MS analysis. The specificity of this receptor-ligand interaction was confirmed by Bio-Layer Interferometry, Surface Plasmon Resonance, and by competition assay during one-to-one interaction. Dissection of PvTRAg36.6 molecule revealed that its CD71 binding activity was residing in its two different regions i.e. ^{119}KESSWYTWLKGTKK^{132} and ^{228}YFSLWKDHRRKELD^{241}. Multiple amino acid residues from these two binding regions of PvTRAg36.6 were found to be involved in this receptor-ligand interaction.

Conclusions: In conclusion, the identification of CD71 as interacting partner for PvTRAg36.6 will provide new directions in *P. vivax* biology and drug development. Currently, the inhibition of protein-protein interactions is becoming a popular tool for the development of therapeutics.

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PATTERN OF BLASTOCYSTIS SPECIES AND HELICOBACTER PYLORI CO-INFECTION IN COMMON LOWER BOWEL DISORDERS, IN A COHORT OF INDIVIDUALS, DAMMAM, SAUDI ARABIA

Ayman El-Badry[1], Abdullatif Al Rashed1[2], Salma Al Jaroodi[2], Ahmed Alqurayn[2]
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Background and Aims: Lower gastrointestinal disorders (LGD) are among the most prevalent health disorders worldwide, with IBS and IBD being the most common. *Blastocystis* spp. and *H.pylori*, pathobionts with controversial pathogenicity, are two of the most globally frequently found microorganisms in human stool samples; they are implicated in gut disorders.

Methods: It is a hospital-based study. Stool specimens and related data were collected from healthy individuals and patients who have LGD. Stool DNA was extracted from all study samples, and PCR and nnPCR were used to detect *Blastocystis* species and *Helicobacter pylori*, respectively. Data were analyzed using SPSS software

Results: Among the study samples, half were positive for single organisms (*Blastocystis* spp. [16.5%], *H.pylori* [36.0%]), while both organisms were co-detected in 2.9% of patients, and 44.6% were negative. Most *H.pylori* cases (96.7%, P<0.001) and *Blastocystis* spp. (63.3%, P=0.21) were associated with no LGD. Cases of co-detection were seen exclusively in patients with no LGD. None of the patients' demographic and clinical data were associated with the occurrence of *Blastocystis* spp. and *H.pylori* infections among study individuals.

Conclusions: In our study, *Blastocystis* spp. and *H.pylori* were highly prevalent; however, their co-detection is an infrequent phenomenon among patients with or without GI diseases. No significant association between *Blastocystis* spp. and *H.pylori* co-infection and GI disease. Patients diagnosed with IBD were less likely to have *H.pylori* or yield *Blastocystis* spp., which may suggest them as protective factors. There was no association between Blastocystis spp. and a diagnosis of IBS.

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CATALYSIS BY SCHISTOSOMA MANSONI SULFIDE:QUINONE OXIDOREDUCTASE INVOLVES QUATERNARY AND CHARGE-TRANSFER COMPLEX FORMATION

Augustin Kabongo[1,2,3,4], Talaam Kiplangat[5,6], Yuki Tayama[5], Acharjee Rajib[5,6,7], Yuichi Matsuo[3,8], Linh Ha[1,5], Samandram Singh[5], Tetsuo Yamashita[9], Euki Yazaki[10,11], Endah Hartuti[3,6,12], Tetsuro Matsunaga[13,14,15], Tomoaki Ida[15], Tomoyoshi Nozaki[16], Takaaki Akaike[15], Tomoo Shiba[17], Jun-Ichi Kishikawa[17], Shinjiro Hamano[5,6], Kiyoshi Kita[2,3,16], Daniel Inaoka[1,2,3,16]
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Background and Aims: *Schistosoma mansoni* inhabits sulfide-rich environments in the host intestine and mesenteric veins, where hydrogen sulfide (H_2S) is abundant and toxic to mitochondrial respiration. While sulfide detoxification in mammals relies on sulfide:quinone oxidoreductase (SQOR), sulfide metabolism in parasitic helminths remains poorly characterized. A putative SQOR gene exists in the *S. mansoni* genome, but its biochemical function and physiological role are unknown. This study aimed to characterize *S. mansoni* SQOR (SmSQOR), elucidate its catalytic mechanism, and determine its expression and relevance across parasite life stages.

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Methods: Recombinant SmSQOR was expressed and purified from bacterial membrane fractions. Enzyme activity was assessed using quinone reduction assays and sulfane sulfur probe 4 (SSP4). Kinetic parameters, substrate specificity, and optimal conditions were determined. UV–visible spectroscopy and site-directed mutagenesis were used to analyze flavin binding, charge-transfer (CT) complex formation, and catalytic residues. SQOR expression, localization, and activity in parasite eggs, cercariae, and adults were examined by western blotting, immunofluorescence, and SSP4-based assays.

Results: SmSQOR is a functional type 2 SQOR with strict dependence on sulfide, quinone, and a sulfur acceptor. Unlike mammalian SQOR, SmSQOR preferentially utilized sulfite and cyanide and formed unusually stable CT complexes. Two cysteine residues were essential for catalysis and CT complex formation. SQOR was expressed in all developmental stages, localized to mitochondria, and enriched in the tegument of adult worms. Several inhibitors effectively suppressed SmSQOR activity.

Conclusions: SmSQOR showed distinct biochemical properties compared to host SQOR, highlighting divergent sulfide metabolism in *S. mansoni*. Its essential role and parasite-specific features support SmSQOR as a potential therapeutic target.

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LOSS OF DDX3 RNA HELICASE IMPAIRS CELLULAR RESPIRATION AND THE TRANSLATION OF KEY COMPONENTS OF MITOCHONDRIAL ENERGY METABOLISM IN LEISHMANIA

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[1]Research Center of the CHU of Quebec-Université Laval, Quebec, Canada, [2]University of Notre Dame, Notre Dame, United States of America, [3]Carlos Chagas Institute, Curitiba, Brazil

Background and Aims: The DDX3 RNA helicase plays key roles in RNA metabolism, translational control, and cell signalling. In *Leishmania*, genetic inactivation of DDX3 (DDX3 KO) led to the accumulation of reactive oxygen species (ROS) and mitochondrial dysfunction. The present study aims to determine the mechanisms through which DDX3 alters expression of proteins/pathways involved in cellular energy production and mitochondrial homeostasis.

Methods: To investigate DDX3-dependent regulation of mitochondrial metabolism, we combined quantitative proteomics, extracellular flux analysis, RNA immunoprecipitation and sequencing, polysome profiling and qPCR analysis. This integrated approach allowed us to highlight important alterations in metabolic pathways associated with cell respiration.

Results: *Leishmania infantum* DDX3 KO parasites displayed a dysfunctional and 'leaky' electron transport chain (ETC) consuming more oxygen, without generating efficient ATP. Proteomic analyses revealed decreased expression of several ETC components in cells lacking DDX3, mostly of complexes I, II and IV. In contrast, glycolysis, TCA cycle and fatty acid beta-oxidation were increased to compensate for the reduced efficiency of oxidative phosphorylation. RNA immunoprecipitation and sequencing revealed potential DDX3 targets, including mRNAs encoding ETC components. Finally, polysome profiling analysis combined to qPCR validated key protein changes in DDX3 KO using mRNA polyribosome association as a readout of translational efficiency.

Conclusions: Our findings demonstrate a central role for DDX3 in regulating mitochondrial energy metabolism by exerting its regulatory control primarily at the translational level. Function of DDX3 in mitochondrial metabolism is novel and still poorly understood. DDX3 is essential for mitochondrial homeostasis in *Leishmania* and represents a promising drug target.

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MICROBIOME–METABOLOME FINGERPRINTS OF CRYPTOSPORIDIUM INFECTION IN NEONATAL CALVES: CLUES FOR DIAGNOSIS AND INTERVENTION

Pedro Pinto[1], William Edwards[1], Przemek Wieckowski[1], Jerome Follet[2], Anastasios Tsaousis[3]
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Background and Aims: *Cryptosporidium parvum* drives early-life diarrhoea in dairy calves, yet the functional disruption of the gut ecosystem is under-defined. We profiled microbiome and metabolome changes across health, asymptomatic infection, and diarrhoea to identify diagnostic signatures and actionable targets.

Methods: Pre-weaned calves (n=134) from 22 dairy farms were classified by qPCR and clinical scoring into healthy, infected–no diarrhoea, and infected–diarrhoeic groups. Faeces underwent 16S rRNA gene sequencing and untargeted ^1H-NMR metabolomics. Diversity metrics, differential abundance, and metabolite quantification were integrated using multivariate models (sPLS/DIABLO) with mixed-effects testing to account for farm-level clustering.

Results: Infection was associated with a stepwise loss of alpha diversity and depletion of butyrate- and propionate-producing taxa (e.g., *Lachnospiraceae, Ruminococcaceae, Bifidobacterium, Faecalibacterium*), with reciprocal enrichment of opportunists including *Fusobacterium* and Enterobacteriaceae. Metabolomes of infected calves showed lower SCFAs and accumulation of fermentation intermediates (lactate, succinate, formate), consistent with impaired anaerobic energy harvest and mucosal stress. Integrated models linked parasite load to modules coupling SCFA deficits with expansion of facultative anaerobes and explained variance beyond age. A cross-validated panel combining a small set of taxa and metabolites separated groups with high accuracy and generalised across farms.

Conclusions: Neonatal cryptosporidiosis in the field is defined by coherent microbiome–metabolome disruption that scales with clinical severity. These signatures offer non-invasive markers for early risk stratification and point to microbiome-informed interventions—dietary SCFA support and restoration of butyrogenic guilds—to complement hygiene and rehydration. The approach is readily adaptable to on-farm monitoring and interventional trials.

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TRYPANOSOMA CONGOLENSE MANIPULATES THE CHEMOSENSORY SYSTEM AND BEHAVIOR OF GLOSSINA PALLIDIPES AND HOST METABOLITES TO FACILITATE THEIR TRANSMISSION.

Merid Getahun[1], Simon Tawich[1], Christopher Weldon[2], Souleymane Diallo[1], Victor Ndunyu[1], Daniel Masiga[1], Rungua Rungua[1], Steeven Belvinos[1], Antoine Barreaux[1], John Ngiela[1]
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Background and Aims: The success of trypanosomes in transitioning between hosts and life stages depends on their ability to manipulate host metabolism, immunity, and behavior. Pathogen-favoring behaviors often link tsetse flies' host behavioral plasticity and sensory adaptations, but the underlying mechanisms remain unclear. *Glossina pallidipes* were more attracted to *Trypanosoma congolense*-infected mice and metabolites from infected cow urine. *T. congolense* infection alters host metabolic pathways, which enhanced fly attraction. Infection modulated across multiple sensory modalities, including olfaction, vision, taste, thermosensation, and immunity in *G. pallidipes*, potentially favoring trypanosome parasite transmission. Trypanosome associated odors doubled the catch of infected *G. pallidipes*. We reveal the molecular interaction between *T. congolense*, *G. pallidipes*, and cattle, highlighting how the parasite manipulates multiple pathways to enhance adaptation, multiplication, and transmission

Methods: A multi-omics approach was used to study *T. congolense* manipulation in tsetse fly and vertebrate hosts. GC-MS was used to study host metabolites altered by infection. To study the effects of infection on the chemosensory system, transcriptome analysis and RT-qPCR were performed on total RNA from the head of a single female *G. pallidipes*. Tsetse flies, both infected and uninfected, were tested for their behavioral responses to infected hosts and infection biomarker metabolites in the lab and field

Results: *Trypanosoma congolense* alters vertebrate host volatile composition, increasing their attractiveness to *Glossina pallidipes*. *T.congolense* infection modulates the chemosensory system of *G. pallidipes,* specifically increasing odorant receptors, IRs, and Grs while downregulating photoreceptors and immunity gene expression

Conclusions: *T. congolense* modulates host chemistry and various sensory modalities to enhance parasite transmission

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CHARACTERISATION OF THE INTERACTION OF SPECIFIC PFEMP1 VARIANTS AND ENDOTHELIAL CELL RECEPTORS DURING CYTOADHESION OF PLASMODIUM FALCIPARUM INFECTED ERYTHROCYTES

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Background and Aims: A major challenge in developing effective treatments and vaccines against *Plasmodium falciparum* malaria is the parasite's ability to undergo antigenic variation on the surface of infected erythrocytes (iEs). These variant surface antigens enable immune evasion and mediate cytoadhesion to other iEs and to the vascular endothelium, a process that is strongly associated with parasite sequestration and the development of severe disease. Cytoadhesion is primarily driven by members of the *P. falciparum* erythrocyte membrane protein 1 (*Pf*EMP1) family, which are encoded by approximately 60 *var* genes per parasite genome and are expressed in a mutually exclusive manner. To date, more than 24 endothelial cell receptors (ECRs), including CD36, ICAM-1 and EPCR, have been identified as *Pf*EMP1 binding partners.

Methods: To overcome the limitations imposed by antigenic variation, we developed the selection-linked integration (SLI) approach to generate parasite transfectants that express a single, defined *var* gene, resulting in a uniform *Pf*EMP1 population on the surface of iEs. Using this strategy, we have established an expanded SLI-*var* transfectant library that enables systematic analysis of *Pf*EMP1–host receptor interactions under both static and flow conditions.

Results: We analyzed binding profiles of twelve *Pf*EMP1 variants, identifying new partners under static and flow conditions using transgenic CHO cells. Static assays revealed novel interactions, while flow assays characterized binding phenotypes, distinguishing static from rolling adhesion under shear stress.

Conclusions: Ongoing work focuses on *Pf*EMP1 interactions with endothelial cells from various vascular beds, including brain, dermal, kidney, intestinal, and lung, to define tissue specific cytoadhesion profiles relevant to malaria pathogenesis.

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EXPLORATION OF TEMPERATURE-DRIVEN AND CIRCADIAN RHYTHMICITY IN THE PARASITE LEISHMANIA MAJOR

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Background and Aims: Most organisms exhibit 24h rhythms. Some are driven by endogenous clocks, and others by acute environmental responses. In parasitic infections, these daily rhythms influence host-pathogen interactions. This is the case of protozoan parasite *Leishmania spp*. *Leishmania* adapts to the host's body temperature, but it is unclear if it also responds to daily temperature cycles in its sandfly vector. Additionally, *Leishmania* infection is regulated by the host's clock, but whether the parasite itself exhibits circadian rhythms remains unknown. We aim to investigate temperature-driven and endogenous rhythmic oscillations in *Leishmania major*.

Methods: Promastigotes were subjected to daily 25°C-30°C temperature cycles, alike what they experience in sandflies. Then, some cultures remained cycling (AT) and some were released into constant conditions (TT). Parasites were sampled every 4h over 48h for RNAseq, and transcripts were analyzed for rhythmic expression. The experiment was later repeated with targeted LC-MS to look for rhythmic abundance of metabolites.

Results: In AT, 10.7% of the transcripts exhibited ~24h rhythmic expression, a significant proportion (permutation analyses). Follow-up qPCR experiments confirmed acute temperature responses. Pathway analyses linked these transcripts to morphological changes, redox homeostasis, and metabolism. In contrast, under TT, a non-significant number of transcripts showed 24h rhythms (3.2%). Meanwhile, the metabolomic analysis revealed a few rhythmic compounds in each group, and related functional assays are being performed.

Conclusions: Our results suggest *L.major* responds rhythmically to temperature cycles, but these may not entrain a transcriptional circadian clock. Further analysis will clarify post-transcriptional rhythmicity. Understanding *Leishmania* rhythms may set a basis for environmental or therapeutic interventions.

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HOST ENVIRONMENT SHAPES FILARIAL PARASITE FITNESS AND WOLBACHIA ENDOSYMBIONTS DYNAMICS

Coralie Martin[1], Frédéric Fercoq[1], Linda Kohl[1], Julien Plisson[1], Frédéric Landmann[2]
[1]Museum National d'Histoire Naturelle, Paris, France, [2]CRBM, Montpellier, France

Background and Aims: Filarial nematodes causing lymphatic filariasis and onchocerciasis depend on the bacterial endosymbiont *Wolbachia* for reproduction and survival. While *Wolbachia* is a validated therapeutic target, the role of host immunity in this symbiosis is poorly understood. Type-2 immune responses influence filarial fitness, yet their impact on *Wolbachia* dynamics across tissues and life stages remains unclear. Using the *Litomosoides sigmodontis* murine model, we investigated how type-2 immunity shapes *Wolbachia* localization and abundance, and how this affects parasite development, fertility, and transmission

Methods: Wild-type and type-2 immune-deficient (Il4rα^{-}/$^{-}$Il5^{-}/$^{-}$) BALB/c mice were infected with *L. sigmodontis*. *Wolbachia* density was measured by qPCR and visualized by fluorescence in situ hybridization. Germline proliferation and apoptosis were assessed by confocal microscopy. To distinguish immune- from bacteria-driven effects, *Wolbachia* was depleted using doxycycline–rifampicin. Development of Wolbachia-positive and -negative microfilariae was tracked in the arthropod vector and mammalian hosts.

Results: Type-2 immunity selectively reduced *Wolbachia* in female germline tissues, sparing somatic populations, leading to impaired oogenesis, defective embryogenesis, and reduced microfilarial output. Antibiotic depletion phenocopied these defects. Transient *Wolbachia*-free microfilariae developed normally in the vector, but upon mammalian infection, they arrested at the L4 stage, failed sexual differentiation, and could not reproduce.

Conclusions: Type-2 immunity indirectly disrupts filarial reproduction by destabilizing the germline–*Wolbachia* symbiosis. While *Wolbachia* is dispensable in the vector, it is essential in mammalian hosts for larval growth, maturation, and reproductive competence. These results reveal a stage-specific dependency shaped by host immunity and reinforce the therapeutic relevance of targeting *Wolbachia* in filarial disease control.

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COMBINED IN VITRO STRATEGIES FOR CHARACTERIZATION OF P. VIVAX TRAG PROTEINS DURING ERYTHROCYTE INVASION IN THE HUMAN HOST

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Background and Aims: *Plasmodium vivax* (Pv) erythrocytic invasion mechanisms remain poorly understood despite representing an exploitable target for malaria elimination strategies. Leveraging orthologous genes in a Plasmodium knowlesi (Pk) laboratory model overcomes the lack of a continuous Pv culture, enabling functional in vitro studies. The PvTRAg multigene family exhibits high, tightly regulated expression throughout the erythrocytic cycle, triggers immune responses in Pv-infected patients, and is potentially implicated in essential parasite processes. Here, we functionally characterize the role of selected PvTRAgs during erythrocyte invasion using complementary *in vitro* approaches.

Methods: Three complementary approaches were used to unravel the role of a set of PvTRAgs during erythrocyte invasion. Erythrocyte binding capacity of different concentrations of recombinant PvTRAgs (recTRAgs) was quantified in reticulocyte-enriched samples. In parallel, inducible knockouts (iKO) targeting *PkTRAg* genes were generated in a DiCre-expressing Pk line, and growth of iKO clones was monitored for six days after gene excision. For final confirmation, Pk/PvTRAgs invasion role is being evaluated after one Pk reinvasion cycle in presence of recPvTRAgs or anti-PvTRAg camelid single-domain antibodies.

Results: RecPvTRAgs preferentially bind to the youngest reticulocytes, typically in a concentration-dependent manner. Disruption of specific *PkTRAg* genes led to up to 40% growth reduction after six days of monitoring. Evaluation of the involment of PkTRAg/PvTRAg in invasion in the Pk model is ongoing.

Conclusions: This study yields new insights into PvTRAgs' role during erythrocytic invasion, while providing knowledge on PkTRAg ortholog function. Our methodology allows for cross-species comparison of ortholog function and establishes the first application of anti-Pv nanobodies in the Pk model.

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NEW FELINE CELLULAR MODELS TO STUDY INTERACTIONS BETWEEN TOXOPLASMA GONDII AND ITS DEFINITIVE HOST AT THE INTESTINAL LEVEL

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Background and Aims: Toxoplasmosis is a zoonotic parasitic disease caused by the protozoan *Toxoplasma gondii*. This parasite can infect any warm-blooded animal, but its unique definitive host is the cat, where it undergoes its sexual reproduction, with the release of oocysts. Despite its importance in *T. gondii's* life cycle, this step remains largely unknown, mainly due to the difficulties of working with cats experimentally. We developed an *ex-vivo* feline cellular model to decipher this host-pathogen interaction at the intestinal level.

Methods: Feline intestinal explants were used to study the mucosal immune environment in different infection conditions, using the RNAScope® technology. Crypt cells were isolated to generate organoids to decipher the importance of mucosal cytokines in *T. gondii's* life cycle. These intestinal tissues were obtained from recently deceased cats in our veterinary hospital, with consent of the owners.

Results: Th1 and Th2 cytokines were detected in explants with variable expression in different culture conditions and in the presence of parasites. Organoids were developed successfully, regardless of post-mortem harvest time and could be infected with *T. gondii* tachyzoites. Ongoing analysis is investigating how host cytokine responses influence the parasite life cycle.

Conclusions: Intestinal explants and organoids can be developed from recently deceased cats to study *T. gondii*'s sexual life cycle. These unique models will give invaluable insights into this very

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specific host-pathogen relationship. A better understanding of this will lead to improve vaccination strategies targeting cats, to prevent animal and human toxoplasmosis.

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Q586B2 IS AN EVOLUTIONARY CONSERVED TRYPANOSOMATID PROTEIN THAT FACILITATES INFECTION THROUGH IMMUNOMODULATION AND REPRESENTS A PROMISING TARGET FOR VACCINE AND THERAPY DEVELOPMENT

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Background and Aims: Pathogenic trypanosomatids, including *Trypanosoma cruzi*, *T. brucei*, and *Leishmania* species, manipulate host immune responses to establish persistent infections. The parasite factors driving this immune regulation remain poorly defined, yet their identification is critical for understanding disease progression and developing new therapies and vaccines.

Methods: We investigated Q586B2, a previously uncharacterized *T. brucei* protein, for its immunomodulatory potential. Its expression across life stages and conservation in Trypanosomatidae were assessed. A Q586B2-deficient parasite line was generated to evaluate effects on fitness, differentiation, parasitemia, and host survival. IL-10 induction by myeloid cells was tested, and Q586B2-specific Nanobodies (also called single-domain antigen-binding fragments of camelid antibodies) were developed to neutralize this effect. Finally, immunization with Q586B2 was followed by challenge with multiple trypanosome species, including *T. cruzi*.

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Results: Q586B2 is expressed in all *T. brucei* life stages and conserved across trypanosomatids. It induces IL-10 in myeloid cells, promoting parasite invasiveness. Deleting the Q586B2 gene (*Tb927.6.4140*) reduced parasitemia, prolonged host survival, and enhanced short stumpy differentiation without affecting *in vitro* fitness. Nanobody neutralization decreased IL-10 levels and parasitemia, and immunization delayed mortality following infection with multiple trypanosome species, including *T. cruzi*.

Conclusions: Q586B2 is a conserved trypanosomatid protein that promotes infection by modulating host immune responses. Targeting it reduces parasite burden and improves host survival, highlighting its potential as a broad-spectrum therapeutic and vaccine target against human diseases such as sleeping sickness, leishmaniasis, and Chagas disease, as well as veterinary infections like nagana, and could open new avenues for antiparasitic drug development.

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ARCHITECTURAL AND REGULATORY PRINCIPLES OF THE APICAL COMPLEX DRIVING MOTILITY AND INVASION IN APICOMPLEXA

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Background and Aims: Apicomplexan parasites depend on a specialized apical complex to drive motility, host cell invasion, and infection establishment. Central to this system is the conoid, a dynamic cytoskeletal structure that coordinates force generation with regulated secretion of invasion factors.

Methods: Recent advances in nanoscale imaging, proteomics, genome editing, and post-translational modification profiling have redefined the apical complex as a modular and highly regulated organellar system rather than a static ultrastructural feature.

Results: High-resolution analyses reveal the conoid as both a motile scaffold of spiraling tubulin fibers and an organizational hub integrating intraconoidal microtubules, vesicular intermediates, and rhoptry organelles to enable repeated invasion events. Molecular tethers linking the conoid to the apical polar ring underscore the importance of mechanical continuity for motility and rhoptry discharge. In parallel, comparative methylome studies identify lysine methylation as a conserved regulatory layer shaping apical complex architecture, conoid dynamics, and glideosome assembly across apicomplexans.

Conclusions: Together, these findings highlight invasion as an emergent property of coordinated cytoskeletal organization, organelle positioning, and reversible biochemical regulation, revealing conserved vulnerabilities that may be exploited for therapeutic intervention.

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EVOLUTION OF RHIPICEPHALUS MICROPLUS GENES INVOLVED IN ACARICIDE RESISTANCE USING HIGH-THROUGHPUT METATRANSCRIPTOMICS

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Background and Aims: *Rhipicephalus microplus* represents a global constraint to sustainable cattle production, with resistance reported against all major classes of acaricides. Most existing studies are narrowly focused, limiting understanding of the broader evolutionary dynamics of resistance. This study presents a population genomic analysis integrating functional and comparative approaches to resolve the evolutionary architecture of acaricide resistance. By analysing a high-resolution RNAseq dataset comprising over 1,000 individuals sampled across multiple geographical regions, we distinguish convergent from lineage-specific adaptations and link allelic variation to phenotypic resistance.

Methods: Resistance-associated mutations and allele frequencies were inferred from RNA-based metagenomic datasets across South Africa. Analyses targeted novel SNP discovery in acaricide target genes: voltage-gated sodium channel (VGSC), octopamine/tyramine receptor, GABA-gated chloride channel, and acetylcholinesterase (AChE2). Gene architectures were resolved for accurate coding-sequence mapping, and high-confidence variants were structurally modelled to assess effects on acaricide binding and docking *in silico* to the protein target.

Results: Known SNPs in protein coding sequences were detected at appreciable frequencies in both resistant and susceptible populations, as well as several novel coding and regulatory SNPs. Structural and phylogenetic analyses revealed life-stage-specific variants and a distinct target isoforms associated with resistance in both immature and adult ticks. For VGSC and AChE2, overexpression and catalytic-site substitutions altered inhibitor docking, supporting functional resistance mechanisms.

Conclusions: Acaricide resistance in *R. microplus* arises from the combined effects of target-site mutations and regulatory variation under strong selective pressure. This genomic framework enhances molecular diagnostics and informs the rational design of next-generation acaricides.

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EAU DE HAIRWORM: A MULTISCALE APPROACH TO PHEROMONE-DRIVEN BEHAVIOUR IN PARASITES

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Background and Aims: Chemical communication plays a central role in animal behaviour, yet pheromone-mediated signalling remains poorly understood in parasitic helminths, despite its potential importance for reproduction and transmission. Experimental progress has been limited by the scarcity of tractable parasite models. Here, we use the parasitic hairworm *Paragordius varius* (Nematomorpha), which develops within terrestrial insects but exits its host to reproduce freely in water, forming characteristic mating knots that suggest complex chemical interactions among individuals. The aim of this study is to characterise chemical signalling in *P. varius* by integrating behavioural assays with molecular profiling, testing for patterns of attraction and deterrence both within and between sexes.

Methods: Behavioural responses to sex-conditioned water were quantified using two-current choice flumes coupled with automated movement tracking. Lipids and metabolites were extracted from male and female worms and analysed using liquid chromatography–high-resolution tandem mass spectrometry (LC–HR–MS/MS) to identify sex-associated chemical profiles. Candidate signalling molecules were further examined using matrix-assisted laser desorption/ionisation mass spectrometry imaging (MALDI-MSI) to visualise their spatial distribution within parasite tissues.

Results: from experimental choice assays and comparative molecular analyses will be presented, describing behavioural responses to conspecific chemical cues alongside molecular profiles associated with reproductive contexts.

Conclusions: This work establishes hairworms as a novel experimental model for parasite chemical ecology and provides new insights into parasite reproductive communication. By linking behaviour with molecular signatures, this study advances understanding of pheromone-mediated interactions in parasites and highlights chemical signalling as a promising target for future comparative research and control strategies.

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PARTNERS IN TIME: COSPECIATION AND HOST-SWITCHING SHAPE THE EVOLUTION OF LEISHMANIA PARASITES

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Background and Aims: *Leishmania* is a neglected tropical parasite infecting millions worldwide, with disease manifestations ranging from mild to severe in humans and other animals. The genus comprises four subgenera—*Leishmania, Mundinia, Sauroleishmania*, and *Viannia*—each with distinct biological traits. Its origin likely dates to the Mesozoic era, with hypotheses pointing to Palearctic, Neotropical, or Supercontinent origins. Understanding Leishmania's evolutionary history is key to clarifying host specificity, geographic distribution, and vector associations.

Methods: We analyzed *Leishmania* evolutionary associations with vertebrate hosts as well as sand fly vectors using a combination of cophylogenetic and maximum parsimony approaches.

Results: A significant phylogenetic congruence was found between *Leishmania* and its vertebrate hosts using PACo (p=0.0001, m^2_{XY}=0.5419) and ParaFit (p=0.0034, ParaFit Global statistic=0.012), with several host-switching and duplication events (p=0.0298), particularly involving *Leishmania*-humans associations. A cospeciation event was also predicted at a higher taxonomic level, corresponding to the divergence of mammals from reptiles and the subsequent separation of the *Leishmania, Viannia* and *Mundinia* subgenera from *Sauroleishmania*. Similarly, significant cophylogenetic signals were observed between *Leishmania* and sand fly hosts using PACo (m^2_{XY}=1.3988, p=0.0001) and ParaFit (ParaFit Global=0.062, p=0.0001) functions. Cospeciation events were again predicted at higher taxonomic levels in the divergence of New World and Old World sand flies, with *Viannia* species and *Leishmania, Sauroleishmania* and *Mundinia* species associated with the latter.

Conclusions: Our findings support the Supercontinent hypothesis and highlight historical biogeography's role in Leishmania diversification. This analysis provides insights into host specificity, vector adaptation, and the maintenance of its digenetic life cycle.

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IMPACT OF CLIMATE CHANGE ON THE SPREAD OF FASCIOLIASIS INTO THE SOUTHERNMOST EXTREME OF SOUTH AMERICA

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Background and Aims: The impact of global warming on the transmission of fascioliasis, a highly pathogenic zoonotic snail-borne disease, was highlighted during the 2010's. However, few studies have since examined the relationship between climate change and increasing occurrence of outbreaks in endemic regions. This gap may reflect the extreme difficulty of assessing the spread of fascioliasis, as it presents the widest latitudinal, longitudinal and altitudinal distribution of any snail-borne disease. In the Americas, where fascioliasis is exclusively caused by *Fasciola hepatica*, the disease is widespread except in southernmost Patagonia, historically considered unsuitable due to the too low temperatures. Nevertheless, recent evidence suggests an ongoing southward expansion of transmission. This study aims to determine whether climate change has facilitated the southward expansion of the fascioliasis endemic area.

Methods: The long-term evolution of climate factors and transmission risk indices in southern Patagonia was evaluated by means of seasonal-trend decomposition and spatial interpolation analyses.

Results: Our findings revealed substantial regional warming and climatic shifts consistent with the observed expansion, establishing a clear link between a changing climate and the spread of fascioliasis transmission risk into extreme latitudes.

Conclusions: These results provide critical insight into local disease dynamics and emphasize the broader implications of climate-driven changes in the distribution of snail-borne diseases. If current climate trends persist, further geographical expansion is likely. Additionally, we provide some recommendations and cautions regarding the interpretation of two widely applied climatic forecast indices. **Funding**. RYC2024-048462-I (MICIU/AEI/10.13039/501100011033 and ESF+); MSCA (ID 101062347, 10.3030/101062347, HorizonEU, EC); PICT-2017-1361 (FONCYT, ANPCyT, Argentina); CIBERINFEC (CB21/13/00056, MICIU/ISCIII and NextGenerationEU); PROMETEO (CIPROM/2024/88, Generalitat Valenciana, Spain).

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DETECTION OF THE ZOONOTIC NEMATODE ANGIOSTRONGYLUS CANTONENSIS IN INDO-PACIFIC FRESHWATER DECAPOD CRUSTACEANS BY REAL-TIME PCR

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Background and Aims: Angiostrongylosis is a parasitic infection causing eosinophilic meningitis, often associated with severe neurological symptoms and sometimes death. It is linked to the presence of larvae of the zoonotic nematode *Angiostrongylus cantonensis* ingested in raw or undercooked meal or sauces. This nematode was first described in China before spreading throughout the Indo-Pacific region. Its complete cycle occurs in rats (definitive hosts) and mollusks (intermediate hosts). Freshwater crustaceans, especially shrimps and crabs, are also known to act as paratenic hosts and transmit the parasite to humans through consumption. Developing detection tools that are as sensitive and rapid as possible for identifying *A. cantonensis* in crustaceans is therefore essential.

Methods: We developed a real-time PCR for the molecular detection of *A. cantonensis* from 2470 DNA extracts of crustaceans collected across the Indo-Pacific region, by the Museum national d'Histoire naturelle over the past twenty years. The qPCR-positive samples were sequenced by targeting cox1 gene.

Results: We detected the parasite in samples from seventeen different locations by qPCR: New Caledonia (4), Japan (1), Solomon Islands (6), La Réunion (2), Mauritius (1), Fiji (1), Vanuatu (1) and French Polynesia (1). The coxI sequences were successfully obtained from two samples and confirmed that they belonged to the Polynesian strain.

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Conclusions: This work enables highly sensitive detection of the parasite in food products commonly consumed in this region, which is crucial because freshwater shrimps and crabs' consumption is the main source of contamination in French Polynesia. The developed technique will be used to support health services in issuing or updating recommendations.

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HIDDEN PARASITE DIVERSITY IN WILD CARNIVORES: CANIDAE AND MUSTELIDAE AS ZOONOTIC RESERVOIRS IN LITHUANIA

Evelina Maziliauskaitė, Evelina Juozaitytė-Ngugu, Donatas Šneideris, Naglis Gudiškis, Eglė Rudaitytė-Lukošienė, Darija Trekailo, Paula Budrytė, Tamara Kalashnikova, Adomas Ragauskas, Dalius Butkauskas, Petras Prakas
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Background and Aims: Carnivoran mammals are globally distributed and play key roles as predators in terrestrial ecosystems. In Lithuania, the most abundant families of wild carnivores are Canidae and Mustelidae, which include both native and invasive species. These carnivores host numerous parasites, including zoonotic agents of public health concern, and anthropogenic environmental changes may enhance transmission pathways among wildlife, domestic animals, and humans. This study aimed to assess the role of Canidae and Mustelidae as intermediate and definitive hosts for various parasite groups, focusing on parasites detected in muscle tissue and the intestine.

Methods: In total, 198 wild carnivores belonging to the families Mustelidae (115) and Canidae (83) were examined. Morphological methods included muscle digestion, microscopic examination, sedimentation, and flotation of intestinal mucosa and feces. Molecular analyses involved PCR amplification (conventional, nested, multiplex) targeting specific genetic regions (nuclear *18S* rRNA, *28S* rRNA, *ITS1*, *ES5*, mitochondrial *12S* rRNA, *cox1* and apicoplast *rpoB*), followed by Sanger sequencing for species identification.

Results: Multiple parasite groups were detected, including helminths (Nematoda, Trematoda, Cestoda) and protists. Muscle tissue contained the zoonotic parasites *Trichinella* spp., *Alaria alata*, and *Neodiplostomum spathula*. Among *Trichinella*, *Trichinella britovi* was the predominant species, while the highly pathogenic *Trichinella spiralis* was also identified. Intestinal samples harbored zoonotic *Toxocara* spp., *Taenia* spp., and *Alaria alata*, as well as *Mesocestoides* spp. Protists of the genus *Sarcocystis* were detected in muscle tissue and intestinal samples, highlighting their potential impact on meat quality and food safety.

Conclusions: These findings highlight the importance of continued surveillance of wild carnivores under changing environmental conditions.

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NANOPORE SEQUENCING TO ASSESS GENETIC DIVERSITY AND HUMAN-DOG TRANSMISSION DYNAMICS OF SOIL-TRANSMITTED HELMINTHS IN VANUATU

Molly Waldron[1], Lucas Huggins[1], Patsy A Zendejas-Heredia[2], Sze Fui Hii[2], Ian Douglas[3], Clare Anstead[4], Prudence Rymill[5], Fasihah Taleo[6], Susana Nery[7], Vito Colella[4]
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Background and Aims: Soil-transmitted helminths (STHs) cause some of the most prevalent neglected tropical diseases worldwide. Despite this, our understanding of STH diversity across hosts and extent of animal contributions to human infections remains limited, largely due to the scarcity of studies using robust molecular methods.

Methods: We identified STHs of public health significance in 2,175 humans and 148 dogs from Vanuatu using multiplex qPCR. A subset (n=148 each) were sequenced using a novel nanopore-based approach targeting the nuclear 18S gene and ITS1-5.8S-ITS2 region to holistically characterise the 'nemabiome'. Given mounting evidence for zoonotic transmission of *Ancylostoma ceylanicum and Strongyloides stercoralis*, we will sequence a large (>1 kb) region of the mitochondrial cox1 gene in these species to assess haplotype variability and infer transmission dynamics between humans and cohabiting dogs.

Results: Across samples, 48.5% of humans and 97.3% of dogs were infected with at least one major STH. Specifically, a prevalence of 8.9% and 64.9% were observed for *A. ceylanicum* and 3.0% and 47.3% for *Strongyloides* spp. in humans and dogs, respectively. Beyond major STHs, nemabiome analyses revealed additional zoonotic species in humans, including two cases of *Eucoleus aerophilus* and one of *A. caninum*. We also elucidated non-target cestodes, trematodes, and unclassified nematodes in people and dogs.

Conclusions: These findings indicate a high abundance of STHs in humans and dogs across Vanuatu, supporting the need for mass-drug administration. Molecular data suggest canine-human transmission may be occurring in endemic areas, however ongoing sequencing at nuclear and mitochondrial loci will further resolve transmission dynamics and inform control approaches.

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LARVAL MIGRATION IS THE KEY DETERMINANT OF HOST IMMUNE RESPONSES IN PORCINE ASCARIS SUUM INFECTION

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Background and Aims: Helminth infections typically induce robust Th2 responses. We previously observed a Th2 response in the lungs during porcine *A. suum* larval migration, and a delayed Th2 response in the intestine after larvae returned. In contrast, type 2 signatures were weak in the liver and blood of *A. suum*-infected pigs. However, it remains unclear how different infection regimens influence migration-associated Th2 responses and the final adult worm burden. This study, therefore, examined dose-dependent local and systemic type 2 responses during larval migration and in the presence of adult worms.

Methods: Conventionally raised German or Belgian landrace pigs were infected with *A. suum* using bolus or trickle inoculations at different doses. Flow cytometry and ELISA characterized type 2, germinal center, and antibody responses in blood and migration-affected tissues. Eosinophils and goblet cells were addressed by histopathology. Adult worm burden was determined at necropsy.

Results: Larval migration caused a systemic type 2 response in high-dose bolus and trickle-infected pigs. When adult worms were present, all initial dose-dependent differences disappeared in blood, highlighting the early phase as the critical window for Th2 imprinting. However, bolus-infected pigs exhibited local dose-dependent Th2, eosinophil, goblet cell, and germinal center responses in mesenteric lymph nodes, whereas these responses were dose-independent in trickle-infected pigs. Despite immunological variations, the adult worm burden was similar across all groups.

Conclusions: Our findings identify larval migration as the key factor influencing the anti-*Ascaris* immune response. Early type 2 responses shape later intestinal immunity, while delayed intestinal Th2 activation likely aids in adult worm establishment and survival.

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PARALLEL ORAL ABSTRACT SESSION
PARALLEL ORAL ABSTRACT 15 : ADVANCES IN DIAGNOSTICS AND BIOMARKER DISCOVERY FOR PARASITIC DISEASES
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ASYMPTOMATIC SCHISTOSOMA MANSONI INFECTION ROBUSTLY ASSOCIATES WITH ALTERED TYROSINE METABOLISM IN THE PLASMA OF SCHOOL-AGED CHILDREN

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Background and Aims: Schistosomiasis elimination relies on controlling morbidity before moving towards transmission interruption, with infection levels measured by microscopy used to define targets. However, microscopy lacks sensitivity in low-prevalence, low-burden settings and post-repeated praziquantel mass drug administration. This study aimed to identify improved infection and morbidity markers for use in subclinical/preclinical *S. mansoni* infections.

Methods: We conducted untargeted LC-MS metabolomics on plasma obtained from school-aged children (SAC) in a rural Cameroon *Schistosoma mansoni*-endemic area, focusing on 'asymptomatic' *S. mansoni* infected SAC (defined by stool egg microscopy and no periportal liver fibrosis by point-of-care ultrasonography) versus rigorously matched *S. mansoni* uninfected controls (egg and circulating anodic antigen (CAA)-negative; no periportal liver fibrosis).

Results: A robust three-phase approach ensured reproducibility. Discovery (n=10 infected vs. n=10 uninfected controls) used univariate (volcano plots, FC≥1.2, p<0.1) and multivariate (PLS-DA)

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analyses, yielding metabolic separation (PERMANOVA R^2=0.029, p=0.588). Targeted validation in an independent cohort (n=23 infected vs. n=14 uninfected controls) applied stricter criteria (FC≥1.5, p<0.05 adjusted), confirming distinction (R^2=0.263, p=0.001). The effect persisted (R^2=0.293, p=0.033) after a polyparasitism adjustment in a third cohort (n=7 infected vs. n=5 uninfected controls with co-endemic malaria, hepatitis B, and/or soil-transmitted helminths; FC≥2, p<0.05), with pathway enrichment highlighting tyrosine metabolism disruptions.

Conclusions: These phases reveal a consistent plasma tyrosine metabolism signature for subclinical/preclinical *S. mansoni* infections, resilient to polyparasitism affects. This non-invasive metabolomics approach offers a sensitive discovery platform for diagnostics, therapeutic targets, and monitoring in evolving low-burden epidemics.

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THE ADDED VALUE OF SERUM CIRCULATING ANODIC ANTIGEN DETECTION FOR THE CLINICAL MANAGEMENT OF SCHISTOSOMIASIS IN A NON-ENDEMIC SETTING

Linda Wammes[1], Christel Van Zeijl-Van Der Ham[1], Stan Hilt[2], Maureen Blokland-Flink[2], Claudia De Dood[1], Danielle De Jong[3], Govert Van Dam[2], Paul Corstjens[3], Pytsje Hoekstra[2], Lisette Van Lieshout[2]
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Background and Aims: Diagnosing imported schistosomiasis remains challenging, mainly due to the lack of a gold standard. A test of cure is also urgently needed, to assess efficacy of current and possible future drugs. Assessment of the *Schistosoma*-specific circulating anodic antigen (CAA) in serum has been demonstrated to confirm active infections in traveler as well as migrant cohorts. CAA levels rapidly decline after antischistosomal treatment in the majority of patients. Here, we present our experiences with serum CAA testing since its introduction as a routine test in our clinical diagnostics setting.

Methods: CAA concentrations measured by the 0.5 mL serum UCP-LF assay were analyzed in a retrospective cohort, covering a four-year period.

Results: From a total of 1130 requests, 560 (49,6%) followed a positive antibody outcome and 570 were specifically requested, mostly for laboratories elsewhere in Europe. Of the antibody-positive initial patient sera tested for CAA (n=496), 146 were positive, leading to a presumed active infection percentage of 29%. For a selection of patients with positive CAA (n=69), multiple serum samples were available. Of these, 34 (49%) turned negative in the first (assumed) post-treatment sample. A number of cases needed additional treatment to negativize CAA.

Conclusions: Serum CAA testing is a promising tool for distinguishing active from past infections and for monitoring treatment responses, which is relevant for the clinical management of imported schistosomiasis. Prospective studies are needed to further confirm the added value of serum CAA for diagnosis and as test of cure.

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SERUM IRON AND INFLAMMATORY BIOMARKERS AMONG PREGNANT WOMEN STRATIFIED BY MALARIA AND ANAEMIA STATUS IN SOUTHERN GHANA

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Background and Aims: Iron deficiency and inflammation commonly coexist among pregnant women in malaria-endemic regions, complicating the interpretation of iron biomarkers. In sub-Saharan Africa, asymptomatic *Plasmodium falciparum* infection during pregnancy may alter nutritional and inflammatory indices, potentially leading to underestimation of iron deficiency when inflammation is not considered. This study investigated the interactions between asymptomatic malaria, inflammation, and iron status among pregnant women attending antenatal clinics in Southern Ghana.

Methods: In this cross-sectional study, participants were categorized into four phenotypes based on PCR-confirmed *P. falciparum* infection and anaemia status: malaria with anaemia *(Mal+Anae)*, malaria without anaemia *(Mal+NoAnae)*, anaemia without malaria *(NoMal+Anae)*, and neither malaria nor anaemia (*controls*). Serum ferritin, soluble transferrin receptor (sTfR), C-reactive protein (CRP), and α-1-acid glycoprotein (AGP) were measured using ELISA. Iron deficiency and iron deficiency anaemia were determined before and after adjusting ferritin for inflammation.

Results: *Mal+NoAnae* had significantly higher ferritin levels than *NoMal+Anae* and *Mal+Anae* ($p<0.05$). Ferritin levels were comparable between *Mal+NoAnae* and controls. sTfR concentrations did not differ significantly across groups. Although CRP differences were not statistically significant, higher levels were observed among *Mal+NoAnae*. AGP was significantly elevated in *Mal+NoAnae* compared with *NoMal+Anae* and *Mal+Anae*. Iron-deficiency prevalence increased from 45.6% to 73.3% after adjusting for inflammation, while iron-deficiency anaemia rose from 37.3% to 55.3%. The increase was particularly marked among women with asymptomatic-malaria.

Conclusions: Asymptomatic-malaria is associated with inflammation-mediated alterations in iron-biomarkers during pregnancy. Adjusting for inflammatory markers substantially increases the estimated burden of iron deficiency, underscoring the need to incorporate inflammation assessment into iron status evaluation in malaria-endemic settings.

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THE REDUCTION OF UNWANTED AMPLICONS FOR PARASITE DETECTION IN METABARCODING OF WILD UNGULATE FECAL SAMPLES

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[1]Public Health Agency of Canada, National Microbiology Laboratory, Guelph, Canada, [2]University of Guelph, Pathobiology, Guelph, Canada

Background and Aims: Metabarcoding is a powerful tool for detecting intestinal parasites, but its effectiveness can be limited by overwhelming amplification of non-target organisms. A novel near-complete 18S rRNA metabarcoding assay, sequenced with Nanopore long-read sequencing, performed well on omnivorous mammals and clinical human samples containing *Blastocystis* spp., *Cryptosporidium* spp., and *Cyclospora* spp. However, sequencing of wild ungulate samples resulted in high proportions of plant and fungal reads that obscured parasite detection. This study aimed to improve the sensitivity and specificity of the 18S metabarcoding assay for characterizing parasite communities in northern ungulates.

Methods: A novel PCR method, Suppression/Competition PCR, was developed to minimize the detection of non-target organisms and metabarcoding was conducted with and without Suppression/Competition PCR for comparison. Nested PCR assays targeting *Cryptosporidium* spp. and *Giardia* spp. were performed in parallel to enhance detection of low-abundance protozoa.

Results: Parasites of interest detected via metabarcoding include *Blastocystis* spp., *Entamoeba* spp., *Eimeria* spp., and various nematodes. Of the samples sequenced under both metabarcoding conditions, 9/15 (60%) yielded 1 or more parasite taxa detected exclusively with Suppression/Competition PCR, as a result of a >99% reduction in plant and fungal reads. Nested PCR detected multiple *Cryptosporidium* species and *Giardia bovis*.

Conclusions: Suppression/Competition PCR substantially improved the performance of 18S metabarcoding on ungulate fecal samples by reducing non-target amplification. Combined with long-read sequencing, this approach enhanced taxonomic resolution and can play a role in parasite surveillance in wildlife, supporting One Health-focused monitoring in northern ecosystems.

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TRYPONE: NEXT-GENERATION CRISPR-LAMP-BASED MOLECULAR DIAGNOSIS OF HUMAN AND ANIMAL TRYPANOSOMOSIS

Andrés Álvarez-Rodríguez[1,2], Bo-Kyung Jin[1], Pieter Thomassen[1], Adrián Muñoz-Aranzueque[1], Unhwa Lee[1], Zeng Li[1,3], Benoit Stijlemans[1,4], Peter Geldhof[2], Stefan Magez[1]
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Background and Aims: Human and animal trypanosomosis caused by *Trypanosoma brucei* remains a major global One Health challenge. While effective control relies on accurate detection of active infections, existing point-of-need (PON) tests fall short of the performance achieved by gold-standard laboratory-based PCR assays. This study aims to develop a sensitive, specific, and field-deployable CRISPR-LAMP-based assay for pan-specific detection of active *Trypanosoma brucei* infections, termed the TrypOne test.

Methods: A CRISPR-Cas12b cleavage assay was developed for sequence-specific detection of Loop-Mediated Isothermal Amplification (LAMP) amplicons targeting the conserved, high-copy RIME retrotransposon of *T. brucei*. The CRISPR and LAMP components were integrated into a single one-pot reaction, constituting the TrypOne test. Following optimization, sensitivity and specificity were determined, and the ability to detect ongoing infection and monitor treatment response was evaluated in a mouse model.

Results: The TrypOne (one-pot CRISPR-LAMP) test enabled pan-*Trypanozoon* detection across *T. brucei* strains of human and animal origin under isothermal 55 °C within 60 min. By integrating CRISPR-Cas12b, it overcame the inherent false-positive limitation of RIME LAMP while detecting ≤1 trypanosome equivalent per reaction, with a naked-eye fluorescent readout. In murine infections, the TrypOne test reliably tracked ongoing parasitaemia and treatment response, achieving 100% specificity and 97.62% sensitivity, and showed concordant performance with the gold-standard TBR PCR.

Conclusions: The TrypOne test enables highly sensitive and specific CRISPR-LAMP-based pan-*Trypanozoon* detection under field-compatible isothermal conditions with a naked-eye fluorescent readout. It provides molecular confirmation of active infections and enables monitoring of treatment

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response, making it a reliable One Health tool for coordinated control of human and animal trypanosomosis.

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A CELL-FREE DIAGNOSTIC ANTIGEN DISCOVERY SYSTEM FOR FOX TAPEWORM INFECTIONS IN HUMANS AND OTHER GREAT APES

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Background and Aims: Alveolar echinococcosis is one of the most severe parasitic zoonoses caused by *Echinococcus multilocularis*. To date, the diagnosis is based on a combined approach of imaging technologies, serology, molecular methods, and histopathology. Furthermore, gorillas are highly susceptible to the disease. In primates, early serological screening is not yet feasible due to a lack of diagnostic markers.

Methods: We designed plasmids for a cell-free recombinant system to screen the total *Echinococcus multilocularis* metacestode secretome. By proteomics, we analyzed the constitutively expressed larval secretome, consisting of 70 proteins. These proteins were produced and directly screened with sera from patients and great apes for novel diagnostic candidates by an optimized proteome array in ELISA-like format.

Results: We were able to construct a novel high-throughput cell-free diagnostic candidate screening system to validate an entire proteomic dataset with minimal time requirements. In an initial attempt, over 64% of the proteins could be produced, which is currently further optimized. For alveolar echinococcosis in humans with a long history in antigen discovery, no novel candidates could be detected in the secretome. For great apes, the system re-discovered an old marker (Em95), which has never been evaluated so far in gorillas.

Conclusions: We have developed a low cost and time optimized, two-day diagnostic screening system for biomarker discovery which can easily be translated to other parasite secretomes or diagnostic pipelines. With costs of approximately 350.- CHF (+-450 USD) per candidate, our pipeline is as cost-efficient as classical recombinant protein production, but without the frustration of finding non-diagnostic proteins.

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DIAGNOSTIC PERFORMANCE OF A PROTOTYPE VISUAL FLOW-S ASSAY IN A KENYAN FIELD SETTING: A LARGE-VOLUME URINE CAA POINT-OF-CARE TEST FOR SCHISTOSOMIASIS

Pytsje Hoekstra[1], Lisette Van Lieshout[1], Maurice Odiere[2], Frederick Rawago[2], Gladys Odhiambo[2], Jay Warrick[3], Cody Carrell[3], Mitch Geiger[3], Dylan Duerre[3], Brianna Mullins[3], Govert Van Dam[1]
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Background and Aims: Accurate, sensitive, and field-deployable diagnostics are essential to support control and elimination of schistosomiasis. Current diagnostics, including microscopy, lack sensitivity, particularly in detecting low intensity infections. Detection of circulating anodic antigen (CAA), a worm-derived biomarker present in serum and urine of *Schistosoma*-infected individuals, can offer an ultra-sensitive alternative but requires pre-concentration that has restricted testing to a laboratory-based platform.

Methods: A novel, low-cost, disposable and field-deployable device (Flow-S) has been developed which allows integration of passive, large-volume pre-concentration of CAA in urine and subsequent lateral flow (LF) detection. The workflow requires less than one minute of hands-on time, no pipetting or centrifugation, and can be performed by minimally trained field-workers. The first prototype used UCP technology to detect CAA on an LF assay, requiring a portable, battery-powered reader. In a ~150-person field study in Tanzania, Flow-S achieved >93% sensitivity and >96% specificity, meeting the WHO TPP requirements for monitoring and evaluation of schistosomiasis (≥60% sensitivity, ≥95% specificity). However, dependence on a reader remains a major limitation. Therefore, Flow-S has been adapted to enable a visual readout, replacing the UCP with a visual label.

Results: The prototype visual Flow-S device will be prospectively evaluated in Kenya across both *Schistosoma mansoni* (N=200) and *Schistosoma haematobium* (N=200) endemic settings. The performance of the Flow-S visual read-out will be assessed against a panel of diagnostics using a composite reference standard and latent class analysis.

Conclusions: Field testing and data analysis will be finalized by July 2026 and final results will be shared at ICOPA in August 2026.

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A NOVEL LATERAL FLOW RECOMBINASE POLYMERASE AMPLIFICATION ASSAY COMBINED WITH A SIMPLE FAECAL EXTRACTION METHOD FOR THE DIAGNOSIS OF STRONGYLOIDIASIS AND HOOKWORM INFECTIONS IN HUMANS

Sze Fui Hii[1], Dinh Ng Nguyen[2], John Kaldor[3], Susana Nery[3], Rebecca Traub[4], Vito Colella[1]
[1]The University of Melbourne, Melbourne Veterinary School, Faculty Of Science, Parkville, Australia, [2]Tay Nguyen University, Faculty Of Animal Sciences And Veterinary Medicine, Buôn Ma Thuột, Viet Nam, [3]University of New South Wales, Kirby Institute, Sydney, Australia, [4]Murdoch University, School Of Veterinary Medicine, Murdoch, Australia

Background and Aims: Soil-transmitted helminth (STH) infections disproportionately affect populations in resource-limited settings. Conventional coproscopic diagnostic methods often lack sensitivity, while molecular assays such as PCR and qPCR, require laboratory infrastructure and technical expertise. In addition, standard DNA extraction procedures using commercial kits are costly. This study aimed to develop and evaluate lateral flow recombinase polymerase amplification (LF-RPA) assays together with a simple, low-cost novel faecal DNA extraction method for the rapid diagnosis of strongyloidiasis and hookworm infections in humans.

Methods: LF-RPA assays targeting *Necator americanus*, *Ancylostoma ceylanicum*, and *Strongyloides stercoralis* were developed and evaluated using DNA extracted from human stool samples. A simplified, low-cost faecal DNA extraction method was then designed and assessed. Diagnostic performance was evaluated using clinical faecal samples and compared with multiplex STH qPCR assays and conventional coproscopic techniques.

Results: A *N. americanus* singleplex LF-RPA and a duplex LF-RPA for *A. ceylanicum* and *S. stercoralis* were successfully developed, demonstrating high diagnostic sensitivity (>85%), 100% specificity, and almost perfect agreement with multiplex qPCR. Using a novel faecal DNA extraction method, sensitivities/specificities reached 93.8%/100% for *N. americanus*, 84.2%/97.5% for *A. ceylanicum*, and 60%/96.2% for *S. stercoralis*. The protocol was significantly more sensitive than sodium nitrate flotation (87% vs. 54.4%).

Conclusions: This novel workflow enabled rapid and cost effective sample processing and result interpretation without complex laboratory equipment. This practical diagnostic platform has strong potential for point-of-care application and improved surveillance in resource-limited endemic settings.

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08-18-2026 4:40 PM - 6:40 PM

EVALUATION OF THE IMPACTS OF A SECOND BLOOD FEEDING ON LUTZOMYIA LONGIPALPIS INFECTED WITH LEISHMANIA INFANTUM

Giulia Brito, Brenda Campos, Willian Campos, Ana Célia Farias, Deborah Mothe, Claudia Brodskyn, Manuela Solcà
Fundação Oswaldo Cruz - Instituto Gonçalo Moniz, Laboratório De Interação Parasito-hospedeiro E Epidemiologia, Salvador, Bahia, Brazil

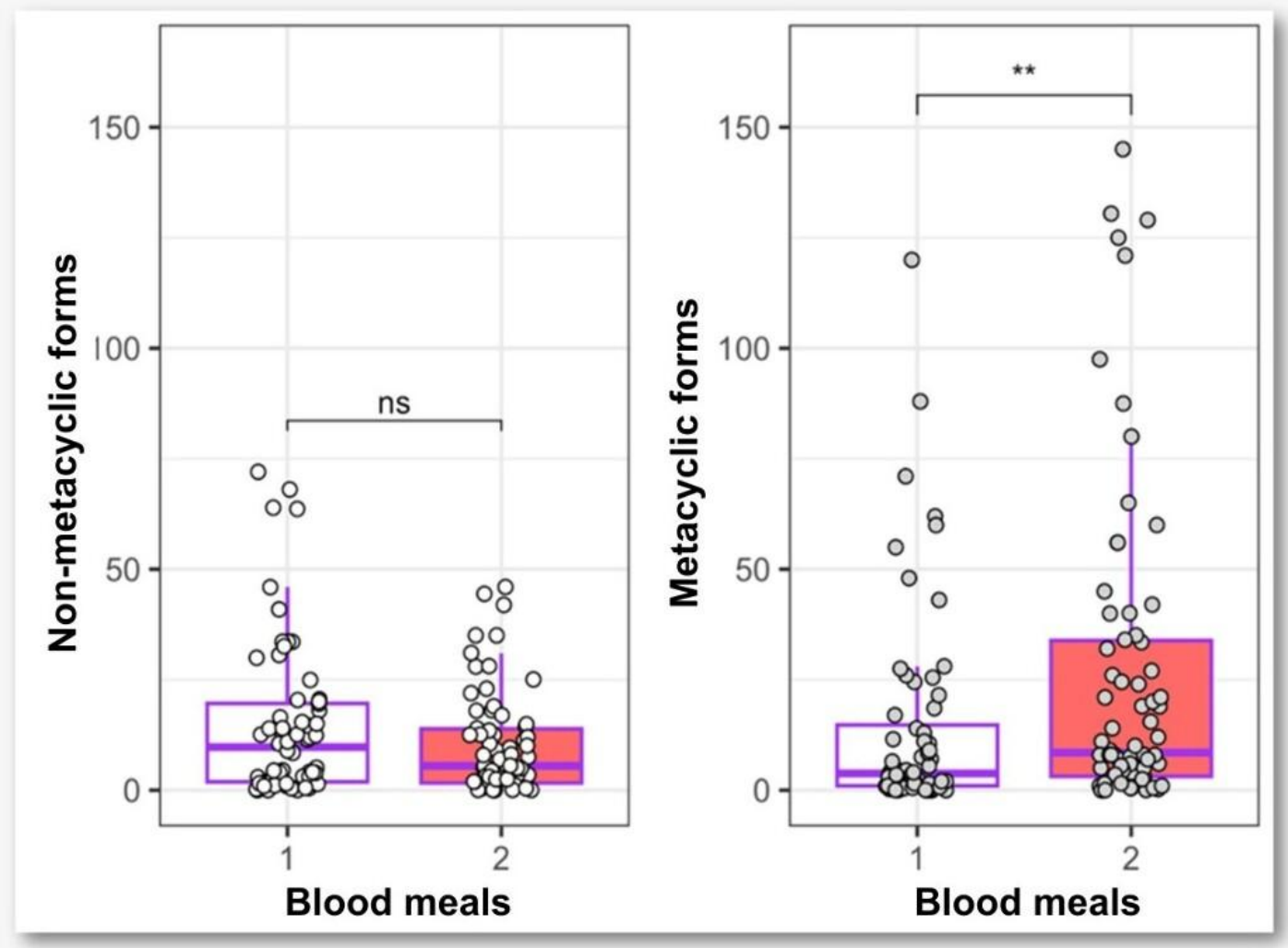
Background and Aims: Blood feeding behavior plays a central role in sand fly vector competence and *Leishmania* transmission. Experimental evidence indicates that additional blood meals influence parasite differentiation within the vector. This study investigated the effects of a second non-infected blood meal in *Lutzomyia longipalpis* females infected with *Leishmania infantum*. Metacyclogenesis, parasite load and survival of the insects were evaluated.

Methods: Five independent experimental cycles were conducted using laboratory-reared *Lutzomyia longipalpis* females artificially infected with *Leishmania infantum*. Blood was provided from healthy dogs of endemic areas for leishmaniasis. After infection, females were either maintained with a single infected blood meal or received an additional non infected blood meal. Midguts were dissected on day twelve post infection to assess infection rates and quantify metacyclic and non metacyclic promastigotes. Infection parameters, parasite differentiation and survival between feeding groups, were analyzed with significance defined as $p<0.05$.

Results: Females that received a second blood meal exhibited a significant increase in the proportion of metacyclic promastigotes compared to those fed once, demonstrating enhanced metacyclogenesis. Parasite loads and infection rates did not differ across experimental cycles. A second blood meal significantly increased insect survival, with a 21% reduction in the risk of death compared with insects that received only one blood meal. These findings show that additional blood feeding alters parasite developmental dynamics within the

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vector.



Conclusions: A second non infected blood meal promotes metacyclogenesis and contributes to longevity of female *Lu. longipalpis*, thereby increasing the transmission potential of *L. infantum*.

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THE BITES OF NON-INFECTED SAND FLIES PROMOTE THE RE-ACTIVATION OF L. MAJOR-CAUSED CUTANEOUS LEISHMANIASIS, ULTIMATELY IMPROVING THE COMPETENCE OF BOTH ANIMAL RESERVOIRS AND SAND FLY VECTORS

Pedro Cecilio[1], Maria Disotuar[1], Eva Iniguez[1], Luana Rogerio[1], Kristina Tang[1], Luiza Dos Santos Felix[1], Johannes Doehl[1], Tiago Serafim[1], Claudio Meneses[1], Caroline Percopo[2], Jesus Valenzuela[1], Fabiano Oliveira[1]
[1]Vector Molecular Biology Section, Laboratory of Malaria and Vector Research, NIAID, National Institutes of Health, Rockville, United States of America, [2]Flow Cytometry Core, Laboratory of Malaria and Vector Research, NIAID, National Institutes of Health, Rockville, United States of America

Background and Aims: Most *Leishmania* parasites are zoonotic agents, meaning that animal reservoirs play an essential epidemiological role in leishmaniasis. However, our understanding of such animal reservoirs, and their interactions with the sand fly vectors is still limited.

Methods: Since in the wild, rodents were incriminated as reservoirs of *Leishmania major* parasites, here, we took advantage of two cutaneous leishmaniasis (CL) models to try to understand whether the bites of non-infected sand flies impact infection/disease.

Results: When we subjected active CL lesions, both in the susceptible BALB/c and the self-healing C57BL/6 models, to a single hour-long exposure event to non-infected sand fly bites, no impact on disease progression/parasite burden was observed. However, when we exposed healed CL lesions in the ears of C57BL/6 mice to a single event of non-infected sand fly bites, a transient pathological exacerbation was observed. This phenotype was reproduced by the injection of sand fly saliva into healed CL lesions, while pathological changes of greater magnitude were observed in the context of multiple exposure events to the bites of non-infected sand flies. Of note, these pathological alterations were accompanied by a significant increase in the ear parasite burden of exposed *versus* control animals. In turn, alterations in sand fly infection quality were also observed; sand flies recovered after the fourth, third, and second exposure events allowed a better development of infection (with proportionality) than those recovered after the first exposure event.

Conclusions: Overall, these results suggest that sand fly-reservoir interactions are essential for the perpetuation of *Leishmania* parasites in the wild.

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DEVELOPMENT OF ATTRACTIVE TOXIC SUGAR BAITS WITH PLANT EXTRACTS AS TOXICANT: AN ATTEMPT AT EFFECTIVE MALARIA VECTOR CONTROL IN ENDEMIC RURAL COMMUNITIES OF NIGERIA.

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Federal University of Agriculture, Abeokuta, Department Of Pure And Applied Zoology, Abeokuta, Nigeria

Background and Aims: Background Attractive toxic sugar bait (ATSB) is a mosquito control strategy which exploits mosquitos' natural attraction to sugar for energy intake and deliver a lethal dose of a toxic substance at the same time. Commercially produced ATSBs are expensive and are not readily available for use in endemic rural communities **Aim**: There is need to develop an efficient, inexpensive, and safe ATSB for controlling mosquito populations.

Methods: Methodology: ATSB was developed using leave extracts of *Polyscias balfourina* and *Duranta erecta*. Bioassays were conducted to determine preferred fruit bait preference for *Anopheles gambiae* from an array of local fruits. The ATSB was formulated by adding the preferred fruit bait with the plant extracts with the highest mosquitocidal effect. The formulated ATSBs were soaked in cotton wicks, placed in bottles and hung inside the experimental cages

Results: Result: Orange and Banana attracted more mosquitoes than other fruits (56% and 27%) after 1hour exposure. *Duranta erecta* recorded a significantly higher mosquitocidal effect compared to *Polyscias balfourina* (100% and 22% respectively) after 24hours. After 48 and 72hrs of exposure the ATSB formulated with Orange-Duranta recorded the highest levels of mortality (50% and 93%), Banana-Duranta ATSB recorded 22% and 83% while semi field cage experiment recorded (77% and 85%) with Orange-Duranta ATSB

Conclusions: Conclusion: Locally formulated plant-based ATSBs, with leave extracts of *Duranta erecta* and orange as bait, are highly effective mosquitocides against *Anopheles gambiae* and offer a promising, low-cost, environmentally safe, and easily adoptable alternative for mosquito control in Nigeria.

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PARALLEL ORAL ABSTRACT 16: ADVANCES IN VECTOR BIOLOGY: BEHAVIOR, COMPETENCE, AND CONTROL OF MOSQUITOES, SAND FLIES, AND TICKS
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TICKS AND TICK-BORNE PATHOGENS DETECTED IN AUSTRIA IN 2025

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Background and Aims: Ticks can transmit pathogens which can cause disease in humans and animals. Aim of this nation-wide study was to survey the tick vector landscape as well as tick-borne pathogens circulating in Austria.

Methods: Ticks were obtained through a citizen science initiative inviting the public to submit ticks found in Austria. After morphological identification a subset of them (n=3838) was tested for tick-borne pathogens by qPCR-based methods.

Results: In 2025 a total of 8,298 ticks were included in the study. The majority belonged to the genus *Ixodes* (n = 7997, 96.4%) followed by *Dermacentor* (n = 254, 3.1%), *Haemaphysalis* (n = 42, 0.5%) and *Hyalomma* (n = 5, 0.1%). Tick species found last year included *I. ricinus*, *I. acuminatus*, *I. hexagonus*, *I. vespertillionis*, *I. frontalis*, *D. marginatus*, *D. reticulatus*, *Ha. concinna* and *H. marginatum*. Tick-borne pathogens detected comprised *Borrelia burgdorferi* sensu lato in about 24% of all ticks, *Rickettsia* spp. in 13%, *Spiroplasma ixodetis* in 10%, *Anaplasma phagocytophilum* in 8%, *Neoehrlichia mikurensis* in 5%, *Borrelia miyamotoi* in 2% and *Francisella tularensis* in <1%. Some pathogens showed a clear association with certain tick species as well as regional differences. All *Hyalomma* ticks tested negative for Crimean Haemorrhagic fever virus.

Conclusions: Citizen science is highly useful for discovering tick species which are otherwise rarely found. Moreover, surveillance including tick-borne pathogens creates knowledge which can be very useful in supporting clinical diagnosis after tick bite. The project is co-funded by the European Union (No. 101132974 – OH SURVector and No. 101183314 – RAISE).

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ARGININE KINASE – A PROMISING TARGET FOR CONTROL OF TICK REPRODUCTION

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Background and Aims: Ticks are obligate ectoparasites that undergo prolonged starvation interrupted by brief periods of intensive blood feeding, during which females ingest large volumes of host blood. Arginine kinase (AK), an invertebrate-specific phosphagen kinase, plays a central role in maintaining ATP homeostasis during these extreme metabolic transitions. This study aimed to characterize arginine kinase from *Ixodes ricinus* (IrAK) and to investigate its role in regulating energy demands during the tick life cycle.

Methods: IrAK expression was analyzed across developmental stages and tissues using quantitative RT-PCR. Functional significance was assessed by RNA interference (RNAi) in adult females, followed by evaluation of feeding parameters and reproductive output. Defective eggs were examined using comparative proteomics and Western blotting. The protective potential of recombinant IrAK (rIrAK) was further tested in host vaccination experiments.

Results: IrAK expression was restricted to the midgut, with highest levels detected during rapid engorgement and immediately after molting. Although IrAK knockdown did not affect feeding success or engorgement weight, it resulted in a severe reproductive phenotype: females produced sterile, darkly pigmented, and morphologically abnormal eggs. Proteomic analysis revealed the absence of several essential proteins involved in energy metabolism, cytoskeletal organization, and ubiquitin-mediated processes. In vaccination trials, hosts immunized with rIrAK exhibited a significant prolongation of tick feeding duration.

Conclusions: IrAK is critical for managing the extreme energy fluctuations characteristic of the tick's "starve-or-gorge" lifestyle, particularly during oogenesis and embryogenesis. Its absence in mammals makes IrAK a promising and selective target for novel tick control strategies. *Acknowledgements: Supported by the Czech Science Foundation, project No. 24-10659.*

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LEISHMANIA SPP. EXPOSURE IN DOMESTIC CATS IN PORTUGAL: A NATIONWIDE STUDY

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Background and Aims: *Leishmania infantum* is endemic in Portugal, where dogs act as the primary domestic reservoir and human visceral leishmaniosis remains a public health concern. Cats are increasingly recognized as susceptible hosts, yet nationwide data on feline exposure remain limited. This study aimed to estimate the seroprevalence of *Leishmania* spp. exposure in domestic cats in Portugal and to assess potential associated risk factors.

Methods: A nationwide cross-sectional study was conducted using feline serum samples submitted for unrelated clinical purposes to a veterinary diagnostic laboratory between December 2024 and March 2025. Samples originated from all mainland districts and the Azores archipelago. Anti-*Leishmania* spp. antibodies were detected using the direct agglutination test (DAT), considering a cut-off titer of 1:400. Associations between seropositivity and explanatory variables were evaluated using univariate analyses followed by multivariable logistic regression.

Results: Overall, 8.9% (96/1080; 95% CI 7.3–10.7) of cats tested seropositive. Marked geographic heterogeneity was observed, with the highest seroprevalence recorded in the Algarve (14.5%). One seropositive cat was detected on Terceira Island, Azores. Region was the only independent predictor of exposure identified, with cats from the Algarve showing increased odds of seropositivity (adjusted OR 1.97; 95% CI 1.24–3.13).

Conclusions: Domestic cats in Portugal are frequently exposed to *Leishmania* spp., with geographic patterns paralleling known canine and human transmission foci. The detection of seropositivity in the Azores suggests possible local transmission and warrants further investigation. These findings support the inclusion of cats in integrated One Health surveillance frameworks. Acknowledgements: Fundação para a Ciência e a Tecnologia (GHTM/UID/Multi/04413/2020 and LA-REAL/LA/P/0117/2020)

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THE CHROMOSOME-SCALE ASSEMBLY OF THE AUSTRALIAN PARALYSIS TICK, IXODES HOLOCYCLUS

Amrita Vijay[1], Thomas Karbanowicz[2], Quentin Gouil[3], Alexander Gofton[4], Shilpa Kapoor[5], Swapnil Tichkule[6], Balu Balan[7], Louise Baker[8], Stefano Gaiarsa[9], Pradip Kumar Roy[7], Clare Anstead[10], Ala Tabor[2], Nathan Lo[11], Stephen Barker[12], Jan Riemer[13], Fabrizia Stavru[14], Davide Sassera[15], Peter Czabotar[16], Tony Papenfuss[16], Aaron Jex[1,17]
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Background and Aims: *Ixodes holocyclus* (Australian eastern paralysis tick) is a medically and veterinary significant ectoparasite capable of producing potent neurotoxins (holocyclotoxins) that induce rapidly ascending flaccid paralysis in companion animals, livestock and humans, often with fatal outcomes. Despite its importance, the molecular mechanisms governing toxin production, host specificity and survival remain poorly understood, primarily due to scarce genomic and transcriptomic resources.

Methods: We built the first chromosomal-scale genome for *I. holocyclus* using Oxford Nanopore long reads, Illumina short reads and Hi-C, and annotated genes with a hybrid de novo transcriptome, resolving alternative splicing with long- and short-read alignments. We scanned UTRs and upstream sequence of complete genes for conserved regulatory motifs, including putative promoters. Comparative genomics (synteny, phylogenomic placement) and population sampling were performed; we sequenced 100 ticks from 32 eastern Australian sites.

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Results: The 1.9 Gb assembly comprises 13 chromosome-level scaffolds, 66% repeats and 93.3% genome BUSCO completeness. Annotation supported 24,447 genes, including 22,811 protein-coding. Transcript evidence resolved isoform complexity, including alternative splicing and variable 5'/3' UTR structures (including UTR splicing), refining transcript boundaries. Synteny with *I. scapularis* and *I. ricinus* identifies conserved “supergene” blocks. Population genomics enables SNP discovery, diversity/structure inference and recombination/LD mapping, quantifying heterogeneity and prioritising candidate regions linked to holocyclotoxin biology, host–parasite interactions and environmental adaptation, consistent with vector capacity hypotheses, in this species.

Conclusions: Together, these genomic and transcriptomic resources represent a significant advancement in tick biology, offering valuable insights into the genetic basis of toxin production, host–parasite interactions and the development of targeted control strategies against tick-borne diseases.

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BLOODLESS: A BLOOD-FREE DIET THAT ENABLES SUSTAINABLE AND SCALABLE REARING OF MULTIPLE ANOPHELES SPECIES FOR VECTOR CONTROL AND RESEARCH

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Background and Aims: Large-scale mosquito production is essential for emerging vector control strategies, including sterile insect techniques and transmission-blocking interventions. Conventional rearing relies on vertebrate blood, raising ethical, regulatory, logistical, and biosafety concerns. We established a network of insectaries to evaluate BLOOLess diet as an alternative for mosquito production, using different anopheline species from several African locations and the Amazon across diverse insectary settings.

Methods: Multiple *Anopheles* species and strains, including *Anopheles gambiae*, *Anopheles coluzzii*, *Anopheles stephensi*, *Anopheles arabiensis*, and *Anopheles darlingi*, were reared for several generation on BLOODless, with the longest-maintained colony being *An. stephensi* for five years, and were compared with blood-fed controls. Outcomes assessed included feeding success, fecundity, fertility, wing length, longevity, larval survival, *Plasmodium* infection prevalence and intensity and microbiota.

Results: BLOODless supported ovarian development, egg maturation, fertility, and successful development into functional adults. Across generations, wing size and general fitness were comparable to blood-fed controls. *Plasmodium* susceptibility was maintained. Longevity was equal or increased in later generations under blood-free feeding. Long-term rearing showed no major effects

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on mosquito fitness, infectivity, or microbiota. Feeding success was high, and larval survival and development were comparable to blood-fed colonies.

Conclusions: BLOODless diet represents a viable, ethical, and sustainable alternative to vertebrate blood for mosquito rearing. The diet supports long-term colony maintenance while preserving key biological and vector competence traits, enabling scalable and responsible mosquito production for malaria research and vector control programs.

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EPIMIC – UNRAVELLING EPIGENETIC AND MICROBIAL DRIVERS OF ADAPTATION IN A SNAIL HOST OF SCHISTOSOMA HAEMATOBIUM

Ruben Schols
ETH Zurich - Eawag, Aquatic Ecology, Dübendorf, Switzerland

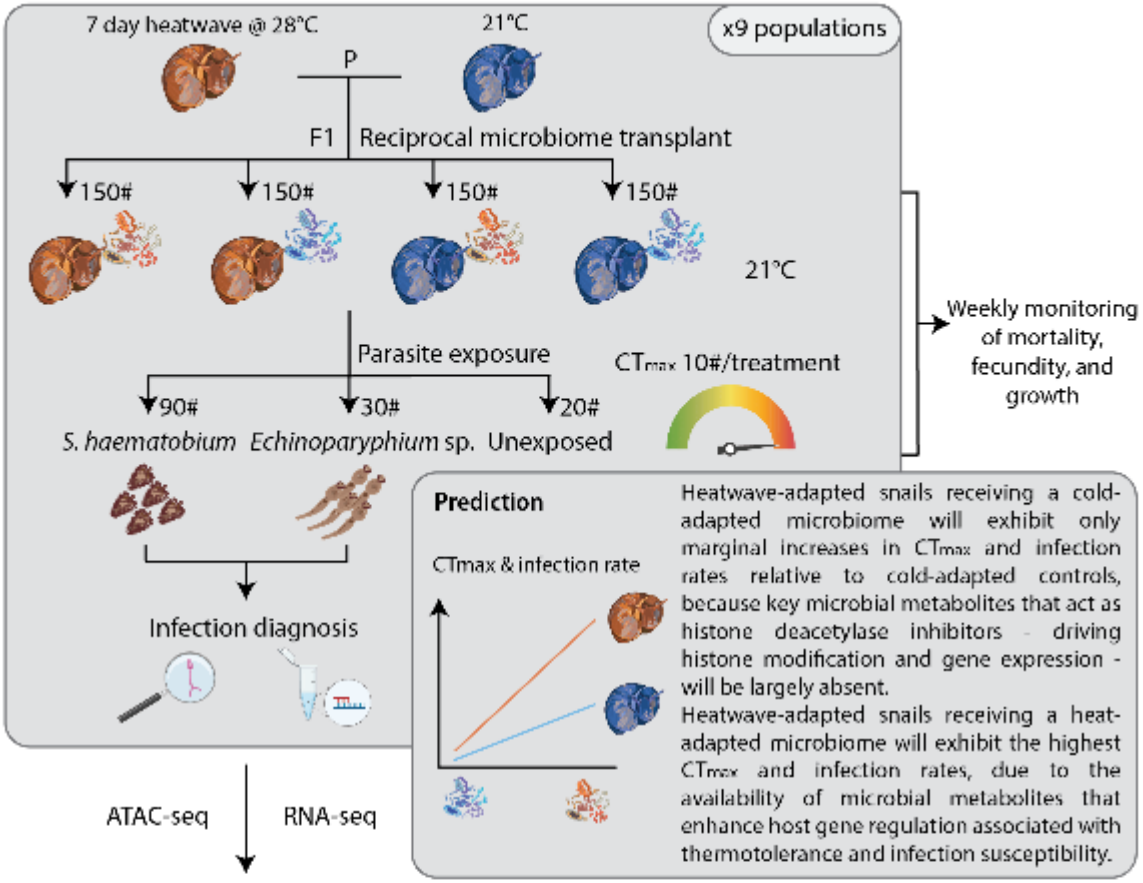
Background and Aims: The causative agent of urinary schistosomiasis, *Schistosoma haematobium*, is classified as a Group 1 carcinogen causing bladder cancer and systemic complications. The recently emerged schistosomiasis endemicity in Corsica (France) is driven by the only *Schistosoma*-compatible snail host present in Europe, *Bulinus truncatus*. Central to this transmission is the snail's immune response. While research has focused on genetic resistance, evidence suggests that the epigenome-microbiome axis could be a non-genetic driver of phenotypic variation. However, its functional role in pathogen resistance and environmental resilience remains poorly understood. EPIMIC proposes that this axis underpins rapid adaptation to climate change while shaping parasite resistance.

Methods: Three hypotheses will be tested: 1) adaptation to warming reduces resistance to trematode parasites by altering immune function, 2) the epigenome-microbiome axis contributes to thermal adaptation, and 3) the epigenome-microbiome axis contributes to parasite resistance by modulating host immune function. A reciprocal microbiome transplant will use 11 populations of *B. truncatus*. Heat- and cold-acclimated snails will exchange microbiomes and tested for thermal tolerance limits and infection resistance. RNA-seq and ATAC-seq will assess gene expression and chromatin

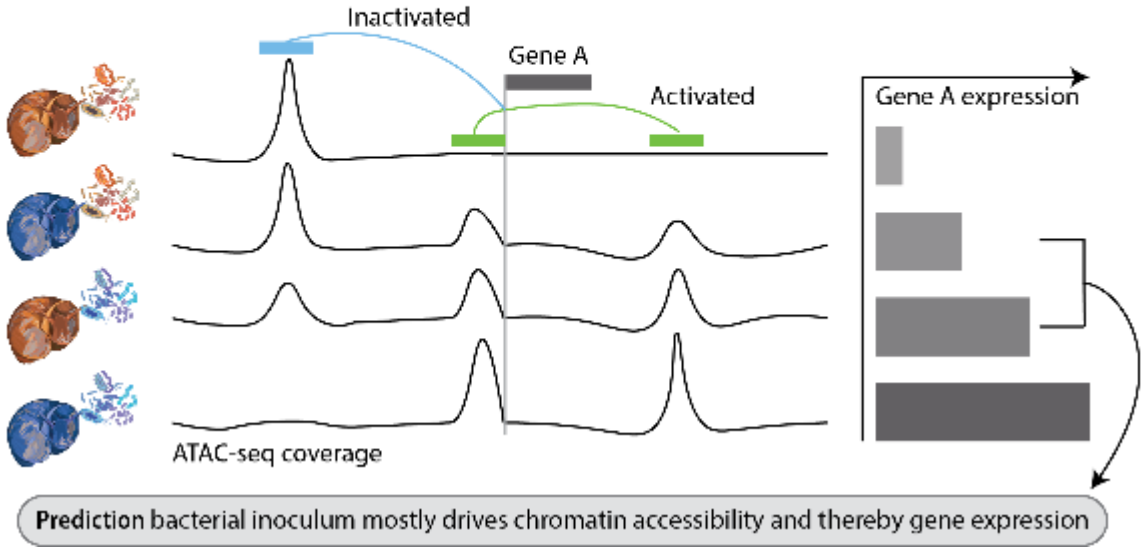
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accessibility, linking cis-regulatory elements to immune genes across treatments.

Reciprocal microbiome transplant experiment involving a heatwave and parasite exposure



Linking cis-regulatory elements to immune genes dependent on microbiome and temperature treatment



Results: It is predicted that microbiome composition, more than host acclimatization, will increase CTmax and infection rates by supplying metabolites that drive epigenetic processes, altering chromatin accessibility and gene expression.

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Conclusions: For the first time in the field of vector biology, the complex datasets associated with histone modification, gene expression and bacterial diversity will be integrated to provide a new fundamental perspective on local adaptation.

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DECODING THE EPIGENETIC BLUEPRINT BEHIND TOXOPLASMA (PRE)SEXUAL COMMITMENT AND CHRONIC PERSISTENCE

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Background and Aims: *Toxoplasma gondii* is a widespread apicomplexan parasite with a complex life cycle characterized by reversible transitions between tachyzoites and bradyzoites to balance acute infection and persistence, as well as an often-overlooked irreversible commitment to the sexual stage. Increasing evidence indicates that these developmental transitions are governed by a coordinated network of transcriptional regulators and chromatin remodeling mechanisms that ensure robust and timely stage conversion.

Methods: This work synthesizes recent advances in the epigenetic regulation of *T. gondii* development, focusing on chromatin accessibility control and transcription factor–mediated fate decisions. In particular, it examines the functional interplay between an ISWI-family chromatin remodeler and the MORC/HDAC3 repressor complex, as well as experimental depletion of the AP2XII-1 and AP2XI-2 transcription factors to probe developmental plasticity in vitro.

Results: We highlight evidence that the ISWI chromatin remodeler and the MORC/HDAC3 complex act synergistically to maintain or shift developmental states through dynamic modulation of chromatin accessibility. Notably, simultaneous depletion of AP2XII-1 and AP2XI-2 reactivates the presexual developmental program in vitro, bypassing the requirement for cat hosts and enabling scalable, ethical investigation of sexual stages. This perturbation also uncovers a previously unrecognized hybrid zoite that co-expresses bradyzoite and merozoite markers, revealing a transitional state that does not conform to classical stage definitions.

Conclusions: Together, the discovery of hybrid developmental states and recent insights into bradyzoite differentiation support a revised model in which *T. gondii* development proceeds along a continuum of epigenetically primed states rather than through rigid binary fate decisions.

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BRC-ANALYTICS: COMBINING AGENTIC AI WITH OPEN INFRASTRUCTURE FOR PATHOGEN GENOMICS

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Background and Aims: The analytical landscape of pathogen research is fragmented—reference genomes, sequencing data, and analysis tools exist in separate silos, hindering transparency and reproducibility. Agentic AI systems create unprecedented opportunities to democratize pathogen genomics by orchestrating complex analyses while preserving full provenance. Here, we introduce BRC-Analytics.org, a platform that combines agentic AI with authoritative data sources and community-curated workflows on freely accessible public infrastructure.

Methods: BRC-Analytics integrates NCBI Datasets, UCSC Genome Browser annotations, and EBI ENA sequence archives with Galaxy-based workflows for quality control, read mapping, variant calling, transcriptomics, and functional annotation. The platform runs on ACCESS-CI infrastructure, requiring no local installations or funding. Agentic AI tools invoke Galaxy's native analysis functions via API, ensuring every analytical step appears in Galaxy's history with full parameter capture.

Results: We demonstrate this approach by re-analyzing published *Candida auris* datasets (transcriptomic and large-scale surveillance). Critically, we show that AI-generated analyses require rigorous validation. In one example, a scientific-sounding AI-proposed method for mapping genes across different genome versions yielded an exceptionally high prediction rate but proved only 1% accurate when validated against NCBI mappings—illustrating both the power and essential safeguards needed when deploying agentic AI.

Conclusions: BRC-Analytics provides true data-to-publication functionality where agentic AI serves as a capable collaborator operating within a framework that enforces transparency—making advanced pathogen genomics accessible to any researcher regardless of computational expertise.

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NEW INSIGHTS INTO POPULATION STRUCTURE OF THE INTESTINAL WORM ASCARIS USING GENOMICS APPROACHES

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Background and Aims: The roundworm *Ascaris* infects hundreds of millions of people globally, leading to morbidity including stunting and wasting in children. It is also a very common infection of domestic pigs, reducing production and causing economic losses. Although separate *Ascaris* species have been described to infect people and pigs, cross infections between hosts and hybridisation events between the two species have been reported. This study aimed to investigate the population structure of *Ascaris* using whole genome sequencing.

Methods: Short-read whole genome sequences were generated from adult *Ascaris* worms from Europe, Africa and Asia and combined with existing *Ascaris* genomic data. A bioinformatics pipeline was developed for *de novo* assembly of complete mitochondrial genomes through host-read depletion or mitochondrial DNA (mtDNA) read enrichment, followed by mtDNA-specific assembly. To exploit nuclear genome data, *Ascaris* genome sequences were mapped to the *Ascaris suum* genome and variant calling was performed. Population structure is currently being investigated.

Results: We generated 149 high-quality *Ascaris* mtDNA assemblies and identified a novel clade (Clade D) associated with human samples from Ethiopia. Our analysis provided further evidence that Clade C comprises of pig-derived samples from Europe. Furthermore, genetic diversity across mitochondrial loci was high, indicating substantial intraspecies variation.

Conclusions: Our methods provide a scalable framework for mtDNA genome reconstruction. The *Ascaris* mtDNA analysis and ongoing work with nuclear genomics will provide further insights into *Ascaris* population structure and evolutionary relationships, important information to understand Ascaris transmission dynamics and maintain effective control measures in endemic regions.

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HEDGEHOGS AS POTENTIAL RESERVOIR HOSTS OF HUMAN CRYPTOSPORIDIOSIS IN DENMARK

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Background and Aims: Human cryptosporidiosis is under surveillance in Denmark since 2023. Calves are recognised as major reservoirs of human cryptosporidiosis in Denmark; however, little is known about small-mammal zoonotic reservoirs. Here, we screened faecal samples from hedgehogs (*Erinaceus europaeus)* for *Cryptosporidium*, including molecular characterisation of positive samples.

Methods: DNAs from faecal samples collected from 42 diseased/dehydrated hedgehogs in four rescue centres across Denmark were screened by real-time PCR for *Cryptosporidium* and *Giardia*. Positive samples were subject to glycoprotein 60 gene (*gp60*) sequencing, and the data were compared with *gp60* surveillance data (human cases).

Results: Sixteen samples were positive, 13 of which were successfully sequenced, revealing subtypes of *Cryptosporidium erinacei* and *Cryptosporidium parvum* (Table 1). The identified subtypes of *C. parvum* are commonly observed in human cases in Denmark. *C. erinacei* has been found in a dozen of human cases, although the specific subtypes of XIIIaA20R10 remains to be seen in humans in Denmark; however, it was previously found in an immunocompetent human individual in the Czech Republic. Only one sample was weekly positive for *Giardia*.

Table 1. Species and subtypes of *Cryptosporidium* identified in hedgehog faecal DNAs

SPECIES	Subtype	N

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Cryptosoporidium parvum	IIcA5G3j	7
	IIaA16G1R1	3
Cryptosoporidium erinacei	XIIIaA20R10	3
TOTAL		13

Conclusions: This is one of very few studies providing *gp60* data from hedgehogs. Species and subtypes of *Cryptosporidium* commonly seen in human cases in Denmark also appear to be common in hedgehogs, suggesting a potential for bidirectional zoonotic transfer between hedgehogs and humans, with a special emphasis on hedgehog rehabilitators treating sick hedgehogs.

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INFLUENCE OF WATER PHYSICOCHEMICAL PROPERTIES AND VEGETATION TYPE ON THE DISTRIBUTION OF SCHISTOSOMIASIS INTERMEDIATE HOST SNAILS IN NELSON MANDELA BAY, SOUTH AFRICA.

Prince Campbell[1], Janine Adams[2], Melusi Thwala[3], Opeoluwa Oyedele[4], Paula Melariri[5]
[1]Nelson Mandela University, Environmental Health, Gqeberha, South Africa, [2]Nelson Mandela University, Institute For Coastal And Marine Research And Department Of Botany, Gqeberha, South Africa, [3]Academy of Science of South Africa, Pretoria, South Africa, [4]University of Namibia, Computing, Mathematical And Statistical Sciences, Windhoek, Namibia, [5]Nelson Mandela University, Health Sciences Research Directorate, Gqeberha, South Africa

Background and Aims: Freshwater snails occupy diverse aquatic environments where distribution is shaped by water physicochemical properties and vegetation structure. Because schistosomiasis transmission depends on compatible intermediate host snails, this study examined physicochemical characteristics, snail distribution, and associated habitats across selected water bodies in Mbombela.

Methods: We conducted a nine-month quantitative cross-sectional survey across wet and dry seasons at eight waterbodies in Mbombela. We measured physicochemical water properties (temperature, pH, dissolved oxygen, conductivity, turbidity, hardness) and macrophyte cover. Snails were systematically sampled, identified to genus, and enumerated. Data were analysed in R (v4.3.1).

Results: Water temperature was significantly higher in the wet season (23.4 °C ± 2.8; $p < 0.001$). EC, salinity, and TDS showed strong positive correlations ($r > 0.99$), while other parameters showed limited seasonal variation. A total of 844 snails were collected, with greater abundance in the dry season (64.7%). Physa dominated (95.9%) and occurred at most sites (87.5%). Schistosomiasis host snails were rare: Bulinus accounted for 0.9% and Biomphalaria 0.6%. Vegetation cover ranged from 5–85% and included submerged, emergent, and floating species. Snail abundance showed weak negative correlations with most physicochemical parameters and vegetation cover.

Conclusions: Despite favourable abiotic conditions, these habitats present a low immediate transmission risk due to the extreme scarcity of host snails. The weak negative environmental correlations, coupled with the dominance of *Physa*, suggest biotic interactions (e.g., interspecific competition) may critically limit host snail populations, hence can be used for biological monitoring. This also highlights the need to integrate community ecology into schistosomiasis surveillance and risk modeling.

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CELLULAR AND PROTEOMIC INSIGHTS INTO TICK MIDGUT ONTOGENY AND BLOOD MEAL DIGESTION

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Background and Aims: The tick midgut is the main site of host-blood storage and intracellular digestion, providing the sole source of energy and nutrients for development and reproduction. Across tick ontogeny and throughout feeding, the midgut epithelium undergoes dynamic remodeling that mirrors changes in the physiological processes in this tissue and serves as the principal interface with tick-borne pathogens. We provide the first longitudinal proteomic profile of the *Ixodes ricinus* midgut across development and the blood meal.

Methods: Label-free quantitative (LFQ) proteomics was performed on midguts from nymphs and adult females. After homogenization and in-solution tryptic digestion, peptides were analyzed on a timsTOF Pro (Bruker) coupled to an Ultimate 3000 RSLCnano system (Thermo Fisher Scientific). Raw data were searched in MaxQuant against the current UniProt *I. ricinus* database and further analyzed in Perseus, Blast2GOPro, and in-house written scripts.

Results: Comparative LFQ profiling revealed protein levels at different feeding and developmental timepoints. Functional annotation highlighted coordinated changes in digestive proteases and inhibitors, vesicle/lysosome-associated pathways, transport and detoxification systems, and proteins linked to the poorly characterized tick lipid metabolism. Manual data curation prioritized main candidates to blood-meal processing and nutrient allocation during the nymph-to-adult transition.

Conclusions: In summary, several transcriptomics data of *I. ricinus* have been published, however only a few studies have investigated the tick proteomes. In contrast to transcriptomics, proteomics provides direct insight into functional tissue states and key enzymatic processes. This work advances understanding of tick midgut biology and highlights molecules with potential as intervention targets for anti-tick drugs or vaccines. *Supported by Czech-Science-Foundation, projects-no.24-10659,26-20632S.*

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OP139

PARALLEL ORAL ABSTRACT SESSION
PARALLEL ORAL ABSTRACT 18 : INTEGRATED PERSPECTIVES ON VECTOR BIOLOGY AND PARASITE TRANSMISSION DYNAMICS
08-19-2026 2:40 PM - 3:40 PM

INVESTIGATING THE MECHANICAL TRANSMISSION OF HELMINTH EGGS BY FLIES IN LABORATORY AND FIELD SETTINGS

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Background and Aims: Synanthropic flies are suspected mechanical transmitters of fecal-borne helminth diseases, yet their role in transmission remains unclear. This study evaluated helminth egg carriage by flies under (i) controlled laboratory conditions and (ii) natural field conditions using validated recovery and detection techniques.

Methods: (i) Forty-eight flies (*Musca domestica*) were experimentally exposed to feces containing 10,000 *Ascaris suum* and 5,000 *Taenia saginata* eggs/g. Three hours later, eggs were recovered from the exoskeletons and digestive tracts and quantified microscopically.
(ii) Flies were collected in rural Zambia at school toilets (n=3), open defecation sites (n=3), and food markets (n=2). Digestive tracts were dissected and pooled (≤20 flies/pool) by genus, sex, and collection site, followed by helminth egg recovery, DNA extraction and qPCR analysis targeting *Ascaris* spp., *Trichuris trichiura*, hookworms, *Taenia* spp., *Schistosoma* spp., and *Strongyloides stercoralis*.

Results: (i) Experimentally exposed flies carried 227 helminth eggs (37/48 positive; mean: 4.7 eggs/fly), including 46 *A. suum* and 181 *T. saginata* eggs. Eggs were predominantly recovered from digestive tracts (33/48 positive; mean: 4.6 eggs/fly) compared to exoskeletons (4/48 positive; mean: 0.1 eggs/fly). Female flies (17/21 positive; mean: 6.7 eggs/fly) carried approximately twice as many eggs as males (19/27 positive; mean: 3.2 eggs/fly).
(ii) In the field, 1,452 flies were collected across 84 pools (50.6% Calliphoridae, 46.6% Muscidae, 2.8% Sarcophagidae) and are currently undergoing molecular analysis.

Conclusions: The potential for flies to internally carry helminth eggs was confirmed under favorable laboratory conditions. Molecular results from field-collected flies, to be presented during the congress, will further clarify their role in helminth transmission.

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PARALLEL ORAL ABSTRACT SESSION
PARALLEL ORAL ABSTRACT 19 : EMERGING REGULATORY MECHANISMS IN HOST–PARASITE AND SYMBIONT INTERACTIONS
08-19-2026 2:40 PM - 3:40 PM

THE EARLY-DIVERGING EUKARYOTE GIARDIA REVEALS THE ORIGINS OF EUKARYOTIC POST-TRANSCRIPTIONAL REGULATORY NETWORKS

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Background and Aims: RNA-binding proteins in eukaryotes (RBPs) regulate splicing, RNA silencing and translational repression. Many eukaryotic RBPs are conserved from yeast to humans yet are absent or rudimentary in prokaryotes, implying an early emergence of “eukaryotic-innovative” RBPs. When, and in what biological context, did these RBPs arise? We hypothesise that their earliest detectable origins lie ~1 billion years before yeast and are preserved in *Giardia*, an early-diverging eukaryote.

Methods: We built a phylogenomic atlas of RBP families across the tree of life to trace the origins of eukaryotic-innovative RBPs. Domain topology, domain co-occurrence and intrinsically disordered region (IDR) mapping were used to analyse architectural features of these RBPs. The Giardia RBPome was characterised using domain- and structure-informed annotation integrated with transcriptomics, proteomics and RNA–protein interactome capture to define canonical and non-canonical (“moonlighting”) RBPs. Direct RNA targets and regulatory networks of representative eukaryotic-innovative RBPs (PUF, DDX3X, EIF4A and PGK) were resolved using enhanced CLIP-seq and RBP immunoprecipitation. Functional roles were tested using CRISPRi-based genetics, and condensate behaviour was assessed using phase-separation induction assays *in vitro* and *in vivo*.

Results: Phylogenomic analyses show that multiple eukaryotic-innovative RBPs are already present in Giardia, supporting an origin ~1 billion years before yeast. Domain topology, co-occurrence and IDR analyses reveal simplified modular architectures that expand in later eukaryotes. *Giardia* encodes

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diverse canonical and non-canonical RBPs with conserved RNA–protein networks, CRISPRi-linked regulatory phenotypes and condensate-like behaviour consistent with properties of their conserved eukaryotic counterparts.

Conclusions: Giardia represents an origin-point model preserving early eukaryotic-innovative RBPs and foundational post-transcriptional regulatory architectures and mechanisms.

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PARALLEL ORAL ABSTRACT SESSION
PARALLEL ORAL ABSTRACT 19 : EMERGING REGULATORY MECHANISMS IN HOST–PARASITE AND SYMBIONT INTERACTIONS
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FROM TAXA TO TRANSCRIPTS: INVESTIGATING THE SNAIL HOLOBIONT IN THE CONTEXT OF HELMINTH INFECTION

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Background and Aims: Schistosomiasis transmission depends on compatible interactions between schistosomes and their snail intermediate hosts, yet the role of the snail-associated microbiome remains poorly defined. This study aimed to characterise microbiota variation across infection outcomes, and to define associated host transcriptional responses.

Methods: We performed 16S rRNA gene sequencing on *Biomphalaria glabrata* under uninfected, infection-failed, and infected states, alongside environmental and parasite samples. Shotgun metagenomics, metabolomics and host RNA-Seq were used to assess microbial functional profiles and host gene expression in *B. glabrata* six weeks post-exposure to *Schistosoma mansoni*.

Results: Alpha diversity was largely conserved, while schistosome exposure was associated with shifts in beta diversity and community composition. Infected snails showed distinct community composition from uninfected and infection-failed profiles. Functional analyses showed enrichment of host-associated nutrient pathways in uninfected microbiota, while infected microbiota showed increased metabolic and self-maintenance functions, including altered tryptophan metabolism. Transcriptomic analyses revealed up-regulation of epithelial repair pathways in infection-failed snails, while patent infections showed changes in structural and metabolic gene expression.

Conclusions: These data show that schistosome infection is associated with coordinated changes in snail microbiota composition and host gene expression that distinguish resistant from susceptible outcomes. Altered regulation of tryptophan-associated pathways in both host and microbiome during infection suggests changes in nutrient dynamics at the host–parasite interface. In contrast, infection-failed snails showed transcriptional signatures linked to epithelial defence, highlighting metabolic and epithelial processes as key features of compatibility.

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PARALLEL ORAL ABSTRACT SESSION
PARALLEL ORAL ABSTRACT 19 : EMERGING REGULATORY MECHANISMS IN HOST–PARASITE AND SYMBIONT INTERACTIONS
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TICK HRF-DEPENDENT FERROPTOSIS PATHWAY TO PROMOTE TICK ACQUISITION OF BABESIA MICROTI

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Background and Aims: *Babesia microti* , a tick-borne zoonotic erythrocytic intracellular parasite, has an unclear mechanism of transmission through ticks. Ferroptosis is an iron-dependent form of programmed cell death that affects pathogen replication in the host. Currently, there is limited research concerning the effect of tick ferroptosis on Babesia infection.

Methods: The tick *Haemaphysalis longicornis* fed on the *B. microti* infected mice was studied. Divalent iron and reactive oxygen species (ROS) levels in the midgut of infected ticks were checked, and mitochondrial structure of midgut cell were observed. Expression of Ferritin 1 and glutathione peroxidase 4 (GPX4) were evaluated by qPCR and Western blotting. The ferroptosis promoter Erastin and the inhibitor Ferrostatin-1 were also used to analyse ferroptosis and *B. microti* load. Tick histamine-releasing factor (HRF) was identifed as a key molecule in the tick ferroptosis pathway by RNAi. Molecue interaction assays were carried out in this works.

Results: The midgut divalent iron and ROS levels were significantly elevated in infected ticks, and the mitochondrial ridges were absent or decreased in size. Along with downregulation of ferritin 1 and GPX4 in infected ticks suggests that Babesia infection promote ferroptosis. The Erastin increased *B. microti* load, while the Ferrostatin-1 decreased load. Interference with tick HRF promotes tick ferroptosis and acquisition of *B. microti* . Molecule interaction showed Babesia's Enolase molecule acting on ticks' HRF molecule promote tick ferroptosis and facilitates Babesia infection.

Conclusions: These findings demonstrate that HRF mediated ferroptosis in ticks facilitates Babesia infection, providing crucial insights into the molecular mechanisms of Babesia transmission via ticks.

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PARALLEL ORAL ABSTRACT 19 : EMERGING REGULATORY MECHANISMS IN HOST–PARASITE AND SYMBIONT INTERACTIONS
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LOSS OF NFIL3 CONFERS RESISTANCE TO CEREBRAL MALARIA

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Background and Aims: NFIL3 is a basic leucine zipper transcription factor with known regulatory roles in circadian rhythm, metabolism, and immunity. Specifically, NFIL3 is important for both lymphocyte development and proper function. Recently, our lab demonstrated that NFIL3 is a regulator of macrophage-mediated inflammation. NFIL3 has been studied in the context of cancer, autoimmune disease, and systemic infection; however, its function in parasite infection remains understudied. Given its roles in inflammation, our lab sought to investigate whether NFIL3 played a role in acute parasitic inflammatory responses. Cerebral malaria is one such response, in which infection by the Plasmodium falciparum parasite, primarily in children, leads to neuroinflammation, blood-brain barrier breakdown, and encephalopathy.

Methods: Our lab uses the Plasmodium berghei ANKA murine infection model to study the pathogenesis of cerebral malaria. We used the NFIL3 KO mouse model we developed to perform survival analyses, parasitemia, and immunophenotyping via flow cytometry.

Results: Known immunophenotypes of NFIL3 KO mice were confirmed in our model using flow cytometry. Loss of NFIL3 resulted in a greater than 60% survival to the cerebral stage of P. berghei infection compared to wildtype controls. Strikingly, the blood barrier remains mostly intact in the knock-out mice. Immune cell proportion and activation will be compared between wildtype and KO mice after infection.

Conclusions: NFIL3 appears to act as a crucial regulator in the immune response to *Plasmodium berghei*. Using KO mice, we demonstrate that loss of NFIL3 prevents blood-brain barrier failure and death. Future characterization will reveal mechanistic insights into NFIL3's role in the pathogenesis of cerebral malaria.

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PARALLEL ORAL ABSTRACT SESSION
PARALLEL ORAL ABSTRACT 20 : FUNDAMENTAL BIOLOGY OF PARASITES: CELLULAR PROCESSES AND NOVEL TARGETS
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CHARACTERISATION OF INHIBITORY NEUROTRANSMITTER RECEPTORS IN SARCOPTES SCABIEI: IMPLICATIONS FOR CONTROL OF SARCOPTIC MANGE IN WOMBATS.

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Background and Aims: *Sarcoptes scabiei* infestation impacts many mammalian hosts and poses a significant welfare challenge for Australian wombats. Control options are limited, with concerns over efficacy, safety, and environmental impacts. We investigated genetic and functional properties of the *S. scabiei* inhibitory neurotransmitter receptor family, the molecular targets of commonly used acaricides including moxidectin and fluralaner.

Methods: Glutamate (GluCl) and GABA (Rdl) receptor genes were annotated from the *S. scabiei* genome and sequenced to obtain full-length cDNAs. Representative cDNAs were expressed in *Xenopus* oocytes, and channel function assessed using two-electrode voltage clamp electrophysiology (TEVC). Genetic diversity was evaluated by sequencing GluCl and Rdl genes from mites collected from 40 wombats across multiple regions and treatment backgrounds.

Results: Twelve inhibitory neurotransmitter genes were identified, including three Rdl and two GluCl channels, which clustered with arthropod receptor orthologs. There were differences in glutamate sensitivity between GluCl1 splice variants (EC_{50} 6.4 ± 1.7 µM vs 36 ± 1.9 µM; $p < 0.0001$). Fipronil and fluralaner inhibited GluCl1 currents, and moxidectin acted as an irreversible agonist. For Rdl2, fipronil and fluralaner showed the expected antagonism, but unexpectedly moxidectin also inhibited channel activity. GluCl and Rdl sequences were highly conserved across mites. A small number of non-synonymous variants in Rdl1 and Rdl2 were detected in one population cluster which warrant functional investigation.

Conclusions: This first characterisation of *S. scabiei* GluCl and Rdl receptors improves understanding of treatment interactions and regional genetic variation. The establishment of functional expression supports future pharmacological analyses and may help guide mange treatment strategies in wombats and screening for novel therapeutics.

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PARALLEL ORAL ABSTRACT 20 : FUNDAMENTAL BIOLOGY OF PARASITES: CELLULAR PROCESSES AND NOVEL TARGETS
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SLEEPING TRYPANOSOMES: GENES INVOLVED IN QUIESCENCE IN TRYPANOSOMA CRUZI EPIMASTIGOTES

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Background and Aims: Background and aims In Chagas disease treatment failure may be associated with quiescent *Trypanosoma cruzi* parasites that survive through reduced metabolic activity. These represent a small fraction of the population and so are difficult to analyze in the mammalian host. We have identified an apparent quiescent population within the insect stage of the parasite. These non-replicative epimastigotes were subject to transcriptomic and proteomic comparison with their replicating companions. This quiescent sub-population was characterised by down-regulation of genes/proteins known to be involved in translation, transcription, metabolism, and DNA replication, and up-regulation of proteins promoting exit from the cell-cycle in other organisms. Differential expression was evident more in the proteome than in mRNA levels, implying a role for translational control in the quiescent phenotype. From the differentially expressed gene list we selected genes we deemed likely to play a major role in quiescence initiation or maintenance for further analysis.

Methods: Methods Parasites were modified to carry tagged or inducible copies of the selected genes and their effect on replication and differentiation was examined. Inducible expression was generated using either pTcINDEX or SHARK ribozymes to create inducible overexpression or knockdown respectively. DNA replication was analyzed using EdU incorporation.

Results: Results Results will be presented from parasites engineered to overexpress or downregulate genes of interest including candidate RNA binding proteins and protein kinases. Some of these proteins have been previously shown to play a role in the transition of replicating slender form *T. brucei* into non-replicative stumpy forms.

Conclusions: will be given based on the results

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PARALLEL ORAL ABSTRACT 20 : FUNDAMENTAL BIOLOGY OF PARASITES: CELLULAR PROCESSES AND NOVEL TARGETS
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UNUSUAL BIOENERGETIC ASPECTS OF THE MITOCHONDRION IN MALARIA PARASITES

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Background and Aims: Malaria remains a major global health burden. *Plasmodium falciparum* causes most malaria-associated morbidity and mortality and its growth and replication in human red blood cells leads to all clinical symptoms. For decades, it has been known that asexual blood-stage parasites primarily rely on glycolysis for ATP production. However, it remains unclear whether the mitochondrion contributes ATP or instead consumes it.

Methods: Here, we demonstrate that a highly divergent ADP/ATP carrier protein (AAC), PfAAC2, localizes to the parasite mitochondrion, and its genetic depletion via TetR-DOZI-aptamer or DiCre-mediated inducible knockout causes rapid developmental arrest.

Results: Addback experiments further reveal that loss of PfAAC2 for just 16 hours, from the ring-stage to the trophozoite-stage, completely abolishes parasite viability. This represents the fastest death phenotype reported for all genetic perturbations of mitochondrial pathways in *P. falciparum*, surpassing even atovaquone, a clinical antimalarial targeting the *bc1* complex. We also knocked down the mitochondrial phosphate carrier, PfMPC, and observed that PfMPC depletion phenocopies that of PfAAC2, suggesting that these two transporters work synergistically. Finally, rescue attempts of PfAAC2 or PfMPC knockdown using a genetic bypass of the mitochondrial electron transport chain facilitated by the yeast dihydroorotate dehydrogenase were unsuccessful, indicating that disruption of PfAAC2 or PfMPC causes broad mitochondrial malfunctions rather than a specific defect in electron transport.

Conclusions: Together, our findings suggest that the PfAAC2-PfMPC system likely facilitates ATP import into the parasite mitochondrion, which serves as the main energy source. This unusual bioenergetic feature represents a promising vulnerability for the development of novel, fast-killing antimalarials.

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PARALLEL ORAL ABSTRACT 20 : FUNDAMENTAL BIOLOGY OF PARASITES: CELLULAR PROCESSES AND NOVEL TARGETS
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A LEISHMANIA RNA BINDING PROTEIN FUNCTIONAL SCREEN IDENTIFIES A RESPIRATORY MASTER REGULATOR

Ewan Parry, Natalia Teles, Rachel Neish, Katherine Newling, Fabiano Pais, Mathieu Cayla, Adam Dowle, Alastair Droop, Sally James, Jeremy Mottram, Pegine Walrad
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Background and Aims: Gene regulation in *Leishmania* species is overwhelmingly post-transcriptional. This elevates the importance of RNA binding proteins (RBPs) in gene expression control for these systems. Building upon the comparative *L. mexicana* RBPome we previously isolated (Pablos et al. MCP, 2019), 70 candidate *trans*-regulators were selected for further investigation. Pablos, LM, Ferreira, TR, Dowle, AA, Forrester, S, Parry, E, Newling, K, PB Walrad, 2019, The mRNA-bound Proteome of Leishmania mexicana: Novel Genetic Insight into an Ancient Parasite, MCP: https://doi.org/10.1074/mcp.RA118.001307.

Methods: A barcoded *L. mexicana trans*-regulator knockout library was created using CRISPR-Cas9, pooled, screened for lifecycle progression, including macrophage and mouse infections, and validated.

Results: Null mutants could not be generated for 60% of RBP genes screened; suggesting a high proportion of RBPs are essential for cell viability. Of the 27 viable RBP null mutants 64% exhibit loss of fitness during lifecycle progression to human-infectious stages, infectivity and/or virulence. Examination of individual null mutants verify our competitive bar-seq screen outcomes that isolate specific RBPs implicit in parasite viability, replication and infectivity. Of these, 3 RBPs were endogenously tagged and immunoprecipitated; including the previously-unidentified *Leishmania* homolog of the Trypanosome respirome master regulator ZC3H40.

Conclusions: We find *Leishmania* ZC3H40 protein binds its essential cofactor ZC3H39 and associates and stabilises RNAs encoding essential mitochondrial proteins as well as both *ZC3H40* and *ZC3H39* mRNA, ensuring metabolic capacity in the amastigote human disease-causing form. This lends significant insight into the relatively uncharacterised *Leishmania* respirome requirements in human-infectious stages and validates the biological relevance of our screen.

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PARALLEL ORAL ABSTRACT 21 : PARASITIC CO-INFECTIONS AND DISEASE INTERACTIONS IN HUMAN POPULATIONS
08-19-2026 2:40 PM - 3:40 PM

ASSESSMENT OF SKIN NTDS IN PARTS OF IMO STATE NIGERIA

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Background and Aims: Skin Neglected Tropical Diseases (NTDs) continue to pose significant public health challenges in Nigeria, especially among the rural populace. This exploratory study aimed at assessing the prevalence, knowledge, attitude, and management practices towards skin NTDs in selected communities across the three geopolitical zones of Imo State, namely Owerri, Okigwe, and Orlu.

Methods: The study was carried out using semi-structured self-administered questionnaires and onsite clinical diagnosis, across the ten randomly selected communities of Oboama, Emii, Umuokanne, Umulolo, Amuro, Ihube, Okwe, Ndianiche, Umuobom, and Eziukwu, located in Owerri, Okigwe and Orlu zone respectively. The data obtained was analyzed using SPSS version 20.

Results: A total of 1,530 participants were surveyed, of whom most were farmers (40.8%), artisans/traders (26.3%), students/pupils (19.6%), civil servants (8.3%), and health workers (5%). The study revealed four major skin NTDs (Buruli ulcer (73.2%), Lymphatic filariasis (26.3%), Onchocerciasis (36.3%), and Leprosy (21.1%). Knowledge about skin NTDs showed 71% males having heard or seen cases of all four diseases versus 29% of female participants ($p = 0.025$). There were major misconceptions related to causation; 72.9% believed that stepping on charms can cause skin NTDs, and 63.3% attributed them to poor hygiene, other causes included sexual intercourse (45.6%), contaminated food (42.2%), fever (30.3%), sun exposure (24.8%), and untreated water (23.1%). Attitude toward skin NTDs was highly influenced by traditional beliefs and stigma. Practices toward treatment were mainly traditional.

Conclusions: These findings highlight the immediate need for culturally sensitive health education and intervention programs to increase awareness, dispel myths, and encourage successful medical treatment strategies as well as innovative preventive strategies

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PARALLEL ORAL ABSTRACT 21 : PARASITIC CO-INFECTIONS AND DISEASE INTERACTIONS IN HUMAN POPULATIONS
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INVESTIGATING VISCERAL LEISHMANIASIS AND HIV-CO-INFECTIONS IN BRAZIL

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Background and Aims: Visceral leishmaniasis (VL) remains a major public health problem in Brazil, accounting for 90% of registered cases in South America. Around 10% of patients are co-infected with HIV; this study will explore this specifically vulnerable population.

Methods: We present preliminary results of an epidemiological analysis of VL in Brazil from 2007 to 2023, based on data from the Sistema de Informação de Agravos de Notificação (SINAN) database. We examine temporal trends in incidence rate (IR) and mortality (case fatality rate, CFR) of patients with HIV co-infection (n=5278), as well as risk factors for mortality using logistic regression models and causal inference methods.

Results: Despite a general decline in the number of newly diagnosed VL patients since 2019, the IR among patients with HIV co-infection shows a marked increase. We also found an increase in the proportion of relapse in this group. VL-HIV co-infected are disproportionally men (77.9%, vs. 63.9% among non-co-infected) and patients are on average older (mean age 38.9 years, vs. 23.34 years). The all-cause CFR of VL-HIV co-infected decreased from 23% in 2017 to 14% in 2023, now comparable to the CFR of non-co-infected individuals which increased, and we describe underlying demographic and geographic patterns over time. Among the risk factors contributing to CFR among VL-HIV co-infected were the presence of severe symptoms, other infectious diseases, as well as treatment with pentavalent antimony. We present estimates of treatment effects based on logistic regression guided by directed acyclic graphs (DAGs).

Conclusions: Results of this study aim to inform public policy.

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PARALLEL ORAL ABSTRACT 21 : PARASITIC CO-INFECTIONS AND DISEASE INTERACTIONS IN HUMAN POPULATIONS
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CHARACTERIZATION OF IRON UPTAKE MECHANISMS IN THE MITOCHONDRIA OF PLASMODIUM FALCIPARUM

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Background and Aims: Malaria remains a major global health challenge, with *Plasmodium falciparum* responsible for the most severe disease outcomes. The parasite contains a single mitochondrion that is essential for survival, particularly during the asexual blood stages. Mitochondrial iron homeostasis is critical for processes such as electron transport, heme biosynthesis, and Fe–S cluster formation. However, the molecular mechanisms governing iron transport into the parasite mitochondrion remain poorly understood, limiting the identification of novel antimalarial targets. The present study aims to characterize the role of a putative mitochondrial iron transporter, mitoferrin, in *P. falciparum* and to assess its importance for parasite growth and mitochondrial function.

Methods: Transgenic *P. falciparum* 3D7 parasite lines were generated using an inducible knockdown system to regulate mitoferrin expression. Parasite growth was monitored using standard growth assays and microscopy. Mitochondrial function and oxidative stress were assessed using fluorescent probes, including mitochondrial superoxide indicators. Biochemical assays, including proteoliposomal reconstitution and biophysical interaction studies, were employed to validate the iron transport function of the protein.

Results: Conditional knockdown of mitoferrin resulted in a significant reduction in parasite growth and viability. Treated parasites showed compromised mitochondrial function and elevated mitochondrial oxidative stress, indicating disrupted iron homeostasis. Biochemical assays supported the role of mitoferrin as a mitochondrial iron transporter essential for parasite metabolism.

Conclusions: Our findings demonstrate that mitoferrin plays a critical role in maintaining mitochondrial iron balance and is indispensable for *P. falciparum* survival. Targeting mitochondrial iron transport pathways may represent a promising strategy for the development of novel antimalarial therapeutics.

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PARALLEL ORAL ABSTRACT 21 : PARASITIC CO-INFECTIONS AND DISEASE INTERACTIONS IN HUMAN POPULATIONS
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ASSOCIATION BETWEEN ART ADHERENCE AND SPECIES-SPECIFIC PARASITIC INFECTIONS AMONG PLHIV IN NORTH-CENTRAL NIGERIA

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Background and Aims: Persistent intestinal parasitic infections (IPIs) contribute to morbidity among adults living with HIV, particularly where ART adherence is suboptimal. This study examined adherence status as a determinant of infection risk and parasite diversity.

Methods: A total of **3,071 HIV-positive adults** receiving care across four LGAs in Benue State were enrolled. Participants were stratified into poor adherence (n=1,056) and good adherence (n=2,015) groups. Stool samples were processed via formalin-ether concentration and wet mount microscopy to detect intestinal parasites.

Results: Marked adherence-related differences were observed. **Approximately one-third of poorly adherent adults (35–38%) harbored at least one parasite**, compared to **only 2–3% in the well-adherent group**. Fifteen parasitic species were identified. Protozoa accounted for >70% of infections in poorly adherent PLHIV, with *Cryptosporidium parvum* (5.1%), *Entamoeba histolytica* (5.0%), *Giardia lamblia* (4.1%), and *Entamoeba coli* (4.0%) most frequent. Coccidian parasites, notably *Cystoisospora belli* (2.5%) and *Cyclospora cayetanensis* (1.6%), were nearly absent in the good adherence group (<0.1%). Helminths, including *Taenia spp.* (5.8%), *Ascaris lumbricoides* (1.4%) and hookworm (2.1%) were significantly more frequent in poorly adherent clients (all **p<0.001**). Mixed infections occurred in ~2–3% of cases in the poor adherence category.

Conclusions: ART adherence strongly predicted both the prevalence and diversity of parasitic infections in this PLHIV population. Targeted parasite control strategies should prioritize PLHIV with poor adherence, integrating routine stool screening, presumptive treatment for opportunistic protozoa, and enhanced adherence counseling to reduce vulnerability to opportunistic enteric disease.

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PARALLEL ORAL ABSTRACT SESSION
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BACTERIAL AND FUNGAL BIOMARKERS IN IBS AND IBD: TRANS-KINGDOM INTERACTIONS, BLASTOCYSTIS CARRIAGE, AND ENTEROTYPE-SUCCINOTYPE STRATIFICATION

Ayman El-Badry[1], Abdelaziz Al-Quran[2], Nehal Hosin[3], Mark Van Der Giezen[4,5], Yohannes Seyoum[5]
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Background and Aims: The gut microbiome, encompassing bacteria, fungi, and protists, is integral to gastrointestinal (GI) health and disease. While bacterial dysbiosis is well documented in irritable bowel syndrome (IBS) and inflammatory bowel disease (IBD), fungal contributions and bacterial-fungal interactions remain underexplored, particularly in non-Western populations. We aimed to provide a comprehensive, multi-kingdom analysis of GI disorders, integrating bacterial and fungal biomarkers, network robustness, enterotype/succinotype stratification, and *Blastocystis* carriage.

Methods: Fecal specimens were collected from Saudi participants with IBS, ulcerative colitis (UC), Crohn's disease (CD), and healthy controls. Fecal specimens were examined microscopically and molecularly for Blastocystis. Copro-DNA was sequenced and amplified for 16S rRNA gene and ITS region. Analyses incorporated bacterial enterotypes, succinotypes, and *Blastocystis* carriage to explore ecological and metabolic stratification.

Results: GI disorder, host factors, enterotype, and Blastocystis each explained bacterial and fungal variation. IBS patients exhibited increased bacterial richness and evenness, UC and CD displayed stronger fungal dysbiosis. Enterotyping identified two Bacteroides-dominant clusters. Succinotyping distinguished D-type and P-type communities; UC was enriched in fragile, bridge-dependent D-type networks, in contrast to short-chain fatty acid (SCFA)- and H_2S-producing P-type networks. *Blastocystis* was detected in 48% of participants, most frequently in controls, associated with the enrichment of butyrate-producing bacteria and distinct fungal genera.

Conclusions: Our study represents the first integrated analysis of bacterial and fungal gut communities in a Saudi Arabian cohort, representing non-Western populations, including IBS, UC, CD, and healthy controls. The observed ecosystem fragility in IBD, particularly UC, offers a novel

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mechanistic perspective and potential translational relevance for diagnostics and personalized microbiome-modulating therapies.

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AN INFLAMMATORY INFERNO: TRICHOMONAS VAGINALIS SPARKS PYROPTOSIS IN ECTOCERVICAL CELLS

Johann Tailor[1,2], Kathryn Morales[1], Maryam Howayer[1], Bryn Baxter[1], Evie Barouch[1], Brayden Young[1], Michiko Adams[1], Angelica Riestra[1]
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Background and Aims: *Trichomonas vaginalis* (Tv) is an extracellular eukaryotic parasite that causes a prevalent sexually transmitted infection called trichomoniasis. Increased inflammation in the urogenital tract during Tv infection is predicted to drive comorbidities like cervical cancer, but the underlying inflammatory mechanism is unclear. Pyroptosis is a lytic cell death that releases proinflammatory cytokines, namely Interleukin-1β (IL-1β) and IL-18, via gasdermin D (GSDMD) plasma membrane pores. We hypothesize that Tv damages ectocervical epithelial cells (Ect1) via GSDMD-mediated pyroptosis.

Methods: To genetically test the involvement of GSDMD, we generated Ect1 GSDMD-knockout (GSDMD KO) cells. Infected Ect1 cells were assayed for cytolysis via lactate dehydrogenase assay and for differential release of proinflammatory cytokines via enzyme-linked immunosorbent assay (ELISA). Furthermore, we sought to identify additional proteins released via Ect1 GSDMD membrane pores during Tv-induced pyroptosis using quantitative proteomics.

Results: We note a 25% decrease in Tv-induced cytolysis of Ect1 GSDMD KO cells, compared to wild-type. Cell supernatant analysis of Tv-infected cells using ELISA revealed a 53% and 25% decrease in IL-1β and IL-18 release, respectively, in GSDMD KO cells compared to wild-type. Quantitative proteomics further demonstrated selective release of small-molecular weight and potential microenvironment-altering proteins via GSDMD membrane pores during Tv infection, validated by western blot analysis.

Conclusions: We show Tv triggers GSDMD-mediated pyroptosis and selective cargo release via GSDMD plasma membrane pores in Ect1 cells. This study provides the first evidence that Tv induces pyroptosis in Ect1 cells as a pathogenic mechanism and highlights that epithelial cells, not just immune cells, can undergo robust pyroptosis during parasitic infection.

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CHARACTERIZING THE TYPE 2 IMMUNE RESPONSE IN THE NEMATODE MODEL HELIGMOSOMOIDES BAKERI

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Background and Aims: A significant gap in knowledge exists regarding the Th2 response to helminth infections. The dynamics of priming, expansion, homing, and memory responses by helminth-specific Th2 cells remain poorly studied due to a lack of available tools. We aim to create a CD4+ TCR transgenic mouse specific for *H. bakeri*, a gastrointestinal nematode, that will enable us to study such cells.

Methods: To facilitate our search for *H. bakeri*-specific CD4+ T cells, we established infection in mice with a restricted T cell repertoire (fixed TCR beta). CD4+ T cells were sorted from the small intestinal lamina propria (SILP) and peritoneal cavity of uninfected and infected mice for single-cell sequencing of gene expression and TCR alpha chains. Selected TCRs were expressed in T cell hybridomas to test their specificity for *H. bakeri* antigens present in excretory-secretory products (HES). TCRs were expressed in primary CD4+ T cells and tested in vivo during *H. bakeri* infection.

Results: TCR sequencing identified expanded T cell clones. We selected our top 4 candidate TCRs, and two of these reacted to HES in vitro. Testing in vivo demonstrated the expansion of T cells retrovirally expressing one of these TCRs upon transfer to infected mice, in the spleen, mesenteric lymph node, and peritoneum. In the SILP, these T cells were only found in infected mice, and not in uninfected controls. These T cells produced type 2 cytokines.

Conclusions: We have identified an *H. bakeri*-specific TCR. Future studies will address how a helminth-specific T cell clone may take multiple polarization fates during infection.

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IDENTIFICATION OF A PARASITIC WORM AS THE FIRST NON-BACTERIAL ORGANISM REPORTED TO BE CAPABLE OF DECONJUGATING BILE ACIDS IN THE MAMMALIAN GUT

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Background and Aims: Bile acids (BAs) enter the small intestine, the niche of the helminth *Heligmosomoides polygyrus bakeri* (*Hpb*), in an amino acid-conjugated form. It is well-established that gut bacteria can deconjugate BAs while mammalian hosts cannot, and it has not been examined whether helminths can do so. Deconjugating BAs reduces toxicity to certain bacteria and alters affinity for host BA signalling receptors. After collecting evidence that *Hpb* deconjugates BAs *ex vivo*, we aimed to **(1) determine whether *Hpb* deconjugates BAs *in vivo* and (2) quantify BA toxicity to *Hpb*.**

Methods: (1) We sterilely infected germ-free mice (which have negligible unconjugated BAs due to lacking a bacterial microbiota) with *Hpb* and later quantified BAs with mass spectrometry. **(2)** We co-incubated *Hpb* with unconjugated or taurine-conjugated BAs, assessing cellular viability via an ATP assay.

Results: (1) We detected significantly less conjugated and more unconjugated BAs in the small intestines of germ-free mice following *Hpb* infection. **(2)** Co-incubation of *Hpb* with physiologically relevant concentrations of the BAs tested resulted in decreased cellular ATP. Notably, an unconjugated BA (CDCA) was significantly less detrimental to *Hpb* than its amino acid-conjugated counterpart.

Conclusions: To our knowledge, our findings are the first description of a non-bacterial organism with BA de-conjugation capabilities. We hypothesize that *Hpb* evolved the ability to deconjugate BAs to reduce toxicity in the worm's environment. Given the potential for BA deconjugation by *Hpb* to modulate microbiome composition and host metabolism/immunity, our ongoing work investigates whether this phenomenon directly impacts parasite health and/or the host response to intestinal helminth infection.

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SAFETY AND EFFICACY OF ALTERNATIVE BENZNIDAZOLE AND NIFURTIMOX REGIMENS FOR ADULTS WITH CHRONIC TRYPANOSOMA CRUZI INFECTION (TESEO): AN OPEN-LABEL, RANDOMISED, NON-INFERIORITY PHASE 2 TRIAL

Igor Almeida[1], Cristina Alonso-Vega[2], Jimy Pinto[3], Gimena Rojas[3], Wilson García[3], Alejandro Palacios[3], Susana Méndez[2], Virgini González[1], Mónica Laserna[3], Isabela Ribeiro[4], María-Jesús Pinazo[2], M Thomas[5], Manuel López[5], Iren Losada[2], Lourdes Ortiz[3], Julio Urbina[6], Sergi Sanz[2], Joaquim Gascon[2,7], Faustino Torrico[3]
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Background and Aims: Benznidazole (BZN) and nifurtimox (NFX), the only treatments for Chagas disease, cause frequent drug-related adverse events (DRAEs) that impair treatment completion. TESEO evaluated alternative BZN and NFX regimens to improve tolerability while maintaining antiparasitic efficacy.

Methods: This open-label, randomised, non-inferiority phase 2 trial enrolled 450 adults with chronic *Trypanosoma cruzi* infection in Bolivia. Six arms (75 participants each) were compared: BZN 150 mg twice daily for 60 days (BZN-60, standard of care [SoC]); BZN 150 mg once daily for 30 (BZN-30) or 90 (BZN-90) days; NFX 240 mg twice daily for 60 days (NFX-60, SoC) or 30 days (NFX-30); or NFX 240 mg once daily for 90 days (NFX-90). Co-primary outcomes were DRAE incidence and sustained parasitological clearance by quantitative PCR (qPCR).

Results: DRAEs affected 273/450 participants (61%; 1052 events), with significantly fewer in the BZN than NFX group (52% vs 69%; p<0·0001). BZN-30 had the lowest DRAE rate (39%; p<0·0001 vs BZN-60 SoC). DRAEs clustered within 10–14 days regardless of duration, suggesting idiosyncratic toxicity. An unexpected lymphopenia-neutrophilia pattern affected 8% of NFX-treated participants. Of 439 qPCR-evaluable participants, 396 (90·2%) achieved PCR negativisation. BZN arms showed higher efficacy than NFX arms overall (p=0·022), ranging from 94·7% (BZN-60 SoC) to 80·8% (NFX-60 SoC). Non-inferiority to SoC was established for BZN-30, NFX-30, and NFX-90, but not BZN-90.

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Conclusions: BZN demonstrated superior tolerability and efficacy compared with NFX. BZN-30 achieved the best safety profile while maintaining non-inferior antiparasitic efficacy, supporting its potential as a revised standard of care. Funding: NIH Grant # U01AI129783.

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ENHANCED DETECTION OF MIXED PLASMODIUM SPECIES MALARIA INFECTIONS BY MULTIPLEX REAL-TIME PCR

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Background and Aims: Human malaria is an important global health problem caused by *Plasmodium* parasites. Five different *Plasmodium* species are responsible for most infections. Microscopy and rapid diagnostic tests often fail to accurately identify mixed species infections (involving two or more *Plasmodium* species), which can lead to improper treatment. This study presents a multiplex real-time PCR designed to accurately detect mixed species infections.

Methods: Species-specific TaqMan assays were designed for *P. falciparum, P. malariae, P. ovale (*subspecies *curtisi* and *wallikeri), P. vivax*, and *P. knowlesi.* Clinical whole blood specimens, specificity control samples, and laboratory-created mixed species samples were used to evaluate diagnostic and analytic performance. Its limit of detection (LOD) and effectiveness at resolving mixed species infections were compared with three other published TaqMan assays.

Results: The LOD of the new test was 0.82 parasites/µl. The test had 100% (50 of 50 samples) diagnostic specificity. The diagnostic sensitivity was 100% for malarial infections overall and 94% (79 of 84 samples) for species-level resolution. The test correctly identified *Plasmodium* species in 13 of 16 (81%) clinical mixed species specimens and 24 of 27 (89%) artificially mixed species samples. The three comparison tests had LODs between 1.36 and >20 parasites/µl and correctly identified 0% to 70% of the mixed species samples.

Conclusions: Compared with the other three TaqMan tests, the new test had the lowest LOD and the best capability to resolve mixed species infections. The new test may be useful for reference diagnostic laboratories requiring sensitive, species-level identification of human-infecting *Plasmodium* species.

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CELL-FREE DNA FROM ECHINOCOCCUS GRANULOSUS IN PATIENT SERUM: A NEW FRONTIER IN HYDATID CYST DIAGNOSIS

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Background and Aims: Hydatid cyst is a zoonotic disease worldwide. The larval stage of *Echinococcus granulosus* is responsible for this disease. In new research, efforts are underway to detect the parasite's cell-free DNA (cfDNA) in the patient's body fluids using molecular methods. This study aimed to propose a method for diagnosing the disease by detecting parasite cfDNA using mitochondrial genes in patients' serum.

Methods: The study included 97 serum samples from patients with pathologically confirmed hydatid cysts. The extracted DNA from serum samples was amplified using conventional and real-time PCR with newly designed primers and probes targeting the mitochondrial genes cytochrome c oxidase I (*cox1*), NADH dehydrogenase subunit I (*nad1*), and the small subunit of ribosomal RNA (*rrnS*).

Results: Among 97 patients, *E. granulosus* cfDNA was successfully detected by conventional PCR in 49 (50.5%; 95% CI: 40.2–60.8) and by Real-time PCR in 89 (91.8%; 95% CI: 84.4–96.4) patients. Overall, the sensitivity and specificity of the three genes was 50.% (95% CI: 40.2–60.8) and 100% (95% CI: 83.2–100.0) for conventional PCR, and 91.8% (95% CI: 84.4–96.4) and 100% (95% CI: 83.2–100.0) for real-time PCR.

Conclusions: Our findings reveal that the primers and probes designed for the *cox1*, *nad1*, and *rrnS* genes of *E. granulosus* are highly specific and sensitive for real-time PCR detection of cfDNA to diagnose hydatid cysts. Therefore, *E. granulosus* cfDNA, as a noninvasive diagnostic marker, can be detected in serum samples from patients with hydatid cysts using real-time PCR. However, this procedure needs more modification to be applicable in the medical laboratory.

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A NOVEL MULTIPLEX SEROLOGICAL APPROACH (MULTICRUZI) FOR MEASURING TREATMENT EFFICACY IN CHAGAS DISEASE

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Background and Aims: Chagas disease (CD), caused by Trypanosoma cruzi, remains a major public health concern in Latin America and an increasing global issue due to migration. An estimated 7–10 million people are infected, with ~12,000 annual deaths. Progress toward shorter, safer, and more effective treatments is hampered by the lack of biomarkers capable of detecting early therapeutic response. DNDi is evaluating the MultiCruzi multiplex serological assay—quantifying antibody reactivity to 15 *T. cruzi* antigens— that has emerged as a promising platform for identifying antibody decline.

Methods: Serum samples from the Phase II BENDITA trial (NCT03378661), which tested six benznidazole regimens and placebo, were analyzed at baseline, 6, and 12 months. MultiCruzi was performed at three dilutions. A validated serial-dilution model was applied to quantify antibody kinetics. DF50—the dilution at which antigen signal decreases by 50%—was calculated for each antigen and time point. Log2-transformed DF50 values were analyzed using linear mixed models to estimate decay rates and detect early treatment effects.

Results: MultiCruzi provided more granular antibody kinetics than ELISA and detected treatment-associated declines by 6 months. In BENDITA adults, ≥79% of individuals receiving benznidazole showed DF50 reductions at 12 months, with significant declines in all benznidazole groups compared with placebo. The DF50 model enhanced visualization of antibody waning and improved discrimination of treatment effects.

Conclusions: MultiCruzi enables detection of treatment-related antibody decline within 6–12 months, providing an early marker of therapeutic response that could accelerate CD drug development and support clinical decision-making.

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A NATIONAL SURVEY OF GASTROINTESTINAL NEMATODES IN AUSTRALIAN DAIRY GOATS USING FAECAL EGG COUNTS AND DEEP AMPLICON SEQUENCING

Endris Ali[1], Abdul Ghafar[1], Ghazanfar Abbas[1], Bahar E Mustafa[1], Sandra Baxendell[2], Elysia Ling[1], Charles Gauci[1], Ian Beveridge[1], Mark Stevenson[1], Abdul Jabbar[1]
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Background and Aims: Gastrointestinal nematodes (GINs) are a major constraint to goat health, welfare and productivity worldwide and represent the highest-priority pathogens affecting goats in Australia. Despite this, contemporary national-scale data on infection burden, species composition and epidemiological drivers in Australian goats are lacking. This study aimed to quantify the prevalence, intensity, species diversity and key risk factors associated with GIN infections at a national level.

Methods: A cross-sectional survey was conducted between November 2023 and July 2024. Faecal samples (*n* = 1,028) were collected from 68 dairy goat herds across Australia and analysed using a modified McMaster technique. Strongyle-positive DNA extracts (*n* = 500) underwent deep amplicon sequencing of the ITS-2 region for species identification. Animal-level prevalence was adjusted for herd effect, and associations between risk factors and faecal egg counts (FEC) were assessed using mixed-effect regression models.

Results: Strongyle infections were detected in 91% of goats and in 100% of herds, exceeding the prevalence of *Trichuris* spp. (13%) and *Nematodirus* spp. (5%). Strongyle FECs were highly overdispersed, with 30% of animals contributing 80% of total egg output. Age, climatic zone, recent deworming and co-infection with *Eimeria* were significantly associated with increased FECs ($p < 0.05$). Metabarcoding identified 2,001 amplicon sequence variants representing 11 strongyle species, with *Haemonchus contortus*, *Teladorsagia circumcincta* and *Trichostrongylus colubriformis* dominating nationally.

Conclusions: This national survey demonstrates a high prevalence and diversity of GINs in Australian dairy goats, dominated by *H. contortus*. These findings underscore the need for coordinated national efforts to achieve sustainable control of GINs in grazing small ruminants.

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EXPANDED HOST RANGE AND GEOGRAPHIC DISTRIBUTION OF TRICHINELLA CHANCHALENSIS IN NORTHERN NORTH AMERICA AND CO-OCCURRING TRICHINELLA SPP.

Cody Malone[1], N Jane Harms[2], Kimberlee Beckmen[3], Raphaela Stimmelmayr[4], Naima Jutha[5], Malik Awan[6], Vladislav Lobanov[7], Emily Jenkins[1]
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Background and Aims: A recently discovered zoonotic nematode, *Trichinella chanchalensis* (T13), was identified in wolverines and an American marten from the Northwest Territories (NWT) and Yukon, Canada. Because traditional diagnostic methods cannot distinguish T13 from *T. nativa*, a broader host range was suspected. We investigated the ecology of T13 by assessing host use and geographic distribution in terrestrial carnivores from Alaska and three Canadian territories.

Methods: Larvae were recovered from muscle tissue of wildlife using the double separatory funnel method and were genotyped using deep amplicon next-generation sequencing on the Illumina MiSeq.

Results: *Trichinella* spp. were detected in wildlife from the Yukon (158/213; 74%), Alaska (53/157; 33.8%), Nunavut (41/50; 82%), and NWT (35/50; 70%). Co-infections with *Trichinella nativa* and *Trichinella* T6 were most common (169/281; 60%), ranging from 33% in Alaska to 87% in the Northwest Territories. No interspecific competition was detected among sympatric taxa. T13 was detected in the Yukon (41/158; 25.9%), Nunavut (9/41; 22%), NWT (2/31; 6.5%), and Alaska (1/51; 2%). T13 occurred predominantly in mixed infections (50/53; 94%) and was identified in two Canada lynx (*Lynx canadensis*), two wolves (*Canis lupus*), and one coyote (*Canis latrans*), representing new host records. T13 was also detected for the first time in Nunavut and Alaska.

Conclusions: This study expands the known host and geographic range of T13 and provides updated estimates of *Trichinella* spp. prevalence, diversity, and infection dynamics across northern North America using a metabarcoding approach. Understanding host-specific patterns of infection is essential for assessing food safety risks and supporting food sovereignty in northern communities.

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HIGH-EFFICACY ANTHELMINTICS FOR TRICHURIS TRICHIURA: COMPARATIVE MODELLING OF THE IMPACT OF THE NOVEL AGENT EMODEPSIDE VERSUS ALBENDAZOLE.

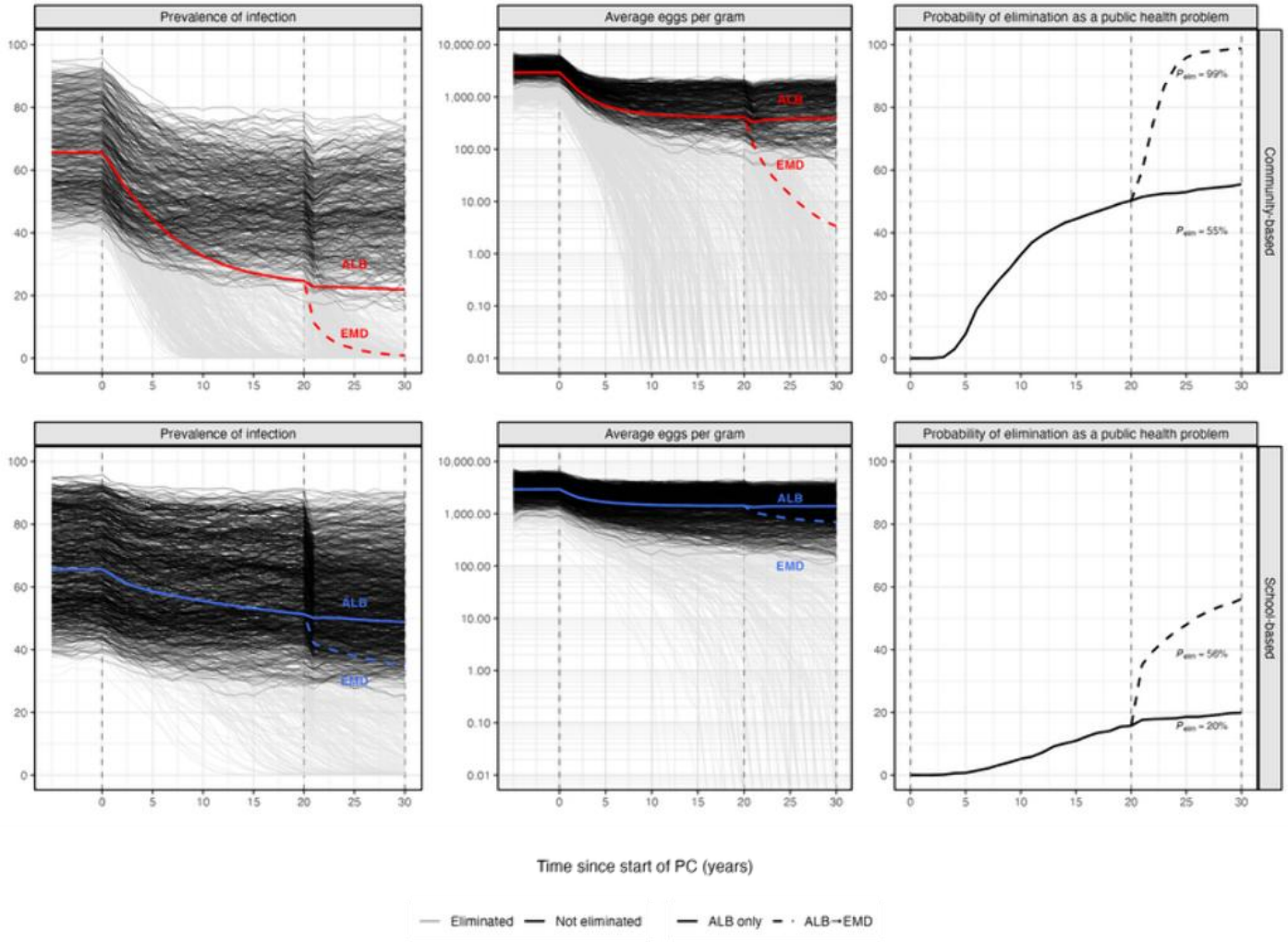
Manfredi Greco[1], Jennifer Keiser[2], Sake De Vlas[1], Luc Coffeng[1]
[1]Erasmus University Medical Center, Department Of Public Health, Rotterdam, Netherlands, [2]Swiss Tropical and Public Health Institute, Allschwil, Switzerland

Background and Aims: *Trichuris trichiura* persists in many soil-transmitted-helminth–endemic settings despite mass drug administration (MDA), partly due to benzimidazoles' limited efficacy. We quantified the impact of switching from benzimidazoles to emodepside (EMD; currently under development) on infection prevalence, intensity, and elimination as a public health problem (EPHP; <2% prevalence of moderate-to-high intensity [MHI] in school-age and pre-school-age children).

Methods: Using the individual-based WORMSIM framework, we simulated the impact of school-targeted MDA (pre-SAC+SAC; 90% coverage) and community-wide MDA (>2 years old; 75% coverage), delivered annually or semiannually by baseline endemicity. Scenarios included 20 years of albendazole (ALB) preventive chemotherapy (PC) followed by 10 years of either continued ALB or switching to EMD. Drug efficacy was assumed to be 50% for ALB (killing patent worms only) and 99% for EMD (killing both pre-patent and patent

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worms).



Results: Relative to continued ALB, switching to EMD produced additional reductions in prevalence and intensity. In high-endemicity settings under semiannual school-based PC, switching to EMD achieved an additional 18-percentage-points reduction in prevalence and 52% in intensity. Incremental benefits were greatest under community-wide delivery (26 percentage-points additional reduction in prevalence; 99% in intensity). EPHP increased by 37 percentage-points under semiannual school-based PC and 43 percentage-points under semiannual community-wide PC after switching to
EMD.

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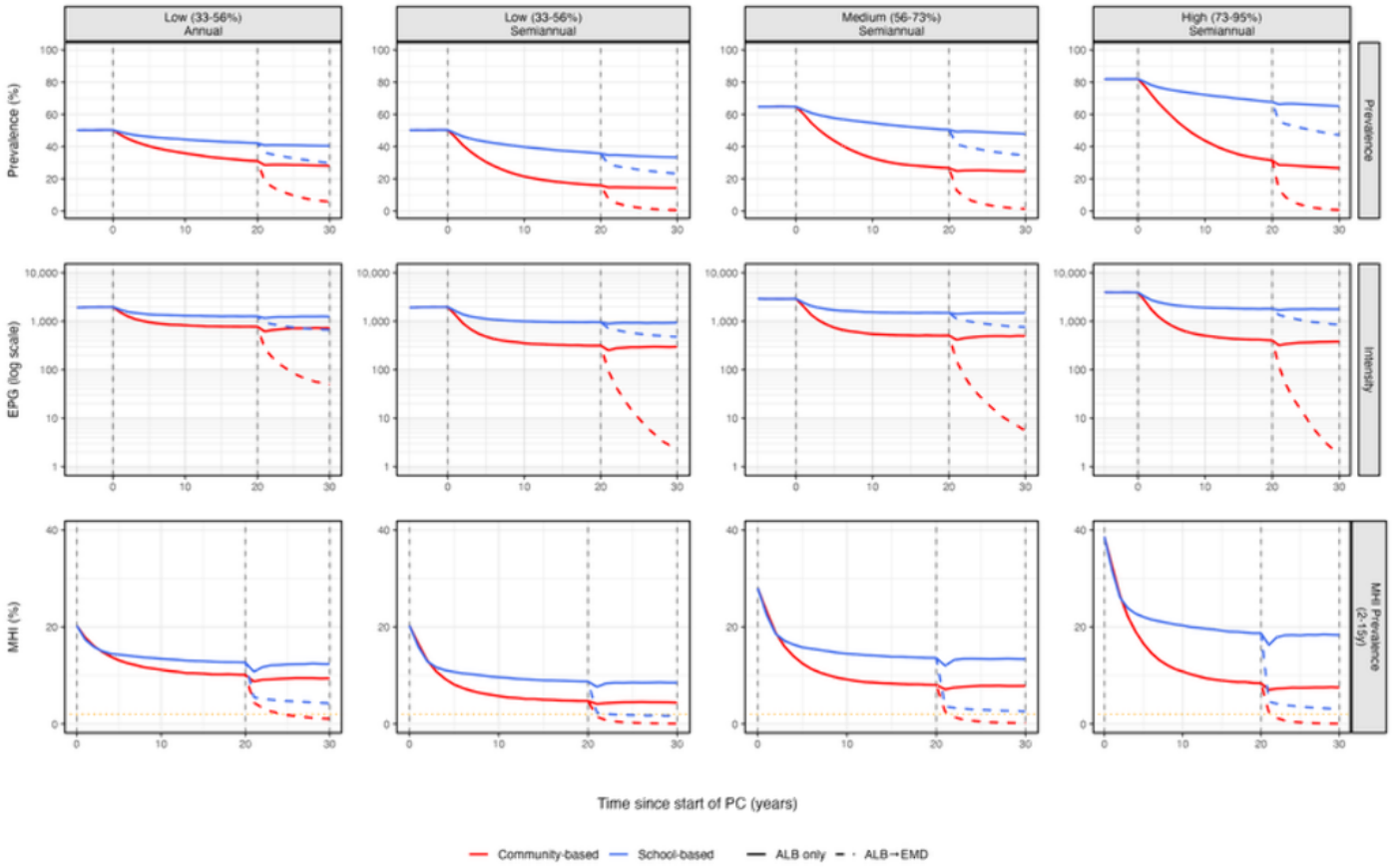


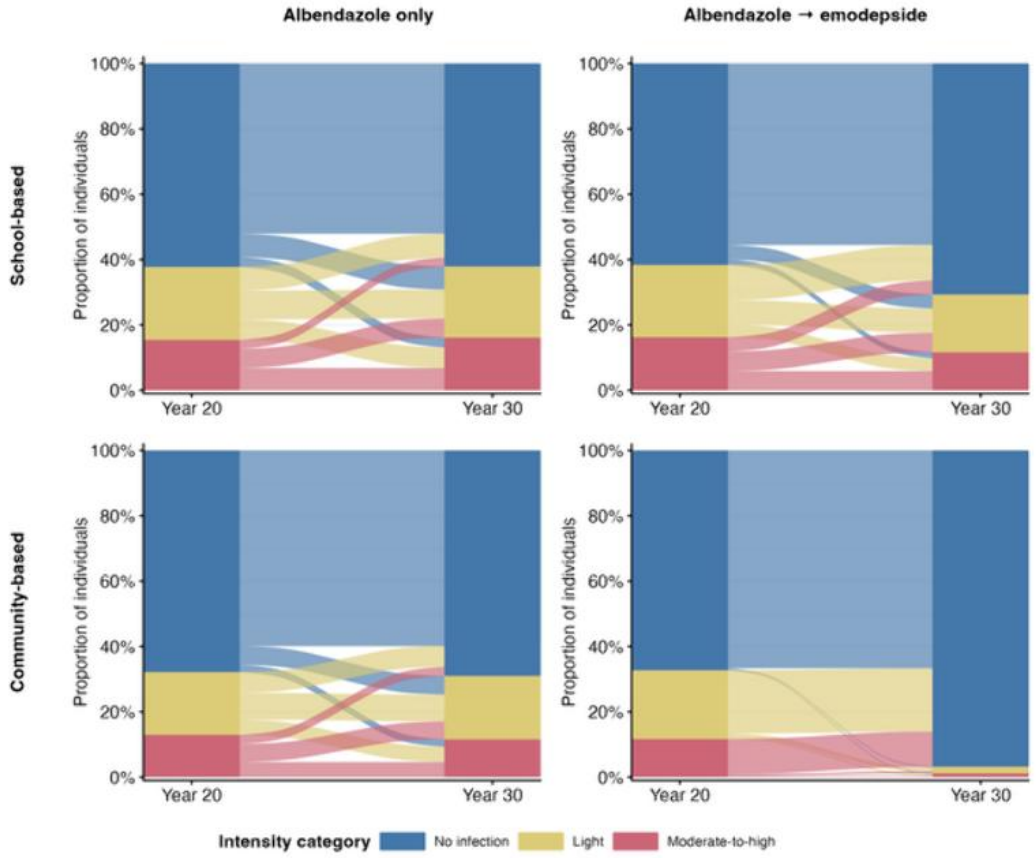
Table 1 | Model-predicted probability of EPHP of *Trichuris trichiura* under school-based and community-based MDA scenarios

MDA strategy (coverage)	MDA frequency	Probability of elimination as public health problem at 30 years					
		Low endemicity		Middle endemicity		High endemicity	
		ALB	EMD	ALB	EMD	ALB	EMD
Trichuris trichiura							
School-based (90%)	Annual	10.2	30.6	6.3	21.1	4.6	12.0
	Semiannual	26.8	68.0	16.9	45.4	12.0	42.0
Community-based (75%)	Annual	30.3	79.9	21.8	66.9	18.7	80.2
	Semiannual	62.0	98.9	47.9	97.2	56.2	98.9

Elimination as a public health problem (EPHP) is defined as medium-to-heavy intensity (MHI) prevalence <2% in children aged 2-15 years, following WHO guidelines. Probabilities are expressed as percentages (%). Rows correspond to MDA strategy and frequency; columns correspond to low/middle/high endemicity settings, each shown for ALB (albendazole only for 30 years) and EMD (albendazole for 20 years followed by emodepside for 10 years) regimens. Values reported at year 30 (end of MDA period).

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Fig. 3 | Intensity-class transitions from Year 20 to Year 30 comparing albendazole-only versus switching to emodepside.



Conclusions: Our findings indicate that emodepside could be a game changer, particularly under community-wide delivery and in high-endemic settings. Given its high efficacy, emodepside is expected to markedly increase the probability of achieving the WHO target of eliminating STH as a PHP, especially in areas highly endemic for *T. trichiura*.

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A NEXT-GENERATION SEQUENCING APPROACH TO ASSESS THE GENETIC VARIABILITY OF STRONGYLOIDES SPP. INFECTIONS IN CHILDREN, DOGS AND LONG-TAIL MACAQUES

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Background and Aims: Strongyloidiasis affects ~600 million people globally but remains one of the most neglected helminthiases due to sub-optimal diagnosis. Severe disseminated disease can be fatal, particularly in immunocompromised individuals. The World Health Organization has prioritised understanding the role of animals in transmission, yet the contribution of reservoirs remains unclear. The aim of this study was to assess the zoonotic transmission risk of *Strongyloides* by investigating infections in humans, dogs, and long-tailed macaques across Cambodia and Papua New Guinea within a One Health framework.

Methods: We used nanopore sequencing to characterise the genetic diversity of *Strongyloides* spp. infecting school-aged children, dogs, and long-tailed macaques in Kampong Chhnang, Cambodia, and humans from Madang Province, Papua New Guinea. Analyses targeted commonly used genetic markers, including 18S-rRNA hypervariable regions and mitochondrial cytochrome c oxidase subunit-1 (*cox1*).

Results: We identified shared *S. stercoralis* haplotypes in humans and dogs from the same villages, alongside a high diversity of unique haplotypes in school-aged children, suggesting that human-to-human transmission may predominate in some settings. We report the first detection of *S. fuelleborni* in non-human primates in Cambodia and demonstrate substantial genetic diversity across primate hosts and geographic regions throughout Asia and Africa, consistent with a cryptic species

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complex. In Papua New Guinea, we identified human infections with both *S. stercoralis* and *S. f. fuelleborni* and discuss historical reports of *S. f. kellyi* in the context of the limited resolution of current genetic markers.

Conclusions: Overall, zoonotic transmission of *Strongyloides* appears context-dependent, and improved genomic resolution will be essential to inform WHO-strongyloidiasis surveillance and elimination strategies.

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UNCOVERING EUROPEAN TRICHINELLA SPIRALIS GENETIC VARIATION AT HIGH RESOLUTION

Ewa Bilska-Zając[1], Annette Johne[2], Davor Balić[3], Laurentiu Ciupescu[4], Weronika Korpysa-Dzirba[1], Aneta Bełcik[1], Tomasz Cencek[1]
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Background and Aims: *Trichinella spiralis* is a nematode responsible for the majority of human trichinellosis cases worldwide. Although it has traditionally been considered a species with very low genetic variability, the recent development of the **Trich-tracker** method (Bilska-Zając et al., 2022) has for the first time enabled detection of subtle differences among isolates. The aim of this study was to investigate the genetic structure of *T. spiralis* populations from different regions of Europe using ddRADseq, analyzing multiple randomly selected loci across the parasite's genome.

Methods: The aim of this study was to investigate the genetic structure of *T. spiralis* populations from different regions of Europe using ddRADseq, analyzing multiple randomly selected loci across the parasite's genome.

Results: Phylogenetic analysis revealed significant genetic diversity among *T. spiralis* isolates from Poland, Germany, Croatia, and Romania. Within certain clades, isolates grouped according to geographic origin, particularly for Poland and Germany, which may reflect local populations and limited gene flow. Isolates from southern Europe (Croatia, Romania) often formed distinct or peripheral branches of the tree, suggesting regional genetic divergence.

Conclusions: The presence of clades containing isolates from different countries and hosts indicates the potential for long-distance parasite dispersal, possibly driven by wildlife migration or human activities. These results confirm that high-resolution ddRADseq data allow the detection of subtle genetic patterns enabling inferences about their genetic relationships that would remain undetected using single molecular markers.

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INTEGRATED GENOMIC, MITOCHONDRIAL AND MICROBIOME INSIGHTS INTO TRICLABENDAZOLE RESISTANCE AND TRANSMISSION OF FASCIOLA

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Background and Aims: *Fasciola hepatica* is a globally distributed zoonotic parasite with major human and livestock impacts. Triclabendazole (TCBZ) is the only drug effective against all parasite stages, but increasing resistance threatens control. We aim at elucidating the resistance mechanisms and transmission by multi-modal data integration.

Methods: We integrated nuclear genomic, mitochondrial, transcriptomic, and gut microbiome data from two geographically distinct endemic regions of Peru and compared TCBZ-S and TCBZ-R *F. hepatica*, evaluated zoonotic transmission and gut microbiome shifts.

Results: Whole-genome sequencing of 473 adult TCBZ-sensitive and -resistant *F. hepatica* from livestock in Cusco and Cajamarca identified highly differentiated loci (FST >99.9th percentile) associated with microtubule stability, cytoskeletal organization, and anti-apoptotic pathways. Distinct regional outlier profiles indicated independent resistance emergence under localized selection with limited gene flow. Transcriptomic comparisons confirmed differential expression of microtubule-associated genes in resistant parasites. Mitochondrial haplotype analysis of 315 animal and human isolates identified five major haplogroups, with resistant human parasites clustering within livestock lineages, providing direct genetic evidence of zoonotic transmission. Metagenomic shotgun sequencing of 30 infected and 60 matched uninfected individuals revealed microbiome shifts linked to infection and treatment response. TCBZ responders showed enrichment of *Firmicutes A*, *Bacteroides spp*., and phospholipid biosynthesis pathways. Non-responders exhibited higher Actinobacteria, and amino acid biosynthesis pathways. Longitudinal analysis demonstrated microbiome restructuring following treatment, associated with therapeutic outcomes.

Conclusions: TCBZ resistance evolves through distinct but functionally convergent mechanisms shaped by parasite genetics, host ecology, and microbiome composition, and highlight integrated genomic-microbiome surveillance to improve diagnostics, guide sustainable drug use, and mitigate transmission of resistant fascioliasis.

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MOLECULAR AND MORPHOLOGIC CHARACTERIZATION OF TRICHURIS TRICHIURA INFECTING FREE-ROAMING AFRICAN VERVETS (CHLOROCEBUS AETHIOPS SABAEUS) ON THE CARIBBEAN ISLAND OF ST. KITTS

Travis Richins[1], Sarah Sapp[1], Jennifer Ketzis[2], Arve Willingham[3], Samson Mukaratirwa[2], Yvonne Qvarnstrom[1], Joel Barratt[1]
[1]CDC: Centers for Disease Control and Prevention, Diagnostic Parasitology Team, Atlanta, United States of America, [2]Ross University School of Veterinary Medicine, One Health Center For Zoonoses & Tropical Veterinary Medicine, Basseterre, Saint Kitts and Nevis, [3]United Arab Emirates University, 3department Of Veterinary Medicine, Falaj Hazza, United Arab Emirates

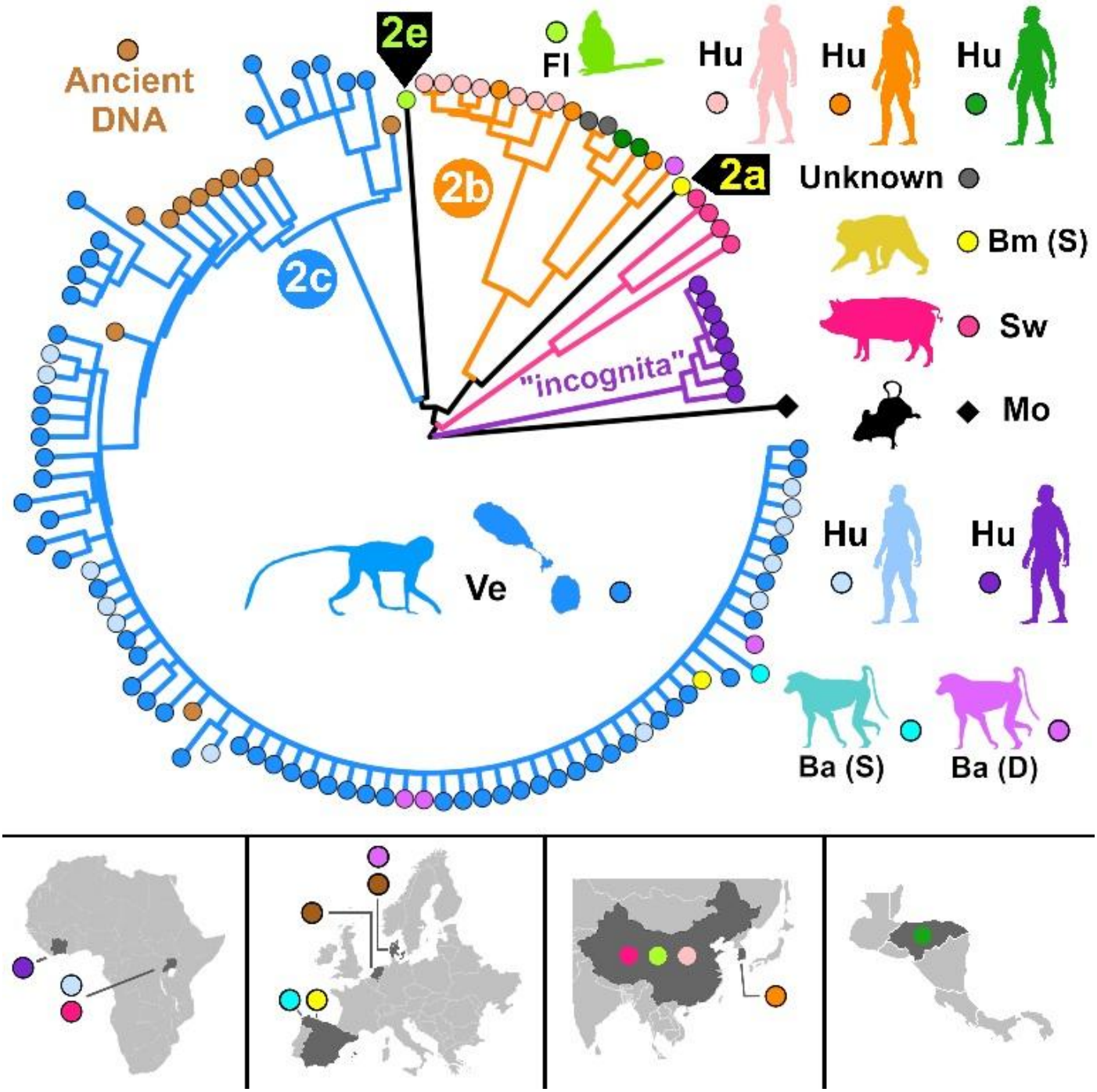
Background and Aims: Human trichuriasis is an important disease caused by the soil-transmitted helminth *Trichuris trichiura*, which typically affects human populations in low socioeconomic situations. Natural hosts of *T. trichiura* include humans and non-human primates (NHPs), giving rise to concerns that NHPs could serve as zoonotic reservoirs of *T. trichiura* in locations where humans and NHPs exist in proximity. Free-roaming vervets (*Chlorocebus aethiops sabaeus*), introduced from Africa to the Caribbean Island of St. Kitts represent cause for concern given their propensity to roam within urban environments.

Methods: To investigate the potential role of vervets as reservoirs of human trichuriasis, we genotyped *T. trichiura* collected from St. Kitts vervets and compare their genotypes to those observed previously from humans and other primates. Thirty-two adult *T. trichiura* and 17 fecal samples containing *T. trichiura* eggs collected from the vervets were subjected to molecular analysis, involving deep sequencing of PCR amplicons targeting the 18S ribosomal DNA hypervariable regions I and IV, and a fragment of the mitochondrial genome spanning part of the small subunit ribosomal RNA gene, the valine transfer RNA gene, and the large subunit rDNA. Morphometric analysis of adult worms demonstrated consistency with previous studies on *T. trichiura* from primates.

Results: Phylogenetic analysis showed that *T. trichiura* from St. Kitts vervets belong to a clade that had recently been implicated in human infections and also been detected in ancient European human

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latrines.



Conclusions: This observation supports that St. Kitts vervets might represent reservoirs for human infection and indicates this tools utility in clustering similar Trichuris infections.

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ZOONOTIC INTESTINAL PARASITES IN DOGS PARTICIPATING IN ANIMAL- ASSISTED INTERVENTIONS: OCCURRENCE AND MANAGEMENT-RELATED RISK FACTORS

Elisabetta Ferraro[1], Marika Grillini[1], Daniela Pasotto[1], Carlotta Valente[1], Patrizia Danesi[2], Barbara Contiero[1], Federica Marcer[1], Giulia Simonato[1]
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Background and Aims: Animal-assisted Interventions (AAIs) involve close contact between dogs and human beneficiaries, often belonging to vulnerable populations. This study aims to identify zoonotic parasites in dogs active in AAIs, evaluate potential risk factors related to their daily management, and provide data to structure sanitary protocols and preventive actions to limit infections, avoid transmission, and guarantee human and animal health.

Methods: One hundred thirteen dogs, nowadays involved in AAIs, were screened for intestinal parasites potentially transmissible through close contact. Tests included copromicroscopy, *Giardia* coproantigen test, molecular analysis (SSU-rRNA/β-giardin genes) on positive samples for *Giardia* assemblage identification, and coproantigen test for intestinal helminths. Owners/handlers completed structured questionnaires regarding daily dog management to identify risk factors through statistical correlation (Chi-squared/Fisher's exact tests).

Results: Twenty-four (21.2%) out of 113 dogs tested positive by copromicroscopy: 11(9.7%) for *Giardia* spp., 10(8.8%) for *Toxocara* spp., and 3(2.7%) for ancylostomatids. *Giardia* antigen test confirmed copromicroscopy results and detected two additional positive dogs. All 13 *Giardia duodenalis* positive samples belonged to the canine C-assemblage. The helminth coproantigen test confirmed 1 dog positive for *Toxocara canis* and 2 positives for ancylostomatids, revealing the coprofagy attitude. Data analysis showed associations between *Giardia* infection and lifestyle factors, including limited outdoor activities and attending dog parks.

Conclusions: The presence of zoonotic helminths and protozoa underscores the importance of health monitoring and promotes more effective daily dog management. Developing standardized sanitary guidelines is crucial for mitigating risks and ensuring the safety of animals and all participants in AAI settings. Supported by MAPS Department, University of Padua, Italy (2024MAPS1SIDPROGETTI-00040)

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FUNCTIONAL ANALYSIS OF A NOVEL LEISHMANIA EFFECTOR TARGETED TO MACROPHAGE MITOCHONDRIA.

Hamlet Adolfo Acevedo Ospina, Albert Descoteaux
INRS-Centre Armand-Frappier Santé Biotechnologie, Laval, Canada

Background and Aims: To colonize macrophages, *Leishmania* manipulates host metabolism to generate a metabolically adapted microenvironment required for replication. We previously reported that *L. donovani* metacyclic promastigotes induce host cell mitochondrial biogenesis and enhance oxidative phosphorylation. Among the factors involved in this process, we identified the *Leishmania* virulence glycolipid lipophosphoglycan and host type I IFN receptor signalling, both critical for *L. donovani* replication.

Methods: To further elucidate the functional consequences of *Leishmania*-induced mitochondrial alterations, we generated a mitoproteome of *L. donovani*-infected macrophages and a functional genomic approach to elucidate the biological relevance of *Leishmania* proteins.

Results: Analysis of the mitoproteomes identified 1644 proteins, 497 of which were classified as high-confidence mitochondrial proteins after cross-referencing with the Mouse MitoCarta 3.0 database. Gene ontology analysis associates these mitochondrial proteins with gene expression and metabolic regulation. Notably, we identified several *Leishmania* proteins within the host mitoproteome, suggesting that *Leishmania* release specific proteins to target host mitochondria. Using a Cas9-T7RNP-engineered *L. donovani* line, we introduced fluorescent tags to confirm the localization of these proteins within the host mitochondrial network and endosomal compartments. The majority of the evaluated *Leishmania* proteins were essential for axenic parasite viability, preventing the generation of double knockouts. Strikingly, one of them was found to be dispensable in culture but essential for intra-macrophage replication.

Conclusions: These findings provide novel insights into the ability of *L. donovani* to colonize macrophages through the action of an effector targeted to host cell mitochondria. Supported by the Canadian Institutes of Health Research

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INFECTIVITY AND REPLICATION OF TOXOPLASMA GONDII IN BOVINE MACROPHAGES FROM SEROPOSITIVE ANIMALS

Sofía Jara-Méndez, Camila Muñoz-Zanzi, Juliette Brante-Bernier, Catalina Alfaro-Godoy, Galia Ramírez-Toloza
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Background and Aims: *Toxoplasma gondii* is a zoonotic, obligate intracellular protozoan, with cats as the definitive hosts and several species, including cattle, as intermediate hosts. Macrophages are the first line of defense against intracellular infections, and recent studies have shown that they can develop memory after some infections or vaccinations. The objective of this study was to analyze the ability of macrophages from cattle infected with *T. gondii* to control the infection.

Methods: CD14+ macrophages were isolated from peripheral blood mononuclear cells of two T. gondii seropositive and two seronegative cattle. The cells were seeded and infected in triplicate with tachyzoites at three multiplicities of infection (MOI): 0.5, 1, and 2. The cells were analyzed by immunofluorescence to calculate two indicators: infection rate (number of parasites per 100 cells) and replication rate (number of parasites per infected cell) at three time points (4, 8, and 24 hours post-infection).

Results: Higher infection rates and lower replication rates were detected in macrophages from seropositive cattle. Significant differences are observed between seropositive and seronegative individuals when analyzing the replication rate at an MOI of 0.5 ($p=0.0345$) and at 4 hours post-infection with the same MOI ($p=0.0138$).

Conclusions: There is a tendency toward higher infection rates and lower replication rates in macrophages derived from cattle seropositive for *T. gondii*, indicating better control of infection.
Acknowledgement: Fondecyt 1231686

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HELMINTH INFECTION ENHANCES HOST SUSCEPTIBILITY TO GAMMAHERPESVIRUS COINFECTION VIA IL-4RA-DEPENDENT CHANGES IN AIRWAY MACROPHAGES LEADING TO SYNDECAN 4 UPREGULATION AND IMPAIRED IFN-I RESPONSE

Georgios Petrellis[1], Marion Rolot[1], Annette Dougall[1], Lionel Lenoir[1], Ariel Madrigal[2], Caroline Wathieu[1], Alisha Chetty[3], Lucia Rodriguez Rodriguez[1], Joan Abinet[1], Alain Chariot[1], Matthias Mack[4], Benedicte Machiels[1], Thomas Marichal[1], Laurent Gillet[1], Hamed Najafabadi[2], William Horsnell[5], Benjamin Dewals[1]
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Background and Aims: Hookworms migrate through the lungs before reaching the intestine, inducing functional changes in airway macrophages (Mϕ). However, it remains unclear how such changes in Mϕ functions could alter the host susceptibility to viral coinfection.

Methods: Here, *Nippostrongylus* infection in mice resulted in markedly enhanced replication of the murid gammaherpesvirus 4 (MuHV-4) in the lung.

Results: *N. brasiliensis* infection resulted in an IL-4Rα-dependent disappearance of SiglecF$^+$ alveolar Mϕ (AlvMs) and recruitment of monocyte-derived Mϕ (moMϕ) in the lung, and MuHV-4 showed increased permissiveness in airway Mϕ in an IL-4Rα-dependent manner. Moreover, MuHV-4 replication in airway Mϕ *ex vivo* when isolated from helminth-infected mice was significantly enhanced and potentiated by IL-4 and IL-13. Single-cell transcriptomic analyses revealed enriched monocyte origin and IL-4 polarization gene signatures in airway and lung Mϕ after helminth infection, together with upregulation of heparan-sulfate proteoglycan syndecan 4 and impaired type I IFN response. Such changes in airway Mϕ were retained up to 42 days after helminth infection as revealed by the analysis of the chromatin accessibility by ATAC sequencing. Strikingly, infection with a gp150$^-$ MuHV-4 virus unable to attach heparan sulfates resulted in significantly reduced susceptibility of mice infected with *N. brasiliensis*.

Conclusions: These results demonstrate that the type 2 cytokines IL-4 and IL-13 drive heparan sulfate proteoglycans in Mϕ and impaired type I IFN response, resulting in enhanced host susceptibility to gammaherpesvirus.

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HELMINTH-DERIVED ANISAXIN A-2S REVEALS A VPAC1–PKA–IL-10 AXIS UNDERLYING MACROPHAGE IMMUNE-MODULATION

Jovana Majstorovic[1], Rhoda Kafui Akpoh[1], Ivona Mladineo[2], Jaroslava Lieskovská[3]
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Background and Aims: Helminth parasites are well known for their ability to modulate host immune responses, often skewing immunity toward anti-inflammatory pathways that facilitate chronic infection. Anisaxin (A-2S), a cecropin-like antimicrobial peptide derived from *Anisakis* spp., has recently been suggested to possess immunomodulatory properties; however, its anti-inflammatory activity and underlying molecular mechanisms remain incompletely defined. Here, we investigated the effects of A-2S on inflammatory responses in murine macrophage-like PMJ-2R cells and elucidated the signaling pathways involved.

Methods: Macrophages were stimulated under pro-inflammatory conditions in the presence or absence of A-2S. Cytokine production was quantified by ELISA, inflammatory gene expression was analyzed by quantitative PCR, and pathway activation was assessed by Western blotting.

Results: A-2S treatment markedly suppressed pro-inflammatory cytokines, including TNFα, while robustly inducing the anti-inflammatory cytokine IL-10 at both transcriptional and protein levels. Mechanistic analyses demonstrated that A-2S acts through activation of the vasoactive intestinal peptide receptor 1 (VPAC1), leading to increased intracellular cAMP and subsequent activation of protein kinase A (PKA). Consistently, a selective VPAC1 agonist reproduced the effects of A-2S, whereas pharmacological inhibition of PKA abolished A-2S–induced IL-10 production. Moreover, blockade of VPAC1 using the selective antagonist PG 97-269 significantly reduced IL-10 levels, confirming the central role of VPAC1 signaling. Neutralization of IL-10 reversed the suppressive effects of A-2S and restored TNFα production, identifying IL-10 as a critical downstream effector

Conclusions: Collectively, these findings identify anisaxin A-2S as a helminth-derived antimicrobial peptide with potent anti-inflammatory activity mediated via a VPAC1–cAMP–PKA–IL-10 axis, highlighting its potential as a parasite-inspired therapeutic strategy for immune-mediated inflammatory diseases.

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LEISHMANIA DONOVANI INDUCES ATP6V0D2 EXPRESSION IN MACROPHAGES LEADING TO MULTINUCLEATION AND LIPID DROPLET FORMATION

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Background and Aims: *Leishmania donovani* infects macrophages and manipulates host cellular functions to promote its survival. Characteristics of infected macrophages include multinucleated giant cell (MGC) formation and lipid droplet formation, whereas the molecular link between these phenomena remains unclear. ATP6V0D2, a subunit of the vacuolar-type ATPase, is upregulated in macrophages upon *L. donovani* infection. This study explores the potential roles of ATP6V0D2 in MGC formation and lipid droplet formation during *Leishmania* infection.

Methods: Bone marrow-derived macrophages (BMDMs) were infected with *L. donovani in vitro*, and formations of MGCs and lipid droplet were examined by staining with Hoechst 33342 and BODIPY 493/503, respectively. Next, involvement of ATP6V0D2 in those formations was examined on the infected BMDMs by RNA interference of the gene.

Results: *L. donovani* infection induced ATP6V0D2 expression in BMDMs and *Atp6v0d2* knockdown reduced MGC formation in the infected BMDMs. The infection also induced increase in total lipid levels as well as the formation of lipid droplets in BMDMs. Though total lipid levels were not markedly suppressed in infected BMDMs by *Atp6v0d2* knockdown, the formation of lipid droplets was suppressed; BODIPY staining signals more dispersed, and the area and number with strong signals decreased.

Conclusions: These findings demonstrate that ATP6V0D2 is a host regulatory factor linking MGC formation and intracellular lipid handling in *L. donovani*–infected macrophages. Since both processes involve membrane dynamics including cholesterol trafficking, upregulated ATP6V0D2 during *L. donovani* infection in macrophages may contribute to changes in membrane kinetics leading to MGC formation and lipid droplet formation.

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ZBTB7B IS IMPLICATED IN MACROPHAGE-INTRINSIC RESPONSES TO INTRACELLULAR PATHOGENS

Julia Luo[1,2], Marine Leroux[3], Marija Landekic[4], Rebecca Bellworthy[1,2], George Dong[3], Saumya Agrawal[5], Michiel De Hoon[5], Danielle Malo[4], Martin Olivier[3,6], David Langlais[1,2,6]
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Background and Aims: Pathogenesis of *Leishmania* requires internalization by, and replication within, innate immune cells, particularly macrophages. ThPOK is a transcription factor necessary for CD4 T lineage determination; however, its role in innate immunity is unclear. Previously, the lab highlighted an essential role of ZBTB7B in intracellular pathogen disease models where macrophages are key for resolving infection via CD4 Th1-mediated IFNγ pathways. Thus, we aim to investigate the role of ThPOK in macrophage responses to *Leishmania major* and other intracellular pathogens.

Methods: We challenged WT and *Zbtb7b*$^{-/-}$ mice subcutaneously with 5x10^{6} *L.major* promastigotes in the right-hind footpad for 6 weeks. Footpad thickness was measured biweekly; parasite burden and flow immunophenotyping was done at endpoint. We then infected *Zbtb7b*^{fl}Cx3cr1^{Cre} mice with *L.major* to assess *Zbtb7b* knockout in mononuclear phagocytes. *In vitro*, we challenged WT and *Zbtb7b*$^{-/-}$ BMDMs with MOI10 *S.typhimurium* for 4 hours and assessed intracellular killing by CFU and transcriptomic differences by CAGE-sequencing.

Results: *In vivo,* we found that *Zbtb7b*$^{-/-}$ mice had significantly larger footpad lesions, higher parasite load, and sustained neutrophilia with fewer footpad MHC2+ macrophages at 6 weeks post-infection (p.i.). Although the footpad lesion phenotype was diminished, there was still significantly more neutrophils and fewer MHC2+ macrophages in the footpad in conditional knockout *Zbtb7b*^{fl}Cx3cr1^{Cre} mice. *In vitro,* we found that *Zbtb7b*$^{-/-}$ BMDMs transcriptionally have decreased enrichment of autophagy pathways and increased intracellular bacterial burden at 4 hours post-infection (p.i.).

Conclusions: These findings suggest a macrophage-intrinsic, T-cell independent effect of *Zbtb7b* in responses to intracellular infection.

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HELMINTH-INDUCED IL-33 DRIVES AN IL-4RA–INDEPENDENT ANTI-INFLAMMATORY MACROPHAGE THAT SUPPRESSES COLITIS

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Background and Aims: Infection with helminth parasites induces alarmin release, including IL-33 which initiates type-2 immunity. In non-permissive hosts, helminth expulsion is often critically dependent on IL-4Rα signaling; indeed, IL-4Ra$^{-/-}$ mice fail to expel the tapeworm *Hymenolepis diminuta*: despite increased intestinal IL-33 indicating recognition of infection. We have previously shown that *H. diminuta* infection protects against DNBS-colitis. Surprisingly, IL-4Rα$^{-/-}$-infected mice also experience less severe colitis. We hypothesised that IL-33 mediates helminth-induced supression of colitis independent of IL-4 signaling.

Methods: BALB/c WT and IL-4Rα$^{-/-}$ mice were orally infected with *H. diminuta*. At day 8 post-infection, colitis was induced with DNBS and disease assessed 72h later. Colon tissue was analyzed by qPCR, ELISA and spectral flow cytometry. Macrophages were depleted using anti-CSF1R , and IL-33/ST2 signaling blocked with HpBARI_Hom2. Bone marrow-derived macrophages were treated with IL-33 and phenotype/function assed. The impact of i.p.-delivered IL-33 treated macrophages on DNBS-induced colitis was examined.

Results: IL-33 expression was significantly increased in the intestine of *H. diminuta*-infected WT and IL4-Ra$^{-/-}$ mice, and was accompanied by expansion of CD206^{+}ST2^{+}IL-10^{+} macrophages. Blocking IL-33/ST2 signaling or depleting macrophages abolished the protective effect of *H. diminuta*-infection in WT and IL-4Rα$^{-/-}$ mice without affecting worm burden. Moreover, blockage of ST2 decreased the numbers of CD206^{+}macrophages. *In vitro*, IL-33 evoked up-regulation of Relm-alpha, Arg1, Gata3 and IL-10 and adoptive transfer reduce colitis severity, confirming the cells regulatory capacity.

Conclusions: In conclusion, *H. diminuta* infection promotes an IL-33–driven, IL-4Rα-independent regulatory macrophage response that suppresses intestinal inflammation. This IL-33–macrophage axis represents a novel mechanism of helminth-mediated immunomodulation and a potential therapeutic target for IBD.

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GASDERMIN D IS ASSOCIATED WITH HUMAN VISCERAL LEISHMANIASIS AND CONTROLS PARASITE BURDEN IN EXPERIMENTAL INFECTION

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Background and Aims: Visceral leishmaniasis (VL) is a severe systemic disease marked by profound immune activation. Gasdermin D (GSDMD) is a central mediator of inflammatory responses; however, its role in VL pathogenesis remains poorly defined. In this study, we investigated the contribution of GSDMD to immune activation and parasite control during *Leishmania infantum* infection using clinical samples and experimental models.

Methods: Serum levels of caspase-1, GSDMD, IL-1β, and IL-18 were quantified in patients with active VL using ELISA and cytometric bead array (CBA). Correlation analyses were performed to evaluate associations between inflammasome-related components and cytokine levels. Bone marrow–derived macrophages (BMDMs) were primed and infected with *L. infantum* to assess GSDMD cleavage by Western blotting, parasite burden by Giemsa staining, and IL-1β production by ELISA. *In vivo* experiments were conducted using Gsdmd-deficient mice to evaluate parasite burden and hepatic inflammatory responses.

Results: Patients with active VL exhibited elevated serum levels of caspase-1, GSDMD, IL-1β, and IL-18, indicating systemic inflammatory activation. Serum GSDMD levels correlated with multiple inflammatory and regulatory cytokines. GSDMD deficiency resulted in increased intracellular parasite burden and reduced IL-1β release in infected macrophages. *In vivo*, Gsdmd-deficient mice displayed higher parasite loads and dysregulated hepatic inflammation.

Conclusions: GSDMD is active in human visceral leishmaniasis and plays a critical role in controlling *L. infantum* replication in mouse models, while modulating inflammatory responses during infection, supporting its role as a key mediator of host defense.

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SHOULD I STAY OR SHOULD I GO? NEW INSIGHTS INTO TRYPANOSOMA CRUZI INVASION AND EGRESS.

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Background and Aims: *Trypanosoma cruzi* is an obligate intracellular pathogen capable of invading virtually any nucleated host cell within minutes of contact. Despite eliciting a strong immune response, infections persist through mechanisms that remain poorly understood. We aimed to determine whether distinct invasion and egress pathways influence the outcome of the infection.

Methods: Experiments employed *T. cruzi* lines expressing mNeonGreen and/or mScarlet fluorescent proteins, genetically modified mammalian cell lines with fluorescently labelled subcellular structures, and multiple live dyes. Real-time epifluorescence microscopy combined with immunostaining was used to monitor infection dynamics.

Results: We observed labelled trypomastigotes entering and leaving a succession of host cells, transiently acquiring layers of host cell membranes, before finally establishing an infection. Entry and exit of these trypomastigotes was by the anterior end (flagellum-first). On occasion, invasion of already infected cells led to their disruption/lysis and release of amastigotes/intermediate infective forms enclosed by host-derived membranes. To assess the role of the flagellum in this invasion/egress process, we have generated parasite cell lines where flagellar activity has been blocked. The impact of these manipulations on parasite invasion and egress will be discussed.

Conclusions: These assays revealed unexpectedly dynamic activity associated with parasite infectivity, challenging the traditional view of *T. cruzi* invasion. Several previously unidentified mechanisms were detected. These findings have broad implications for understanding mammalian cell entry strategies, egress, transmission, and persistence within the host. The genetically modified parasite lines we have generated will facilitate dissection of the processes involved.

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A DISTINCT VIRAL STRATEGY: LEISHMANIA RNA VIRUS 2 (LRV2) REDUCES LEISHMANIA CUTANEOUS PATHOGENICITY

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Background and Aims: Endosymbiotic viruses of the *Totiviridae* family, such as *Leishmania* RNA Virus 2 (LRV2), infect several *Leishmania* species. While *Leishmania* RNA Virus 1 (LRV1) is associated with disease severity and treatment failure in the Viannia subgenus, the biological impact of *Leishmania* RNA Virus 2 (LRV2) remains largely unexplored. *Leishmania* parasites release extracellular vesicles (EVs) and use them to communicate and secrete virulence factors—pathways also hijacked by LRV2 for transmission. Here, we investigated whether LRV2 alters cutaneous pathogenicity and how it disseminates among *Leishmania* species.

Methods: The effect of LRV2 on pathogenicity was assessed in vivo using a BALB/c mouse model; infections with endogenous LRV2-positive *L. major* (Uzbekistan strain) were compared to parasites cured of LRV2 using 2'-C-methyladenosine treatment (*L. major* LRV2-). EVs were isolated from LRV2-positive and -negative parasites and characterized by nanoparticle tracking analysis, electron microscopy, and qRT-PCR. EV-mediated transmission of LRV2 was assessed in vitro and in vivo across multiple *Leishmania* species to assess viral infectivity and maintenance over time.

Results: LRV2-positive parasites (Uzbekistan strain) induced significantly reduced footpad inflammation compared to LRV2-negative parasites, revealing a pathogenic profile distinct from the lesion-exacerbating phenotype associated with LRV1. EVs efficiently infected multiple *Leishmania* species, with variable levels, and the viral persistence was highly strain dependent. In vivo, newly infected parasites with LRV2 display infection outcomes distinct from the Uzbekistan LRV2-positive strain. Notably, these newly infected parasites induced greater inflammation.

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Conclusions: Our findings uncover a distinct transmission strategy and pathogenic profile for LRV2 and establish EVs as critical mediators of endovirus dissemination in *Leishmania*.

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HOST CELL REMODELLING BY THE MALARIA PARASITE IS COORDINATED BY THE AP2-HCR DNA BINDING PROTEIN

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Background and Aims: *Plasmodium* species that infect red blood cells (RBCs) can significantly modify the permeability, rigidity, and cytoadherence properties of these terminally differentiated RBCs. This host-cell remodelling process is achieved through the export of numerous effector proteins into the RBC cytosol and membrane, which is central to the virulence associated with *P. falciparum*. These effector proteins are expressed within a very short time frame and are likely co-regulated by single or similar transcription factors.

Methods: We have characterised an essential Apicomplexan AP2 (ApiAP2) DNA-binding protein in P. falciparum (PfAP2-HCR; host cell remodelling) during the intraerythrocytic developmental cycle. PfAP2-HCR peaks in its expression 40 hours post-red blood cell invasion (h.p.i) and codes for two ApiAP2 DNA-binding domains. An inducible gene-knockout approach revealed that PfAP2-HCR is essential for early-ring-stage development, during which parasites cease to develop beyond the late-ring stage. RNA-seq experiments revealed that PfAP2-HCR is critical for the expression of genes encoding proteins essential for the formation of the Plasmodium Translocon of Exported Proteins (PTEX) complex and host-cell remodelling. ChIP-seq experiments further revealed PfAP2-HCR binding to the promoters of genes encoding the majority of these host cell remodelling proteins, suggesting that these genes are direct regulatory targets of PfAP2-HCR.

Results: We provide conclusive evidence that PfAP2-HCR is a key transcriptional regulator controlling proteins essential for the transport of exported proteins and host-cell remodelling.

Conclusions: These findings position PfAP2-HCR as a central transcriptional hub that drives the expression of key pathogenic factors required to remodel a terminally differentiated host cell for parasite survival and growth.

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TRYPANOSOMA CRUZI TRANS-SIALIDASES, A POWERFUL VIRULENT FACTOR THAT CAN BE TARGETED FOR THE DEVELOPMENT OF A CHAGAS DISEASE VACCINE

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Background and Aims: Trans-sialidases (TS) is a surface protein encoded by the largest Trypanosoma cruzi gene family, with most genes encoding inactive enzymes. Active TS (aTS) transfers sialic acid from host glycoconjugates to parasite mucins, a parasite strategy to evade host immune responses. Shed in the blood of the infected host, aTS also mediates several biological effects and because of its essential role during infection, it has been tested recurrently as a vaccine candidate against Chagas disease. Active TS also contains a C-terminal immunogenic domain with amino acids repeats known as SAPA (shed acute phase antigen).

Methods: By disrupting all aTS genes using CRISPR-Cas9, we generated a parasite cell line (aTS-KO) that displays undetectable levels of TS activity.To test the role of the SAPA domain, we immunized mice with recombinant TS proteins or RNA sequences with and without repeats.

Results: Although aTS disruption did not affect the parasite capacity to invade cells in vitro, parasite egress from the cell was strongly impaired. Importantly, aTS-KO is completely unable to establish infection in vivo and acts as a attenuated parasite strain that offers 100% protection against a challenge infection. In addition to confirming the immunodominance of SAPA repeats, after challenging immunized animals with *T. cruzi*, we showed that the presence of the repeats resulted in increased production of anti-inflammatory cytokines such as IL-10.

Conclusions: By promoting anti-inflammatory responses, the SAPA domain is part of the virulence properties of TS that favors parasite survival. As a vaccine component, the presence of the SAPA domain may help promoting a balanced immune response.

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MODELING TRICHINELLA SPIRALIS-INDUCED MUSCLE REGENERATION SHIFTS USING SKELETAL MUSCLE ORGANOIDS

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Background and Aims: Three-dimensional (3D) skeletal muscle organoids provide a physiologically relevant platform to study host–pathogen interactions. *Trichinella spiralis* uniquely reprograms skeletal muscle cells into nurse cells through processes resembling muscle regeneration and repair. This study aimed to establish a human skeletal muscle organoid model to investigate alterations in muscle development and regeneration induced by *T. spiralis*.

Methods: Human skeletal muscle organoids (hSMOs) were generated from induced pluripotent stem cells (iPSCs), recapitulating myogenic progression from satellite cells to mature myofibers. Following infection with *T. spiralis* newborn larvae, myogenic gene expression and histopathological changes were assessed by qRT-PCR and H&E staining. The effects of *T. spiralis* excretory–secretory (ES) products on tissue remodeling were analyzed using mass spectrometry–based proteomics.

Results: iPSC-derived hSMOs matured into myofibers expressing key myogenic markers. Infection induced marked structural remodeling, with reduced expression of late myogenic regulators (MYOD, MYOG) at 3 and 15 days post infection (dpi) and increased satellite cell proliferation (PAX7) at 10 dpi, indicating reactivation of muscle regeneration. Proteomic analysis revealed differential protein expression following ES product treatment, notably the upregulation of extracellular matrix remodeling proteins, consistent with a microenvironment permissive for nurse cell formation.

Conclusions: This study establishes a robust 3D human skeletal muscle organoid model to investigate *Trichinella*-induced muscle remodeling and regeneration. The findings advance understanding of trichinellosis pathogenesis and provide a valuable platform for studying skeletal muscle regeneration and identifying therapeutic targets.

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MODULATING THE LIVER ENVIRONMENT: UNDERSTANDING THE DISCRETE INTERACTIONS BETWEEN A LIVER PARASITE AND TISSUE-RESIDENT SPECIALIZED CELL TYPES

Miriam Garner[1,2], Dan Price[1], Krystyna Cwiklinski[3], Marc Faber[1], Philip Mccarron[2], Dave Bartley[1], Brian Quinn[2], Mark Robinson[2], David Smith[1]
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Background and Aims: Understanding how helminth parasites modulate host responses within the liver has been limited by the lack of physiologically relevant models, particularly for tissue-resident liver cell populations. Organoid-based technologies offer a powerful approach to interrogate species- and tissue-specific host–parasite interactions. Here, we established ruminant liver-derived primary cultures and three-dimensional (3D) liver organoids for modelling cellular and molecular interactions between the liver fluke *Fasciola hepatica* and the host liver.

Methods: Liver tissue was collected from experimentally infected sheep at 8- and 25-weeks post-infection and analysed to define host responses. In parallel, primary liver progenitor cells were isolated from cattle and sheep and cultured as 3D liver organoids to investigate cell-type-specific responses to parasite excretory/secretory products (ESPs) and compare these with *in vivo* tissue responses.

Results: Differential expression analysis of *F. hepatica*-infected tissue revealed pathways associated with epithelial remodeling, fibrosis, and reduced hepatic metabolic function. Bovine and ovine liver organoids were successfully established, capable of serial passage and cryopreservation. When cultured in growth medium, organoids consisted predominantly of ductal cholangiocytes and retained the capacity to metabolize triclabendazole. Organoids and isolated primary cells were subsequently challenged with ESPs for transcriptomic analysis.

Conclusions: This study provides the first comprehensive characterization liver cell responses to *F. hepatica* in a relevant species model and establishes a novel platform for identifying parasite-derived drivers of pathology and informing vaccine target discovery.

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ARTEMISININ RESISTANT MALARIA PARASITES RELY ON REGULAR MEALTIMES

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Background and Aims: The spread of malaria parasites resistant to the most common antimalarial drugs, the artemisinins, is of increasing concern. Artemisinin resistance involves reducing haemoglobin digestion to inhibit the activation of the drug and lower its toxicity. However, haemoglobin is a source of the nutrients that fuel blood stage replication, forcing resistant parasites to rely more on acquiring nutrients directly from the blood, following the host's digestion of meals. Indeed, if parasites cannot replicate on time, their within-host survival and between-host transmission are compromised. We predict that impaired haemoglobin digestion by artemisinin-resistant parasites exacerbates the importance of aligning replication to the host's rhythmic meals.

Methods: We used the rodent malaria, *Plasmodium chabaudi* to test this prediction, by comparing the replication rate and transmission potential of an artemisinin-sensitive genotype to that of an isogenic artemisinin-resistant genotype throughout infections where parasites were either aligned, or misaligned with their host's daily rhythms.

Results: As expected, misalignment to host rhythms reduced replication for both genotypes, but artemisinin-resistant parasites suffer more from being out of synch with the host's feeding rhythms.

Conclusions: The parasites' dependence on host feeding rhythms, and specifically the higher dependence of artemisinin-resistant parasites, may provide novel ways to control malaria and curb the spread of artemisinin-resistance.

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THE EFFECTS OF INTERACTION WITH THE PATHOBIONT, AIEC, ON THE BEHAVIOR OF ENTAMOEBA HISTOLYTICA

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Background and Aims: In asymptomatic infection, *Entamoeba histolytica* (*Eh*) colonizes on colonic mucus where it interacts and feed on indigenous microbiota. However, in malnourished or immunocompromised subjects, *Eh* binds to and enzymatically digests the mucus barrier and invades colonic tissues to cause intestinal amebiasis. In intestinal dysbiosis, Enterobacteria bacteria predominates and is highly associated with the pathobiont, adherent-invasive *E. coli* (AIEC). It is currently unknown if dysbiosis alters the virulence of *Eh*. In this study, we determine if *Eh*-AIEC interaction can alter parasite virulence and pathogenesis.

Methods: To assess the effects of microbiota on virulence factor gene expression, *Eh* were incubated with AIEC, a nonpathogenic DH5 alpha *E. coli*, or without bacteria and analyzed for expression of *cysteine proteases (CP-A) 1-5, Cox-1,* and *Gal/GalNac lectin*. To determine effects on *Eh* mucus ingestion (mucophagy), similar coincubation conditions were used, and amoebae were then plated on LS174T cells (mucus-producing). Mucophagy was quantified by immunofluorescence using a fluorescent lectin antibody and analysis by imaging flow cytometry.

Results: Our data indicate while *EhCP-A1-5* expression were modestly increased, co-incubation with AIEC markedly upregulated the expression of *Cox-1* and *Gal/GalNac lectin* as compared to DH5 alpha *E. coli*. Mucophagy was not significantly different among these groups.

Conclusions: These data suggest a trend towards greater *Eh* Gal/GalNAc adherence and altered cysteine protease and Cox-1 (PGE_2) upregulation following co-incubation with AIEC. Studies are ongoing to determine whether mucophagy, secretion of CPs with high enzymatic activity, mucus degradation and cytotoxicity are modulated following AIEC-*Eh* interaction.

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ASSESSMENT OF EARLY CUTANEOUS MIGRATION AND VASCULAR PENETRATION OF STRONGYLOIDES VENEZUELENSIS

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Background and Aims: *Strongyloides venezuelensis*, a skin-penetrating parasitic nematode in rodents, initiates infection through infective third-stage larvae (iL3) that penetrate the skin and subsequently reach target organs via circulation. While skin penetration is known to occur rapidly, the subsequent transition from the skin into blood vessels, an essential step for migration to the lungs, remains poorly understood. This study aimed to clarify the early cutaneous migration and vascular approach of *S. venezuelensis* iL3 at minute-scale resolution.

Methods: Ten-week-old male C57BL/6 mice were percutaneously infected with 5,000 iL3 applied to shaved abdominal skin. Skin samples were collected at 10, 20, and 35 minutes post-infection and examined using histology and scanning electron microscopy (SEM). Furthermore, fluorescent labelling of iL3 was tested to evaluate feasibility for future intravital imaging.

Results: Dermal penetration was observed within the first 10 minutes post-infection. By 20 minutes, larvae were distributed within the dermal interstitium, consistent with active tissue migration. At 35 minutes, the total number of larvae decreased, while remaining larvae were located deeper in the dermis and near vessel-associated regions, suggesting progression towards vascular entry. SEM findings supported early superficial localisation and showed minimal tissue disruption, consistent with controlled interstitial migration rather than destructive invasion. Tetramethylrhodamine isothiocyanate (TRITC) labelling was limited by DMSO-associated toxicity, whereas carboxyfluorescein succinimidyl ester (CFSE) labelling preserved larval viability and produced stable fluorescence.

Conclusions: This study provides a minute-scale view of early *S. venezuelensis* cutaneous migration. CFSE-labelled iL3 represent a suitable tool for future *in vivo* imaging to capture real-time vascular invasion dynamics.

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DRUGGABLE ENZYMATIC REGULATORS OF INVASION, EGRESS, AND PROTEOSTASIS IN BABESIA DIVERGENS

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Background and Aims: *Babesia divergens* is a tick-transmitted apicomplexan parasite causing severe bovine babesiosis and emerging human disease. Recent functional genomic studies have identified key proteases and kinases controlling parasite invasion and egress from red blood cells, yet independent validation and molecular pathway resolution remain limited. Here, we aimed to complement genetic approaches by combining recombinant enzyme biochemistry, enzyme-selective chemical inhibition, and multi-omics analyses to define protease-, kinase-, and proteostasis-driven regulatory networks governing *B. divergens* invasion, intracellular development, and egress.

Methods: Parasites were cultured ex vivo in bovine erythrocytes and subjected to selective inhibitor treatments to achieve chemical knockdown of aspartyl proteases (BdASP3a/b), calcium-dependent protein kinase BdCDPK4, and the parasite proteasome. Recombinant enzymes were expressed and biochemically characterized to assess activity, specificity, and inhibitor sensitivity, supported by 3D structure studies. Phenotypes were quantified by flow cytometry and microscopy. Downstream molecular effects were analyzed using comparative phosphoproteomics and HUNTER N-terminome profiling. Parasite ultrastructure was examined by ultrastructure expansion microscopy and transmission electron microscopy with immunolabeling

Results: Recombinant BdASP3 proteases and BdCDPK4 were biochemically characterized to verify selective inhibition. In parasite cultures, the corresponding compounds inhibited native BdASP3a/b and BdCDPK4 and phenocopied invasion and egress defects, respectively; BdCDPK4 inhibition

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uncoupled nuclear replication from membrane rupture, inducing multinucleated intracellular stages. Phosphoproteomic and HUNTER N-terminomic analyses revealed disrupted calcium-dependent signaling and proteolytic processing, while expansion microscopy and TEM immunomicroscopy confirmed preserved cellular architecture.

Conclusions: Targeted chemical inhibition of protease-, kinase-, and proteasome-based regulators of cell signaling and proteostasis impairs invasion, intracellular replication, and egress of *Babesia divergens* during intraerythrocytic development.

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MULTIGENERATIONAL STUDIES IN PLASMODIUM BERGHEI DEMONSTRATE THE POWER OF PARASITE GENE DRIVES TO CONTROL MALARIA.

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Background and Aims: New approaches to combating malaria are essential in an environment of increasing drug and insecticide resistance. Gene drives are simple genetic tools with the potential to genetically modify large natural populations in relatively short periods of time. The malaria parasite's life cycle and genetics make it extremely promising target for gene drives, with the potential to modify or eliminate entire parasite populations. The aim of this study is to explore the potential of parasite-based gene drives to alter parasite populations in the mouse malaria model *Plasmodium berghei*.

Methods: We created a range of *P. berghei* gene drives targeting neutral loci, sex-specific development genes, virulence genes, and drug resistance mutations. Gene drive efficiency and spread in moderate sized parasite population (~10 000 parasites) over multiple sexual cycles were measured using fluorescence and ddPCR assays. Results were analysed in the context of predictive mathematical models to determine the potential for gene drives to spread in much larger natural populations.

Results: *P. berghei* gene drives are copied into all offspring with greater than 98% efficiency; including gene drives targeting multiple loci. Forced outcrossing of sex-specific drives results in population collapse within a single transmission cycle. Over multiple cycles of transmission to wild type parasites, the gene drives spread efficiently and alter the genetic make up of entire populations at rates that suggest effective spread in natural populations.

Conclusions: Parasite gene drives efficiently modify *P. berghei* parasite populations in lab-based studies, demonstrating the potential of these gene drives as tools for combating malaria.

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LEISHMANIA EXPLOITS THE MACROPHAGE ENDOPLASMIC RETICULUM-SHAPING PROTEIN CLIMP63 TO MODULATE MITOCHONDRIAL BIOGENESIS AND BIOENERGETICS

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Background and Aims: *Leishmania* is a vacuolar protozoan parasite that colonizes and proliferates within mammalian macrophages. Upon internalization by macrophages, *Leishmania* creates parasitophorous vacuole (PVs) where it multiplies. Previous studies revealed that *Leishmania*-harboring PVs continuously interact with the macrophage endoplasmic reticulum (mER). In the present study, we aimed at investigating the contribution of the mER-shaping protein CLIMP63 to the relationship between *Leishmania* and its host cell.

Methods: We infected murine bone marrow-derived macrophages with either *L. donovani* or *L. amazonensis* metacyclic promastigotes and we used a combination of experimental approaches, including confocal and electron microscopy, RNA interference, as well as extracellular flux assays.

Results: Shortly after internalization of the parasites, CLIMP63 redistributed and formed aggregates in close proximity to *Leishmania*-harboring PVs. Using *Leishmania* mutants defective in the synthesis of the virulence glycolipid lipophosphoglycan (LPG), we found that LPG is responsible for CLIMP63 aggregation as well as its dissociation from the macrophage mitochondrial network. Depletion of CLIMP63 impaired *L. donovani* replication and expansion of *L. amazonensis*-harboring communal PVs. In contrast, depletion of another ER-shaping protein, Reticulon 4, had no effect on *Leishmania* replication. Lastly, we found that CLIMP63 is required for *Leishmania*-induced mitochondrial biogenesis, cristea synthesis, as well as increased mitochondrial respiration and ATP production.

Conclusions: We previously demonstrated the importance of mitochondrial biogenesis for *Leishmania* to colonize macrophages. Our results suggest that CLIMP63 contributes to the ability of *Leishmania* to colonize macrophages partly through its role in modulating host cell mitochondrial properties.

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VARIATION IN SURFACE PROTEIN EXPRESSION DRIVES TRYPANOSOMA CRUZI HETEROGENEITY AND HOST-CELL INTERACTION DURING INFECTION

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Background and Aims: *Trypanosoma cruzi* possesses ~3,000 genes encoding virulence (vir) factors associated with pathogenesis. However, the spatial organization of these genes and the functional consequences of their expression and sequence variability during infection remain poorly understood.

Methods: We combined PacBio HiFi DNA and Pore-C to assemble a high-resolution genome. To investigate vir expression dynamics, we performed transcriptomics and proteomics to examine gene expression during infection and are performing single-cell RNA sequencing (scRNA-seq) on parasites recovered from mouse infections. Furthermore, we employed genome-wide yeast surface display (YSD) screens of *T. cruzi* proteins against host antibodies and cardiomyocyte cell receptors to identify the molecular targets of parasite surface proteins.

Results: The genome assembly provided a map of vir gene expansion and distribution. Quantitative proteomics showed their expression across life stages, and identified upregulation of trans-sialidases in trypomastigotes. Using transcriptomic and proteomic analyses, we observed that vir gene expression varies significantly across rounds of infection, creating heterogeneous parasite populations with distinct invasion capacities. The YSD antibody screen against *T. cruzi* proteins identified antibody targets and showed limited overlap in binding sites, likely reflecting host surface variation. A host cell surface receptor YSD screen is in progress to identify parasite proteins involved in host cell invasion and tissue tropism.

Conclusions: These findings suggest that *T. cruzi* surface protein variation is a key driver of infection persistence. The ability of *T. cruzi* to generate heterogeneous populations with variable surface proteins likely facilitates both immune evasion and widespread tissue colonization. This work was partially published in NatureCommunications 2025; includes unpublished data.

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UNCOVERING A NOVEL INTERACTION OF HUMAN MALARIA PARASITE PLASMODIUM VIVAX WITH RED BLOOD CELL MEMBRANE PROTEIN AND LIPID RAFT

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Background and Aims: Malaria remains a major global health challenge, with *Plasmodium vivax* being a predominant pathogen in subtropical regions. Traditionally, the Duffy antigen was considered the sole receptor for *P. vivax* invasion; however, emerging evidence indicates alternative invasion pathways. The Pv-fam-a gene family, which encodes tryptophan-rich antigens (PvTRAgs), is a promising candidate for exploring such mechanisms. Among them, PvTRAg26.3 elicits both cellular and humoral immune responses, offering important insights into host–parasite interactions. **Objective**
To characterize parasite ligand–host receptor interactions in *P. vivax* infections.

Methods: Using pull-down and label transfer assays followed by liquid chromatography–mass spectrometry (LC-MS), we identified Stomatin—a lipid raft protein in the human red blood cell membrane—as a specific interaction partner of PvTRAg26.3. Further assays, including solid-phase binding, biolayer interferometry (BLI), membrane lipid strip binding, and microscale thermophoresis (MST), validated this interaction and examined associated lipid partners.

Results: PvTRAg26.3 demonstrated robust red blood cell binding, confirmed by flow cytometry. LC-MS analyses revealed Stomatin as a key partner, while BLI quantified the interaction with high specificity, showing a sub-micromolar dissociation constant. Lipid strip binding and MST confirmed strong interactions with phosphatidic acid and phosphatidylglycerol in the micromolar range.

Conclusions: This study identifies Stomatin and specific lipid partners as critical mediators of PvTRAg26.3 interactions, highlighting a sophisticated host-recognition strategy of *P. vivax*. These findings provide new perspectives on parasite invasion pathways and may inform novel intervention strategies in the fight against malaria.

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ENTAMOEBA HISTOLYTICA STIMULATES THE RELEASE OF HMGB1 FROM COLONIC EPITHELIAL CELLS VIA SECRETORY AUTOPHAGY

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Background and Aims: Aim: *Entamoeba histolytica* (*Eh*) is a protozoan parasite that causes amoebiasis, accounting for 10^6 deaths annually. During disease pathogenesis, it invades the gut, triggering a robust early pro-inflammatory response with significantly elevated HMGB1 secretion during initial contact. However, the precise molecular pathway by which colonic cells respond to *Eh* at initial contact and induce rapid HMGB1 secretion remains unexplored. This study aims to define the pathway regulating early HMGB1 secretion from colonic cells in response to *Eh*.

Methods: Methods: LS174T colonic cells were exposed to live *Eh* to examine the dynamics of HMGB1 secretion. ATP antagonists, autophagy inhibitors, and GW4869, an exosome biogenesis inhibitor, were used to define regulatory pathway. HMGB1 localization was assessed by confocal microscopy and exosome isolation and HMGB1 secretion was validated in human colonic organoids and mouse colonic loop studies.

Results: Results: Following live *Eh* contact with colonic cells, high HMGB1 secretion occurred within 5 minutes. HMGB1 secretion was ATP-dependent and regulated by secretory autophagy enumerated with use of autophagy inhibitors. Confocal microscopy revealed HMGB1 was pre-packaged within LC3-positive autophagosomes and inhibition of multivesicular body (MVB) maturation with GW4869 attenuated HMGB1 secretion. Exosome isolated from *Eh*-stimulated colonic cells contained HMGB1 which confirmed that HMGB1 was pre-loaded into exosomes following autophagosome–MVB fusion and released via exocytosis. Early HMGB1 secretion was conserved in human colonic organoids and in mouse colonic loop studies in response to live *Eh* stimulation.

Conclusions: Conclusion: These findings uncover a novel secretory autophagy pathway for HMGB1 secretion in colonic cells following *Eh* contact in disease pathogenesis.

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A COMPARATIVE ASSESSMENT OF HOST EPITHELIAL RESPONSES TO DIFFERENT NEMATODE PARASITES USING ORGANOIDS

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Background and Aims: Gastrointestinal nematodes (GINs) infect billions of humans, livestock and companion animals globally, with control increasingly threatened by widespread anthelmintic resistance and limited vaccine availability. Many GIN species exhibit strict tissue tropism within the gastrointestinal tract; for example, *Teladorsagia circumcincta* infects the ovine abomasum, whereas *Trichostrongylus colubriformis* colonizes the duodenum. This study used tissue-specific ovine abomasal and duodenal organoids to model epithelial responses to live infective larvae and parasite excretory/secretory products (ESPs), enabling comparative investigation of species- and life stage–specific host–parasite interactions.

Methods: Abomasal and duodenal organoids were generated from primary ovine epithelial stem cells and challenged with live L3 larvae or ESPs derived from L3, L4, and adult stages. Host responses were assessed using microscopy to evaluate structural changes and bulk RNA sequencing to characterize transcriptomic responses. Comparative analyses were performed across GIN species, life stages, and host tissue types.

Results: Distinct interaction profiles were observed across parasite species. *T. circumcincta* induced the strongest structural and transcriptional responses, whereas *Haemonchus contortus* showed minimal interaction. Larval stages of *T. circumcincta* and *T. colubriformis* produced pronounced host responses. Notably, *T. circumcincta* triggered comparable responses in gastric and intestinal organoids, while responses to *T. colubriformis* were more duodenal organoid-specific, consistent with tissue niche specialization. Transcriptomic analyses revealed rapid epithelial immunomodulation, particularly associated with inflammatory, stress and EMT-response pathways, driven by infective larval ESPs.

Conclusions: Tissue-specific organoids provide powerful reductionist platforms for dissecting epithelial–GIN interactions. We are using these models to identify immunomodulatory ESP components driving host responses, for rational nematode vaccine antigen discovery.

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BUILDING PREDICTIVE MODELS FOR EFFICIENT DRUG AND VACCINE DISCOVERY AGAINST LEISHMANIASIS

Sarah Hendrickx[1], Fanny Escudié[2], Eric Chatelain[2], Benoit Stijlemans[3], Guy Caljon[1]
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Background and Aims: Despite the ongoing quest to find new drugs and vaccines for both visceral (VL) and cutaneous leishmaniasis (CL), only a handful of new drug leads entered clinical development during the last decade. As efficient drug discovery relies strongly on the availability of predictive models, substantial efforts have been made in the past years to refine existing models to be translational to human.

Methods: Combining different *in vitro* assays (amastigote susceptibility, time-to-kill) with *in vivo* bioluminescent (BLI) reporter technology and PK analyses allows us to not only follow-up parasite clearance upon drug treatment, but also to predict treatment failure and relapse. By expanding our *in vivo* model arsenal to include parasite transmission by *Lutzomyia longipalpis*, vaccine challenge studies can now be included as well.

Results: On the one hand, the use of different reference drugs and experimental compounds in the BLI BALB/c mouse model for VL was able to show drug-specific efficacy and a strong predictive value for clinical failure. On the other hand, application of our transmission model for CL has proven its value in vaccine challenge studies.

Conclusions: The in-house established battery of integrated *in vitro* and *in vivo* assays proves to be useful for predicting clinical drug efficacy and supporting the development of new drugs and vaccines.

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SURVEILLANCE OF SINGLE NUCLEOTIDE POLYMORPHISMS CORRELATED TO MACROCYCLIC LACTONE RESISTANCE IN DIROFILARIA IMMITIS FROM CLIENT-OWNED DOGS ACROSS THE UNITED STATES

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Background and Aims: *Dirofilaria immitis* is a parasitic filarial nematode and the causative agent of heartworm disease. Heartworm disease is predominantly managed via macrocyclic lactone (ML) based chemoprophylactics. Through opportunistic sampling, genotypically and phenotypically confirmed ML-resistant *D. immitis* isolates have been isolated in the Lower Mississippi River Valley region (LMRV); however, the pervasiveness of resistant isolates in the USA has not been evaluated.

Methods: This study aimed to evaluate the geographic distribution and prevalence of genotypically ML-resistant heartworms in client-owned dogs across the USA over a 3-year period. Owner consent was obtained to collect microfilaremic blood samples from heartworm-positive dogs from participating clinics. Veterinarians completed a questionnaire on the known history of each dog. Microfilariae were filtered from blood, DNA extracted utilizing the QIAGEN QIAamp DNA Micro Kit and samples sequenced by the Génome Québec Innovation Centre to determine allele frequencies at nine SNP sites previously correlated with ML resistance. The highly predictive 2-SNP model was used to identify genotypically susceptible, mixed, and resistant populations.

Results: A total of 310 microfilaremic blood samples were collected from 45 geographically diverse veterinary clinics located in 22 states. Computational analysis indicated 111 (35.8 %) were genotypically susceptible, 96 (31.0 %) were genotypically resistant, and 103 (33.2 %) were genotypically mixed.

Conclusions: The genotypically mixed and ML-resistant infections were located within and outside of the endemic LMRV, as far north as Michigan, which indicates canine populations outside of the LMRV are at increased risk for transmission of potentially ML-resistant heartworm infections than previously hypothesized.

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APPARENT PRAZIQUANTEL-RESISTANT DIPYLIDIUM CANINUM IN DOGS FROM CHILE SUCCESSFULLY TREATED WITH NITROSCANATE

Cristian Alvarez Rojas[1], Lucía Peters[2], Paulina Gómez Fett[1], Camilo Arriaza-Onel[1], Nallinne Vergara[1], Colomba Arjona[3]
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Background and Aims: *Dipylidium caninum* is a zoonotic cestode of dogs and cats that has been effectively controlled using praziquantel. However, recent reports from the United States and Europe have described apparent praziquantel-resistant infections. This study aimed to document similar cases in Chile and to evaluate clinical responses to an alternative cestocidal treatment.

Methods: Eleven canine cases from Chile with persistent shedding of *D. caninum* proglottids despite repeated praziquantel-based treatments were investigated. Dogs were subsequently treated with oral nitroscanate (50 mg/kg), and parasitological clearance was assessed based on the cessation of proglottid shedding following treatment.In a subset of cases, genetic characterization of *D. caninum* was performed using PCR amplification and sequencing of the mitochondrial cytochrome c oxidase subunit I (cox1) gene to confirm species identity.

Results: Nitroscanate achieved complete parasite clearance after a single oral dose in seven dogs, while two to three doses were required in the remaining four cases. All cases had failed to respond to praziquantel-based regimens prior to nitroscanate administration. Cox1 sequence analysis confirmed that all isolates corresponded to the canine-associated lineage of *D. caninum*.

Conclusions: These findings are consistent with the emergence of apparent praziquantel-resistant *D. caninum* in South America. Limited access to alternative cestocidal drugs, including nitroscanate, represents a major challenge for clinical management in Chile. Integrated flea control and molecular surveillance are essential to confirm resistance and guide effective control strategies.

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INTEGRATING ARTIFICIAL INTELLIGENCE AND INTERDISCIPLINARY APPROACHES IN ANTI-PARASITIC DRUG DISCOVERY: INSIGHTS FROM NIGERIAN POLYHERBAL ANTIMALARIAL RESEARCH

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Background and Aims: Parasitic infections, especially malaria, remain a major global health burden, with sub-Saharan Africa mostly affected despite extensive chemotherapeutic and environmental interventions. The emergence of *Plasmodium* resistance to frontline antimalarial drugs and insecticide resistance in mosquito vectors continually undermines progress. This necessitates new therapeutic strategies. Medicinal plants, originating from traditional knowledge,have long been a valuable source of anti-parasitic agents, and building on this tradition,alongside drug design tools (DDTs), continue to offer promising leads for novel antimalarial development. **Aim**:To evaluate the antimalarial potential of a polyherbal mixture and demonstrate how integrating DDTs, artificial intelligence (AI), and interdisciplinary collaboration can accelerate anti-parasitic drug discovery.

Methods: Infusions of the medicinal plants were prepared using different solvents of polarities. The extracts were assessed for antimalarial activity using suppressive and curative assays in mice infected with chloroquine-sensitive *Plasmodium berghei* NK-65 strain. Some lead scaffolds from the medicinal plants are designed with computational tools and structural modification under investigation

Results: The polyherbal extracts exhibited significant chemosuppressive and curative antiplasmodial activities($p < 0.05$)comparable to chloroquine, which are promising, demonstrating strong therapeutic potential.

Conclusions: While traditional pharmacological evaluations remain essential, the complexity of parasite biology and phytochemical interactions limits rapid optimization. DDTs and AI offer a transformative complement by predicting drug–parasite interactions, identifying active phytochemicals, and optimizing compound combinations. Coupled with interdisciplinary collaboration across parasitology, computational biology, pharmacology, medicinal chemistry, and AI integration can accelerate the translation of polyherbal findings into clinically relevant antimalarial candidates. This combined approach strengthens the pipeline for next-generation anti-parasitic drug discovery and contributes to global health equity.

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CHEMICAL INHIBITION OF CDPK-ASSOCIATED SIGNALING ARRESTS EGRESS IN BABESIA DIVERGENS

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Background and Aims: *Babesia divergens* is a major cause of bovine babesiosis in Europe and an emerging zoonotic pathogen, yet the molecular mechanisms controlling parasite signaling pathways remain poorly understood. Egress from red blood cells is essential for parasite propagation and disease progression, and in related apicomplexans, calcium-dependent protein kinases (CDPKs) are central regulators. BdCDPK4 has been implicated in *B. divergens* egress, but its precise role and signaling are unknown. This study aimed to use selective bumped kinase inhibitors (BKIs) as chemical probes to investigate kinase-dependent egress mechanisms and characterize the resulting phenotypic, molecular, and ultrastructural effects.

Methods: *B. divergens* was cultured *ex vivo* in bovine erythrocytes and treated with BKIs. Parasite development and egress were quantified by flow cytometry, and egress-arrested multinucleated stages were selected for downstream analyses. Inhibitor-induced phosphorylation changes were profiled by comparative phosphoproteomics using phosphopeptide enrichment and data-independent acquisition mass spectrometry. Parasite ultrastructure in treated and untreated parasites was compared using ultrastructure expansion microscopy (U-ExM).

Results: Inhibitor BKI-1294 blocked parasite egress without preventing nuclear replication, causing accumulation of multinucleated intracellular stages. Phosphorylation changes affected signaling proteins, consistent with perturbation of a CDPK-associated regulatory network. U-ExM comparisions demonstrated that cellular and cytoskeletal architecture in inhibited parasites remained intact, indicating that egress blockade was not due to a structural collapse.

Conclusions: Our findings provide the first integrated phenotypic, molecular, and ultrastructural characterization of egress inhibition in *B. divergens*. The study establishes a framework for dissecting

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kinase-dependent signaling and identifies CDPK-associated pathways as candidates for future functional studies to clarify their role in parasite egress.

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OVERCOMING TRYPANOSOMA CRUZI PERSISTENCE USING A MECHANISTICALLY DISTINCT DRUG COMBINATION

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Background and Aims: Benznidazole is the front-line drug used against *Trypanosoma cruzi* infection. However, treatment involves prolonged dosing regimens (60-90 days), limited efficacy and toxic side-effects. Parasites that persist in mice after treatment failure are in a transient non-replicative state, and host cells that remain infected generally contain only a single parasite. We hypothesised that combination therapy, based on a proteasome inhibitor (GNF6702) and benznidazole, a drug that generates mis-folded/damaged proteins, might result in enhanced efficacy leading to the elimination of persisters.

Methods: Studies were carried out using *T. cruzi* expressing a bioluminescent-fluorescent fusion protein. This allows murine infections to be detected and imaged at single cell resolution. Drug efficacy was assessed by highly sensitive bioluminescence imaging of infected mice, with *ex vivo* imaging used to monitor parasite dissemination in organs and tissues.

Results: Combination treatment of infected cell monolayers with benznidazole and GNF6702 resulted in sterile clearance, under conditions where monotherapy failed. Oral treatment of chronically infected mice for 10 days with sub-efficacious doses of both drugs resulted in a 100% cure rate, in the absence of adverse effects. Elimination of persisters was mediated by the complementary activity of both drugs, rather than by pharmacokinetic interactions.

Conclusions: Short-duration co-administration of well-tolerated sub-efficacious oral doses of the parasite-selective proteasome inhibitor GNF6702 (30 mg/kg) and the pro-drug benznidazole (10 mg/kg) produces parasitological cure in a well-validated experimental model of chronic Chagas disease. This represents a promising advance for treatment of a major Neglected Disease for which there have been no newly approved therapeutics for >50 years.

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EVALUATION AND CHARACTERIZATION OF THE EFFECT OF STYRYL-ISOXAZOLES AGAINST GIARDIA DUODENALIS

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Background and Aims: Infection with *Giardia duodenalis* affects humans and other vertebrates worldwide. Current treatments for giardiasis rely mainly on benzimidazoles and 5-nitroheterocyclic compounds such as metronidazole, furazolidone, and nitazoxanide; however, therapeutic failures are increasingly reported, highlighting the need for new antiparasitic agents.

Methods: We synthesized 15 isoxazole derivatives featuring a methyl group at position 3 (Isox1) and a nitro group at position 4 (Isox2). At position 5, a styryl moiety was variously substituted with hydroxyl, nitro, phenyl, or halogen groups (Isox3–Isox15). Compounds with the highest anti-*Giardia* activity were further evaluated in vitro by electron microscopy and flow cytometry, in vivo using an experimental giardiasis model in gerbils, and for biotoxicity in Artemia salina nauplii and Caco-2 cell monolayers.

Results: All styryl-substituted derivatives (Isox3–Isox15) showed enhanced cytotoxicity against cultured trophozoites. Isox3 and Isox6, both bearing hydroxyl substituents, were the most active compounds. Isox12, containing two methyl groups, was the least active, whereas Isox13, with hydroxyl and nitro substituents, displayed intermediate efficacy. Isox2, Isox3, and Isox13 reduced trophozoite burden in infected gerbils, consistent with their in vitro activity. Isox3 demonstrated selectivity toward trophozoites over nauplii and Caco-2 cells. Its cytotoxic effects included cell deformation, flagellar retraction, surface disintegration, and the formation of autophagy-like perinuclear and cytoplasmic vacuoles. Additionally, Isox3 induced partial S-phase arrest, increased reactive oxygen species production, and apoptosis-like cell death.

Conclusions: These findings identify styryl isoxazoles, particularly Isox3, as promising candidates for further preclinical development as alternative treatments for giardiasis.

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2% AND 3% VORICONAZOLE EYE DROPS FOR SEVERE INFECTIOUS KERATITIS: STABILITY, OCULAR TOLERANCE AND PHARMACOKINETICS IN RABBITS

Marine Cavelier[1], Romy Razakandrainibe[2], Henri Gondé[3], Christophe Arnoult[4], Tony Pereira[5], Sabine Lefevre[6], Océane Verde[2], Maxime Quirke[7], Rémi Varin[3], Marc Muraine[8], Loic Favennec[9], Julie Gueudry[10]
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Background and Aims: Severe infectious keratitis, particularly *Acanthamoeba* keratitis and fungal keratitis, is a potentially blinding corneal infection. Management remains difficult due to the lack of standardized treatment. Topical voriconazole 1% is frequently used but requires very frequent administration, which may compromise adherence and clinical efficacy. This study aimed to develop higher-concentration voriconazole eye drops and to assess their stability, ocular tolerance, and pharmacokinetics *in vivo*.

Methods: Two ophthalmic voriconazole formulations (2% and 3%) were prepared and stability was evaluated. Ocular tolerance and pharmacokinetics were assessed in a rabbit model. Animals were randomized into three treatment groups (1%, 2%, 3%; n=5/group) and received one eye drop per hour for 12h daily over three days. Voriconazole concentrations were quantified in ocular tissues and plasma, and compared with 1% results.

Results: Both formulations remained stable for at least three months (refrigerated and frozen conditions) and for one month after opening. Mean voriconazole concentrations in the corneal stroma/endothelium were 1.43±0.96ng/mg (1%), 3.23±1.27ng/mg (2%, $p<0.05$) and 3.78±2.79ng/mg(3%, $p<0.05$). In the iris, means were respectively in the 1%, 2%, 3%-group 0.68±0.53ng/mg, 1.39±0.85ng/mg ($p<0.05$) and 1.74±0.90ng/mg ($p<0.01$). Concentration in the vitreous was 0.07±0.12µg/mL(1%), 0.06±0.04µg/mL (2%, $p=0.47$), and 0.10±0.03µg/mL (3%, $p<0.01$). Plasma concentrations remained low across all groups (0.06±0.02µg/mL (1%), 0.07±0.04µg/mL (2%),

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0.14±0.08µg/mL (3%)). Mild conjunctival hyperemia was observed in 1/10 eyes (2%), 4/10 eyes (3%) without intolerance with 1%.

Conclusions: 2% and 3% voriconazole eye drops achieve improved ocular tissue exposure with minimal systemic absorption and acceptable ocular tolerance. These findings support further investigation as potential alternatives to standard topical voriconazole therapy.

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EXPOSURE TO ECHINOCOCCUS SPP. IN CANADIANS: POPULATION-BASED SEROLOGICAL FINDINGS AMID CHANGING ECOLOGICAL CONDITIONS

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Background and Aims: In Canada, the zoonotic cestodes Echinococcus canadensis and Echinococcus multilocularis maintain their life cycles in wild canids and ungulates or small mammals, with humans acting as accidental hosts. Shifts in climate, ecological conditions and wildlife host distribution may alter human exposure risk. Both infections have long latency periods (5–15 years) and often require prolonged clinical management, yet population-level exposure to *Echinococcus* spp. in the general Canadian population remains poorly characterized. Objective To estimate the prevalence of antibodies to *Echinococcus* spp. in the Canadian population using nationally representative data.

Methods: Human sera and accompanying data were obtained from Cycle 5 of the Canadian Health Measures Survey (2016–2017), which sampled 5,086 individuals aged 3–79 years across 16 sites in 10 provinces, representing urban and rural settings. Samples were tested for anti-*Echinococcus* spp. IgG using an in-house ELISA developed at the National Reference Centre for Parasitology. This screening assay detects antibodies to *Echinococcus* spp. but does not differentiate between species. Confirmatory Western blot testing and Bayesian adjustment for diagnostic misclassification are ongoing.

Results: Preliminary analyses indicate that 1.2% of participants were seropositive, increasing to 2.1% when equivocal results were classified as positive. These findings provide the first population-based serological evidence of human exposure to *Echinococcus* spp. in Canada and establish baseline data for future comparison amid changing ecology and climate.

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Conclusions: This presentation will highlight need for cautious interpretation of serological screening data in low-prevalence settings and underscore the importance of improved diagnostics and better integration of One Health data to inform surveillance prioritization and decision-making.

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THE FRENCH ALVEOLAR ECHINOCOCCOSIS REGISTRY: EPIDEMIOLOGICAL AND CLINICAL DATA ON 1081 PATIENTS FROM 1982-2024

Jenny Knapp[1], Florent Demonmerot[1], Houssein Gbaguidi-Haore[2], Carine Richou[3], Dominique Angèle Vuitton[1], Coralie Barrera[1], Anne-Pauline Bellanger-Clerget[1], Solange Bresson-Hadni[1], Laurence Millon[1]
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Background and Aims: In France, surveillance of alveolar echinococcosis (AE) is performed by the National Reference Centre for Echinococcoses (NRC-E), mostly through active investigation from the NRC-E and a voluntary approach from physicians. Data are collected on a dedicated information system.

Methods: From 1982 to 2024, epidemiological and clinical information were collected on 1081 patients with an AE diagnosis made in France.

Results: Patients' mean age at diagnosis was 61. Patients residence at diagnosis was located in the at-risk regions in France in 82% of cases, in a rural area in 51.5%. and 25% of AE cases have agricultural activities. Picking activities was declared by 80.1% of patients, gardening by 76.6%, owning a pet by 76.3%, fox contact by 41.5% and hunting activity by 15.5%. Asymptomatic patients at diagnosis markedly increased over time (30% <2000 vs 56% >2014). Antiparasitic therapy was administered to 92% of patients, Albendazole for 91% of the molecules administrated. Surgery was practiced for 40% of patients, with curative surgery preferred in this last 10 years, liver transplantation representing 3% of the interventions. Liver involvement represents the majority of AE cases and primary extra-hepatic localization 2% of patients. Immunosuppressed (IS) patients, represented 25.1% the last 10 years.

Conclusions: From this 43-years back investigation, an increasing number of asymptomatic patients at diagnosis, a large administration of albendazole therapy, a plebiscite of curative surgeries and an increasing number of IS AE patients was observed in France from 1982 to 2024.

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PARASITIC ZOONOSES IN CANADA: INFORMING THE PUBLIC THROUGH EMPOWERMENT-BASED MESSAGING

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Background and Aims: Several parasitic zoonoses are endemic or emerging in Canada, with transmission patterns influenced by climate change and ecological disruption. Communicating these risks to the public is essential but challenging, as fear-based messaging can lead to unintended behaviors such as avoidance of pets, greenspaces, or wildlife. Aims To develop accessible video clips on parasitic zoonoses of human and animal importance for the Canadian public, and to describe both the results of a public consultation and implications for science communication drawn from this interdisciplinary collaboration.

Methods: An interdisciplinary team collaborated with technopedagogical advisors to develop four short video clips on *Toxoplasma gondii*, *Baylisascaris procyonis*, *Echinococcus* spp., and *Toxocara* spp. Two animation styles (coloured vector and sketch) were disseminated as 30-second example clips via social media groups including producers, veterinarians, and members of the public. Audience attention and perceived credibility were assessed using a brief online multiple-choice survey.

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Results: Forty-one respondents completed the survey. Both animation styles were perceived as equally credible, while the coloured vector style was rated as better at retaining audience attention. Several considerations emerged from this collaboration process. Vector-style graphics with bright colours and carefully designed animal representations were selected to avoid fear-inducing imagery. In addition, each parasite was presented through a simple storyline using a persona to support clear communication.

Conclusions: In a context where public information on parasitic infections is largely text- or infographic-based, short video clips represent a complementary approach to communicate zoonotic risks and preventive behaviors while informing and empowering the public without amplifying fear.

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IS ALBERTA A NEW ENDEMIC FOCUS FOR ALVEOLAR ECHINOCOCCOSIS?

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Background and Aims: Alveolar echinococcosis (AE) is a potentially fatal zoonosis caused by *Echinococcus multilocularis (Em)*, an intestinal tapeworm of canids.Human AE presents as a continuously growing tumour-like larval mass, located in the liver with local or metastatic spread to distant organs. Human AE is endemic in Europe and Asia but until recently was virtually absent from North America (except for Alaska). We report an exponential increase in cases within Alberta since 2013

Methods: Liver biopsy specimens from patients with suspicious histopathological or radiological lesions were tested by multiplex PCR assay targeting *nad2* gene of *E. multilocularis* and *cox1* gene of *E.canadensis* and *E.granulosus sensu lato* genotypes *(*G1, G3, G5, G6, G7, G8 and G10). Em serology was performed at University of Berne, Institute for Infectious Diseases, Switzerland A case was defined by a positive histopathology and positive Em PCR with or without positive Em serology. Each positive result was mapped according to a patient submitting location as per laboratory records.

Results: There was a total of 51 cases between 2013 and 2025 as compared to 0-4 cases per each Canadian province to date. Case numbers averaged 2.5 per year between 2013 and 2023, followed by a steep increase to 12 per year in 2024 and 2025. Majority of cases originated from Central Alberta with just under 40% located in metropolitan Edmonton

Conclusions: The unprecedented increase of Alberta acquired AE cases poses an emerging individual and public health threat, and warrants an urgent reassessment of our approaches to this zoonosis, working within a OneHealth framework.

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HOST-PARASITE DYNAMICS OF STRONGYLOIDES IN BANGLADESH: WHO INFECTS WHOM?

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Background and Aims: Strongyloidiasis, caused by the helminths *Strongyloides stercoralis* and *S. fuelleborni*, is a pervasive yet underestimated soil-transmitted nematode infection. This study investigates the genetic relationships of *Strongyloides stercoralis* and *S. fuelleborni* parasites in humans, dogs, and rhesus macaques in Bangladesh to clarify their zoonotic potential and evolutionary history.

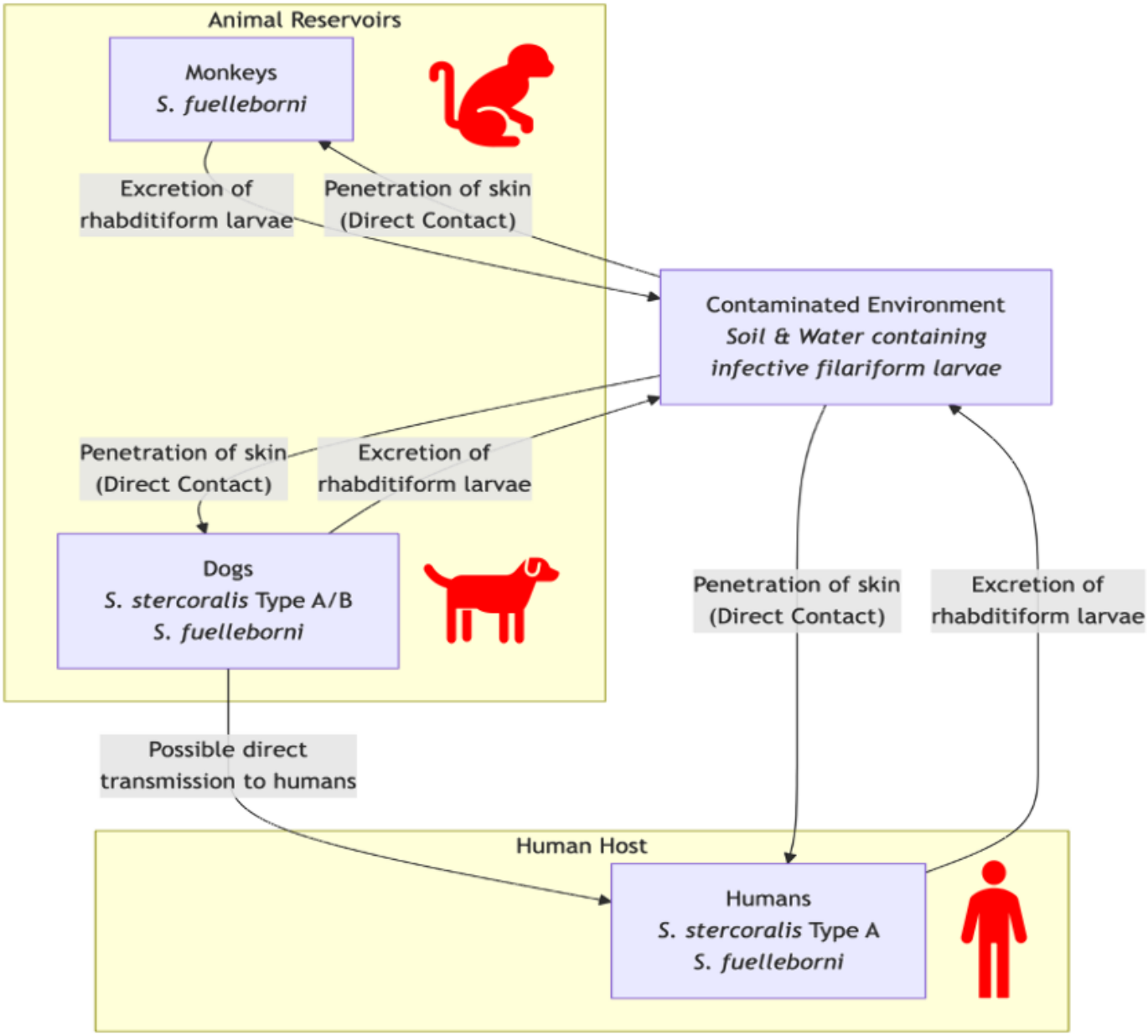
Methods: Stool samples from 550 humans, 430 dogs, and 74 primates were analyzed. High infection prevalence was found: 27.7% in humans, 50.8% in dogs, and 64.3% in macaques. Genomic analysis identified two types of *S. stercoralis*: one exclusive to dogs and another found in both humans and dogs.

Results: Nuclear genome data revealed significant genetic differentiation between the human- and dog-derived *S. stercoralis*, indicating they are distinct populations. However, evidence of rare cross-infection events confirms they remain capable of zoonotic transmission. Mitochondrial genome analysis suggested a history of genetic introgression between these populations. The evolutionary evidence points to an origin in canids, with the parasite later adapting to humans after dog domestication, leading to host-specific differentiation without full speciation. Furthermore, a high incidence of *S. fuelleborni* was detected across all three host species, revealing a complex, multi-host transmission cycle previously underestimated in Asia.

Conclusions: Population genomic analyses indicate that human- and dog-derived S. stercoralis in Bangladesh are genetically distinct but capable of occasional cross-infection. The evolutionary trajectory underscores the zoonotic potential and highlights the need to reassess the role of S.

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fuelleborni as a significant human parasite in Asia.



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20+ YEARS OF MASS DRUG ADMINISTRATION AGAINST UROGENITAL SCHISTOSOMIASIS IN ZANZIBAR: IS PRAZIQUANTEL STILL EFFICACIOUS?

Stefanie Knopp[1], Norbert Van Dijk[2], Tom Pennance[3], Naomi Ndum[1], Mohammed N. Ali[4], Khamis R. Suleiman[4], Aidan M. Emery[3], Bonnie Webster[3], Luc Coffeng[2], Said M. Ali[4]
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Background and Aims: On the Zanzibar islands, Tanzania, mass drug administration (MDA) with praziquantel against *Schistosoma haematobium* infections is implemented regularly since the early 2000s. Elimination of schistosomiasis as a public health problem has been achieved in most areas and interruption of transmission is anticipated. The RESIST project investigates whether any MDA-driven selection of praziquantel resistance is occurring and contributes to persistent transmission in hotspots.

Methods: The efficacy of praziquantel treatment was assessed in two schools in Pemba from October 2024 to March 2025. Cross-sectional surveys were conducted in the week before and two weeks after school-based MDA with praziquantel (40 mg/kg). In each survey, up to five urine samples per participant were collected and examined with urine filtration microscopy. Statistical analyses were based on a per protocol approach.

Results: Before MDA, the *S. haematobium* prevalence was 14.3% (62/435) in School 1 and 37.8% (233/617) in School 2. Post-MDA, the prevalence was 0.5% (2/435) and 9.6% (59/617), respectively. Egg reduction rates (ERR) were 99.8% (90% confidence interval (CI): 99.2-100%) in School 1 and 94.8% (90% CI: 88.5-98.7%) in School 2. Evaluation by (non-)inferiority testing showed adequate efficacy in School 1 and inconclusive efficacy in School 2.

Conclusions: The efficacy of praziquantel in Zanzibar remains higher than the 90% threshold for optimal efficacy set by the World Health Organization. Yet, the inconclusive efficacy results for School 2 revealed by our analyses require more in-depth investigation. Future molecular work will show whether the *S. haematobium* miracidia isolated from egg-positive individuals post-MDA carry genetic markers of reduced praziquantel susceptibility.

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MAPPING THE EPIDEMIOLOGY OF EPILEPSY AND ONCHOCERCIASIS-ASSOCIATED EPILEPSY IN MAHENGE, TANZANIA

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Background and Aims: Epidemiological evidence suggests that *Onchocerca volvulus* may directly or indirectly contribute to the development of Onchocerciasis-Associated Epilepsy (OAE). This study from Mahenge focuses on assessing the overall burden of epilepsy, including cases linked to OAE.

Methods: A door-to-door cross-sectional epilepsy screening survey was conducted between August and November 2021, covering 34 villages in the Mahenge. Screening used a two-stage approach: first, community health workers administered a standardised five-item questionnaire; then, a physician evaluated individuals with suspected epilepsy to confirm or refute the diagnosis. OAE were identified using established clinical criteria. Descriptive statistics were used to analyse the data, supplemented by bivariate and multivariate techniques to explore the association.

Results: Fifty-six thousand six hundred four people were screened, and 1,946 tested positive. Of these, 1,809 (93%) were available for confirmation, and 1,194 (66%) were confirmed to have epilepsy. This gives a point prevalence of 21.1 cases per 1,000 population (95%CI: 19.9–22.3). Six hundred and ten of the positive (31.3%) were diagnosed with other health conditions, mostly recurrent febrile convulsions (17%) and dizziness/syncope (13%). Of the confirmed cases, 447 (37.4%) were eligible for OAE assessment, of whom 338 (75.6%; 95%CI: 71.4–79.5) were confirmed as OAE, and 132 individuals (11.0%; 95%CI: 9.3–13.0) presented with nodding syndrome (NS). Of them, 37.8% were not receiving antiseizure medication, while 47.3% were using phenobarbital.

Conclusions: Mahenge has a high epilepsy burden, with many cases linked to OAE/NS, suggesting ongoing onchocerciasis transmission and an urgent need to improve epilepsy care access.

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HIGH-RESOLUTION CLIMATE MODELLING OF FASCIOLOSIS RISK IN AUSTRALIA: A ONE HEALTH EARLY-WARNING FRAMEWORK

Rana M. Athar Ali[1], Mark A. Stevenson[1], Leah Tyrell[2], Nichola E.D. Calvani[3], Travis Beddoe[4], Grant Rawlin[5], Terry Spithill[4], Neil Young[1], Abdul Jabbar[1]
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Background and Aims: Fasciolosis, caused by the liver fluke *Fasciola hepatica*, is a climate-sensitive parasitic disease that threatens livestock health, productivity and farm profitability, with broader implications for food security, rural livelihoods, and sustainable food systems. The disease is endemic in Victoria, Australia, where environmental and climatic conditions favour parasite transmission at the livestock–environment interface. This study aimed to quantify historical trends and project future fasciolosis risk across Victoria under changing climate conditions.

Methods: High-resolution (5 km^2) climate data were retrieved for 40,504 grid points across Victoria to validate a Growing Degree Days (GDD) model for estimating fasciolosis risk over the past 50 years (1975–2024) and to project future risk centred at 2050 and 2090 under medium (RCP 4.5) and high (RCP 8.5) representative concentration pathways.

Results: Linear regression analysis demonstrated a strong relationship between observed fasciolosis prevalence and modelled risk values ($R^2 = 0.94$, $p < 0.003$). Historical analyses revealed substantial interannual variability, with consistently higher risk in eastern Victoria, followed by western and northern regions, and pronounced seasonal peaks in autumn and spring. Under future climate scenarios, risk increased spatially by up to two-fold in Barwon and three-fold in the Great South Coast, particularly under the 2090 RCP 8.5 scenario. Risk remained persistently high in Gippsland, with southern areas comprising approximately 35% of the region, while Greater Melbourne showed a modest 5% increase relative to a baseline risk of 45%.

Conclusions: These findings provide spatially explicit evidence to support climate-responsive surveillance, risk-based control strategies, and integrated animal health and environmental policy development.

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COORDINATED HUMORAL AND CELLULAR RESPONSES SHAPE BIOMPHALARIA GLABRATA SUSCEPTIBILITY TO SCHISTOSOMA MANSONI

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Background and Aims: Immune factors in snails of the genus *Biomphalaria* are critical for combating *Schistosoma mansoni*, the predominant cause of human intestinal schistosomiasis. Independently, many of these factors play an important role in, but do not fully define, the compatibility between the model snail *B. glabrata*, and *S. mansoni*. Here, we demonstrate an association between four previously characterized humoral immune molecules: BgFREP3, BgTEP1, BgFREP2 and Biomphalysin. These factors coordinate to initiate hemocyte-mediated destruction of *S. mansoni* sporocysts by producing reactive oxygen species.

Methods: Protein pull-down assays demonstrate that FREPs, TEP1 and biomphalysin form complexes in *B. glabrata* hemolymph. Recombinant *Bg*FREP3 and *Bg*TEP were used as bait to assess protein-protein associations between the recombinant bait proteins and hemocyte lysate/hemolymph from M-line and BS-90 *B. glabrata*. Associated proteins were analyzed by SDS-PAGE and identified by LC-MS/MS. Anti-FREP3 or anti-TEP1 antibodies to show that FREP3 and TEP1 bind to the parasite surface.

Results: We demonstrate an association between four previously characterized humoral immune molecules: BgFREP3, BgTEP1, BgFREP2 and biomphalysin. We also identify unique immune determinants in the plasma of *S. mansoni*-resistant *B. glabrata* that associate with the incompatible phenotype. These factors coordinate to initiate hemocyte-mediated destruction of *S. mansoni* sporocysts by producing reactive oxygen species. The inclusion of BgFREP2 in a BgFREP3-initiated complex that also includes BgTEP1 almost completely explains resistance to *S. mansoni* in this model.

Conclusions: Our study unifies many independent lines of investigation to provide a more comprehensive understanding of the snail immune system in the context of infection by this important human parasite.

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ESSENTIAL ROLE OF THE TRAMP–CSS COMPLEX IN PLASMODIUM BERGHEI LIVER AND BLOOD STAGE INFECTION

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Background and Aims: *Plasmodium* parasites, like other Apicomplexa, are obligate intracellular organisms that rely on specialized invasive stages, known as zoites, to infect host cells. These stages exhibit a polarized and elongated morphology that enables efficient cell invasion mediated by the apical complex, a conserved structure composed of secretory organelles essential for host cell adhesion and entry. *Plasmodium* sporozoites sequentially invade mosquito salivary glands, mammalian blood vessels in the skin, and the hepatic parenchyma, ultimately entering hepatocytes to generate thousands of erythrocyte-invasive merozoites. Despite the critical importance of these early infection steps, their molecular basis remains poorly understood. Here, using a rodent malaria model and *in vivo* conditional mutagenesis, we investigate the role of the TRAMP and CSS (TC) complex in *Plasmodium berghei* sporozoite and merozoite infectivity.

Methods: To elucidate the function of PbCSS and TRAMP in sporozoite and merozoite stages, we generated a conditional knockout parasite line using the rapamycin-inducible DiCre recombinase system.

Results: Conditional gene excision of *PbCSS* and *TRAMP* did not significantly affect sporozoite invasion of mosquito salivary glands, nor sporozoite motility or cell wounding activity. However, conditional knockout (cKO) sporozoites showed impaired hepatocyte invasion and intracellular development in hepatoma cells. Similarly, cKO merozoites failed to establish blood-stage infection *in vivo*, indicating that the Pb TC complex is essential for erythrocyte invasion.

Conclusions: The essential and multi-stage role of the TC complex in hepatocyte and erythrocyte infection highlights its potential as a vaccine target and provides new insights into the molecular mechanisms underlying *Plasmodium* invasion of distinct host cell types.

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TRANSCRIPTION FACTOR-ASSOCIATED POSITIONING OF THE ORIGIN RECOGNITION COMPLEX AT TRANSCRIPTION UNIT BOUNDARIES IN TRYPANOSOMA BRUCEI.

Gabriel Almeida Da Silva[1], Grace Gill[1], Catarina De Almeida Marques[1], Joana Faria[2], Richard Mcculloch[1]
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Background and Aims: African trypanosomatids, including the sleeping sickness parasite *Trypanosoma brucei*, must accurately duplicate their genome prior to cell division to ensure faithful inheritance. Although DNA replication is tightly regulated in all eukaryotes, several core components of the replication machinery have diverged in these parasites. The *T. brucei* origin recognition complex (ORC) lacks Orc6, contains highly diverged subunits, and associates with an S-phase–specific factor, Orc1B. In most eukaryotes, the mechanism by which ORC is recruited to chromatin remains unclear. In *T. brucei*, replication origins are restricted to strand switch regions (SSRs), which define the boundaries of multigenic transcription units, suggesting a functional link between transcription and replication initiation. We hypothesized that ORC recruitment to SSRs may be mediated through interactions with transcription factors.

Methods: To test this, we mapped the ORC proximal interactome using TurboID-based proximity biotinylation combined with quantitative mass spectrometry.

Results: Three ORC subunits (Orc1B, Orc2, and Orc4) were fused to TurboID and expressed in live parasites. Across datasets, 39 common proximal proteins were identified, including 12 associated with transcription. We validated interactions between ORC subunits and four transcription-related factors: TATA-binding protein (TBP), Bromodomain factor 2 (BDF2), the H2A.Z-binding ortholog YEA2 (Yaf9/Gas41), and the SPARC component PWWP1. Integration of replication timing and ChIP-seq data revealed differential enrichment at SSRs, with TBP preferentially associated with early-replicating regions.

Conclusions: These findings provide the first evidence in eukaryotes of direct ORC–transcription factor interactions, revealing a mechanistic link between multigenic transcription regulation and DNA replication initiation in *T. brucei*.

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OP211

PARALLEL ORAL ABSTRACT SESSION
PARALLEL ORAL ABSTRACT 30 : REGULATORY AND CELLULAR ARCHITECTURE OF HOST–PARASITE INTERACTIONS
08-20-2026 4:40 PM - 6:40 PM

LEISHMANIA INFANTUM INFECTION OF HUMAN HEPATIC ORGANOIDS AND MONOCYTES IN AN ORGAN-ON-A-CHIP SYSTEM

Svenja Stenzel[1], Danyel Rahimi[1], Helena Fehling[1], Melanie Lütkemeyer[1], Gülsah Gabriel[2], Iris Bruchhaus[1], Hanna Lotter[1]
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Background and Aims: Visceral leishmaniasis is a neglected tropical disease caused by *Leishmania* parasites and is associated with high morbidity and mortality. Following host invasion, parasites are engulfed by phagocytic cells, including circulating monocytes, which act as carrier and host cells, enabling their dissemination to the spleen and liver. Although the liver can partially control the infection, chemokine-mediated immune cell recruitment may exacerbate disease progression and liver damage. However, the pathophysiological effects of *Leishmania* infection on hepatocytes and their interactions with immune cells, particularly monocytes, remain largely unexplored. This study aims to characterize these interactions using human hepatic organoids in an organ-on-a-chip system.

Methods: Three-dimensional hepatic organoids were generated from primary human hepatocytes of three donors and characterized by immunofluorescence analysis. Organoids were co-cultured either with monocytes and *L. infantum* or with monocytes pre-infected with *L. infantum* in an organ-on-a-chip system. The infection-induced immune response was assessed using ELISA and multiplex cytokine assays.

Results: Human hepatic organoids showed functional characteristics of hepatocytes, as demonstrated by specific hepatocyte markers, including MRP2, A1AT, and CYP3A4. Infection with *L. infantum* in the organ-on-a-chip model induced regulation of cytokines conductive to parasite clearance, including upregulation of protective cytokines (TNF, IL-1b, and IL-18) and downregulation of disease-promoting cytokines (IL-10). CXCL1 was also downregulated after infection in both hepatic organoids and monocytes, mirroring responses observed in murine models.

Conclusions: The liver organ-on-a-chip co-culture model allows the simulation of *Leishmania* infection and mimics infection-specific immune responses. The model represents a promising tool for studying host-pathogen interactions.

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PARALLEL ORAL ABSTRACT SESSION
PARALLEL ORAL ABSTRACT 30 : REGULATORY AND CELLULAR ARCHITECTURE OF HOST–PARASITE INTERACTIONS
08-20-2026 4:40 PM - 6:40 PM

THE GENETIC BASIS OF SNAIL HOST SPECIFICITY IN SCHISTOSOMES

Frédéric Chevalier[1], Benjamin Gourbal[2], Winka Le Clec'H[1], Marina Mcdew-White[1], Grace Ann Arya[1], Philip Loverde[3], André Théron[2], Guillaume Mitta[2], Timothy Anderson[1]
[1]Texas Biomedical Research Institute, San Antonio, United States of America, [2]Hosts Pathogens Environments Interactions UMR5244, University Of Perpignan, Perpignan, France, [3]University of Texas Health Science Center, San Antonio, United States of America

Background and Aims: Interactions between parasitic trematodes and their aquatic snail hosts provide a classic example of gene-for-gene co-evolution. Trematode infections typically castrate snails, selecting for costly host defenses, while parasites have evolved mechanisms to overcome these defenses. We used a genetic approach to identify parasite genes involved in circumventing snail defenses in the *Biomphalaria glabrata–Schistosoma mansoni* system.

Methods: We performed genetic crosses between parasites from two schistosome populations (SmBRE and SmLE) that differ in host specificity: both infect BgBRE snails, but only SmLE infects BgBS90 snails. F1 progeny were unable to infect BgBS90, while infectivity was restored in a subset of F2 progeny, consistent with recessive inheritance. We used a bulk segregant approach to identify the genomic region(s) involved. We sequenced pooled F2 parasites emerging from BgBRE or BgBS90 snails, and compared allele frequencies between the pools.

Results: We identified a 1.9 Mb genomic region on chromosome 3 showing strong enrichment in SmLE alleles linked to compatibility with BgBS90 snails. This Quantitative Trait Locus (QTL) region spans 61 genes. We are prioritizing candidate genes under the QTL based on cell-specific gene expression of parasite larvae and on functional impacts of mutations.

Conclusions: Identification of interacting parasite and snail genes is essential to better understand host-parasite co-evolution at the molecular level. We demonstrated the power of bulk segregant analysis to reveal these genes in schistosomes. This fast and cost-efficient approach can significantly accelerate the understanding of the genetics of biomedically relevant traits in parasites.

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PARALLEL ORAL ABSTRACT 30 : REGULATORY AND CELLULAR ARCHITECTURE OF HOST–PARASITE INTERACTIONS
08-20-2026 4:40 PM - 6:40 PM

PREDICTIVE ECOLOGICAL MODELING AND MACHINE-LEARNING FRAMEWORKS FOR URBAN MICROBIOME AND PARASITIC BIOSURVEILLANCE

Suleiman Aminu, Abdulaziz Ascandari, Rachid Daoud
Mohammed VI Polytechnic University (UM6P), College Of Chemical Sciences And Engineering (ccse), Chemical And Biochemical Sciences (cbs), Benguerir, Morocco

Background and Aims: Built environments such as hospitals, sewage systems, ambulances, and public transport represent interconnected microbial networks influencing pathogen and parasite persistence. However, predictive ecological modeling remains limited. This study introduces a genome-resolved, AI-driven framework linking microbial community composition, ecological resilience, and biosurveillance risk, supporting early detection of contamination and parasitic transmission under the One Health paradigm.

Methods: Using >760 Illumina metagenomes, >10,000 MAGs were reconstructed and annotated for antimicrobial resistance and virulence markers (CARD, VFDB). Random Forest models classified environments (balanced accuracy = 0.97 ± 0.01). Synthetic donor–recipient simulations (α = 0–1) and entropy trajectories quantified ecological resilience, dominance, and Minimal Detectable Contamination (MDC).

Results: Hospital sewage acted as a dominant microbial donor (MDC = 0.2–0.3), while hospital interiors exhibited the greatest resilience (MDC ≥ 0.8). Entropy analyses revealed compositional tipping zones (α ≈ 0.3–0.5). The integrated model captured directional source–sink dynamics, predicting cross-environment contamination and ecological vulnerability.

Conclusions: This genome-resolved AI framework transforms metagenomic data into predictive ecological diagnostics. By combining machine-learning classification with synthetic contamination simulations, it enables quantitative assessment of microbial and parasitic risk, guiding biosurveillance strategies for resilient, infection-resistant urban infrastructures. **Data Availability** All analytical scripts and workflow configurations are openly available at https://github.com/SuleimanAminu/genome-resolved-urban-microbiome-biosurveillance (DOI: 10.1093/bioinformatics/btaf548)

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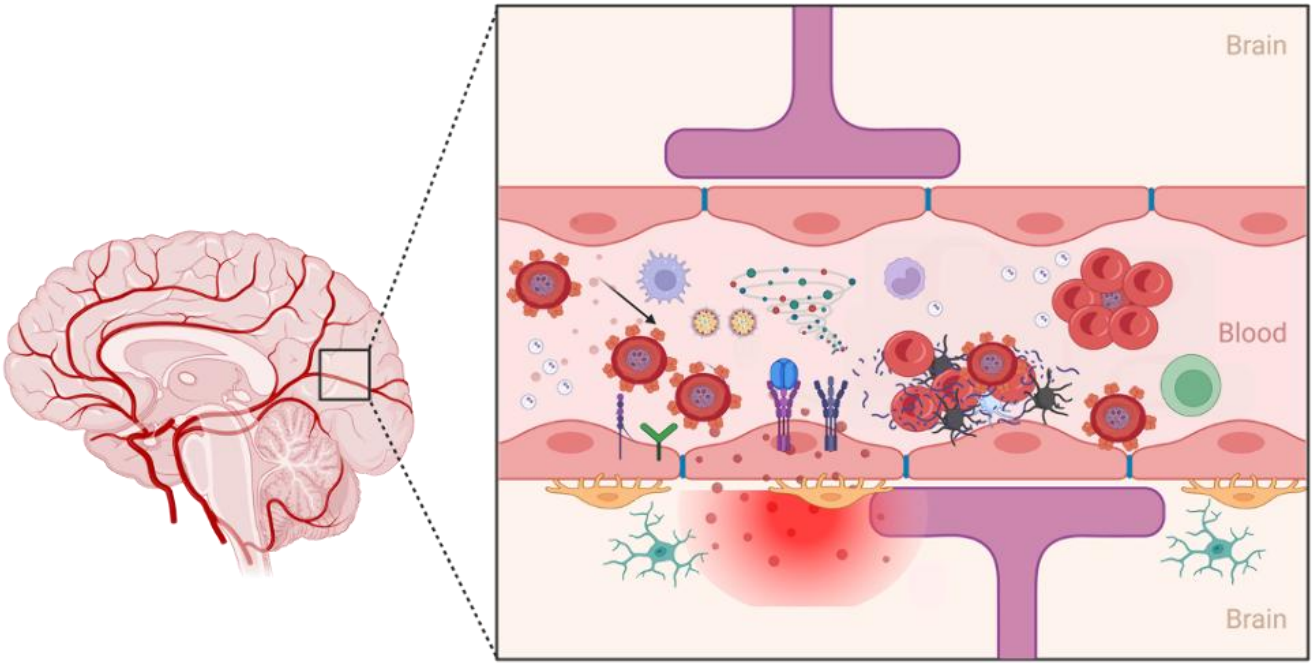
PARALLEL ORAL ABSTRACT SESSION
PARALLEL ORAL ABSTRACT 30 : REGULATORY AND CELLULAR ARCHITECTURE OF HOST–PARASITE INTERACTIONS
08-20-2026 4:40 PM - 6:40 PM

INVESTIGATING THE ROLE OF HUMAN MIRNAS IN THE DISRUPTION OF BLOOD-BRAIN BARRIER INTEGRITY DURING PLASMODIUM FALCIPARUM INFECTION

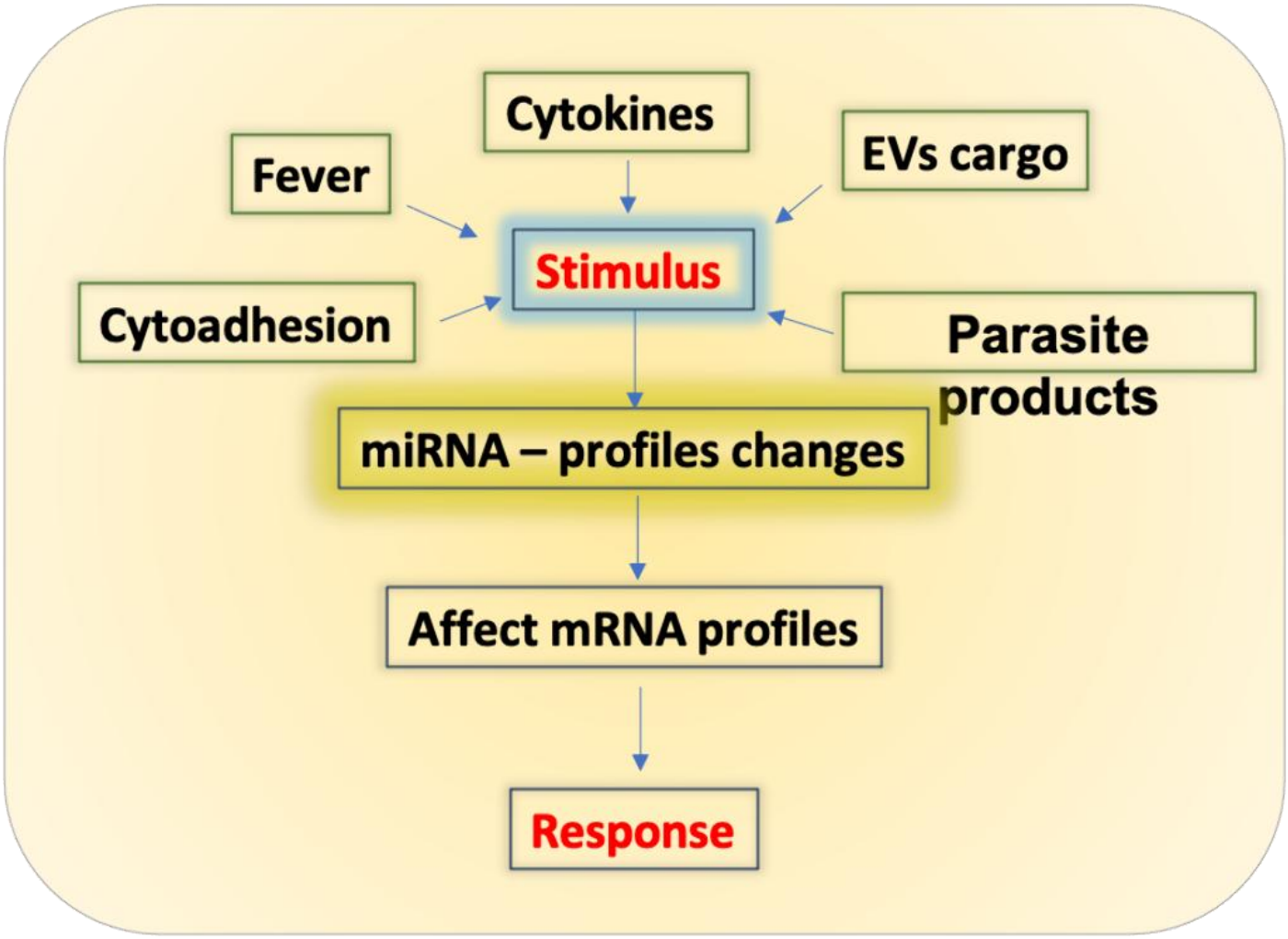
Nahla Galal Metwally[1], Maria Del Pilar Martinez Tauler[2], Hanifeh Torabi[1], Viktor Michaelis[1], Marlena H Kemper[1], Thomas Jacobs[3], Iris Bruchhaus[1]
[1]Bernhard Nocht Institute for tropical medicine, Host Parasite Interaction, Hamburg, Germany, [2]Bernhard Nocht Institute for tropical medicine, Interface, Hamburg, Germany, [3]Bernhard Nocht Institute for tropical medicine, Protozoan Mmunology, Hamburg, Germany

Background and Aims: *Plasmodium falciparum (P. falciparum) is* the malaria parasite species associated with the highest mortality. Cerebral malaria leads to a dramatic loss of blood-brain barrier (BBB) integrity, which can cause brain oedema, hypoxia, and an elevation in intracranial pressure, ultimately leading to coma and death. Therapeutic miRNAs are emerging, but their role in severe malaria complications remains unclear, despite their regulation of 60% of human genes. This project examines miRNA changes during *P. falciparum* infection in a human BBB model.

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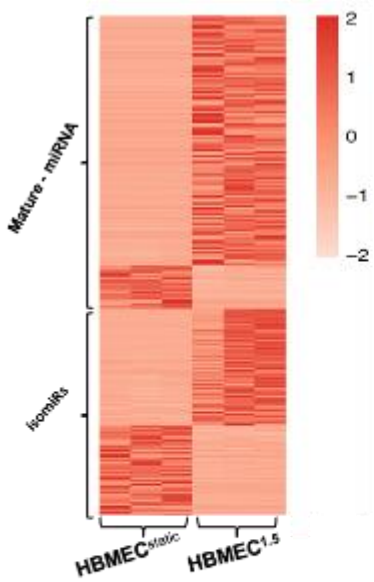


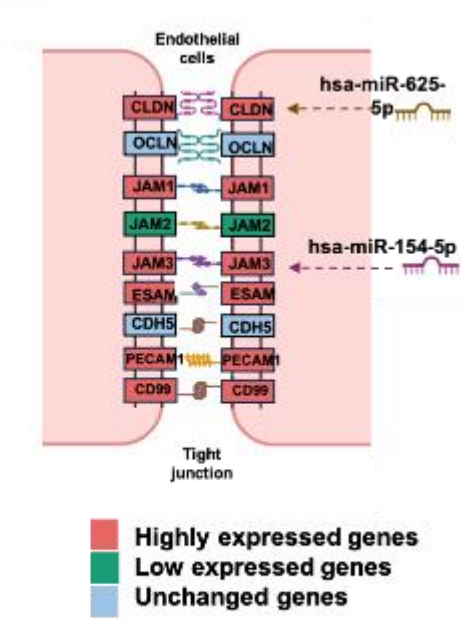
Methods: Cellular miRNA and mRNA sequencing were performed after exposing human brain endothelial cells (ECs) to shear stress, fever, uninfected RBCs, TNFα, and infected RBCs (Rings or trophozoites). Extracellular vesicles were also purified. NGS sequencing was conducted. For data analysis, we used CLC Genomics, IPA, KEGG, and Reactome.

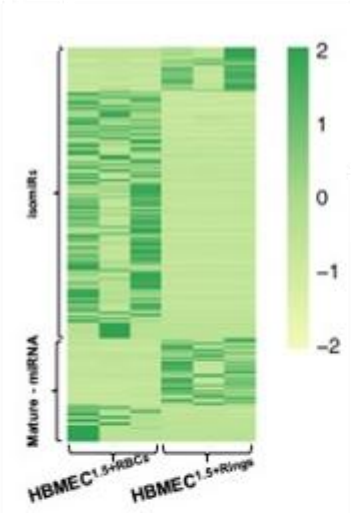
Results: We demonstrated that shear stress significantly altered the expression of 18 miRNAs and increased tight junction gene expression. TNFα stimulation upregulated various immune pathways, including cytokine signalling pathways. Exposure to ring-stage iRBCs altered the expression of eight miRNAs, including miR-4509, -142-5p, -144-3p, -342-3p, -497-5p, -103b, -4485-3p, and 4289. Exposure to trophozoite-stage iRBCs altered the expression of 20 seed family miRNAs, including hsa-miR-6886-5p, 148a-5p, 758-5p, and 493-5p. The mRNA profiles remained stable in the presence of cytoadhesion. TEER measurements indicated significant changes in ECs' tight junctions in response

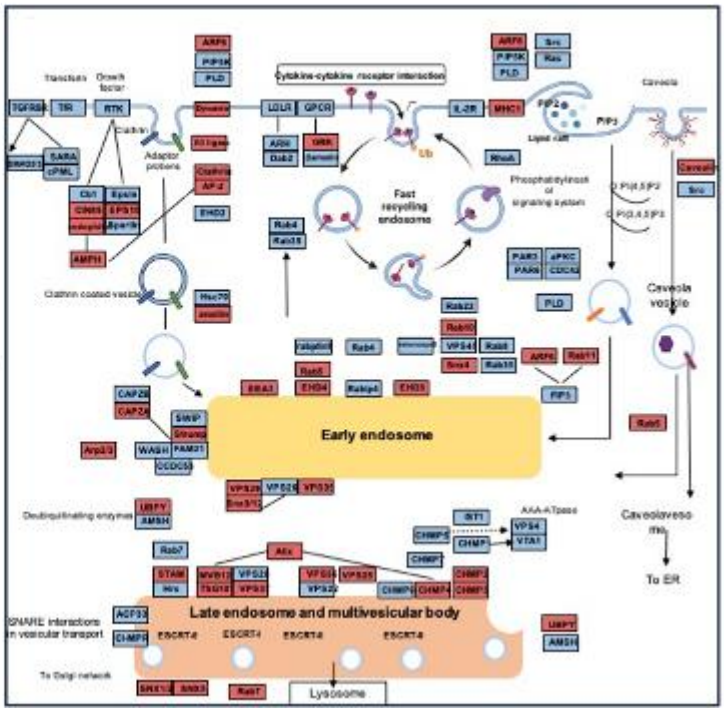
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to TNFα, but stability during









cytoadhesion.

Conclusions: We present a diverse dataset of human brain EC miRNA profiles under various stimuli during malaria. These findings show host miRNAs respond to stimuli, activating downstream signalling in ECs.

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OP215

PARALLEL ORAL ABSTRACT SESSION
PARALLEL ORAL ABSTRACT 30 : REGULATORY AND CELLULAR ARCHITECTURE OF HOST–PARASITE INTERACTIONS
08-20-2026 4:40 PM - 6:40 PM

OMICS PROFILING OF ACANTHAMOEBA AFTER CONTACT WITH HUMAN CORNEA EPITHELIAL CELLS

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[1]Medical University of Vienna, Institute For Specific Prophylaxis And Tropical Medicine, Vienna, Austria, [2]University of Alcala, Madrid, Spain, [3]Medical University of Vienna, Proteomics Core Facility, Vienna, Austria, [4]Medical University of Vienna, Institute For Hygiene And Applied Immunology, Vienna, Austria

Background and Aims: *Acanthamoeba* spp. are ubiquitous protists and the causative agents of *Acanthamoeba* keratitis, a painful and sight-threatening corneal infection that occurs mainly in contact lens wearers. The lytic activity of *Acanthamoeba* on human cells is contact-dependent. In a previous study, we have shown that the expression profiles of lytic proteases change already very early after cell-cell contact. In this study, we aimed to evaluate the impact of contact with human corneal epithelial cells (HCECs) on the proteome of *Acanthamoeba* at the early stage of interaction.

Methods: We incubated a pathogenic strain of *Acanthamoeba castellanii* with HCECs at different multiplicities of infection and investigated the transcriptomic, proteomic and secretomic landscapes before and after cell-cell contact using tandem mass tag (TMT) technology and RNA sequencing.

Results: The exploration of transcript abundances identified 12,208 differentially expressed genes, with 2,366 upregulated and 1,705 downregulated. Expression profiles from 17 transcripts possibly involved in virulence showed comparable trends to those obtained by RNA sequencing. Through TMT-based analysis, we identified 13,873 proteins in the dual-species proteome of which 3,828 were significantly upregulated and 2,834 significantly downregulated. In the total secretome, 422 proteins were significantly upregulated and 474 were significantly downregulated. Integrating transcriptomic data with proteomic and secretomic datasets revealed both, unique and overlapping molecular features.

Conclusions: The generated transcriptomic, proteomic and secretomic datasets provide a valuable overview of the regulation events that occur in the specific context of the early interaction between *Acanthamoeba* and HCECs.

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Poster on Board Abstracts

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SHIFT01-001

POSTER ON BOARD SHIFT 01
PARASITE ECOLOGY, BIODIVERSITY, AND TAXONOMY / ECOLOGY OF PARASITISM IN CHANGING ECOSYSTEMS

PREVALENCE AND LARVAL DEVELOPMENT OF A TREMATODE PARASITE IN AN INTERTIDAL BIVALVE ASSOCIATED WITH THERMAL VARIATIONS IN THE NATURAL ENVIRONMENT

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Background and Aims: Climate change influences host–parasite interactions. The transmission of trematode larvae (cercariae) from primary to secondary hosts is climate-sensitive, and higher temperatures often increase cercarial output. The bivalve *Perumytilus purpuratus*, widely distributed along the Chilean and Argentine intertidal zone, harbors a species of larval trematode, of the family Fellodistomidae. Its prevalence in southern South America is reported, and larval development across host populations under contrasting sea surface temperature (SST) regimes is compared.

Methods: The prevalence of Fellodistomid was assessed in *P. purpuratus* collected from multiple locations in Chile and Argentina across different seasons. Intramolluscan larval development and cercarial release were compared between bivalve populations exposed to contrasting SST regimes. Patent and pre-patent prevalence (i.e., the proportions of infected mussels that did and did not release cercariae, respectively), cercarial output, and sporocyst maturity were statistically compared across locations and seasons with differing SST regimes.

Results: The trematode was more prevalent in Pacific coast mussels. Infection dynamics, including cercarial release and maturity, were associated with lower temperatures, contrary to expectations. Larval maturity peaked at ~13°C, corresponding to regional minima and shifting geographically with the seasons.

Conclusions: The fellodistomid infects *P. purpuratus* between 30° and 49°S in southern South America. Its prevalence and cercarial release correlate with lower regional temperatures, with an optimal larval development temperature of approximately 13°C—matching the area's typical annual intertidal minimum. This suggests the parasite may be vulnerable to global warming rather than benefiting from it.

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SHIFT01-002

POSTER ON BOARD SHIFT 01
PARASITE ECOLOGY, BIODIVERSITY, AND TAXONOMY / ECOLOGY OF PARASITISM IN CHANGING ECOSYSTEMS

COMPREHENSIVE MOLECULAR SURVEY OF TREMATODES IN DOMESTIC AND WILD DEFINITIVE HOSTS FROM CROATIA: COMPLETING THE EUROPEAN PICTURE

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[1]Croatian Veterinary Institute, Zagreb, Croatia, [2]Croatian Veterinary Institute, Veterinary Department Vinkovci, Vinkovci, Croatia

Background and Aims: Trematodes are widespread parasites of major vertebrate groups, with several species of veterinary and public health relevance. To date, no comprehensive molecular survey of trematodes from domestic and wild definitive hosts has been conducted in Croatia, and comparable data remain limited at the European level. This study aimed to provide a large-scale, molecular overview of trematode diversity in Croatia and to place these findings in a broader European context.

Methods: Trematodes from domestic and wild animals were collected over a ten-year period and morphologically identified whenever possible. A total of 276 individual trematodes were analysed molecularly. DNA was extracted and species identification was supported by sequencing fragments of the mitochondrial cytochrome c oxidase subunit I (COI) gene, the nuclear ITS 2 region and the 28S rDNA gene.

Results: In martens and polecats, *Euryhelmis zelleri* and *Euryhelmis* sp. were confirmed, while *Mesostephanus microbursa* was detected in badgers. Golden jackals harboured *Alaria alata, Metagonimus romanicus, Mesostephanus microbursa, Apophallus* sp., *Brachylaima* sp. and *Echinochasmus* sp., whereas red foxes were infected with *Metorchis bilis, M. microbursa, M. romanicus* and *A. alata*. In brown bears, only *Dicrocoelium dendriticum* was detected, which was also found in sheep, goats and cattle together with *Fasciola hepatica* and *Calicophoron daubneyi*. *Fascioloides* magna was confirmed in wild boar, fallow deer, red deer and roe deer. Genetic diversity was detected only in *A. alata* and *D. dendriticum*.

Conclusions: This study provides the first comprehensive molecular overview of trematode diversity in domestic and wild definitive hosts from Croatia, contributing valuable data to the European context.

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SHIFT01-003

POSTER ON BOARD SHIFT 01
PARASITE ECOLOGY, BIODIVERSITY, AND TAXONOMY / ECOLOGY OF PARASITISM IN CHANGING ECOSYSTEMS

ECO-EPIDEMIOLOGIE OF TOXOPLASMA GONDII IN SMALL MAMMALS FROM COTONOU, BENIN: PREVALENCE, MURINE VIRULENCE AND CO-INFECTION PATTERNS WITH TRYPANOSOMA LEWISI

Jonas Etougbetche[1], Lokman Galal[2], Gauthier Dobigny[3], Aurélien Mercier[2]
[1]University of Abomey-Calavi, Laboratory Of Applied Biology Research, Polytechnic School Of Abomey-calavi, Cotonou, Benin, [2]Université de Limoges, Epimact U1094 Inserm Umr270 Ird Usc1501 Inrae, Limoges, France, [3]Université de Montpellier, Cbgp, Ird, Cirad, Inrae, Institut Agro, Montpellier, Madagascar

Background and Aims: Toxoplasmosis is a widespread parasitic infection caused by the protozoan *Toxoplasma gondii*. The parasite shows a wide genetic and host diversity, thus resulting in a wide panel of eco-epidemiological and clinical profiles. Small mammals, play a key role in the maintenance of the *T. gondii* life cycle. In order to a better picture of toxoplasmosis reservoir-associated risks in Benin, we focused on *T. gondii* ecology in domestic small mammal populations.

Methods: We determined the molecular prevalence and genetic diversity of *T. gondii* as well as the patterns of *T. gondii* infection in 632 commensal small mammals from Cotonou city. We tested the virulence of an African and European *T. gondii* lineages in invasive and native rodent species and finally, we explored statistical interactions between *T. gondii* and *Trypanosoma lewisi*.

Results: An overall prevalence of 15.2% was found, and seven genotypes, all belonging to the *Africa 1* lineage, could be retrieved from black rats and shrews. Our results pointed towards the need to screen both brain and heart and to take into account host abundance in order to paint a more comprehensive picture of *T. gondii* circulation in wild rodents. A total resistance of wild *Mus musculus domesticus* to *T. gondii Africa 1* lineage, virulent for laboratory mice. In contrast, native *Mastomys natalensis* were partly sensitive (54% mortality). Statistical analyses showed a clear and significant segregation between the *T. gondii* and *Trypanosoma lewisi*.

Conclusions: Overall, our results highlight the interest of associating field approaches and experimental studies for understanding *Toxoplasma* eco-epidemiology in urban socio-environments.

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SHIFT01-004

POSTER ON BOARD SHIFT 01
PARASITE ECOLOGY, BIODIVERSITY, AND TAXONOMY / ECOLOGY OF PARASITISM IN CHANGING ECOSYSTEMS

EFFECT OF UPWELLING INTENSITY ON THERMAL PERFORMANCE DESCRIPTORS IN FIRST INTERMEDIATE HOSTS AND THEIR LARVAL PARASITES

Manuel García-Huidobro Moreno, Marcela Aldana
Universidad Santo Tomás, Centro De Investigación E Innovación Para El Cambio Climático, Santiago, Chile

Background and Aims: This study aimed to understand the combined effects of temperature and resource availability on parasite transmission, a critical gap given climate change. Using a natural mosaic of coastal upwelling intensities in central Chile, it investigated the interaction between these factors in the mussel *Perumytilus purpuratus* and its trematode parasites. The central hypothesis was that higher environmental productivity (resource availability) would enhance both host metabolic performance and parasite output (cercarial emergence and survival). The research employed a Thermal Performance Curve (TPC) framework to quantify these responses across a temperature gradient.

Methods: Mussels were collected from three locations with varying upwelling intensity. Parasite prevalence was determined, and experiments measured: 1) thermal performance of cercarial emergence from infected hosts, 2) cercarial survival at set temperatures, and 3) thermal performance of host metabolism (oxygen consumption) for both parasitized and unparasitized individuals. Key TPC descriptors (e.g., optimal temperature, critical maximum) were calculated and statistically compared among sites and parasitism states.

Results: Cercarial survival was highest at cold temperatures from the strong upwelling site. For hosts, parasitism increased thermal tolerance (CTmax) and warming tolerance, but only in the weak upwelling location, indicating a location-specific interaction.

Conclusions: The findings demonstrate that temperature and resource availability interact complexly to shape host-parasite dynamics. The effects of parasitism on host thermal resilience are context-dependent, mediated by local environmental productivity. This study underscores that environmental mosaics, like upwelling zones, are crucial for testing and predicting how multiple stressors will alter ecological interactions under global change.

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SHIFT01-005

POSTER ON BOARD SHIFT 01
PARASITE ECOLOGY, BIODIVERSITY, AND TAXONOMY / ECOLOGY OF PARASITISM IN CHANGING ECOSYSTEMS

EFFECTS OF TRACE METALS ON TREMATODE LARVAL PERFORMANCE IN THE HUMBOLDT CURRENT MARINE SYSTEM: WILL INFECTIONS DECLINE UNDER GLOBAL WARMING?

M. Teresa Gonzalez[1], Natalia Leiva[1], Gabriela Munoz[2], Rodrigo Vidal[3]
[1]University of Antofagasta, Instituto Ciencias Naturales "alexander Von Humboldt", ANTOFAGASTA, Chile, [2]University of Valparaiso, Facultad De Ciencias, Valparaiso, Chile, [3]University of Santiago of Chile, Facultad De Ciencias, SANTIAGO, Chile

Background and Aims: Parasites play a key role in structuring intertidal ecosystems by regulating host populations, a role that can be affected by environmental stressors such as metal pollution and global warming. Trematodes have free-living stages highly sensitive to environmental conditions, which can alter cercarial production, transmission, survival, and longevity. The second free-swimming larval stage, cercariae, is crucial for infecting definitive hosts (birds). The northern Chilean coastal ecosystem faces severe degradation from over a century of industrial mining, with records of elevated metal concentrations. This study experimentally examines the combined effects of metal exposure and temperature (~14 and 25°C) on the survival and longevity of *Himasthla* sp. cercariae emerged from their first intermediate host, *Echinolittorina peruviana*.

Methods: In the laboratory, individually collected snails (rocky area, 23°S) were placed in separate wells and kept dry for 6 h. Then, seawater was added to each well to get the emergence of cercariae. Recovered cercariae were exposed to different metal treatments (Cd, Zn) and to controls, and mortality was recorded every 6 hours until all larvae died. Experiments were repeated three times.

Results: At 24°C, both Cd (0.03 mg/L) and Zn (0.54 mg/L) independently reduced cercarial survival and longevity, while their mixture had no significant effect. At 14°C, only Cd affected parasite performance, indicating that temperature modulates metal toxicity in this system.

Conclusions: These results demonstrate that environmental stressors interact with thermal conditions to influence parasite survival, which may, in turn, affect host regulation in intertidal ecosystems. **Financial Support: Fondecyt project N°1241647.**

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SHIFT01-006

POSTER ON BOARD SHIFT 01
PARASITE ECOLOGY, BIODIVERSITY, AND TAXONOMY / ECOLOGY OF PARASITISM IN CHANGING ECOSYSTEMS

METABOLIC RESPONSES TO TEMPERATURE AND OXYGEN AVAILABILITY IN THE PARASITIC NEMATODE EUSTRONGYLIDES SP.

Maryane Gradito, Timothy Clark
Deakin University, School Of Life And Environmental Sciences, Geelong, Australia

Background and Aims: Many helminth species receive little research attention beyond their initial description. Nematodes of the genus *Eustrongylides* are globally distributed parasites with complex life cycles. Based on experiments in the 1940s, *Eustrongylides* sp. are thought to use predominantly aerobic metabolism across temperatures despite spending most of their lives deep within fish tissue. Here, we used modern respirometry approaches to investigate the *in vitro* physiological performance of *Eustrongylides* sp. using oxygen consumption as a proxy for aerobic metabolic rate.

Methods: Using a SensorDish® reader (PreSens), we measured oxygen consumption of individual nematodes across a temperature gradient (10-35°C) in sealed chambers. We also conducted a preliminary examination of oxygen-use strategies by measuring metabolic responses to declining oxygen availability to determine whether parasites exhibit a critical oxygen tension (Pcrit) or function as oxyregulators.

Results: Metabolic rate of the worms was strongly temperature-dependent, increasing significantly with rising temperature. The thermal sensitivity of metabolism was equivalent to that seen in the fish host, with a temperature coefficient (Q_{10}) of 1.59. Very low metabolism at 10°C suggested that the nematodes may be essentially dormant below this temperature. Preliminary experiments suggested that parasites maintain their oxygen consumption rate down to relatively severe hypoxia, as the oxygen uptake rate did not parallel environmental oxygen as would be expected from an oxyconformer.

Conclusions: This study provides new physiological insights into the thermal dependence of aerobic metabolism in *Eustrongylides*, a widely distributed parasitic nematode, and contributes to a broader understanding of parasite functional ecology, particularly in the context of a warming world.

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SHIFT01-007

POSTER ON BOARD SHIFT 01
PARASITE ECOLOGY, BIODIVERSITY, AND TAXONOMY / ECOLOGY OF PARASITISM IN CHANGING ECOSYSTEMS

GASTROINTESTINAL PARASITE FINDINGS IN WILD CARNIVORES FROM CERRO EL MELÓN, CENTRAL CHILE: COPROLOGICAL DETECTION OF TOXASCARIS-LIKE EGGS IN PUMA CONCOLOR AND LEOPARDUS COLOCOLA

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Background and Aims: Wild felids play an important ecological role as top predators and may act as hosts for a wide diversity of gastrointestinal parasites. However, parasitological studies in free-ranging wild felids from Chile remain limited, particularly in remote Andean ecosystems. This study aimed to characterize gastrointestinal parasite findings in fecal samples collected from wild carnivores inhabiting Cerro El Melón, Valparaíso Region, Chile.

Methods: Fecal samples from wild carnivores were collected in collaboration with the NGO Felinos del Cerro Caqui, which monitors local wildlife through camera traps. Recorded species included *Leopardus jacobita*, *Leopardus colocola* and *Puma concolor*. Samples were analyzed using flotation and sedimentation coprological techniques. Parasitic structures were microscopically examined and morphologically characterized.

Results: Coprological examination revealed larvated eggs morphologically compatible with *Toxascaris* sp. in samples attributed to *Puma concolor* and *Leopardus colocolo*. Molecular analyses for species confirmation are currently in progress. Notably, samples had been collected approximately one year before laboratory examination, yet viable larvae were still observed within the eggs, suggesting prolonged environmental resistance and potential infectivity. Camera trap records confirmed the coexistence of multiple wild carnivore species within the study area, supporting the potential circulation of gastrointestinal parasites among sympatric hosts.

Conclusions: These findings contribute new information regarding gastrointestinal parasites infecting wild carnivores in central Chile and highlight the importance of integrating wildlife monitoring and parasitological surveillance in conservation programs. The persistence of viable larvated eggs may have epidemiological relevance for parasite transmission in Andean ecosystems.

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SHIFT01-008

POSTER ON BOARD SHIFT 01
PARASITE ECOLOGY, BIODIVERSITY, AND TAXONOMY / ECOLOGY OF PARASITISM IN CHANGING ECOSYSTEMS

UPDATING CURRENT KNOWLEDGE ON THE SEASONALITY OF INTERMEDIATE SNAIL HOSTS OF FASCIOLA HEPATICA IN THE SOUTHERN TABLELANDS OF NEW SOUTH WALES COMMA AUSTRALIA

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Background and Aims: *Fasciola hepatica* is dependent on temperatures >10°C and adequate moisture for several stages of larval development, plus the presence of their intermediate lymnaeid snail hosts to maintain the life cycle. Livestock producers in the Southern Tablelands of New South Wales leverage the seasonal pause in the life cycle between the first frost in May, and last frost in September, to guide treatment schedules. Whilst conducting a drug resistance trial in July 2024, numerous lymnaeid snails were discovered when average daily temperatures were <7°C, challenging the foundations underpinning integrated parasite management (IPM) strategies. This study set out to update knowledge on the seasonality of lymnaeid snail populations in the region to help refine current *F. hepatica* risk periods and inform new IPM strategies.

Methods: To achieve this, twelve sites across six farms endemic for *F. hepatica* were sampled bi-monthly during 2025. Collected snails were counted and morphologically speciated to determine the average abundance and diversity of susceptible hosts. Snails were first visually inspected for *F. hepatica* larval infection, then confirmed with qPCR.

Results: One site significantly deviated from the historical seasonal abundance pattern, recording the study's highest abundance of lymnaeid snails (N = 240) in July (Austral winter), when the mean weekly temperature was <8°C. The invasive snail species *Pseudosuccinea columella* was most abundant in July at three sites.

Conclusions: This study provides the first seasonal monitoring of the invasive snail *P. columella* and their increased abundance in winter suggests that the infective risk period for *F. hepatica* extends beyond the historical dormancy window.

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SHIFT01-009

POSTER ON BOARD SHIFT 01
PARASITE ECOLOGY, BIODIVERSITY, AND TAXONOMY / ECOLOGY OF PARASITISM IN CHANGING ECOSYSTEMS

FRECUENCY OF TRYPANOSOMATIDS IN WILD RESERVOIRS FROM THE METROPOLITAN AREA OF BUCARAMANGA, SANTANDER, COLOMBIA: THEIR IMPORTANCE IN PERI-URBAN AREAS

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Background and Aims: Trypanosomatids are protozoan of major global importance for both public and veterinary health. Their transmission and persistence across diverse ecosystems are strongly influenced by the presence of wild and synanthropic animals, which may act as reservoirs and disseminators of infection. This study aimed to determine the infection frequency and diversity of trypanosomatids in wild and synanthropic mammals and reptiles from the Metropolitan Area of Bucaramanga, Santander, Colombia.

Methods: Blood samples were collected from 94 animals, including 76 mammals from the orders Rodentia (n = 2), Didelphimorphia (n = 20), Carnivora (n = 12), Pilosa (n = 5), and Primates (n = 37), as well as 18 reptiles. Parasite detection was performed by blood culture in NNN medium and by molecular methods targeting the 18S rRNA and HSP70 genes.

Results: Blood culture detected trypanosomatid infection in 7.89% (6/76) of mammals. Molecular analysis using the 18S rRNA marker identified *Trypanosoma* spp. infection in 9.21% (7/76) of mammals and 22.2% (4/18) of reptiles, whereas *Leishmania* spp. was detected by HSP70 in 10% (2/20) of Didelphimorphia. Among reptiles, the highest frequency of *Trypanosoma* spp. infection was observed in *Boa constrictor* (22.2%), while among mammals, *Cebus versicolor* showed the highest frequency (9.21%). *Leishmania* spp. was detected exclusively in *Didelphis marsupialis*.

Conclusions: The high frequency of *Trypanosoma* spp. infection observed in reptiles highlights their potential role as cryptic hosts in the maintenance and transmission of trypanosomatids in the region. Likewise, this study confirms the role of primates and marsupial species as important reservoirs of these flagellates in urban areas.

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SHIFT01-010

POSTER ON BOARD SHIFT 01
PARASITE ECOLOGY, BIODIVERSITY, AND TAXONOMY / ECOLOGY OF PARASITISM IN CHANGING ECOSYSTEMS

ENHANCED OPISTHORCHIS VIVERRINI EDNA DETECTION SENSITIVITY USING A MULTI-TARGET ASSAY FOR WATER SAMPLES

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Background and Aims: Opisthorchiasis, caused by the fish-borne liver fluke *Opisthorchis viverrini* (Ov), is a major public health problem in endemic Southeast Asian countries and a leading cause of cholangiocarcinoma. Although fecal examination and treatment are standard control measures, reinfection remains common in high-risk environments due to dietary habits. We apply environmental DNA (eDNA) analysis for opisthorchiasis surveillance, which enables identification of high-risk areas and fish for consumption. To enhance detection sensitivity in field settings where Ov eDNA levels are low, we developed and implemented a multi-target qPCR assay, to improve the accuracy of environmental risk mapping.

Methods: A multi-target qPCR assay was developed targeting 4 genes, COI, Cytb, ITS1 and ITS2. After the analytical sensitivity was tested, it was applied in field samples. Environmental water samples from 56 sites in OV prevalent areas in Thailand were used. The detection rate of multi and single target qPCR assays were compared.

Results: The multi-marker qPCR showed quite high analytical sensitivity as 10 fg. The multi-target qPCR showed higher sensitivity than single-marker assays for OV eDNA detection with field samples, 11/56 sites (19.6%) and 5/56 sites (8.9%) respectively. The field sensitivity for OV eDNA showed equivalent results to the analytical detection limit.

Conclusions: The multi-target qPCR assay demonstrated improved sensitivity for detecting O. viverrini (Ov) eDNA. Enhanced Ov eDNA detection enables more accurate identification of

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environmental contamined sites and therefore infection risk, thereby facilitating targeted (focal) control interventions.

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SHIFT01-011

POSTER ON BOARD SHIFT 01
PARASITE ECOLOGY, BIODIVERSITY, AND TAXONOMY / ECOLOGY OF PARASITISM IN CHANGING ECOSYSTEMS

ECOLOGICAL NICHES AND CLIMATIC DETERMINANTS OF FASCIOLA GIGANTICA DISTRIBUTION IN SOUTH AND NORTH WAZIRISTAN, PAKISTAN

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Background and Aims: Fascioliasis caused by Fasciola gigantica, is an important parasitic disease responsible for significant economic losses in ruminants due to decreased productivity and liver condemnation at slaughter. The aim of the present study was to determine the prevalence of Fasciola gigantica infection and to predict the ecological niches and climatic variables supporting fascioliasis in South Waziristan and North Waziristan of Pakistan.

Methods: With the goal to determine the presence for Fasciola gigantica infection, 253 liver samples and 4300 fecal/blood samples were taken from sheep and goat herds within the study area. Ecological and meteorological factors were examined for correlation with the occurrence of infection, and an environmental risk map was created to forecast active transmission zones.

Results: Fasciola gigantica was detected in 46 of 253 liver samples and 700 of 4300 fecal/blood samples. Fascioliasis was more common in sheep than in goats, and it was most common in females older than three. North and South Waziristan had the highest transmission potential, according to environmental risk mapping. Fasciola gigantica infection was substantially correlated with mean monthly diurnal temperature range (Bio2), yearly precipitation (Bio12), and Normalized Difference Vegetation Index (NDVI) ($p < 0.05$).

Conclusions: The findings of this study demonstrate the most suitable ecological niches and climatic variables influencing the risk of fascioliasis caused by Fasciola gigantica in South Waziristan and North Waziristan, Pakistan. These results may serve as a reference for developing effective control and prevention strategies for fascioliasis in the region.

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SHIFT01-012

POSTER ON BOARD SHIFT 01
PARASITE ECOLOGY, BIODIVERSITY, AND TAXONOMY / ECOLOGY OF PARASITISM IN CHANGING ECOSYSTEMS

COLPODELLIDS THAT DON'T SUCK: NOVEL DIVERSITY OF FREE-LIVING BACTERIVOROUS COLPODELLIDS, RELATIVES OF APICOMPLEXA

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Background and Aims: Colpodellids, along with the algae *Chromera* and *Vitrella*, are the closest free-living relatives of apicomplexan parasites. Collectively, these organisms possess lifestyles that range from primary production, through predation and parasitism. They share an apical complex that has been repurposed for different activities. Most apicomplexans are obligatory intracellular parasites that invade their host cells using the apical complex. All described colpodellids live aerobically and prey on microbial eukaryotes by a process called myzocytosis, in which attachment to the prey is followed by formation of a microtubule ring derived from the apical pseudoconoid that delimits the connection between the predator and prey. This has similarities to the mechanisms by which apicomplexa invade host cells, and led to hypotheses that the ancestor of Apicomplexan parasites might have been a eukaryovorous predator.

Methods: We have isolated a colpodellid lineage that differs from all other known colpodellids; they are predominantly free-living bacterivores that are capable of living both aerobically and anaerobically. We used transcriptome/genome sequencing, FIB-SEM, TEM and expansion microscopy to study these lineages.

Results: At least one endobiotic lineage, infecting invertebrates, was identified within the clade of bacterivorous colpodellids. The behavioral, morphological, and molecular characterizations of these novel colpodellids, including the apical complex structures will be described in detail. We demonstrate the bacterivory using both culturing experiments and expansion microscopy.

Conclusions: These results add to the existing diversity of life histories within the Chrompodellids-Apicomplexa lineage and suggest that within Colpodellids, there might have been a parallel evolution of parasitism to that of Apicomplexa.

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SHIFT01-013

POSTER ON BOARD SHIFT 01
PARASITE ECOLOGY, BIODIVERSITY, AND TAXONOMY / ECOLOGY OF PARASITISM IN CHANGING ECOSYSTEMS

ARE TRYPANOSOMES SUITABLE HOSTS FOR RNA VIRUSES?

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Background and Aims: RNA viruses infect several trypanosomatid lineages and can profoundly influence parasite biology and pathogenicity. However, despite intensive study, no RNA viruses had been characterized from the genus Trypanosoma. We aimed to test whether this apparent absence reflects intrinsic host incompatibility or ecological constraints limiting viral acquisition.

Methods: We analyzed a cultured strain of Trypanosoma platydactyli, a parasite of the common wall gecko transmitted by sand flies, for viral infections using dsRNA enrichment, next-generation sequencing, genome annotation, and phylogenetic analyses. Viral morphology was examined by transmission electron microscopy.

Results: Three RNA viruses were identified in a single T. platydactyli strain: one leishmaniavirus and two narnaviruses. The leishmaniavirus exhibited genomic organization typical of the genus and phylogenetic affinity to the viruses from Old World Leishmania, consistent with shared vector ecology. The two narnaviruses had independent evolutionary origins but clustered within trypanosomatid-associated clades, indicating historical virus exchange among co-infecting flagellates.

Conclusions: These findings demonstrate that trypanosomes are compatible hosts for diverse RNA viruses. The scarcity of viral reports from medically important trypanosomes likely reflects ecological barriers—particularly the strictly hematophagous feeding behavior of their vectors—rather than intrinsic cellular resistance. Trypanosomes transmitted by vectors with mixed or sugar-feeding habits represent promising targets for future viral discovery, underscoring the central role of ecology in shaping virus–host associations among protists. The work was supported by the EU's Operational Program 'Just Transition' LERCO CZ.10.03.01/00/22_003/0000003.

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SHIFT01-014

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PARASITE ECOLOGY, BIODIVERSITY, AND TAXONOMY / ECOLOGY OF PARASITISM IN CHANGING ECOSYSTEMS

EMERGING POLLUTANTS RESHAPE MARINE PARASITISM: IMPACTS OF NANOPLASTICS AND ARTIFICIAL NIGHT LIGHTING ON TREMATODE TRANSMISSION DYNAMICS

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Background and Aims: Anthropogenic stressors increasingly shape marine ecosystems, yet their effects on parasite transmission remain poorly understood. We examined how nanoplastics (NPs) and artificial light at night (ALAN) influence cercarial emergence, behaviour, survival, and transmission of marine trematodes infecting *Zeacumantus subcarinatus* snails.

Methods: Infected snails were exposed to NPs (0, 5, 20 mg/L) for three months to quantify effects on *Maritrema novaezealandensis* cercarial emergence, survival, and infectivity to second intermediate amphipod hosts. Parallel ALAN experiments tested three light types (warm white LED, cold white LED, UV fluorescent) to evaluate impacts on cercarial output, snail phototaxis, cercarial light attraction, and transmission success across four trematode species.

Results: High NP exposure (20 mg/L) caused significant reductions in cercarial emergence and markedly shortened cercarial lifespan, while infectivity remained unchanged. ALAN produced strong species-specific responses: cercarial output, and snail and cercarial phototaxis varied among trematode species. For *M. novaezealandensis*, transmission to amphipods was highest under UV light and lowest under cold white light.

Conclusions: Nanoplastics and ALAN affect trematode transmission by altering cercariae emergence, lifespan, and infectivity to the next host. Since trematodes contribute substantially to coastal food-web structure, even subtle disruptions to their transmission stages may generate cascading ecological effects. Integrating parasitology into ecotoxicology and light-pollution research is essential for predicting ecosystem responses to global change.

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SHIFT01-016

POSTER ON BOARD SHIFT 01
PARASITE ECOLOGY, BIODIVERSITY, AND TAXONOMY / ECOLOGY OF PARASITISM IN CHANGING ECOSYSTEMS

ASCENT OF CAMALLANIDAE TO ANISAKIDAE ZOONOTIC NEMATODES

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Background and Aims: i)The findings of recently established tribe Indospineziinea have critically indicated infiltration of characteristics of members of Anisakidae into the characteristics of Camallanoidea. ii)To attempt major revision of systematics of recently discovered worms with camallanoid and raphidascaridoid characteristics.

Methods: Fresh roundworms from Central West coast of India and Gangetic and Ken-Betwa riverine ecosystems in Madhya Pradesh were studied. A major revision of the worms with camallanoid characteristics and anisakid features like a ventriculus, ventricular appendix and intestinal ceca but with three pairs of buccal teeth atop cephalic complex was undertaken.

Results: The emergence of physalopterid character, a post-cephalic collar in Indospinezia (Jaiswal and Malhotra, 2017), and a post-anal collar in raphidascaridoid, Rostellascaris spinicaudatum; while a post-cephalic collar in another worm bearing anisakid characters, namely, Pronakid sp. were noticed. Finally, in worms with two pairs of buccal teeth, 21-ribbed cephalic armature, a post-cephalic and post-anal collar were observed.

Conclusions: Rotundocollarette capoori of Anisakidae with 6 buccal teeth emerged as climax species ahead of worms with 4 buccal teeth. The erroneous interpretation of Goezia spinulosa under Anisakidae without providing an invincible proof of the buccal tooth in second stage larva has been highlighted, while this is confirmed in Indospinezia. Goezia spinulosa is thus marked species inquirendae. (Yadav and Malhotra). The current nematodes were equipped with firstly, the highest number of 6 buccal teeth around i) oral, ii) genital and iii) excretory pores, while next to these in hierarchy were the roundworms with i) oral and ii) excretory pores.

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SHIFT01-017

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PARASITE ECOLOGY, BIODIVERSITY, AND TAXONOMY / ECOLOGY OF PARASITISM IN CHANGING ECOSYSTEMS

STRUCTURAL VARIATION IN THE GENOMES OF THE YELLOW-FEVER MOSQUITO AND THEIR RELATIVES

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Background and Aims: *Aedes aegypti* is the primary vector for pathogens such as chikungunya, dengue, yellow-fever, and Zika viruses. It is a globally invasive mosquito, found in the tropics and sub-tropics, thus exposing millions of people to these diseases. Curiously, this mosquito exhibits different bionomics across its range and have been designated into two subspecies—in its native, sub-Saharan African range, *Ae. aegypti formosus* (Aaf) is primarily found in sylvatic or natural areas, whereas everywhere else in its invasive range, *Ae. aegypti aegypti* (Aaa) is found in urban areas in and around human dwellings. Furthermore, the subspecies differ in their preferred blood-host where females of Aaf feed generally on mammals and females of Aaa preferentially feed on humans.

Methods: To understand the genomic basis for these phenotypic differences, we used the PacBio HiFi sequencing platform and HiC sequencing to assemble and annotate chromosome-scale genomes of both Aaf and Aaa and compared them against other high-quality genome assemblies. We used both nucleotide and gene order synteny to detect how each assembly differs from one another. Furthermore, we characterized the genes present in structurally variant sites to pinpoint which genes (if any) may contribute to phenotypic differences.

Results: Overall we found several notable inversions relative to the reference genome present in Aaf that is not present in the other taxa.

Conclusions: These inversions may contain genes implicated in the behavioral and ecological differences between Aaf and Aaa.

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PARASITE ECOLOGY, BIODIVERSITY, AND TAXONOMY / ECOLOGY OF PARASITISM IN CHANGING ECOSYSTEMS

INVASIVE SPECIES AS SENTINELS. THE PARASITE FAUNA OF RACCOONS AS A DIAGNOSTIC TOOL FOR CIRCULATING MEDICAL AND ECOLOGICAL RELEVANT PATHOGENS.

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Background and Aims: In Europe, the invasive raccoon is considered a potentially significant carrier of infectious diseases and parasites, with a significant negative impact on native ecosystems. Its preference for urban areas inevitably leads to increased contact with humans, pets, and livestock, making the raccoon a potential carrier of zoonotic diseases.

Methods: 387 raccoons were caught and examined during regular hunting. The raccoons were dissected to record morphometric data, stomach contents. Parasitological examinations were conducted using morphological, genetic, and serological methods.

Results: 23 different parasite species were identified, five of which are human pathogens, 18 of which are new for the parasite fauna of raccoons in Europe. The human pathogenic raccoon roundworm *Baylisascaris procyonis* is the most common parasite species.Life cycles of detected endoparasites indicate sustained use of predominantly animal food sources. The results suggest that species of conservation concern are at significant local risk from raccoon predation in certain areas.Two raccoons were tested positive for *Mycobacterium bovis* in real-time PCR and bacteriological culture.

Conclusions: The study reveals an extremely diverse and adaptable parasite fauna and demonstrates its essential role as intermediate and definitive hosts for ectoparasites and endoparasites. The identified endoparasites reveal ecologically significant food webs and point to potential conflicts from a conservation perspective. It further shows that raccoon populations could be endemic carriers of *M. bovis*. The ongoing spread of raccoons increases the potential health risk to wildlife, livestock, and humans and underscores the need for an interdisciplinary One Health approach.

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SHIFT01-019

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PARASITE ECOLOGY, BIODIVERSITY, AND TAXONOMY / ECOLOGY OF PARASITISM IN CHANGING ECOSYSTEMS

CRYPTOSPORIDIUM SPECIES AND GENOTYPE DIVERSITY IN WILD BIRDS ALONG AGRICULTURAL LANDSCAPES: ECOLOGICAL INSIGHTS FROM A ONE HEALTH APPROACH

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Background and Aims: Anthropogenic activities such as the intensification of agriculture can restructure parasite communities by modifying host exposure and transmission routes. Wild birds are essential hosts for the study of parasite-ecology and biodiversity within One Health context. The purpose of this study was to describe the diversity of Cryptosporidium species and genotypes in wild birds sampled from different agricultural landscapes.

Methods: Wild birds (n=674) were collected along a gradient from organic to conventional farming with pesticide application. PCR amplification of the SSU rRNA 18s gene was used to detect *Cryptosporidium* in faecal samples. Only samples with an unequivocal genus-and species-level determination were incorporated. Additional molecular typing was carried out by gp60 gene sequencing when the amplification was successful, to determine the genotypes.

Results: At species level, 13 birds were identified as *Cryptosporidium* positive. Four species were detected; C. galli (n = 6), *Cryptosporidium baileyi* (n = 1), *C. andersoni* (n = 1) and *Cryptosporidium parvum* (n = 5). Molecular characterization of *C. parvum* isolates by subtyping revealed three genotypes: IIaA15G2R1 (n = 3), IIaA14G3R1 (n = 1) and IIcA5G3 (n = 1). These genotypes are frequently found in human infections, and suggests the existence of zoonotic interface. The presence host-adapted avian species and zoonotic genotypes indicates that *Cryptosporidium* circulation is complex in wild bird communities.

Conclusions: Wild birds host a diverse assemblage of *Cryptosporidium* species and genotypes, including host-adapted avian taxa and zoonotic genotypes. This diversity reflects complex parasite ecology in agricultural landscapes. The presence of human-associated genotypes suggests that wild birds may act as reservoirs, contributing to environmental maintenance and potential transmission at the wildlife–human interface.

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SHIFT01-020

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PARASITE ECOLOGY, BIODIVERSITY, AND TAXONOMY / ECOLOGY OF PARASITISM IN CHANGING ECOSYSTEMS

FIRST REPORT OF ISOLATION, MOLECULAR CHARACTERIZATION, AND CRYOPRESERVATION OF TRYPANOSOMA MINASENSE CHAGAS, 1908 (PROTOZOA; TRYPANOSOMATIDA)

Maria Paula Machado Silva[1], Filipe Abreu[2], Marlon Da Silva[1], Deivid Magalhães[2], Maria Eduarda Almeida[2], Maria Eduarda Santos[2], Fernanda Alves[1], André Luiz Roque[1]
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Background and Aims: *Trypanosoma minasense* is a frequent parasite of neotropical non-human primates which isolation with molecular characterization and cryopreservation has not been reported.

Methods: A *Callithrix penicillata* from Coronel Murta/MG, Brazil, presented flagellated forms morphologically compatible with *T. minasense* on direct microscopic examination. Blood samples were cultured in axenic NNN/Schneider and NNN/RPMI-1640 media (300 µL each). After 11 days, approximately 50 µL from each culture were inoculated together into VERO cell cultures supplemented with Schneider medium. Five days later, the first parasites were observed in the supernatant of the co-culture (POP1). One week after the initial detection, parasites were inoculated into an axenic NNN/Schneider tube (POP2). After approximately one month of monitoring, parasites from both the co-culture and the axenic culture were each transferred to new VERO cell flasks, this time supplemented with Schneider added with Medium 199 (POP3 and POP4, respectively). Parasite pellet was obtained by centrifugation for DNA extraction, followed by Nested PCR amplification targeting the 18S SSU rDNA region. Products were sequenced by the Sanger method and analyzed using the BLAST algorithm.

Results: POP3 could not be maintained, whereas POP4 exhibited profuse parasite growth. The isolate obtained from this population was cryopreserved and deposited in the Collection of *Trypanosoma* from Wild and Domestic Mammals and Vectors (www.coltryp.fiocruz.br; C1050). Molecular analysis revealed 100% coverage and identity with *T. minasense* reference sequences.

Conclusions: This is the first report of the isolation and cryopreservation of this species in a biological collection, providing a reference strain that enhances the prospects for future studies.

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PARASITE ECOLOGY, BIODIVERSITY, AND TAXONOMY / ECOLOGY OF PARASITISM IN CHANGING ECOSYSTEMS

DIVERSITY LOSS AND CO-INVASION: A MULTI-BASIN STUDY OF GAMMARID-PARASITE INTERACTIONS

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Background and Aims: Biological invasions drive global biodiversity change, with host-parasite interactions serving as key indicators of community restructuring. We compared helminth species richness between native and invasive gammarids to reveal patterns of co-invasion and diversity loss.

Methods: Helminth communities were analyzed from gammarids sampled in 2020–2025 across two European drainage basins: the Black Sea (Danube River; western Slovakia) and the Baltic Sea (Pomerania; Dunajec River). The research included 5 invasive species (Ponto-Caspian, Balkan, and American migrants) and 4 native species, totaling 7,363 host individuals.

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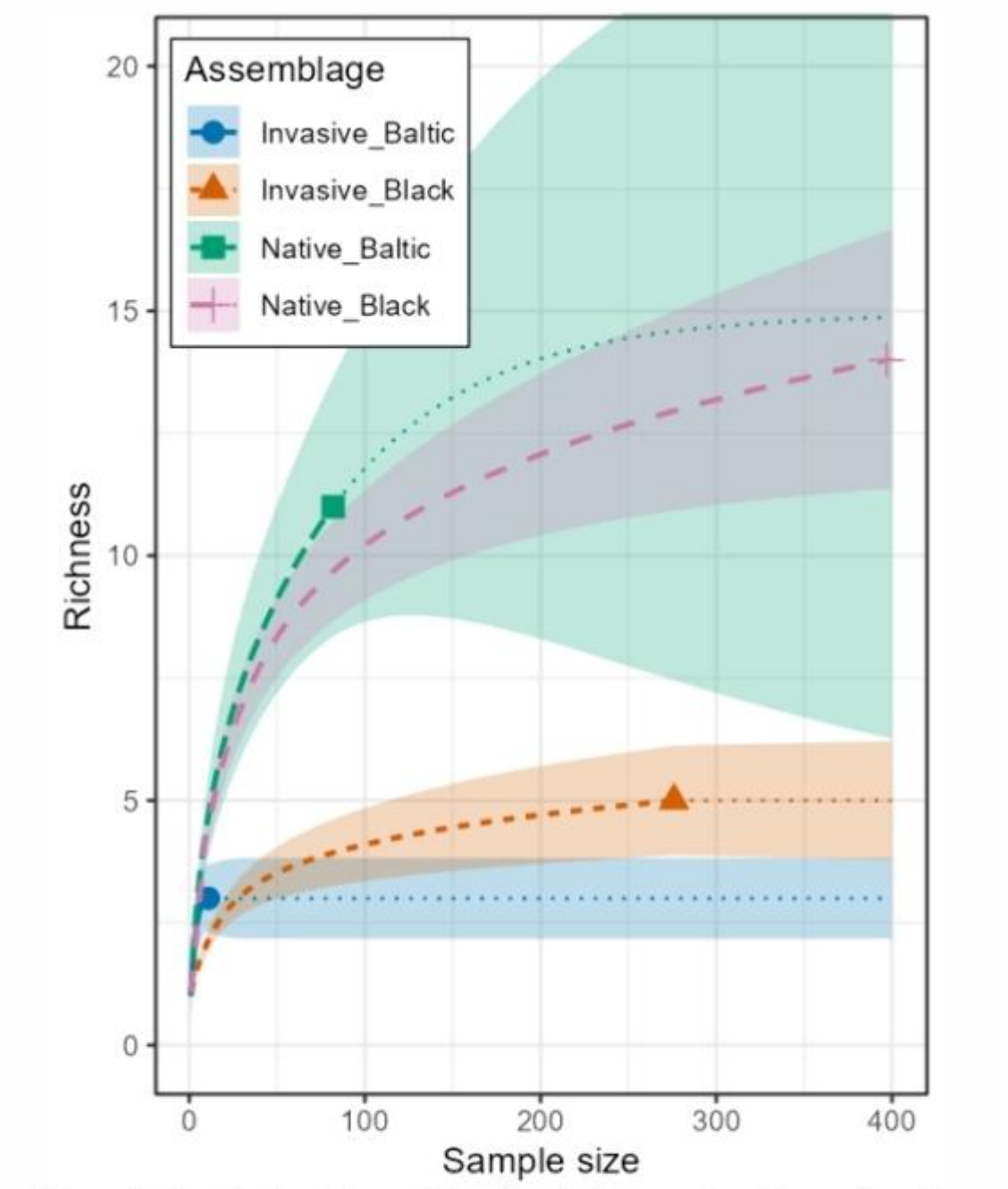


Figure 1. Rarefaction/extrapolation of helminth species richness in native vs invasive gammarids from the Black Sea (Danube; western Slovakia) and Baltic Sea (Pomerania; Dunajec River) basins; ribbons = 95% CIs, points = observed richness.

Results: We identified 20 helminth taxa using morphological and molecular methods, including 6 Trematoda, 7 Cestoda, 5 Acanthocephala, 1 Nematoda, and 1 Nematomorpha. Invasive hosts exhibited significantly lower species richness (3–5 species) than natives (11–14 species) (Fig. 1). The study highlights contrasting patterns of parasite co-invasion across European watersheds. The Danube acts as a fluvial corridor facilitating co-invasion, where 60% of the invasive parasite fauna

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(*Pomphorhynchus bosniacus*, *Plagioporus skrjabini*, and *Maritrema dikerogammari*) consists of co-introduced taxa. In contrast, the Baltic region shows an absence of co-invasive helminths, reflecting the "filter effect" of long-distance and marine dispersal routes.

Conclusions: Despite successfully displacing native amphipods, invasive species acquire few native parasites and maintain significantly low prevalence and abundance. Consequently, invasive gammarids do not actively participate in local transmission cycles, acting as a 'sink' for native helminths. Combined with the displacement of native amphipod populations, this process leads to an overall reduction in native parasite diversity within invaded ecosystems. This study was supported by grants APVV-24-0140 and SAS SoE/2025/61/MSCA4Uk.

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SHIFT01-022

POSTER ON BOARD SHIFT 01
PARASITE ECOLOGY, BIODIVERSITY, AND TAXONOMY / ECOLOGY OF PARASITISM IN CHANGING ECOSYSTEMS

DIVERSITY-DISEASE RELATIONSHIP DEPENDS ON CONTEXT FOR AN ENVIRONMENTALLY TRANSMITTED PARASITE

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Background and Aims: Anthropogenic environmental change, biodiversity loss, and infectious diseases are three of the greatest challenges facing humanity, having consequences for public health, conservation, societies and economies. Moreover, these challenges are all linked to each other, but the complexity of natural systems obscures our ability to understand the direction and magnitude of their relationships and their interactions through observational work alone. This project empirically disentangles the complex relationships between biodiversity, parasitism, and human-driven environmental changes using *Daphnia* and the parasite *Pasteuria ramosa*.

Methods: We experimentally disentangle the effects of biodiversity on disease dynamics. We investigate negative (dilution) and positive (amplification) effects of biodiversity on parasite epidemics in populations of *D. magna and D. pulex*. We further study the impact of parasitism on diversity through competition mediation. We also determine how warming temperature (such as that predicted due to climate change) and nutrient enrichment (such as that due to agricultural/industrial runoff) may influence these effects.

Results: We find that under nutrient enriched conditions, mixed-species groups experience parasite amplification, however under poor food availability, the epidemic declines. We did not see evidence for parasite-mediated competition, and temperature did not impact competitive interactions or epidemic dynamics.

Conclusions: These results reveal the complexities of the biodiversity-disease relationship and demonstrate that environmental variables may play a role in determining its outcome. This project furthers our understanding of disease ecology and improves our ability to predict their response to environmental change.

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SHIFT01-023

POSTER ON BOARD SHIFT 01
PARASITE ECOLOGY, BIODIVERSITY, AND TAXONOMY / ECOLOGY OF PARASITISM IN CHANGING ECOSYSTEMS

CONTRASTING SCHISTOSOME TRANSMISSION DESPITE CRYPTIC AND SPATIALLY STRUCTURED BIOMPHALARIA DIVERSITY IN THE LAKE ALBERT REGION

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Background and Aims: *Biomphalaria* snails are intermediate hosts of *Schistosoma mansoni,* and their distribution predicts where intestinal schistosomiasis may occur. Here, we investigated the genetic diversity and distribution patterns of *Biomphalaria* spp. in the Lake Albert region and assessed their role in schistosome transmission.

Methods: We sequenced a partial cytochrome *c* oxidase subunit one (*COX1*) fragment for phylogeographic analyses (n = 190). Snails were screened for schistosome infection using cercariae shedding, diagnostic PCRs, and *COX1* sequencing.

Results: In total, 29,670 *Biomphalaria* snails were collected, 90% of which were in the non-endemic upland sites. *Biomphalaria sudanica*, *Biomphalaria pfeifferi* and *Biomphalaria stanleyi* were identified, with the former two species sharing haplotypes between lake and upland sites. We also report presence of a cryptic species *B.* cf. *pfeifferi* that awaits further description. Molecular assays detected higher infection prevalence of *S. mansoni* (1.9%) and *S. rodhaini* (1.12%) in *Biomphalaria* spp. (n = 1,619) than cercarial shedding (0.66%). *Schistosoma mansoni* was restricted to lake and adjacent stream sites and occurred mainly in *B. sudanica*, whereas *S. rodhaini*, predominantly infecting *B*. cf. *pfeifferi*, showed a broader upland distribution, allopatric to *S. mansoni*. Infection prevalence in *B. pfeifferi sensu stricto* was low (0.59%, n = 1,364). The wet season (October and November) had the highest *S. mansoni* prevalence.

Conclusions: We argue that lack of *S. mansoni* in upland sites reflects a more nuanced situation, possibly driven by human water contact behaviour and hygiene practices, than simply the absence of suitable host snails. We suggest taxonomic revision of the *B. pfeifferi* complex.

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SHIFT01-024

POSTER ON BOARD SHIFT 01
PARASITE ECOLOGY, BIODIVERSITY, AND TAXONOMY / ECOLOGY OF PARASITISM IN CHANGING ECOSYSTEMS

TITLE: MOLECULAR DETECTION AND PREVALENCE OF BLASTOCYSTIS SPP. IN URBAN AND RURAL POPULATIONS OF SOUTHEAST ASIA

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Background and Aims: Blastocystis spp. is a common intestinal protozoan with uncertain pathogenicity, linked to gastrointestinal disorders. Prevalence and subtype distribution vary geographically. This study aimed to compare the prevalence and genetic diversity of Blastocystis spp. in urban versus rural communities in Southeast Asia using molecular tools.

Methods: Stool samples were collected from 600 participants (300 urban, 300 rural) in Vietnam and Thailand. DNA was extracted and screened for Blastocystis using PCR targeting the SSU rRNA gene. Positive samples were sequenced and subtyped using established molecular phylogeny methods. Epidemiological data were collected via questionnaires.

Results: Overall prevalence was 24.5%, with significantly higher infection rates in rural areas (32.7%) compared to urban areas (16.3%). Subtypes ST1, ST3, and ST4 were most common; ST3 predominated in urban settings, while ST1 was more frequent in rural populations. Mixed subtype infections were detected in 8% of rural samples.

Conclusions: The higher prevalence and genetic diversity of Blastocystis in rural areas suggest environmental and socio-demographic influences on transmission. Subtype distribution patterns may reflect different zoonotic or human-adapted transmission cycles. These findings underscore the need for targeted public health interventions in high-risk communities.

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SHIFT01-025

POSTER ON BOARD SHIFT 01
PARASITE ECOLOGY, BIODIVERSITY, AND TAXONOMY / ECOLOGY OF PARASITISM IN CHANGING ECOSYSTEMS

ECOLOGICAL DRIVERS OF PARASITE PREVALENCE ESTIMATES IN FISH COMMUNITIES OF LAKES

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Background and Aims: Parasite distribution patterns and drivers of infection vary in time and space, making understanding the underlying mechanisms highly complex. Comparative studies based on empirical data must therefore consider the factors of variation involved in estimating infection parameters in natural populations. Using a multi-scale approach, our aimed to explore the sources of variation in the estimation of infection prevalence, focusing on black spot disease in littoral freshwater fish communities.

Methods: We assessed infection prevalence in littoral freshwater fish communities from fifteen lakes in Québec, Canada, using different sampling methods in order to investigate potential method bias, and inspected relationship of infection data with multiple environmental drivers.

Results: Our results show that infection prevalence is spatially heterogeneous across the landscape with evidence of infection hotspots and coldspots. Method-related sampling biases led to significant variations in prevalence estimates and spatial patterns of disease occurrence. Our results also indicated that low sampling efforts tend to overestimate the prevalence of infection in the landscape, and that the sampling effort required to estimate infection prevalence depends on the sampling method employed. Physico-chemical characteristics of the sites and local fish community structure were found to be the best predictors of infection at smaller spatial scale. Furthermore, our results suggest dilution effects due to obstruction and compatibility barriers limiting cercarial survival. Several relationships between infection prevalence and environmental predictors revealed non-linearity, suggesting complex interactions.

Conclusions: Examining infection prevalence data at various spatial scales allowed to uncover many sources of variation revealing the importance of context-dependencies and scale-dependencies in host-parasite interactions.

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SHIFT01-026

POSTER ON BOARD SHIFT 01
PARASITE ECOLOGY, BIODIVERSITY, AND TAXONOMY / ECOLOGY OF PARASITISM IN CHANGING ECOSYSTEMS

BREEDING FOR SHEEP RESISTANCE TO GASTROINTESTINAL NEMATODES: EFFECTIVENESS AGAINST A MULTIRESISTANT PARASITE AND CONSEQUENCES ON LARVAL ECOLOGY

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Background and Aims: In France, genetic selection of sheep resistance to gastrointestinal nematodes (GIN) is currently based on indoor artificial infections, using an anthelmintic susceptible *Haemonchus contortus* strain. However, when natural infection occurs in pastures, sheep are increasingly exposed to drug-resistant or multidrug-resistant parasites. This study explored the potential impact of this new challenge on the effectiveness of selection for sheep resistance, and the consequences of sheep resistance and parasite drug resistance on ecology of free-living stages.

Methods: Eighty-two ewe lambs of Romane breed from lines selected on their resistance or susceptibility to GIN were used. Three successive artificial infections were performed with a susceptible strain of *H. contortus* (currently used for selection) or an eprinomectin-resistant strain. Sheep were kept indoors throughout the experiment. Multiple traits were monitored, including parasite faecal egg counts (FEC) and haematocrit (HT). Coprocultures were performed to assess larval development and resistance to desiccation.

Results: The resistant sheep line exhibited lower FEC and haematocrit loss than the susceptible line whatever the parasite's strain. Neither sheep line nor parasite strain influenced larval development. However, larvae obtained from the resistant sheep line showed a better resistance to dryness compared with those from the susceptible line.

Conclusions: In this study, the effectiveness of selection for sheep resistance to GIN was not compromised by drug-resistant strains. Host resistance did not seem to induce compensatory effects on the larval development. However, preliminary results suggest a significant impact on larval resistance to desiccation.

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SHIFT01-027

POSTER ON BOARD SHIFT 01
PARASITE ECOLOGY, BIODIVERSITY, AND TAXONOMY / ECOLOGY OF PARASITISM IN CHANGING ECOSYSTEMS

THE POTENTIAL ROLE OF WORMS OF ZOONOTIC SIGNIFICANCE AS ENVIRONMENTAL SINKS

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Background and Aims: Anisakine worms frequently parasitized coral reef-associated Sillago sihama and freshwater catfish, Bagarius bagarius. To investigate response of zoonotic worms, Anisakis typica in B.bagarius and marine fish (Goa), as against metals and elements (analysed by EDXMA technique) and their use as significant biomarkers of public health. To investigate the pattern of extracellular vesicles on the surface of helminthes and their functional relations

Methods: The sites of collections of A. typica were explored in India at the coastal Jetti at Porvorim, Panjim, North Goa at 15.505082°N, 73.834789°E for latitude and longitude, and Riverine Ganges at 26.117°N for latitude and 80.661°E longitude, respectively, 19Kms away from 'Grande' Island discovered on March 5, 2024.

Results: The metals and elements in decreasing order of concentrations were, Cr < S < Al < AS < U < Cu < Pb. The association between A. typica and Sillago sihama demonstrated surrogate relationship to drain in highly toxic heavy metals like Uranium from ambience in the micro-environment of nematodes and thus acted as benefactors for S. sihama. Extracellular vesicles were distributed within the conducting duct extending from the base of dorsal tooth atop cephalic complex of A. typica ventrally across to the excretory pore. These were extracted (Mladineo, 2023) and segregated.

Conclusions: The hypothesis of metal filter being functional at the worm-biometal influx interface was illustrated. Uranium and Iron got stored after crossing past barriers and avoided toxicity influence. Apparently, nematode's tissues, functionally operated as 'environmental sink' which could relieve host fish's tissues from unwarranted duress under biometallic stress.

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SHIFT01-028

POSTER ON BOARD SHIFT 01
PARASITE ECOLOGY, BIODIVERSITY, AND TAXONOMY / ECOLOGY OF PARASITISM IN CHANGING ECOSYSTEMS

"MORPHOLOGICAL TRANSFORMATIONS AND ADAPTATIONS IN BIOINVASIVE SPECIES OF ANISAKIDAE"

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Background and Aims: The diversity in parasites in fishes of India, varied to the order of climate's versatile variability along the gradients in hydrological attributes. To investigate the status of worms of Cucullanidae (i.e. Dacnitoides sp.) and Dioctophymidae (i.e. Eustrongylides sp.) as biomarkers of bioinvasion. To study the influence of environmental attributes in regulating dynamics of invasive species as confirmed by molecular analysis.

Methods: The roundworms extracted from Bagarius bagarius in river Ganges and Mastacembelus armatus in Sagar (Madhya Pradesh) were subjected to analysis to resolve adaptive correlations with environmental variants

Results: Emergence of a new variety of cucullanids with intestinal ceca but none of the other anisakidae characteristics were encountered. Apparently under the influence of environmental attributes, and the transforming changes therein, the new worms possessed specialized cephalic armature equipped with 21- ribbed circular armature; buccal teeth 2 pairs. Physalopterid multiple papillae occurred around a centrally placed larger globular structure; with excretory pore atop head, post-cephalic collarette, cephalic alae, and two unequal dissimilar alate spicules with a spoon-shaped extremity in male worms, and vulva pre-equatorial in females. Therefore, though mutation occurred yet natural selection disfavoured adaptation of the 2 characters other than intestinal ceca.

Conclusions: The mixing of species occurred under the influence of climate change between Cucullanidae from river Ganges at Prayagraj as they traversed through the subhumid region in Uttar Pradesh and semi-arid- subhumid region in Madhya Pradesh. Natural selection facilitated adaptation of intestinal ceca clad Dacnitoides sp. under Anisakidae with molecular evidence.

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SHIFT01-029

POSTER ON BOARD SHIFT 01
PARASITE ECOLOGY, BIODIVERSITY, AND TAXONOMY / ECOLOGY OF PARASITISM IN CHANGING ECOSYSTEMS

MOLECULAR MECHANISM OF CLK/JH SIGNAL TRANSDUCTION AXIS REGULATING PHOTOPERIODIC DIAPAUSE IN AEDES ALBOPICTUS

Xiaohong Zhou
Southern Medical University, Guangzhou, China

Background and Aims: *Aedes albopictus*, a major vector for dengue, Zika, and chikungunya, has rapidly spread globally, adapting to diverse climates through diapause, a key survival strategy enabling arbovirus persistence across years. While tropical populations remain active year-round due to stable photoperiods, subtropical/temperate populations exhibit photoperiod-dependent diapause plasticity. This study investigates the genetic and molecular mechanisms underlying diapause behavior in *Ae. albopictus*.

Methods: Using Guangzhou (diapause-capable) and Haikou (non-diapause) strains of *Ae. albopictus*, intra-population/inter-population hybridization experiments and transcriptomic weighted gene co-expression network (WGCNA) analyses were performed. The function of the selected genes was further verified by RNAi and hormone drop supplementation experiments.

Results: Hybridization experiments revealed maternal influence on offspring diapause rates. WGCNA analyses idenfied 16 gene co-expression modules, with one module (13,645 genes) most strongly associated with diapause, involving pathways like insect hormone synthesis and biological clocks. Key genes, including clock gene (Clk) and juvenile hormone acid methytransferase (JHAMT),were functionally validated. Clk knockout abolished photoperiodic diapause plasticity, reducing diapause rates by 98.36% under short-day conditions. RNAi targeting AalbJHAMT suppressed diapause, partially rescued by JHIII supplementation, confirming its pivotal role.

Conclusions: A number of target molecules and pathways involved in the central-neuroendocrine system and closely related to the diaphaetic behavior of *Ae. albopictus* were screened. Clk plays an important role in the diapause of *Ae. albopictus*. These findings highlight genetic and molecular bases for diapause regulation, offering insights for mosquito control strategies targeting diapause inhibition.

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SHIFT01-031

POSTER ON BOARD SHIFT 01
PARASITE ECOLOGY, BIODIVERSITY, AND TAXONOMY / CLIMATE CHANGE AND PARASITE DISTRIBUTION

TRACING MALARIA THROUGH TIME: CLIMATE CHANGE AND THE LEGACY OF PALEOPARASITOLOGY

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Background and Aims: Paleoparasitology is a fascinating field that explores the ancient connections between humans, animals, and their parasites. By examining archaeological remains such as coprolites, mummies, and burial site sediments, researchers can reconstruct patterns of parasitic infections and demonstrate how these patterns have influenced history amid environmental and climatic changes.

Methods: Dental samples were collected from skeletons from the ancient site of Shahr Sokhteh (3550–2300 BC) in southeastern Iran. The study aimed to investigate the impact of climate change over time on the presence and genome of malaria parasites. For this purpose, ancient DNA methods, including next-generation sequencing and metagenomic profiling, were used. Paleoclimatological studies were also conducted to investigate the possible relationship between past environmental fluctuations and the presence of pathogens.

Results: Due to the age of the skeletons, it was not possible to recover the malaria parasite genome; however, dental plaque revealed remnants of oral flora bacteria dating back about 5,000 years. Archaeological evidence suggests significant climatic events, including the drying up of lakes and rivers. These changes suggest that environmental fluctuations in the past, similar to today, could have influenced the spread of vectors and parasite-host interactions.

Conclusions: Paleoparasitological findings indicate that climate change has affected the distribution of parasites and their vectors throughout history. Understanding these ancient patterns provides a framework for predicting future challenges, as current changes in vector ecology closely resemble past trends. Thus, paleoparasitological studies, by integrating archaeological, biomolecular, and climatic data, provide a novel viewpoint for understanding and mitigating emerging parasite threats in today's changing world.

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SHIFT01-032

POSTER ON BOARD SHIFT 01
PARASITE ECOLOGY, BIODIVERSITY, AND TAXONOMY / CLIMATE CHANGE AND PARASITE DISTRIBUTION

THE IMPACT OF CLIMATE CHANGE ON ARTHROPOD VECTORS AND SELECTED PARASITES IN ALPINE PASTURES

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Background and Aims: Climate change will increase the impact of vector-borne diseases on human and animal health worldwide, either through direct effects on vector biology and transmission cycles or indirectly through socioeconomic mechanisms that influence human and animal susceptibility to these diseases and our ability to prevent and control them. In addition, internal parasites, particularly helminths (such as liver fluke: Fasciola hepatica), significantly affect the health and fitness of domestic and wild animals and often undergo life stages in which they live outside the main host and are exposed to external environmental conditions. Climate—and climate change—is therefore a key factor in infection patterns. To prepare for these risks, we need to assess the current state and predict the potential impacts of climate change on vectors and parasites.

Methods: From May to October 2025, monthly samples of vectors, mollusks, and larval stages of liver flukes/eDNA were taken from four pre-selected alpine pastures and four associated valley pastures in the province of Tyrol, Austria.

Results: More than 750 snails were collected, most of which were *Galba tranculata* (intermediate host of the liver fluke *Fasciola hepatica*). Moreover, various tick and mosquito species were sampled. Both molecular specification and screening for vector-borne pathogens are currently underway.

Conclusions: The ultimate goal of the project is to assess the current distribution, abundance, and specific habitat conditions of arthropod vectors, vector-borne pathogens, intermediate hosts (snails), and selected parasites, as well as changes in these factors in relation to climate change, particularly with regard to shifts in altitude and seasonality.

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SHIFT01-033

POSTER ON BOARD SHIFT 01
PARASITE ECOLOGY, BIODIVERSITY, AND TAXONOMY / CLIMATE CHANGE AND PARASITE DISTRIBUTION

PREVALENCE AND MOLECULAR CHARACTERIZATION OF POTENTIALLY PATHOGENIC FREE-LIVING AMOEBAE IN SOILS OF URBAN PARKS AND SQUARES IN WARSAW, POLAND

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Background and Aims: Free-living amoebae (FLA) are ubiquitous protozoan found in diverse environmental niches, some of which can cause severe human infections such as *Acanthamoeba* keratitis and fatal encephalitis. Despite their potential public health implications, data on the prevalence and diversity of FLA in urban soils are limited, particularly in Central Europe. In this study, we investigated the occurrence, species composition, and pathogenic potential of FLA isolated from soil samples collected from parks and squares frequently visited by the public in Warsaw, Poland.

Methods: A total of 23 soil samples were collected from parks and city squares frequently visited by residents. Samples were processed using standard culture-based isolation techniques. PCR amplification targeted the 18S rRNA gene using genus-specific and universal primers. Phylogenetic analysis was conducted to determine genotype assignment, particularly within the *Acanthamoeba* T-genotype classification system.To assess potential pathogenicity, thermotolerance assays were performed.

Results: FLA were detected in 78.2% (18/23) of soil samples. *Acanthamoeba* spp. predominated, with most isolates assigned to the T4 genotype, the genotype most frequently associated with human infections worldwide. Other identified genera included *Platyamoeba*, *Stenamoeba*, and *Allovahlkampfia*. Nearly half of the isolates demonstrated growth at 37°C, and a subset tolerated 42°C, suggesting potential virulence traits.

Conclusions: This study provides the first molecular characterization of FLA from urban soils in Warsaw and highlights the presence of potentially pathogenic genotypes in recreational environments. The findings underscore the need for environmental monitoring programs and increased awareness of exposure risks, particularly among contact lens users and immunocompromised individuals.

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SHIFT01-034

POSTER ON BOARD SHIFT 01
PARASITE ECOLOGY, BIODIVERSITY, AND TAXONOMY / CLIMATE CHANGE AND PARASITE DISTRIBUTION

RANGE EXPANSION OF PARELAPHOSTRONGYLUS TENUIS (MENINGEAL WORM) IN NORTH AMERICA – THE BIOWEAPON OF WHITE-TAILED DEER

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Background and Aims: In North America, white-tailed deer (WTD, *Odocoileus virginianus*) are asymptomatic carriers and definitive hosts of meningeal worm (*Parelaphostrongylus tenuis*), which is fatal in other sympatric cervids serving as aberrant hosts. Historically, the distribution of meningeal worm was limited to eastern North America, but changes to climate and land use have favoured the expansion of WTD further west and north. Our study was the first effort since the 1990s to determine the distribution of *P. tenuis* in Saskatchewan and assess exposure risk to moose and caribou of significance for conservation and Indigenous harvest.

Methods: WTD heads were submitted by hunters and examined for adult meningeal worm. PCR amplification and Sanger sequencing of the CO1 gene confirmed their identity. Records of aberrant host mortalities from the Canadian Wildlife Health Cooperative were analysed for apparent and confirmed *P. tenuis* infections. Next, we will use molecular methods to identify protostrongylid larvae shed in WTD feces.

Results: Between 2021-2024, 35/330 (10.6%) craniums from WTD in central and eastern Saskatchewan—some overlapping with boreal caribou habitat—had a mean of 2 (range: 1-10) *P. tenuis*, confirmed via sequencing (99.8% identity to sequences from Manitoba). Between 2015-2024, 91 aberrant hosts had confirmed or suspected *P. tenuis* infections.

Conclusions: Our study supports the expansion of the westernmost distribution limit of *P. tenuis* in Canada, and spillover into aberrant hosts. Future work will investigate whether meningeal worm is circulating in Saskatchewan and Alberta by analyzing WTD feces in previously nonendemic areas for the presence of larvae.

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SHIFT01-035

POSTER ON BOARD SHIFT 01
PARASITE ECOLOGY, BIODIVERSITY, AND TAXONOMY / CLIMATE CHANGE AND PARASITE DISTRIBUTION

DIAGNOSIS OF CUTANEOUS LEISHMANIASIS BY MICROSCOPY AND PCR: CASE OF A 13-YEAR-OLD GIRL FROM THE THIES REGION OF SENEGAL

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Background and Aims: This is a 13-year-old girl with negative immunological status who was referred to us by the dermatology department at Fann Hospital for a painless ulcerated lesion on her left forearm, which had been developing for three months.

Methods: A serous sample was taken from the lesion for smear and PCR testing. The slides were stained with a 10% Giemsa solution before being read under a microscope with a 100x objective lens. For PCR testing, DNA extraction was first performed using the Quick DNA miniprep Plus kit. Next, nested PCR was performed with the CSBIX-R and CSB2-F primers for the first amplification, and the 13Z and LIR primers for the second amplification. The positive controls used were*: Leishmania tropica, Leishmania infantum, and Leishmania major*, and water without Rase was used as a negative control. The amplification product was revealed after migration on a 1.5% agarose gel and reading under ultraviolet light.

Results: Microscopy revealed amastigote forms of Leishmania, and PCR was used to conclude that the disease was caused by *Leishmania major*.

Conclusions: Leishmaniasis is still present in Senegal with a potential residual transmission focus.

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SHIFT01-036

POSTER ON BOARD SHIFT 01
PARASITE ECOLOGY, BIODIVERSITY, AND TAXONOMY / CLIMATE CHANGE AND PARASITE DISTRIBUTION

MODELLING THE RANGE CHANGE DYNAMICS OF HOST-MACROPARASITE SYSTEMS UNDER CLIMATE CHANGE

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Background and Aims: Macroparasites, such as helminths, mosquitoes, and ticks, can impact host fitness and population dynamics, both directly and through the diseases they transmit. Climate change is expected to lead to macroparasite range shifts as habitats become more suitable towards the poles and less suitable towards the equator. These shifting distributions can have negative impacts on host-macroparasite population persistence and survival; however, mathematical models that can accurately predict such spatiotemporal changes are lacking.

Methods: Here, we develop trait-based partial differential equations to model the range change dynamics of environmentally transmitted macroparasites under climate change. Using MATLAB to numerically simulate climate warming scenarios, we focus our analyses on a single-host-single-macroparasite system and track how the macroparasite progresses throughout various life stages, dies and reproduces; traits which are temperature dependent.

Results: We outline in which scenarios macroparasites and/or their hosts are expected to undergo range contractions vs expansions, as well as which scenarios would lead to increases or decreases in disease burden and extinction risk.

Conclusions: Our framework lays the foundation for predicting climate change impacts on the changing spatial distributions of parasites and parasitic diseases and will help managers develop proactive plans for mitigating the subsequent impacts on human, animal and environmental health.

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SHIFT01-037

POSTER ON BOARD SHIFT 01
PARASITE ECOLOGY, BIODIVERSITY, AND TAXONOMY / CLIMATE CHANGE AND PARASITE DISTRIBUTION

CLIMATE CHANGE AND ITS INFLUENCE ON THE DISTRIBUTION OF PARASITIC DISEASES IN CAMEROON

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Background and Aims: BACKGROUND Climate change has emerged as a major global challenge with far-reaching consequences for human, animal, and environmental health. Changes in temperature, rainfall patterns, and the frequency of extreme weather events are reshaping ecosystems worldwide, particularly in tropical regions. Cameroon, with its wide range of ecological and climatic zones, is especially vulnerable to these changes. Many parasites and their vectors are highly sensitive to environmental conditions, making climate change a critical factor influencing their survival, development, and geographical distribution.

Methods: This study synthesizes epidemiological data, climate record, and published literature to examine the relationship between climate variability and the spatial distribution of parasitic diseases across Cameroon's major ecological zones.

Results: The results highlight growing public health challenges, especially for rural and underserved communities where access to healthcare, safe water, and sanitation remains limited. Climate-driven changes in parasite distribution threaten to undermine current disease control efforts and increase the burden on already stretched health systems. Understanding the links between climate variability and parasitic disease patterns is essential for strengthening surveillance, guiding targeted interventions, and developing climate-adaptive public health strategies to reduce future risks in Cameroon. These insights can support policy development, community-based prevention programs, and integrated responses linking environmental management with long-term public health planning across vulnerable regions nationwide.

Conclusions: By understanding and anticipating climate-related changes in parasite distribution, Cameroon can better prepare for future health risks and develop more effective control and prevention strategies.

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SHIFT01-038

POSTER ON BOARD SHIFT 01
PARASITE ECOLOGY, BIODIVERSITY, AND TAXONOMY / CLIMATE CHANGE AND PARASITE DISTRIBUTION

GENETIC CHARACTERIZATION OF ENTEROCYTOZOON BIENEUSI IN DOMESTIC AND WILD ANIMALS IN CHILE

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Background and Aims: *Entercocytozoon* bieneusi is a unicellular microorganism that habitates the guts of animals and humans causing diarrhea and gastrointestinal issues. Especially domestic animals that are in close contact with humans are of interest, for their possible role as reservoir species. Nonetheless, wild animals also should be considered to better understand their role in the propagation of the domestic and wild cycle. We aimed to characterize the genetic diversity and zoonotic potential of detected *Enterocytozoon bieneusi* genotypes.

Methods: In this study we detected the presence of this parasite in different animal species, such as birds of prey, pumas, cattle, dogs and sealions, using fecal samples for DNA amplification using a specific nested PCR for *E. bieneusi* internal transcribed spacer (ITS) region of the rRNA gene and subsequent sequencing.

Results: *Enterocytozoon bieneusi* was detected in all host species. Shelter dogs showed the highest prevalence, with 85 of 165 animals positive (51.52%), all corresponding to genotype PtEb IX. In cattle, 14 of 115 animals were infected (12.17%), mainly calves (n = 11), with three adult cows positive. Among wildlife, prevalence was 16.67% (2/12) in birds of prey, 9.33% (7/75) in black rats (*Rattus rattus*), 6.25% (2/32) in pumas, and 4.55% (1/22) in sea lions. In black rats, zoonotic genotypes EbCar2 and BANEB3 were detected.

Conclusions: *Enterocytozoon bieneusi* is widely distributed among domestic and wild hosts in Chile, with shelter dogs and synanthropic rodents likely acting as key reservoirs. The detection of zoonotic genotypes supports ongoing cross-species transmission and highlights the need for integrated One Health surveillance.

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SHIFT01-039

POSTER ON BOARD SHIFT 01
PARASITE ECOLOGY, BIODIVERSITY, AND TAXONOMY / CLIMATE CHANGE AND PARASITE DISTRIBUTION

SEABIRD TICKS (IXODES URIAE) AND PATHOGEN CIRCULATION IN ANTARCTIC PENGUIN COLONIES

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Background and Aims: Antarctica has long been considered an isolated environment with limited pathogen pressure; however, increasing evidence indicates that infectious agents circulate within Antarctic ecosystems. The seabird tick *Ixodes uriae* is one of the few hematophagous arthropods occurring in Antarctica and is commonly associated with seabird breeding colonies, including penguins. Due to its blood-feeding behaviour and circumpolar distribution, this species may contribute to pathogen maintenance and transmission in polar environments. The aim of this study was to investigate the occurrence of selected microorganisms in *I. uriae* ticks and their penguin hosts in maritime Antarctica.

Methods: Ticks were collected from colonies of three *Pygoscelis* penguin species during two austral summer field campaigns. Additionally, biological samples were obtained from adult birds and chicks to assess pathogen presence in both vectors and hosts. Molecular screening targeted selected bacterial and protozoan microorganisms previously associated with seabirds and tick-borne transmission. Obtained sequences were compared with previously published data from Antarctic and sub-Antarctic regions.

Results: Preliminary analyses confirmed the presence of microbial DNA in both ticks and penguin samples, suggesting active circulation of microorganisms within Antarctic colonies. Comparative analyses revealed similarities as well as regional differences in detected genetic variants among Antarctic localities.

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Conclusions: These findings provide new insights into host-vector-pathogen interactions in Antarctic ecosystems and highlight the need for further studies on pathogen transmission dynamics in polar regions.

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SHIFT01-040

POSTER ON BOARD SHIFT 01
PARASITE ECOLOGY, BIODIVERSITY, AND TAXONOMY / CLIMATE CHANGE AND PARASITE DISTRIBUTION

MOLECULAR DETECTION AND PREVALENCE OF BLASTOCYSTIS SPP. IN URBAN AND RURAL POPULATIONS OF SOUTHEAST ASIA

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Background and Aims: Blastocystis spp. is a common intestinal protozoan with uncertain pathogenicity, linked to gastrointestinal disorders. Prevalence and subtype distribution vary geographically. This study aimed to compare the prevalence and genetic diversity of Blastocystis spp. in urban versus rural communities in Southeast Asia using molecular tools.

Methods: Stool samples were collected from 600 participants (300 urban, 300 rural) in Vietnam and Thailand. DNA was extracted and screened for Blastocystis using PCR targeting the SSU rRNA gene. Positive samples were sequenced and subtyped using established molecular phylogeny methods. Epidemiological data were collected via questionnaires.

Results: Overall prevalence was 24.5%, with significantly higher infection rates in rural areas (32.7%) compared to urban areas (16.3%). Subtypes ST1, ST3, and ST4 were most common; ST3 predominated in urban settings, while ST1 was more frequent in rural populations. Mixed subtype infections were detected in 8% of rural samples.

Conclusions: The higher prevalence and genetic diversity of Blastocystis in rural areas suggest environmental and socio-demographic influences on transmission. Subtype distribution patterns may reflect different zoonotic or human-adapted transmission cycles. These findings underscore the need for targeted public health interventions in high-risk communities.

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SHIFT01-041

POSTER ON BOARD SHIFT 01
PARASITE ECOLOGY, BIODIVERSITY, AND TAXONOMY / CLIMATE CHANGE AND PARASITE DISTRIBUTION

THE DEVELOPMENT OF AVIAN MALARIA CO-INFECTIONS IN EXPERIMENTALLY EXPOSED VECTORS

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Background and Aims: Co-infections are widespread in natural populations, can influence both host-parasite and vector-parasite interactions, and pose significant threats to health. However, information about the interactions between these infections within vectors and their potential impact on the development of sporogonic stages, remains scarce and poorly understood.

Methods: We investigated the sporogony, transmission, and virulence of the locally transmitted *Plasmodium relictum*, *Plasmodium elongatum*, and co-infections of these parasites in *Culex* mosquitoes using DNA and microscopic examination methods. Mosquitoes were experimentally infected by allowing them to take a blood meal from canaries carrying either a single parasite species or from birds infected with two species, resulting in double infection, and were maintained until the parasite reached the sporozoite stage.

Results: Our findings reveal that both parasite species can complete sporogonic development within the same vector and can be simultaneously transmitted to a healthy avian host through a single mosquito bite. Additionally, the study shows distinct effects of single and co-infections on mosquito survival, demonstrating specific interactions between parasites during sporogonic development in the vector. Mosquitoes infected with co-infection experienced lower mortality than those infected with *P. elongatum* alone. This could be due to a potential suppressive effect of *P. relictum* on the development of *P. elongatum* within the mosquito, thereby reducing harm to the vector.

Conclusions: This information deepens our understanding of avian malaria co-infections and underscores their potential impact on transmission efficiency, vector fitness, and the ecological dynamics of disease spread. This research was funded by a grant S-MIP-24-85 from the Research Council of Lithuania.

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SHIFT01-042

POSTER ON BOARD SHIFT 01
PARASITE ECOLOGY, BIODIVERSITY, AND TAXONOMY / WILDLIFE RESERVOIRS AND ZOONOTIC SPILLOVER

BOTHROPS ALTERNATUS SNAKES INFECTED BY TRYPANOSOMA CRUZI AND TRYPANOSOMA RANGELI IN MATO GROSSO DO SUL STATE, BRAZIL

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Background and Aims: Can mammalian trypanosomes infect reptiles? To answer this, blood samples were collected from *Bothrops alternatus* snakes (n=17) rescued in the wild and maintained in captivity for up to twelve years, and parasitological analyzed for *Trypanosoma* sp. detection.

Methods: Positive cultures were characterized twice: first aliquot (initial growth), and isolate (after mouse *in vivo* passage) or sediment, the latter when cultures failed to grow. Swiss mice were experimentally infected and followed up for 65 days post infection (dpi). Cultures and mice's blood clots were characterized by sequencing analysis of 18S rDNA PCR products.

Results: Microscopic direct examinations were negative. Three positive cultures (17.6%) were obtained, and one was successfully isolated (Ba1). The first aliquots were characterized as *Trypanosoma cruzi* TcII in Ba1 and Ba3 and *Trypanosoma rangeli* A in Ba2 individuals, this latter confirmed in the sediment characterization. Both the first aliquot and strain showed infection by *T. rangeli* lineage A in Ba1, pointing to an original mixed infection. The experimentally infected mice showed a prepatent period of 9±2 and parasitemia lasting 15±9, peaking at 17±3. Despite *T. cruzi* detection in blood clot (BC) from one mouse 12 dpi, hemocultures and/or BC demonstrated *T. rangeli* infection in mice 12, 30 and 65 dpi, exhibiting the same pattern observed in the snake primary culture.

Conclusions: This is the first report of *T. rangeli* infection in reptiles and *T. cruzi* in *B. alternatus* and substantially expands our understanding: the zoonotic *T. cruzi* and *T. rangeli* retain significant ecological plasticity, uncovering new questions regarding their evolutionary pathways.

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SHIFT01-043

POSTER ON BOARD SHIFT 01
PARASITE ECOLOGY, BIODIVERSITY, AND TAXONOMY / WILDLIFE RESERVOIRS AND ZOONOTIC SPILLOVER

GENOMIC AND MORPHOLOGIC CHARACTERIZATION OF STRONGYLOIDES NASUA CAUSING FATAL STRONGYLOIDIASIS IN COATIS (NASUA NASUA)

Benjamin Dewals[1], Dominika Lastik[2], Huan Zhao[3], Ophélie Piedfort[1], Françoise Marechal[1], Meriem Flih[1], Michela Deiana[4], Edward Morris[2], Oliwier Maziarski[2], Dora Buonfrate[4], Richard Bradbury[5], Vicky Hunt[2]
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Background and Aims: The parasitic nematode *Strongyloides* sp. infects a large range of host species, including ungulates, carnivores, non-human primates, and humans, causing mostly asymptomatic chronic infections. However, hyperinfection syndrome may occur resulting from self-amplifying autoinfection with parthenogenetic female parasites.

Methods: We report here the first genomic description of *Strongyloides nasua* which caused fatal strongyloidiasis in two coatis (*Nasua nasua*) in a wildlife rescue centre in Peru, South America. The two animals were housed in the centre for a few months when clinical signs occurred, ultimately leading to a fatal outcome. Post-mortem examination showed cachectic animals, inflamed intestine with enlarged Peyer's patches and mesenteric lymph nodes. Histological analysis revealed severe parasitic enteritis with the presence of numerous nematodes at various developmental stages entrapped in the intestinal mucosa.

Results: Direct microscopic examination of the nematodes revealed key morphological characteristics of parasitic female *Strongyloides*. Finally, combining *18S rRNA* and *cox1* genotyping together with short- and long-read whole genome sequencing, our data revealed that *S. nasua* represents a member of a potentially new group in the genus *Strongyloides*, closely related to *S. stercoralis* and *S. procyonis*.

Conclusions: The *S. nasua* genome was associated with expansion and contraction of orthogroups associated with hatching, development and the female germline indicating potential diversification or specialisation of associated processes in *S. nasua*.

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SHIFT01-044

POSTER ON BOARD SHIFT 01
PARASITE ECOLOGY, BIODIVERSITY, AND TAXONOMY / WILDLIFE RESERVOIRS AND ZOONOTIC SPILLOVER

GENETIC DIVERSITY OF BLASTOCYSTIS IN FARMED WILD RED DEER (CERVUS ELAPHUS) FROM SOUTHERN SPAIN

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Background and Aims: Wild red deer farming is a common practice for meat production in Spain, the world's second-largest venison exporter. Zoonotic infectious diseases in these populations remain poorly understood, potentially increasing public health risks. *Blastocystis* is a widespread zoonotic enteric protist with high genetic diversity, broad host range, and uncertain pathogenicity. To address these gaps, we investigated the occurrence, genetic diversity, and colonisation patterns of *Blastocystis* in a semi-extensively managed red deer population in southern Spain.

Methods: Faecal samples (*n*=319) from 247 individuals were collected between 2012-2014 and analysed employing next-generation amplicon sequencing.

Results: *Blastocystis* was detected in 14.7% of samples, and thirteen previously described subtypes (ST5, ST10, ST14, ST21, ST23-ST26, ST30, ST32, ST42-ST44), along with one novel subtype, were identified. The validity of the novel subtype was confirmed through phylogenetic and pairwise distance analyses of full-length *ssu* rRNA gene sequences generated by Oxford Nanopore sequencing. All *Blastocystis*-positive samples exhibited 4-16 subtype combinations, with up to 44 unique variants, showing substantial intra-subtype variability. A longitudinal study of five deer revealed both persistent and intermittent colonisation patterns among various subtypes. Potentially zoonotic ST10a and ST14, as well as ST21, ST25, ST43, and ST44, appeared in 90–100% of samples longitudinally analysed.

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Conclusions: Farmed wild red deer harbour high *Blastocystis* subtype diversity and show evidence of long-term colonisation and complex transmission dynamics, underscoring the need to better understand the ecological role of *Blastocystis* in the cervid gut microbiome. **Acknowledgement** This work is supported by the European Cooperation in Science and Technology (COST) Action *Blastocystis* under One Health, CA21105.

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SHIFT01-045

POSTER ON BOARD SHIFT 01
PARASITE ECOLOGY, BIODIVERSITY, AND TAXONOMY / WILDLIFE RESERVOIRS AND ZOONOTIC SPILLOVER

PREVALENCE AND GENOTYPES OF MICROSPORIDIA IN DOMESTIC AND WILD CATS POPULATIONS FROM SPAIN

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Background and Aims: Microsporidia are obligate intracellular parasites with a wide distribution and well-recognized zoonotic potential. Cats can act as hosts and potential environmental reservoirs, shedding viable spores in their feces. However, data on their prevalence in feline populations remains limited. We investigated the occurrence and genetic diversity of *Enterocytozoon bieneusi* and *Encephalitozoon* spp. in fecal samples from wildcats and domestic cats collected in urban and peri-urban environments in Spain.

Methods: Fecal samples from stray cats (*n*=200), indoor cats (*Felis catus*) (*n*=90) and wildcats (*Felis silvestris*) (*n*=60), were collected between 2022-2025. Microsporidia were detected and genotyped by molecular (PCR and Sanger sequencing) methods.

Results: Overall, 2.6% (9/350) of the samples were positive for microsporidia, including stray cats 2.5% (5/200) and indoor cats 4.4% (4/90). All wildcats tested negative. Stray cats were infected with *Enterocytozoon bieneusi* genotype D (*n*=3), *E. bieneusi* genotype PtEbIX (dog-adapted) (*n*=1), and *Encephalitozoon cuniculi* genotypes III+IV (mixed infection) (*n*=1). Indoor cats carried *E. bieneusi* genotype D (*n*=3) and *Enc. cuniculi* genotypes I+IV (mixed infection) (*n*=1). *E. bieneusi* genotype D and *Enc. cuniculi* genotypes I/III/IV are zoonotic, and *E. cuniculi* is also associated with feline clinical disorders (*e.g.*, uveitis, meningoencephalitis, nephritis).

Conclusions: Despite their low global prevalence, microsporidia remain significant emerging zoonotic agents in cats. Both indoor and stray cats can act as environmental reservoirs for zoonotic microsporidia, particularly in urban settings where they cohabit with humans. Our results underscore the need to expand molecular surveillance in feline populations to better understand the parasite's genetic diversity and its implications for public and animal health.

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SHIFT01-046

POSTER ON BOARD SHIFT 01
PARASITE ECOLOGY, BIODIVERSITY, AND TAXONOMY / WILDLIFE RESERVOIRS AND ZOONOTIC SPILLOVER

BLOOD TRYPANOSOME DIVERSITY IN WATER FROGS OF THE PELOPHYLAX COMPLEX IN FRANCE

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Background and Aims: In the context of the ongoing spread of the invasive marsh frog *Pelophylax ridibundus* across Western Europe, studying its haemoparasites is of major interest. Anurans are known to host a wide range of blood parasites, including haemogregarines, haemococcidia, trypanosomes, and microfilariae. However the overall prevalence of haemoparasite infection through *Pelophylax* spp. had never been so far investigated in France. As part of an ongoing research project on biological invasions, we thus assessed the diversity of these parasites in *Pelophylax*.

Methods: Frogs were captured by hand during the night and blood samples were collected from the femoral veins. Thin blood smears were prepared in the field, further stained in the lab, and examined under an optical microscope for parasite screening and measurements. DNAs were extracted from blood of infected frogs for PCR. Target specific primers were used for the amplification of 18S rRNA and COI genes among haemoparasites and 16S and RAG1 genes among *Pelophylax*. Resulting sequences from amplicons sequencing were analysed for phylogenetic inferences.

Results: *Pelophylax* was found to be infected by all four groups of haemoparasites that were characterised at both the morphometric and molecular levels. Overall, results revealed a high diversity of trypanosome species infecting all frogs species of the *Pelophylax* complex in France.

Conclusions: Our study provides important baseline data and enhances our understanding of haemoparasite distribution in both native and invasive *Pelophylax* frogs. Based on trypanosome phylogenetic relationships and *Pelophylax* taxononomy, results also revealed complex host-parasite interactions suggesting *P. ridibundus* as a potential host for haemoparasite spillover and/or spillback.

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SHIFT01-047

POSTER ON BOARD SHIFT 01
PARASITE ECOLOGY, BIODIVERSITY, AND TAXONOMY / WILDLIFE RESERVOIRS AND ZOONOTIC SPILLOVER

OCCURRENCE AND GENETIC CHARACTERIZATION OF SARCOCYSTIS SUIHOMINIS AND SARCOCYSTIS MIESCHERIANA IN WILD BOARS IN POLAND

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Background and Aims: Wild boars can be intermediate hosts for *Sarcocystis miescheriana* and the zoonotic *Sarcocystis suihominis*. Humans become infected with *S. suihominis* by eating raw or undercooked meat. Herein we investigated the occurrence of *Sarcocystis* spp. in wild boars hunted for human consumption in Poland.

Methods: Diaphragms were collected from 241 wild boars during two hunting seasons; a multiplex-PCR protocol targeting the mtDNA *cox1* gene was applied; the diaphragm of wild boars testing positive for *S. suihominis* underwent sarcocysts isolation and molecular characterization.

Results: Out of 241 wild boars, 216 (90%) were positive for *S. miescheriana* DNA, with 8 (3.3%) also co-infected with *S. suihominis*. Diaphragms from 3 wild boars positive for both *S. suihominis* and *S. miescheriana* were processed, yielding 37 sarcocysts. Two out of 37 sarcocysts tested positive for *S. suihominis* and underwent molecular characterization, revealing 98.9–100% identity with *S. suihominis* on the 18S rRNA gene and 98.7% identity on the cox1 mtDNA gene. Thirteen of the remaining sarcocysts were also characterized molecularly: 12 were identified as *S. miescheriana*, while one produced 1053 bp cox1 mtDNA sequence and 923 bp 18S rDNA sequence with <89.1% and 95.6% homology, respectively, to any known *Sarcocystis* species.

Conclusions: This is the first study identifying *Sarcocystis* spp. circulating in wild boars intended for human consumption in Poland. It reveals a non-negligible zoonotic infection rate of *S. suihominis*, high prevalence of *S. miescheriana*, and the presence of a putative novel *Sarcocystis* sp. Our findings indicate that the risk to consumers of undercooked wild boar meat cannot be ignored.

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SHIFT01-048

POSTER ON BOARD SHIFT 01
PARASITE ECOLOGY, BIODIVERSITY, AND TAXONOMY / WILDLIFE RESERVOIRS AND ZOONOTIC SPILLOVER

POST-MORTEM FINDINGS OF SERRATOSPICULUM SP. AND CAPILLARIA SP. EGGS IN A PEREGRINE FALCON (FALCO PEREGRINUS): PATHOLOGICAL AND PARASITOLOGICAL FINDINGS FROM CHILE

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Background and Aims: Respiratory helminths in wild raptors are poorly documented in South America despite their potential effects on wildlife health and conservation. *Serratospiculum* spp. are important spirurid nematodes affecting the respiratory tract and air sacs of birds of prey, whereas reports of respiratory in peregrine falcons (*Falco peregrinus*) remain scarce for Chile. This study aimed to describe the pathological and parasitological findings associated with respiratory nematodes in a free-ranging peregrine falcon from Chile.

Methods: A complete necropsy was performed following standard wildlife pathology procedures, with emphasis on the respiratory system. Adult nematodes recovered from the air sacs and respiratory tract were manually extracted and preserved in ethanol for morphological and future molecular analyses. Parasites were examined under light microscopy using available taxonomic keys. Additionally, a tracheal wash was performed and microscopically evaluated for parasitic structures.

Results: Numerous adult nematodes morphologically compatible with *Serratospiculum* sp. were observed within the respiratory tract and air sacs, associated with severe airsacculitis. Microscopic examination supported their preliminary identification as spirurid nematodes. To the best of our knowledge, this represents the first documented report of *Serratospiculum* sp. in Chile. Tracheal wash examination additionally revealed eggs morphologically compatible with *Capillaria* sp., suggesting possible respiratory co-infection.

Conclusions: These findings expand current knowledge regarding respiratory helminths infecting wild raptors in South America and highlight the importance of integrating necropsy, parasitological examination, and molecular tools for wildlife disease surveillance and parasite characterization.

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SHIFT01-049

POSTER ON BOARD SHIFT 01
PARASITE ECOLOGY, BIODIVERSITY, AND TAXONOMY / WILDLIFE RESERVOIRS AND ZOONOTIC SPILLOVER

TREMATODES IN EURASIAN CURLEWS (NUMENIUS ARQUATA) IN FRANCE: IDENTIFICATION, CIRCULATION, AND IMPACT OF THESE PARASITES IN A "ONE HEALTH" APPROACH.

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Background and Aims: Eurasian Curlews were analysed as part of a National Management Plan (PNG) in France (2015–2021). The objectives were to i) conduct an inventory of macro-parasites in this host ; ii) understand the impact of these parasites on health and populations of birds ; iii) understand the parasite cycles (periods and sites of contamination); iv) assess the presence of species with a potential zoonotic risk; v) trace migratory routes and reconstruct the social networks of birds (exchange of parasites).

Methods: For each bird, gizzard, digestive tract, cloaca, and Fabricius' bursa were analyzed individually, focusing initially on trematodes. Parasites were isolated and then identified morphologically and molecularly. Among the birds studied, slightly more than half were females (56%), and most (86%) were juveniles or pre-adults.

Results: Of the 47 birds studied, 41 were positive for a least one trematode species, and most with at least two or more species (55.32%). The results obtained highlight the presence of six different genera: *Leucochloridium*, *Microphallus*, *Maritrema*, *Plagiorchis*, *Prosthogonimus*, and *Himasthla*. No adults or eggs of avian schistosomes, potential agents of cercarial dermatitis in humans, were found.

Conclusions: This first study conducted on the Eurasian curlew in France, whose populations have recently been classified as "Vulnerable" and "Critically Endangered" on the national and regional Red Lists, has revealed the presence of numerous parasites that will enrich databases and knowledge about these trematodes and their hosts, their circulation in France and Europe, but also provide a better understanding of their impact on bird health.

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PARASITE ECOLOGY, BIODIVERSITY, AND TAXONOMY / WILDLIFE RESERVOIRS AND ZOONOTIC SPILLOVER

VECTOR-BORNE PATHOGENS IN EUROPEAN POPULATIONS OF AMERICAN MINK (NEOGALE VISON)

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Background and Aims: The American mink (*Neogale vison*), an invasive species in Europe, harbors a wide range of pathogens, several of which possess zoonotic potential. The aim of this study was to detect vector-borne pathogens (VBPs) in European populations of American mink to assess their current distribution and evaluate the threat to humans, as well as to companion and wild animals.

Methods: Spleen samples of 358 American mink from seven European countries that were collected between 2008 and 2024 were analyzed for the presence of VBPs (hemoplasmas, piroplasmids, Trypanosomatidae, *Hepatozoon* spp., Filarioidea, *Bartonella* spp., *Rickettsia* spp., and Anaplasmataceae). For haemoplasmas and *Hepatozoon* spp. a phylogenetic analysis was additionally conducted after Sanger sequencing.

Results: The overall pathogen prevalence in our study was 40%, the most prevalent ones being *Mycoplasma* spp. (39%), followed by *Babesia gibsoni* (1%), *Babesia canis*, *Theileria capreoli*, *Hepatozoon canis*, *Hepatozoon ayorgbor*, *Hepatozoon ophisauri*, *Hepatozoon* sp., and *Trypanosoma pestanai* (each 0.3%). While 95% of the animals were infected with one pathogen only, only 5% showed an infection with two pathogens.

Conclusions: While most pathogens detected are associated with carnivores (i.e., hemoplasmas, *Babesia* spp., *H. canis*, and *T. pestanai*), there were also unexpected findings. Notably, three of the four *Hepatozoon* species detected were reptile-specific. Furthermore, *T. capreoli* was detected; while usually associated with cervids, this pathogen has previously been identified in carnivores as well. These findings highlight the role of the American mink as a possible reservoir for VBPs, potentially facilitating interspecies transmission within European ecosystems.

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PARASITE ECOLOGY, BIODIVERSITY, AND TAXONOMY / WILDLIFE RESERVOIRS AND ZOONOTIC SPILLOVER

NEW INSIGHTS OF PATHOGENS FOUND INSIDE TICKS ON AFRICAN WILDLIFE, FROM THE SUBGENUS AFRIXODES (ACARI: IXODIDAE, IXODES)

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Background and Aims: The sub-genus *Afrixodes*, native to Africa and India, comprises about sixty species. At present, very little is known about this group, and much information is lacking on ecology, taxonomy, description of stages, barcoding, etc. Only four genera of pathogens (*Anaplasma, Babesia, Ehrlichia* and *Rickettsia*) have been reported in only seven species classified as vectors (*Ixodes aulacodi*, *I. colasbelcouri*, *I. muniensis* and *I. rasus)* and putative vectors (*I. ceylonensis*, *I. pilosus* and *I. rubicundus)*. To improve knowledge of this taxon, this study surveys pathogens in tropical African wildlife to provide an overview of host-parasite-pathogen interactions.

Methods: This study is based on 120 *Afrixodes* specimens, females and nymphs, collected between 1995 and 2021 from wildlife in seven African countries. Ticks were identified morphologically and non-destructive DNA extraction was conducted on specimens. DNA sequences were obtained by NGS (Illumina) after design of new primers; both to determine the tick species and to target pathogens commonly found in Africa (*Anaplasma, Ehrlichia, Rickettsia*, Relapsing Fever Group *Borrelia*), targeting two different genes per genus.

Results: The preliminary results show new records of pathogens present in *Afrixodes* with *Anaplasma* spp., found inside two ticks collected on a *Philantomba monticola* (duiker), and *Borrelia* spp. found in nine ticks from *Phataginus tricuspis* (pangolin), *Crocidura nigrofusca*, *Crocidura* sp., *Paracrocidura schoutedeni* (shrews) and *Deomys ferruginus* (rodent), in three different countries.

Conclusions: These initials results demonstrate the significance of a One Health approach by highlighting the importance of studying wildlife in the spillover of tick-borne diseases in Africa.

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PARASITE ECOLOGY, BIODIVERSITY, AND TAXONOMY / WILDLIFE RESERVOIRS AND ZOONOTIC SPILLOVER

FROM A NOVEL HOST TO A PUBLIC HEALTH ALERT: FIRST REPORT OF PATHOGENIC LEISHMANIA, PLASMODIUM, AND MICROFILARIAL CO-INFECTION IN A NIGERIAN NILE CROCODILE (CROCODYLUS NILOTICUS) IN IBADAN, NIGERIA

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Background and Aims: This case report documents the first molecularly confirmed concurrent infection with *Leishmania spp*., *Plasmodium spp*., and microfilariae in a 10-year-old female Nile crocodile (*Crocodylus niloticus*) from Nigeria. The animal presented with anorexia, cutaneous nodules and lethargy, and died shortly after evaluation.

Methods: The study adopted haematology, DNA extraction, sequencing and phylogenetic analysis.

Results: Haematology revealed a non-regenerative macrocytic anaemia (PCV 22%), leukocytosis (10,100/µL) with marked neutrophilia, and thrombocytopenia (140,000/µL). Blood smear and buffy coat microscopy confirmed a systemic triple infection, revealing extracellular *Leishmania* promastigotes, intra-erythrocytic *Plasmodium* parasites, and abundant unsheathed microfilariae. Post-mortem examination identified generalized pallor, granulomatous pneumonia, and fibrinonecrotic gastritis. Phylogenetic analysis of the 18S rRNA gene identified the *Leishmania spp*. within a clade containing the human-pathogenic *L. donovani* and *L. major* complexes.

Conclusions: This case provides the first evidence of pathogenic *Leishmania* in a West African reptile and highlights crocodilian susceptibility. The findings raise significant concerns about the potential role of reptiles as reservoir hosts in the enzootic cycle of leishmaniasis, reinforcing the critical importance of a One Health approach in surveillance of emerging parasitic zoonoses.

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PARASITE ECOLOGY, BIODIVERSITY, AND TAXONOMY / WILDLIFE RESERVOIRS AND ZOONOTIC SPILLOVER

PARASITES OF RATS IN MALAYSIA: ZOONOTIC IMPLICATIONS FOR HUMANS

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Background and Aims: Rodents are capable of spreading diseases to ani-mals and as well as to humans. Wild rats in Malaysia (such as *Rattus rattus* and *Rattus norvegicus*) harbor a high prevalence of zoonotic parasites, posing severe public health risks. Key parasites include protozoa like *Toxoplasma gondii* and *Giardia spp.*, intestinal helminths (e.g., *Hymenolepis nana*), and ectoparasites like fleas and mites.

Methods: Ten rats were caught in the compound surrounding a Malaysian research Insitute using the conventional mouse trap and post mortem was conducted for disease investigation. Samples of blood, faeces, skin scraping, sera and organs were collected from each rat and sent for various disease investigations including parasitic, viral and bacterial.

Results: The results show that seven rats harboured mites (Demodex sp) from skin sam-ples while parasite worm eggs such as Strongyle and Strongyloides sp. were also recovered from the intestines of four rats.

Conclusions: In this study, seven out of the ten rats was positive for Demodex sp mites. Demodex is a genus of tiny parasitic mites that live in or near hair follicles of mammals. It can be concluded that wild rats can be responsible for the spread of many diseases, either directly, through contamination of human food with their urine or feces, or indirectly, by way of rodent fleas and mites as vectors. Therefore, precautions need to be taken in order to prevent the spread of diseases especially for workers involved in animal care.

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PARASITE ECOLOGY, BIODIVERSITY, AND TAXONOMY / WILDLIFE RESERVOIRS AND ZOONOTIC SPILLOVER

ZOONOTIC PARASITES IN GOLDEN JACKALS: A THREAT TO PUBLIC HEALTH?

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Background and Aims: Golden jackals are reservoirs of zoonotic parasites. As intermediate hosts, jackals can act as bioindicators for circulating parasites, while as definitive hosts, they contribute to environmental contamination. To evaluate the impact of golden jackals residing in suburban areas on public health, the presence of *Toxoplasma gondii*, *Echinococcus spp.*, *Spirometra spp.*, *Toxocara spp.* and Ancylostomatidae was determined.

Methods: Hearts and gastrointestinal tracts from legally hunted animals were obtained. Heart apex was homogenized; intestines were cut into sections and opened longitudinally. Helminths and feces were collected, the mucosa scraped and examined by sedimentation. Parasite eggs were recovered by flotation and sequential mesh filtration. DNA from tissues and eggs was extracted and specific PCR assays were run.

Results: Samples from 26 animals were examined. *T. gondii* gDNA was detected in eleven (42.3%), followed by Ancylostomatidae in nine (34.6%), *Toxocara spp.* in four (15.3%) and *E. canadensis* and *E. multilocularis*, each in one animal (3.85%). *Spirometra spp.* were not detected.

Conclusions: The detection of *E. multilocularis* is of the greatest public health concern, given the severity of alveolar echinococcosis. In contrast, Ancylostomatidae, which were detected in more than one third of the samples, are generally associated with relatively mild clinical conditions. The substantial presence of *T. gondii* however, reflects a high degree of exposure to the parasite, which may pose a risk to public health. This research was supported by the Science Fund of the Republic of Serbia, #2424, Environmental Monitoring of Food and Waterborne Parasites – PARASITE_HUNTER.

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PARASITE ECOLOGY, BIODIVERSITY, AND TAXONOMY / WILDLIFE RESERVOIRS AND ZOONOTIC SPILLOVER

VECTOR-BORNE DISEASES IN WILD CANID POPULATIONS IN QUEENSLAND, AUSTRALIA

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Background and Aims: Vector-borne diseases (VBDs) are an increasing concern within Australia and worldwide, presenting significant challenges due to complex interactions among various pathogens and their hosts. In Queensland, Australia, limited research on vector-borne pathogens makes it difficult to define the status of these diseases and their potential impact on animals and humans. Recent findings, such as the emergence of *Ehrlichia canis* in free-roaming dogs in Aboriginal communities, highlight the need to reassess its epidemiology. This is particularly urgent given the encroachment of wild dogs and red foxes into urban areas, where they may act as reservoirs for pathogens.

Methods: This study examined over 200 wild canid carcasses, with blood, spleen, and attached tick samples collected for molecular detection of vector-borne pathogens such as *Rickettsia* spp., *Babesia* spp., and *E. canis*.

Results: Neither *E. canis* nor *Babesia* spp. were detected. However, *Candidatus* Rickettsia jingxinensis was identified in three *Haemaphysalis longicornis* tick pools, *Rickettsia* spp. in one *Ixodes holocyclus* tick pool and *Coxiella burnetii in another I. holocyclus* tick pool. *Hepatozoon canis* was identified in a fox, and *Dirofilaria immitis* was detected in 12 foxes and one wild dog, with one fox exhibiting a concurrent infection of *H. canis*, *D. immitis* and its endosymbiont *Wolbachia*.

Conclusions: These findings suggest that wild canids and their associated ticks in Queensland harbour a diverse range of vector-borne pathogens, some with zoonotic potential. The detection of multiple pathogens highlights the importance of continued surveillance to better understand the role of wild canids in VBD epidemiology.

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PARASITE ECOLOGY, BIODIVERSITY, AND TAXONOMY / WILDLIFE RESERVOIRS AND ZOONOTIC SPILLOVER

METAZOAN PARASITE FAUNA OF THE AMERICAN MINK (NEOGALE VISON) IN COMPARISON WITH THE CLOSELY RELATED EUROPEAN MINK (MUSTELA LUTREOLA) IN EUROPE

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Background and Aims: The American mink is native to North America and was introduced to Europe at the beginning of the twentieth century. Newly listed as an invasive alien species (IAS), it is suspected to have negative impacts on native ecosystems by serving as a vector for pathogens, as a predator of native species and as a competitor to native species such as the highly endangered European mink. The aim of this study was to reveal carried parasites of American mink in Germany/Europe and to compare them with that of the European mink.

Methods: 50 American mink were examined by dissection. Isolated parasites were identified morphologically and prevalence, intensity and abundance of parasite infestation was calculated. A comprehensive literature search was conducted to compare the parasite infestation between the two mustelid species *N. vison* (invasive) and *M. lutreola* (native).

Results: Among the 50 American mink examined, 9 parasite species could be identified, of which the nematode species *Capillaria aerophila* and *C. putorii* were found for the first time in American mink in Germany. Literature search resulted in the identification of 65 parasite species in 12 European countries for American mink and 37 parasite species in six European countries for European mink.

Conclusions: The present study shows that the American mink carries a more diverse parasite fauna than the European mink. Also due to its greater body size and high adaptability it may have negative impact on native species by disease transmission and predation and seems to be in direct competition to the disappearing European mink.

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PARASITE ECOLOGY, BIODIVERSITY, AND TAXONOMY / WILDLIFE RESERVOIRS AND ZOONOTIC SPILLOVER

ECTOPARASITE DIVERSITY ON THE COMMON VAMPIRE BAT (DESMODUS ROTUNDUS) IN SAN LUIS POTOSÍ, MEXICO: A ONE HEALTH PERSPECTIVE

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Background and Aims: Background: The common vampire bat (*Desmodus rotundus*) is the principal rabies reservoir in Latin American livestock. Its ectoparasites—flies, mites, and ticks—are potential vectors of pathogens like *Bartonella* spp. Assessing their diversity is essential to evaluate spillover risks at the livestock-wildlife-human interface under a One Health framework. **Objective:** To identify ectoparasite diversity on *D. rotundus* from two regions of San Luis Potosí, Mexico, during national rabies control campaigns.

Methods: Materials and Methods: Captures occurred February–November 2024 and June 2025 under Mexican regulation NOM-067-ZOO-2007. Sixty-three bats (31 males, 32 females; 22 juveniles, 41 adults) were examined. Ectoparasites were identified using morphology, PCR, and Sanger sequencing of the 16S rRNA gene, and histopathology of wing tissue.

Results: Results: Ectoparasite prevalence reached 30.1% (19/63 bats). Sixty ectoparasites were collected. Bat flies (Diptera: Streblidae) were dominant, with *Trichobius parasiticus* representing 95% (57/60) of all specimens, followed by *Strebla mirabilis* at 5% (3/60). In addition, mites from the families Macronyssidae and Spinturnicidae, and the soft tick *Alectorobius yumatensis* were identified. Histopathology revealed an embedded parasitic structure with a chitinous exoskeleton and digestive tract, compatible with a mite.

Conclusions: Conclusions: The detection of a consistent community of bat flies, mites, and a soft tick across study sites indicates a stable host-parasite association. Detection of *A. yumatensis*, a vector of relapsing fever group Borrelia, and the high prevalence of streblid flies, warrant sustained surveillance within rabies control programs to monitor vector-borne pathogens.

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PARASITE ECOLOGY, BIODIVERSITY, AND TAXONOMY / WILDLIFE RESERVOIRS AND ZOONOTIC SPILLOVER

INTESTINAL PARASITES AND GUT MICROBIOTA ASSOCIATIONS IN WILD PHILIPPINE BATS: IMPLICATIONS FOR WILDLIFE RESERVOIRS AND ZOONOTIC RISK

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Background and Aims: Bats are important reservoirs of diverse pathogens, yet intestinal parasite infections in wild bats often occur without apparent clinical symptoms. Their effects on the gut environment and potential zoonotic risks remain poorly understood. This study aimed to investigate the prevalence of intestinal parasites in Philippine bats, examine their associations with the gut microbiota, and assess potential zoonotic risks.

Methods: In February 2025, 30 bats were captured in the Orani region of the Philippines, and DNA was extracted from gut contents. PCR detection and phylogenetic analysis were performed. In addition, 16S rRNA amplicon sequencing and shotgun metagenomic analysis were conducted to characterize gut microbiota structure and bacterial functional profiles. Intergroup comparisons based on parasite infection status were performed, with host species and other host-related characteristics included as covariates using univariate and additive multivariable models.

Results: *Cryptosporidium* species, including the zoonotic pathogen *C. parvum*, were detected in 10 individuals. Although β-diversity differed between *Cryptosporidium*-infected and uninfected bats, the infection effect was not significant after species adjustment, indicating that host species was the primary driver of community-level differences. At the taxon level, *Mycoplasma* sp. was the only bacterial taxon associated with *Cryptosporidium* infection; however, it was detected in only three individuals, suggesting a limited and possibly species-specific association. Shotgun metagenomic analysis of selected samples additionally confirmed other intestinal parasites, including *Giardia*.

Conclusions: Wild bats in the Philippines act as reservoirs of *Cryptosporidium* with zoonotic potential. Gut microbiota composition was primarily structured by host species, while *Cryptosporidium* infection showed limited, taxon-specific associations.

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PARASITE ECOLOGY, BIODIVERSITY, AND TAXONOMY / INNOVATIONS IN PARASITE TAXONOMY AND SYSTEMATICS

MITOCHONDRIAL GENOME OF STRONGYLOIDES FROM SWINE SUPPORTS EVOLUTIONARY HOST-SWITCHING EVENT BETWEEN RODENT AND UNGULATE HOSTS

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Background and Aims: *Strongyloides* is a diverse genus of soil-transmitted nematodes infecting humans, pets, and livestock. Recent phylogenetic studies have unveiled evolutionary relationships among mammalian-infecting species; however, some taxa, including *Strongyloides* from swine, remain underrepresented in molecular research. The present study sought to address this.

Methods: An environmentally collected fecal specimen from Bangladesh was positive for *Strongyloides* by microscopy. The host was confirmed as swine by PCR and Sanger sequencing of the mitochondrial 12S and 16S rRNA genes. Worms from this sample underwent Illumina whole-genome sequencing followed by mitochondrial genome assembly using a combined reference mapping and *de novo* assembly approach. A mapping-based assembly of the 18S rRNA gene supported a tentative identity of *Strongyloides ransomi*. A fragmented mitochondrial genome comprising two large contigs was recovered from the Illumina data, including the full suite of 12 *Strongyloides* protein-coding genes plus the 12S and 16S rRNA genes. A concatenated sequence of these 14 genes facilitated construction of a maximum likelihood phylogeny including other mammalian-infecting *Strongyloides* for reference.

Results: The phylogeny supported two major clades within the genus: the carnivore clade (including *S. stercoralis*) and the *S. fuelleborni/papillosus* clade. This tentative *S. ransomi* clustered as a strongly supported sister taxon to the rodent parasite *S. venezuelensis*, both occupying a basal position in the latter (fuelleborni/papillosus) clade.

Conclusions: This work allowed representation of swine *Strongyloides* in a mitogenomic analysis and supports an evolutionary host-switching event between rodent and ungulate hosts. This data provides a foundation for future studies investigating host adaptation and diversification within the *Strongyloides* genus.

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PARASITE ECOLOGY, BIODIVERSITY, AND TAXONOMY / INNOVATIONS IN PARASITE TAXONOMY AND SYSTEMATICS

PHYLOGENETIC INSIGHTS INTO HOST SPECIFICITY AND BIOGEOGRAPHY IN ECHINOSTOMATIDAE

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Background and Aims: Species within Echinostomatidae (Digenea) are globally diverse trematodes that infect a wide range of vertebrate hosts as adults, using mollusks as first intermediate hosts and predominantly gastropods as second intermediate hosts. Their diversity and complex life histories make them useful for investigating host use, evolutionary history, and parasite ecology. Given the broad distribution and diversity of this family, a phylogenetic framework incorporating taxa across geographic regions is needed to generate hypotheses regarding patterns of host association and diversification.

Methods: Specimens were collected from North America, South America, and Africa from both snail and avian hosts, and these data were combined with available sequences from GenBank to reconstruct a family-level phylogeny. We sequenced (28S and ND1) from 8 putative species based on morphological identifications. Patterns of host use, species diversity, and biogeography were inferred from the resulting tree.

Results: Two notable patterns emerged. First, phylogenetic patterns of host use suggest that apparent host specificity in echinostomes may reflect ecological opportunity and transmission dynamics, where consistent host encounters across life-cycle stages may constrain realized host use more strongly than strict physiological or immunological compatibility. Second, multiple African–South American phylogenetic associations were observed, particularly among lineages associated with species of *Biomphalaria* snails, a pattern also reported in Schistosomatidae and suggestive of broader biogeographic connections across trematode lineages.

Conclusions: These results provide new insight into the evolutionary history and host associations of echinostomes and generate hypotheses regarding the processes shaping parasite diversity across regions.

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PARASITE ECOLOGY, BIODIVERSITY, AND TAXONOMY / INNOVATIONS IN PARASITE TAXONOMY AND SYSTEMATICS

FROM BARCODES TO GENOMES: NGS-BASED INSIGHTS INTO THE TAXONOMY OF COTYLURUS

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Background and Aims: Traditional taxonomy of Digenea, based on morphological traits and a limited number of molecular markers, is hampered by morphological convergence and the presence of cryptic species. These challenges are particularly evident in the genus *Cotylurus*, whose representatives are morphologically similar yet genetically diverse. Complete mitochondrial genomes represent a promising tool for resolving digenean taxonomy. However, their application is limited by the requirement for large amounts of high-quality DNA. We present results of applying Next-Generation Sequencing to investigate mitochondrial genome variability in *Cotylurus*.

Methods: We optimized a DNA isolation that enables whole-genome data acquisition through de novo sequencing. DNA was isolated from freshly collected single specimens derived from definitive hosts or from metacercariae collected from intermediate hosts. Samples meeting DNA quantity requirements were subjected to NGS sequencing, and the resulting data were analyzed using Geneious software.

Results: NGS data were obtained from seven *Cotylurus* taxa representing different lineages. Quality control confirmed high-quality datasets, with approximately 50 million paired-end reads (150 bp) per sample, appropriate GC content (~39%), and negligible proportions of ambiguous bases, indicating reliable library preparation and no evidence of contamination. These data provide a solid basis for genome analyses. Preliminary results reveal that genetic variability in the COI gene differs significantly from variability in other mitochondrial regions.

Conclusions: Our findings highlight the potential of genomic approaches to refine species boundaries within Digenea and the limitations of standard barcoding markers. This study contributes to assessing whether genomic data can clarify the complex taxonomy and support the development of improved standards in digenean systematics.

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PARASITE ECOLOGY, BIODIVERSITY, AND TAXONOMY / INNOVATIONS IN PARASITE TAXONOMY AND SYSTEMATICS

PSILOCYBIN AS A MULTI-PATHWAY COUNTERMEASURE TO TOXOPLASMA GONDII-INDUCED BEHAVIOURAL MODIFICATION: A CONVERGENT EVIDENCE SYNTHESIS

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Background and Aims: Chronic T. gondii infection affects 30–50% of the global population with documented behavioural modifications across dopaminergic, serotonergic, and inflammatory pathways (Flegr, 2007). No countermeasure framework exists. This synthesis examines whether psilocybin addresses this modification.Chronic T. gondii infection affects 30–50% of the global population with documented behavioural modifications across dopaminergic, serotonergic, and inflammatory pathways (Flegr, 2007). No countermeasure framework exists. This synthesis examines whether psilocybin addresses this modification.

Methods: Literature synthesis across parasitology and neuropharmacology mapped T. gondii modification mechanisms against psilocybin receptor data, focusing on TrkB affinity and neuroinflammatory modulation.

Results: Psilocybin shows 1,000-fold greater TrkB receptor affinity than SSRIs, driving neurogenesis while producing anti-inflammatory effects, addressing both neuroinflammation and neurochemical disruption in chronic infection. Whole mushrooms with multiple tryptamine analogues produce an entourage effect across broader receptor networks than single compounds.Psilocybin shows 1,000-fold greater TrkB receptor affinity than SSRIs, driving neurogenesis while producing anti-inflammatory effects, addressing both neuroinflammation and neurochemical disruption in chronic infection. Whole mushrooms with multiple tryptamine analogues produce an entourage effect across broader receptor networks than single compounds.

Conclusions: Psilocybin's profile maps precisely onto modification by chronic T. gondii. Controlled studies in seropositive versus seronegative populations with behavioural and neuroinflammatory measures are warranted.Psilocybin's profile maps precisely onto modification by chronic T. gondii. Controlled studies in seropositive versus seronegative populations with behavioural and neuroinflammatory measures are warranted.

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THE NANOSUIT METHOD AS A RAPID, UNIVERSAL ALTERNATIVE TO CONVENTIONAL SEM PREPARATION: PRACTICAL APPLICATION ACROSS DIVERSE PARASITIC TAXA

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Background and Aims: Scanning electron microscopy (SEM) is a powerful tool for investigating the ultrastructure of small parasitic organisms. However, conventional SEM preparation requires labor-intensive, multistep procedures—namely chemical fixation, dehydration, drying, and metal coating. These steps often induce shrinkage, deformation, or significant loss of discrete specimens (e.g., spores and small protozoans). The recently developed NanoSuit method enables SEM observation under high vacuum by forming a thin polymer membrane on the specimen surface, thereby preserving structural integrity and moisture with minimal handling.

Methods: Here, we report the practical applicability of the NanoSuit protocol across a diverse array of parasitic taxa, including arthropods, nematodes, platyhelminths (aspidogastrids, digeneans, cestodes), protozoans (apicomplexans, ciliates), and myxozoans. Ethanol-fixed specimens were rehydrated and immersed in the NanoSuit solution for approximately 15 minutes, followed by SEM observation under high vacuum without conventional drying or metal coating.

Results: The protocol enabled high-resolution visualization of diagnostic morphological features with minimal artifacts across all examined groups. Notably, the simplified workflow significantly mitigated the loss of unattached or minute specimens, such as spores, which are typically washed away during conventional dehydration sequences. Fine surface topography of delicate organisms was well preserved.

Conclusions: The NanoSuit method provides an extremely simple and rapid workflow, significantly reducing preparation time and preventing sample loss compared with conventional techniques. Our results demonstrate that this method offers a versatile and effective alternative for SEM-based morphological studies, expanding the methodological toolkit for integrative taxonomy and rapid morphological diagnosis in parasitology.

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PARASITE ECOLOGY, BIODIVERSITY, AND TAXONOMY / INNOVATIONS IN PARASITE TAXONOMY AND SYSTEMATICS

RESOLVING THE TAXONOMIC IDENTITY OF A CAMEL FILARIAL NEMATODE: AN INTEGRATIVE REDESCRIPTION OF DERAIOPHORONEMA EVANSI

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Background and Aims: Background: Filarioses are common nematode infections in camels (Camelus spp.). The most significant disease is caused by Deraiophoronema evansi, which impacts camel reproductive function, working ability, and productivity. The taxonomy of this onchocercid is equivocal, and its phylogenetic relationships within Onchocercidae are ambiguous.

Methods: Methods: We analyzed D. evansi specimens from camels and examined their morphology. For comparative material, we analyzed fresh specimens from camels in Iran and specimens from Egypt deposited in the Muséum National d'Histoire Naturelle (MNHN) collections. Multi-locus sequence analyses based on seven genes (two mitochondrial genes cox1 and 12S ribosomal DNA (rDNA) and five nuclear genes 18S rDNA, 28S rDNA, MyoHC, rbp1, and hsp70) of these filarioids and six genes of Wolbachia (16S rDNA, ftsZ, dnaA, coxA, fbpA, and gatB) were analyzed.

Results: Results: Deraiophoronema evansi (Lewis, 1882) Romanovitch 1916 combinatio nova (n. comb.)(syn: Dipetalonema evansi) was described on the basis of morphological characteristics and its genetic divergence from congeners. Molecular characteristics of the new species revealed its close evolutionary relationship with Setaria sp. Wolbachia endosymbiont was not present in D. evansi.

Conclusions: Conclusions: We provide new molecular and morphological data on D. evansi, increasing the number of valid genera of Setariinae to four and setting up the taxonomic information regarding this species of veterinary importance.

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A NEW SPECIES OF SPAULIGODON (NEMATODA: PHARYNGODONIDAE) PARASITE OF THECADACTYLUS RAPICAUDA (SQUAMATA: PHYLLODACTYLIDAE) IN THE BRAZILIAN AMAZON

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Background and Aims: During a study on the helminth parasite community in the turnip-tailed gecko *Thecadactylus rapicauda* from the Brazilian Amazon, we found nematode specimens in the large intestine assigned to the genus *Spauligodon* that possess unique traits that classify them within a new taxon. In the present work, we aimed to describe this new species.

Methods: A total of 40 specimen hosts were collected, anesthetized, and necropsied to search for helminths (SISBIO: Nº 48102-2). Adult nematodes were killed and preserved in 70% ethanol. Specimens of both sexes were examined under a light microscope and a scanning electron microscope.

Results: We identified morphological features essential for species diagnoses, such as the lateral alae present in males, the lateral line present in females, the absence of spicule, eggs fusiform without knobs, and postbulbar vulva. The prevalence of infection was 15% (six infected hosts), range of infection 7–42, mean abundance 3.15, mean intensity 21.

Conclusions: Based on the distribution of other *Spauligodon* species recorded in the Neotropical region, as well as their different characteristics, the specimens from our study have unique traits that suggest that the nematodes described here belong to a new species. *Spauligodon caxiuana* n. sp. is the first species of the genus described in Brazil and the third record of the genus parasitizing Brazilian lizards, representing the 56th species of the genus, and the fifth record of the genus in the Neotropical realm.

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MITOGENOMICS OF TRICHURIS (NEMATODA: TRICHOCEPHALIDA: TRICHURIDAE) BASED ON A SAMPLING MORPHOLOGICAL CLUSTER STRATEGY.

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Background and Aims: Trichuris *spp.* are worldwide monoxenous geohelminths that infect strictly mammals. Within the genus, there are about 114 recognized species, of which, three are of medical and economic importance: *Trichuris trichiura* (etiological agent of trichuriasis), *T. suis* (zoonotic trichuriasis), and *T. muris* (a model organism in parasitology). However, despite their significance, there is no phylogeny or systematic rearrangement for the genus. To resolve this problem, we propose grouping species into morphological clusters for a phylogenetic sampling strategy.

Methods: First, we conducted a systematic literature review focused on the diversity of *Trichuris* spp. and the morphological characters described in their species. Furthermore, through a meta-analysis, we found key morphological diagnostic traits and constructed morphological clusters for phylogenetic reconstruction. Finally, phylogenetic analyses were performed employing the complete mitochondrial genome of representatives of our clusters.

Results: The clustering identified 10 morphological groups, while our preliminary phylogeny shows an arrangement of different well-supported clades, showcasing 8 independent clades belonging to 5 morphological clusters associated with host families (1.- Bovidae, 2.-Suidae, and 3.- Hominidae, 4.- Holarctic Cricetidae, 5.- Heteromyidae, 6.- Neotropical Cuniculidae, Ctenomyidae, 7.-European-African Muridae, and 8.-Asian Muridae).

Conclusions: Five out of ten clades were reconstructed using a morphological sampling strategy; although in most cases the clades are consistent with morphological arrangements, discrepancies are present because of inconsistent morphological descriptions. Future work involves continuing strategic sampling of the missing clusters to complete a predictive phylogeny of the genus, thereby allowing us to establish a solid morphological comparative framework for species descriptions.

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BROAD VERTEBRATE HOST SAMPLING HIGHLIGHTS DEEP PHYLOGENETIC STRUCTURE IN STRONGYLOIDES

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Background and Aims: The parasitic nematode genus *Strongyloides* infects a wide range of vertebrate hosts; yet molecular studies have largely focused on a limited subset of mammal-associated taxa and have relied on short genetic markers. Parasites infecting non-mammalian hosts and divergent lineages have been underrepresented, leaving the structure of the genus unresolved. This study aimed to establish a phylogenetic framework for *Strongyloides* by integrating nuclear loci and mitochondrial genome sequences from parasites sampled across a broad diversity of vertebrate hosts.

Methods: *Strongyloides* isolates were obtained from reptiles, amphibians, birds, and mammals across geographic regions. For newly collected isolates, sequence reads were generated using an Illumina MiSeq platform. Conserved single-copy nuclear gene markers were concatenated and used for multi-locus phylogenetic analysis using maximum-likelihood methods. Mitochondrial genome sequences were assembled from the same datasets and analyzed in parallel.

Results: Nuclear gene phylogenetic analyses resolved three major clades within *Strongyloides*: two mammalian-associated clades (A and B) and one amphibian–reptile-associated clade. An avian-infecting isolate from chicken did not cluster in these clades. Divergence among these lineages indicated ancient splits. Within mammalian-associated clade B, three subgroups were identified: the *papillosus* group, a rodent-associated lineage (the *myopotami* group), and a primate-associated

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lineage (the *cebus* group). An isolate from a Gabonese galago clustered in the *stercoralis–procyonis* group but was distinct from *S. stercoralis*.

Conclusions: These results demonstrate phylogenetic structuring within *Strongyloides* and underscore the importance of integrating non-mammalian lineages, New World taxa, and parasites from understudied host groups. Inclusion of parasites infecting strepsirrhine primates, such as galagos, is critical for resolving evolutionary relationships.

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PETABASE-SCALE SURVEY PROVIDES AN UNPRECEDENTED FRAMEWORK FOR ANALYZING MICROSPORIDIAN DIVERSITY ACROSS HOSTS, HABITATS, AND GEOGRAPHY

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Background and Aims: Microsporidia are a diverse and poorly characterized group of obligate intracellular parasites that infect animals. They pose significant risks to human health, especially in immunocompromised individuals, and in livestock. While microsporidia are common, especially in wild animals, their detection and characterization have been limited by their small size and cryptic nature.

Methods: Here, we use a recently developed resource of assembled publicly available sequencing data to systematically explore microsporidian diversity.

Results: Using a sequence-homology search, we identified 22,520 microsporidian-positive samples and approximately 1,300 novel microsporidian species. Notably, we report the first detection of microsporidia in several animal phyla, including Xenacoelomorpha (acoelomorph flatworms) and Onychophora (velvet worms). We then reconstruct a phylogeny of all known and previously unknown (n = 1,945) microsporidian 18S rRNA sequences to reveal extensive microsporidian diversity across animal hosts and environments. A complementary multi-gene phylogeny based on 464 genomes supports the recognized six-clade framework while improving the resolution of relationships within clades. We further examine global patterns of geographic distribution, habitat association, and host specificity.

Conclusions: Overall, this petabase-scale survey substantially expands our understanding of microsporidian diversity and provides new insights into their evolutionary breadth and ecological range.

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PARASITE ECOLOGY, BIODIVERSITY, AND TAXONOMY / INNOVATIONS IN PARASITE TAXONOMY AND SYSTEMATICS

ATRIOPHALLOPHORUS DEBLOCK & ROSE, 1964 (TREMATODA, MICROPHALLIDAE) IN GASTROPOD SNAILS FROM LITHUANIAN WATERS

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Background and Aims: The records of the larval stages of microphallid trematodes in European freshwaters are rare. During a study of trematode diversity in freshwater ecosystems in Lithuania, specimens of gastropod snails *Bithynia tentaculata* and *Lithoglyphus naticoides* were found to be naturally infected with the larval stages of species belonging to the family Microphallidae Travassos, 1920. In this study we utilize novel molecular sequence data from larval microphallids and new chromosome set structure data to estimate their phylogenetic affinities and identity.

Methods: Morphology of larval stages was studied on live specimens and preliminarily identification was made to the family level. ITS2 and partial 28S rDNA regions were sequenced and used for phylogenetic analyses. In addition, the diploid chromosome sets were studied. Slide preparations of mitotic chromosomes were made using an air-drying technique.

Results: Phylogenetic analyses including sequence data revealed three species-level genetic lineages and suggested a congeneric relationship of these three to a hitherto reported species of the genus *Atriophallophorus*. One isolate from *B. tentaculata* was identified as *A. minutus*. The remaining isolates represent two unidentified species of *Atriophallophorus*. Karyological observations support the close taxonomic affinity between species and provide strong evidence for assigning them to Microphallidae. Diploid karyotypes consist of 18 chromosomes, with a predominance of meta- and submetacentric elements

Conclusions: Our results indicate unexpected and hitherto unrecognized species diversity in the genus *Atriophallophorus* and provide further confirmation of the opinion that an unpredictable number of trematode species remain undescribed.

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CERCARIA OF GYRABASCUS OPPOSITUS (ZDZITOWIECKI 1969) (PLEUROGENIDAE): UNEXPECTED FINDING FROM LITHOGLYPHUS NATICOIDES SNAIL USING MOLECULAR AND KARYOLOGICAL TOOLS

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Background and Aims: Trematodes from the genus *Gyrabascus* Macy, 1935 are parasites of bats, rodents and marsupials. *Gyrabascus oppositus* was described as *Parabascus oppositus* by Zdzitowiecki from bats in Poland. The life cycle of *G. oppositus* is unknown, as is the life cycle of any other species of *Gyrabascus*. During a study of larval trematodes developing in gastropod snail *Lithoglyphus naticoides,* Xiphidiocercaria sp. 2 (Odening, 1971) of unknown taxonomic position from subgroup *Cercariae virgulae* was found. Both karyological and molecular phylogenetic analysis allowed us to discuss its phylogenetic relationships and species identity.

Methods: Naturally infected *L. naticoides* were collected from Kaunas water reservoir, Lithuania. Photomicrographs of live cercaria were taken for measurements and further identification. Samples of intramolluscan larval stages were fixed for karyological and molecular analysis. Comparative sequence analysis was made using ITS2 and 28S rDNA. Conventionally stained mitotic chromosome sets were obtained using an air-drying technique.

Results: Newly generated 28S sequences of *Xiphidiocercaria* sp. 2 (Odening, 1971) from *L. naticoides* cluster together with GenBank sequences of adult *Gyrabascus* spp. and were identical with those of adult *G. oppositus* from bats, *Nyctalus noctula*, *N. leisteri* and *Pipistrellus nathusii*. Examination of mitotic metaphases revealed the modal diploid number 2n = 22.

Conclusions: Our finding is the first to report *L. naticoides* serving as the first intermediate host for *G. oppositus*. Karyological and morphological data are consistent with the results of molecular analysis and support the assignment of the species to the family Pleurogenidae. This project was funded by the Research Council of Lithuania, agreement No S-MIP-25-46

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TREMATODES IN LITHOGLYPHUS NATICOIDES (GASTROPODA): UPDATING HISTORICAL DATA ON SPECIES IDENTITY

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Background and Aims: The gravel snail, *Lithoglyphus naticoides*, is native to the western part of the Black Sea. Now, this snail is common in various freshwater ecosystems of Europe. It harbors a unique spectrum of cercariae that disperse along with snails and can be pathogenic to vertebrates. However, a number of cercariae are still known under provisional names and are not associated with adult stages of digeneans. The use of DNA sequencing has enabled us to solve problems inherent in classical approaches.

Methods: Combined analysis of morphological and molecular data was performed, based on our newly generated ITS2 and partial 28S rDNA data. We provide review on historical data, followed by examples of the challenges each group of trematodes has relative to the goal of the accurate identification of species.

Results: Based on the results of comparative molecular analysis, we were able to identify 11 larval digeneans to the genus or species level and elucidate some life cycles. Thus, it was revealed that cercariae of cercariaeum type represent two lissorchiid species - *Palaeorchis incognitus* and *Asymphylodora* sp.; *Spelotrema* sp. or Microphallidae gen. sp. has been proven to be *Atriophallophorus* sp.; *Xiphidiocercaria* sp. – *Maritrema* sp.; *Xiphidiocercaria* sp. 2 (Odening, 1971) - *Gyrabascus oppositus;* Tetracotyle metacercariae – *Cotylurus cornutus*; Lophofurcocercaria - *Sanguinicola* sp.

Conclusions: Our molecular studies have filled many gaps and provided new data crucial for understanding parasite diversity in *L. naticoides* and their transmission strategies in nature.

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PARASITE ECOLOGY, BIODIVERSITY, AND TAXONOMY / INNOVATIONS IN PARASITE TAXONOMY AND SYSTEMATICS

LONG-TERM ACTIVITY AND VIABILITY OF BIVESICULID LARVAE IN DIFFERENT CULTURE MAINTENANCE MEDIA

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Background and Aims: Digenean cercariae are nonfeeding and rely on intrinsic energy reserves, and so generally survive hours to a few days after release from their snail host. The physiological and developmental underpinnings for this stage remain to be studied, as this short lifespan limits the potential scope of experimental work in laboratory conditions. One possible solution may be to maintain cercariae in culture media. This investigation aims to identify a medium for longer-term maintenance of bivesculid cercariae, and to evaluate the effectiveness of culturing cercariae under laboratory conditions.

Methods: Cercariae were recovered by crushing and dissection from dark cerith snails (Cerithium atratum) collected along the Fort Lauderdale Intracoastal Waterway. Cercariae were exposed to either Fetig's Medium, Mitochondrial Isolation Medium, or Mitochondrial Respiration Medium, with seawater serving as a control. Cercarial activity and survival were assessed daily and compared.

Results: Cercariae remained active for up to 25 ± 0.03, 8 ± 0.01, and 7 ± 0.02 days in Fetig's Medium, Mitochondrial Isolation Medium (250 mM sucrose,0.5 mM Na_2EDTA,10 mM Tris,1 g/LBSA;pH 7.4 at 0ºC), and Mitochondrial Respiration Medium (0.5 mM EGTA,3 Mm $MgCl_2 \bullet 6H_2O$,60 mM K-lactobionate,20 mM taurine,10 mM KH_2PO_4,20 mM HEPES,110 mM sucrose,1 g/LBSA;pH 7.1 at 20ºC), respectively; cercariae maintained in seawater alone survived for approximately two days. Declines in activity and viability were associated with signs of senescence, characterized by tail-loss and degradation of eyespots.

Conclusions: We conclude that maintaining cercariae in culture media significantly extends their period of activity. We discuss possible opportunities for studies requiring extended cercarial viability, including investigations of cercarial physiology, behavior, and metacercarial encystment.

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PARASITE ECOLOGY, BIODIVERSITY, AND TAXONOMY / EMERGING PARASITIC DISEASES

PATHOBIOME DIVERSITY AND DYNAMICS IN CULTURED BIVALVES FROM THE EBRO DELTA: INTEGRATING METABARCODING AND TARGETED DETECTION OF EMERGING MARINE PROTIST DISEASES

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Background and Aims: Emerging marine diseases caused by protist parasites represent a growing threat to bivalve aquaculture worldwide, particularly under changing environmental conditions and increasing global trade. In the Ebro Delta (NW Mediterranean), one of the main shellfish production areas in Spain, protists parasites are of special concern due to their association with notifiable diseases, such as the recently reported *Perkinsus olseni* infecting the Mediterranean mussel. Thus, protist parasites have a high potential relevance for regional aquaculture sustainability and ecosystem health. We aimed to characterize the annual dynamics of the protist pathobiome in cultured bivalves and surrounding water, and to assess the occurrence and distribution of key pathogenic species, with special focus on *P. olseni*.

Methods: We applied a multi-approach integrating metabarcoding, targeted molecular methods and histology. Multivariate analyses were conducted to explore differences among sample types and seasons, and to evaluate relationships between environmental gradients, parasite assemblages and parasite load. For *P. olseni*, phylogenetic and haplotype diversity analyses were also performed.

Results: Pathobiome composition differed among sample types and showed seasonal variation, with water harbouring higher diversity and bivalves displaying more host-specific assemblages. OIE-listed pathogens followed host-associated patterns, including *Bonamia and Haplosporidium* in oysters, and *Marteilia* and *Perkinsus* in mussels. *P. olseni* occurred year-round, with peaks in summer and autumn. Histology revealed variable infection intensities associated with tissue damage, supporting molecular detections and suggesting differential host responses linked to parasite load.

Conclusions: Our results highlight the value of integrating broad and targeted approaches, to understand emerging marine diseases and improve surveillance in bivalve aquaculture systems.

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SHIFT01-074

POSTER ON BOARD SHIFT 01
PARASITE ECOLOGY, BIODIVERSITY, AND TAXONOMY / EMERGING PARASITIC DISEASES

COCHLIOMYIA HOMINIVORAX, A RE-EMERGING PARASITE IN MEXICO: IMPACT ON ANIMAL AND HUMAN HEALTH

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Background and Aims: The New World Screwworm (NWS) myiasis is a re-emerging parasitic disease caused by *Cochliomyia hominivorax*, a Calliphorid fly that lays eggs in wounds and mucus membranes of warm-blooded animals. Once larvae hatches, they start feeding from live tissues making the wound larger and deeper while more *C. hominivorax* and other secondary myasis flies are attracted, producing secondary infections that may be lethal if treatment is not applied. The NWS was eradicated from Mexico more than 30 years ago by an intensive program using sterile flies. However, NWS infestations appeared in Panama in 2023 and expanded Northward to Central America in one year. The aim of this work was to analyze the impact of reintroduction of NWS on animal and human health in Mexico.

Methods: Databases from the Animal Health agency (Senasica) and the National System of Epidemiology (SNVE) from November 2024 to January 2026 were reviewed and analyzed.

Results: Since its reintroduction, about 15 thousand cases of NWS have been reported by Senasica in 18 states, including Nuevo Leon and Tamaulipas, two Northern states that border with Texas, USA. The most affected species in Mexico are cattle, followed by dogs, pigs, horses, sheep, goats, cats, and birds., although few cases in wild animals have also been detected. In addition, myiasis by NWS have been presented in 117 humans from Southern Mexico.

Conclusions: The impact of NSW on animal and human health and actions of governmental authorities combined with education and training programs to control and eradicate this pest from the country will be presented.

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SHIFT01-075

POSTER ON BOARD SHIFT 01
PARASITE ECOLOGY, BIODIVERSITY, AND TAXONOMY / EMERGING PARASITIC DISEASES

CANINE STRONGYLOIDOSIS IN SWITZERLAND: FROM AN EXOTIC TO AN EMERGING DISEASE

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Background and Aims: *Strongyloides stercoralis* is an intestinal nematode that primarily affects humans and dogs in warm regions worldwide and is considered rare in Central Europe. Clinical presentations range from asymptomatic to life-threatening, particularly in young dogs or in cases of impaired immunity. We report the recent occurrence of strongyloidosis in dogs in Switzerland, together with the molecular characterisation of the parasites involved.

Methods: Faecal samples were analysed using routine flotation and Baermann techniques. When larvae morphologically consistent with *S. stercoralis* were observed, PCR assays targeting the mitochondrial *cox1* gene, and the hypervariable regions I and IV of the 18S rDNA were performed, followed by amplicon sequencing.

Results: Since 2017, strongyloidosis has been diagnosed in 11 individual dogs, and in several dogs from a kennel (n=36) associated with one of these cases. Affected breeds included Yorkshire Terrier, Pomeranian, Chihuahua, West Highland White Terrier, French bulldog, Boston Terrier and American Staffordshire Terrier. Dogs age ranged from two months to 11 years. Eight of the dogs were imported from Italy, France, Belgium, Croatia, or Portugal. Molecular characterisation revealed the *cox1*-haplotypes HP16 and HP20 in 7 and 4 dogs, respectively. All isolates corresponded to haplotype VI (HVR-I), and haplotype A (HVR-IV), the latter being assumed to characterise the zoonotic *S. stercoralis* population.

Conclusions: Breeding kennels and animal trade seem to play an important epidemiological role in the dissemination of *S. stercoralis*. Given its clinical relevance, zoonotic potential, and difficulties with treatment, increased awareness for *S. stercoralis* of diagnosticians and clinicians is required.

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SHIFT01-076

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PARASITE ECOLOGY, BIODIVERSITY, AND TAXONOMY / EMERGING PARASITIC DISEASES

PARASITES AS ECOLOGICAL SIGNATURES: STRUCTURE AND HOST-ASSOCIATED DIVERSITY OF ENDOPARASITE COMMUNITIES IN FEW TUNISIAN TELEOSTS

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Background and Aims: Parasites are key players in marine ecosystems, acting as ecological tags revealing host ecology, trophic interactions, and food-web connectivity. Although ecologically significant, parasites along the Tunisian coast are fairly studied.

Methods: Between February and September 2014, a total of 180 fish specimens, representing six species from the families Carangidae, Mullidae, and Sparidae, were collected from the Gulf of Tunis. Endoparasites were recovered from the digestive tract, stained with Borax carmine, illustrated for morphological analysis, and assigned to their respective host microhabitats. Epidemiological indices, including prevalence, mean intensity, and mean abundance, were calculated to characterise host–parasite interactions.

Results: A total of 25 parasite taxa were recorded: 20 digeneans, 2 nematodes, 2 larval cestodes, and 1 acanthocephalan. *Trachurus trachurus* harboured the most diverse helminth assemblage (9 parasites). Infracommunity analysis revealed that 38% of fish hosted a single parasite species, 33% two species, and 13% three species, with only a few individuals harbouring four, five, or six species. With the exception of the digenean *Robphildollfusium fractum*, *Wardula capitellata*, *Holorchis micracanthum, H. pycnoporus, Gymnotergestia* sp., and the nematode *Anisakis spp.*, infection indices showed low variation with host size. Comparisons with other Mediterranean regions indicate that six digenean species were recorded in new fish hosts in this study: *Gymnotergestia* sp., *Ectenurus lepidus*, *Timonia mediterranea*, *Opecoeloides furcatus*, *Diphterosomum brusinae*, and *Lecithocladium excisum*.

Conclusions: These results provide a comprehensive overview of host-associated diversity and community structure of endoparasites in few Tunisian teleosts, documenting patterns of host specificity and reporting new fish–parasite associations for Tunisia and the broader Mediterranean.

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SHIFT01-077

POSTER ON BOARD SHIFT 01
PARASITE ECOLOGY, BIODIVERSITY, AND TAXONOMY / EMERGING PARASITIC DISEASES

EMERGING AND RE-EMERGING PARASITES IN MEDITERRANEAN AQUACULTURE

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Background and Aims: Fish naturally host a wide range of parasites in their ecosystems, including both specialist and generalist species. Although disease-free fish are ideally selected for aquaculture, health problems may still arise, particularly in farming systems supplied with external water sources. **The aim of this report is to highlight** the occurrence of re-emerging and emerging parasites capable of causing disease outbreaks in Mediterranean farmed fish.

Methods: Between **2020 and 2025**, to support fish farmers in limiting parasitic disease outbreaks, fish from several farming systems in the Mediterranean area were submitted to our Fish Pathology Unit for diagnostic purposes. All fish were subjected to a **complete parasitological examination**, and when necessary, **histological evaluation was also performed**.

Results: During the observational period, several parasite species belonging to different taxa were isolated and identified. Based on our observations, the most impactful groups were **monogenean flukes**, specifically *Neobenedenia girellae* in *Sparus aurata*, *Benedenia seriolae* and *Zeuxapta seriolae* in *Seriola dumerilii*, and *Ktariella polyorchis* in *Argyrosomus regius*. Moreover, in *S. aurata*, a species of *Caligus* was also recently detected during a disease outbreak.

Conclusions: Overall, the current epidemiological scenario of parasitic diseases in aquaculture can be considered relatively reassuring with regard to the emergence of novel pathogens. Nevertheless, the diversification of farmed species increases the likelihood of new host–parasite interactions, while global climate change is rapidly altering the epidemiology of several parasitic diseases by influencing parasite distribution, life cycles, and transmission dynamics.

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SHIFT01-078

POSTER ON BOARD SHIFT 01
PARASITE ECOLOGY, BIODIVERSITY, AND TAXONOMY / EMERGING PARASITIC DISEASES

GENOMIC AFFINITIES BETWEEN PLASMODIUM SIMIUM AND A HISTORICAL SAMPLE OF PLASMODIUM FROM THE SADO VALLEY, PORTUGAL

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Background and Aims: *Plasmodium vivax* is believed to have arrived in the Americas with Portuguese and Spanish colonizers, since vivax malaria was endemic in southern Europe until the mid-last century. We analyzed Giemsa-stained blood smears prepared in the 1950s from malaria patients in the Sado Valley, Portugal — a region with autochthonous transmission until that period.

Methods: DNA was extracted from thirteen blood slides of patients with P. vivax infections using the Qiagen QIAamp Investigator kit and subjected to qPCR diagnostic before genomic sequencing. Two approaches for parasite DNA enrichment were compared: selective whole-genome amplification (sWGA) and hybrid capture.

Results: With sWGA, the highest quality sample (Portugal 3) yielded 11.7 million reads, an average depth of 18.7x, and 14,522 biallelic SNPs (8,763 of them with depth ≥5x). Analyses using smartPCA, ADMIXTURE, and f4 statistics revealed a clear genetic affinity between Portugal 3 and *Plasmodium simium* from the Atlantic Forest of Brazil. Although this sample does not exhibit the mitochondrial SNPs characteristic of P. simium, we observed the deletion of 3,010 base pairs in the PvRBP2a gene, which is characteristic of this parasite.

Conclusions: These results suggest that the Portugal 3 sample may represent an intermediate form between *P. simium* and *P. vivax*, which could have existed in Europe before reaching Brazil, where it underwent changes until becoming *P. simium*. Another possibility is that Portugal 3 is a lineage of *P. simium* without the characteristic mitochondrial SNPs, introduced to Europe from the Atlantic Forest.

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SHIFT01-079

POSTER ON BOARD SHIFT 01
PARASITE ECOLOGY, BIODIVERSITY, AND TAXONOMY / EMERGING PARASITIC DISEASES

DELETIONS OF HISTIDINE-RICH PROTEIN 2/3 GENES IN NATURAL PLASMODIUM FALCIPARUM POPULATIONS FROM CAMEROON AND INDIA: ROLE OF ASYMPTOMATIC AND SUBMICROSCOPIC INFECTIONS

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Background and Aims: There are growing reports on the prevalence of non-falciparum species and submicroscopic infections in sub-Saharan African countries but little information is available from Cameroon.

Methods: A hospital-based cross-sectional study was carried out in four towns (Douala, Maroua, Mayo-Oulo, and Pette) from three malaria epidemiological strata (Forest, Sahelian, and Soudanian) of Cameroon. Malaria parasites were detected by Giemsa light microscopy and polymerase chain reaction (PCR) assay. Non-falciparum isolates were characterized and their 18S gene sequences were BLASTed for confrmatory diagnosis.

Results: PCR assay detected malaria parasites in 82.4% (98/119) patients, among them 12.2% (12/98) were asymptomatic cases. Three Plasmodium species viz. P. falciparum, P. ovale curtisi and P. vivax, and two co-infection types (P. falciparum+P. vivax and P. falciparum+P. ovale curtisi) were found. The remaining infections were mono–infections with either P. falciparum or P. ovale curtisi. All non–falciparum infections were symptomatic and microscopic. The overall proportion of submicroscopic infections was 11.8% (14/119). Most asymptomatic and submicroscopic infection cases were self-medicated with antimalarial drugs and/or medicinal plants. On analysis, P. ovale curtisi sequences were found to be phylogenetically closer to sequences from India while P. vivax isolates appeared closer to those from Nigeria, India, and Cameroon. No G6PD-d case was found among non-falciparum infections.

Conclusions: This is frst study from Cameroon reporting presence of putative drug resistance mutations in Pv infections, especially in the pvdhps gene, and also outlined the absence of a G6PD-defciency trait in patients.

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SHIFT01-080

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PARASITE ECOLOGY, BIODIVERSITY, AND TAXONOMY / EMERGING PARASITIC DISEASES

EMERGENCE AND DISTRIBUTION OF ANGIOSTRONGYLUS VASORUM IN WILD CANIDS FROM THE CANADIAN MARITIMES

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Background and Aims: *Angiostrongylus vasorum* is an emerging cardiopulmonary nematode of domestic and wild canids with increasing geographic distribution globally. Information on its occurrence in Atlantic Canada remains limited. This study represents the first large-scale investigation of *A. vasorum* in wild canids from the Canadian Maritimes and aimed to determine its prevalence, worm burden, and co-infection patterns in foxes and coyotes from Prince Edward Island (PEI) and Nova Scotia (NS).

Methods: A total of 517 wild canids (138 foxes and 379 coyotes) were examined from PEI (n = 234) and NS (n = 283). Lungs and hearts were examined using established dissection methods. Adult nematodes were recovered, identified morphologically, and prevalence, worm burden, and co-infections with *Crenosoma vulpis* and *Capillaria aerophila* were recorded by host species and province.

Results: *Angiostrongylus vasorum* was detected in foxes and coyotes from both provinces. In NS, prevalence ranged from 7% in coyotes to 40% in foxes, while in PEI prevalence was 31% in coyotes and 21% in foxes. Worm burdens ranged from 1–64, with the highest burdens observed in NS coyotes. Co-infections involving *A. vasorum* were more frequent in foxes, including triple-species infections, particularly in NS.

Conclusions: This study provides the first large-scale evidence that *A. vasorum* is established in wild canids in the Canadian Maritimes. The observed prevalence and co-infection patterns highlight potential implications for parasite transmission to domestic dogs and underscore the need for continued surveillance in the region.

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SHIFT01-081

POSTER ON BOARD SHIFT 01
PARASITE ECOLOGY, BIODIVERSITY, AND TAXONOMY / EMERGING PARASITIC DISEASES

PHYLOGENETIC CHARACTERIZATION OF TAENIA SOLIUM IN JAPAN: ON THE ORIGINS OF THE PORK TAPEWORM

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Background and Aims: The origin(s) of the pork tapeworm, *Taenia solium*, remain controversial. Its morphological similarity to other *Taenia* species in Africa has led to the "Out of Africa" hypothesis, which proposes that host-switching to humans occurred in sub-Saharan Africa, followed by parasite dispersal accompanying human migration. However, subsequent genetic analyses have shown that *T. solium* is most closely related to *T. arctos*, a species distributed in the near-Arctic region. Here, we present genetic findings supporting the case for revisiting the "Out of Africa" hypothesis based on phylogenetic analyses of cysticerci recovered from two wild boars in Japan, a country generally regarded as non-endemic for cysticercosis.

Methods: Six cysticerci were collected from two wild boars and their mitogenome sequences were obtained using an Illumina MiSeq platform. A haplotype network analysis was performed using mitochondrial *cox1* and *cytb* sequences. Subsequently, a Bayesian phylogenetic tree was constructed using mitochondrial 12 protein-coding genes to infer the phylogenetic relationships among *T. solium* haplogroups.

Results: Six complete mitogenomes of 13,712 bp were successfully obtained. Haplotype network analysis revealed that *T. solium* in Japan was genetically most distant from other haplogroups reported from Asia, Africa/America, and Bhutan, showing p-distances of 1.79–2.05%. Furthermore, phylogenetic analysis revealed that the Japanese haplotype diverged prior to the divergence between the Asian and African/American haplogroups which possibly occurred in the Early to Middle Pleistocene.

Conclusions: The observed genetic diversity challenges the traditional "Out of Africa" hypothesis for *T. solium*. Modern humans might have acquired this zoonotic tapeworm in multiple regions of the Palearctic from previous definitive hosts.

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SHIFT01-082

POSTER ON BOARD SHIFT 01
PARASITE ECOLOGY, BIODIVERSITY, AND TAXONOMY / EMERGING PARASITIC DISEASES

INVESTIGATING THE PRESENCE OF LEISHMANIA RNA VIRUS (LRV) IN LEISHMANIA DONOVANI IN SRI LANKA

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Background and Aims: *Leishmania* RNA virus (LRV) is a double-stranded RNA virus residing in the cytoplasm of certain *Leishmania* species. These viruses are classified in the family *Totiviridae* and are composed of a 5.3 kb double-stranded RNA genome, an RNA-dependent RNA polymerase, and a capsid protein. Based on gene regulation and sequence differences, these viruses are classified as LRV1 in New World species (*L. guyanensis*) and LRV2 in Old World species (*L. major*). Studies have shown that the presence of LRV in some strains increases disease severity by promoting parasite replication, disease pathology, and lesion metastasis. *L. donovani* isolates from Sri Lanka have not yet been investigated for the presence of LRV; therefore, this study aims to determine the presence of LRV in cutaneous leishmaniasis (CL) lesions.

Methods: Samples were collected from four CL patients using slit-skin smears and lesion aspirates, and cultured in M199 medium. DNA was extracted from stationary-phase *Leishmania* promastigotes for species identification, and PCR was performed using *L. donovani*–specific primers. Products were visualized on 2% agarose gel containing ethidium bromide under UV light. RNA was extracted from parasite cultures, followed by cDNA synthesis. Semi-nested PCR was performed using LRV-specific primers, and the amplicons were visualized on 2% agarose gels.

Results: All four samples tested positive for *Leishmania donovani*, with LRV detected in two isolates. Subculturing appeared to influence LRV load in the parasite isolates.

Conclusions: These findings indicate the presence of LRV in *L. donovani* isolates from Sri Lanka, highlighting the need for further studies, including sequencing, to confirm these findings.

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POSTER ON BOARD SHIFT 01
PARASITE ECOLOGY, BIODIVERSITY, AND TAXONOMY / EMERGING PARASITIC DISEASES

COLON IMPACTION RELATED TO TRIODONTOPHORUS SERRATUS AND CYLICOCYCLUS SPP. INFECTION IN A HORSE

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Background and Aims: Colic syndrome represents one of the most frequent and life-threatening conditions in equine medicine, and gastrointestinal nematode infections may act as important predisposing factors for intestinal motility disorders. This study aimed to investigate a case of large colon impaction associated with strongyle infection in a 10-year-old male Mangalarga horse.

Methods: The animal was referred to the School of Veterinary Medicine and Animal Science (FMVZ/UNESP) for exploratory celiotomy after presenting acute abdominal pain unresponsive to clinical treatment. Preoperative hematological evaluation revealed normocytic normochromic anemia and leukopenia. During surgery, severe impaction of the large colon was identified; the affected segment was emptied by lavage and siphoning, which allowed the visualization and recovery of numerous nematodes. Fecal samples and parasites obtained from the intestinal content were submitted to the Parasitic Diseases Service of FMVZ/UNESP for coproparasitological examination using the Gordon & Whitlock technique and for morphological identification.

Results: Fecal egg count revealed 1,750 Strongylida-eggs per gram of feces. The recovered specimens were morphologically compatible with *Triodontophorus serratus* and *Cylicocyclus* spp. Five hours after surgery, the horse defecated, and additional parasites of the species *Oxyuris equi* were identified in the feces. Anthelmintic treatment with oral moxidectin was instituted, together with postoperative supportive therapy. Hematological parameters progressively returned to normal after surgery and deworming.

Conclusions: This report reinforces that helminth infections may contribute to gastrointestinal impactions and severe colic episodes in horses and highlights the importance of accurate parasitological diagnosis and timely anthelmintic therapy as part of clinical management and prevention of recurrent colic in equine patients.

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SHIFT01-084

POSTER ON BOARD SHIFT 01
PARASITE ECOLOGY, BIODIVERSITY, AND TAXONOMY / EMERGING PARASITIC DISEASES

IN VITRO HYBRIDIZATION AND EXPERIMENTAL EVOLUTION TO ELUCIDATE LEISHMANIA HYBRID ADAPTATION TO DIFFERENT ENVIRONMENTS

Viviane Louzada-Flores, Gerald Spath, Isabelle Louradour
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Background and Aims: *Leishmania* spp. are protozoan parasites transmitted by phlebotomine sand flies, causing neglected diseases. The genus comprises over 20 species with diverse tissue tropisms and vertebrate hosts. *Leishmania* parasites can undergo facultative sexual reproduction inside vectors, forming hybrids. Hybridization is recognized as crucial in evolution, leading to modified virulence, altered tropism, and potential drug resistance. However, mechanisms of hybrid survival, adaptation to different environments, and the impact of parental genetic distance remain unknown. To address these questions, we experimentally produce different hybrid types and expose them to relevant environments (culture, sand flies, and hamsters) to assess their genome and transcriptome prior and after adaptation.

Methods: Using *in vitro* hybridization, we are generating hybrids with varying genetic distances between parents ("self", intra- and inter-specific hybrids). Hybrids and parental strains are inoculated into culture (20 passages), sand flies (*Lutzomyia longipalpis*), and golden hamsters. Phenotypic parameters (growth, virulence, infectivity, tissue tropism, morphology) are monitored, and DNA/RNA are collected pre- and post-adaptation.

Results: We generated multiple hybrid lines, including highly divergent species (*L. infantum*/*L. tarentolae*, *L. infantum*/*L. tropica*, *L. infantum*/*L. donovani*, and *L. donovani*/*L. donovani* hybrids), indicating no species barriers to hybridization. Hybrids compensate genomically through: (i) chromosome loss causing mosaic aneuploidy, and (ii) preferential mono-parental allele expression. Fitness assessment in three environments is ongoing.

Conclusions: While genome plasticity and post-transcriptional regulation are central to hybridization, our study will decipher genetic distance impacts on hybrid adaptability. Furthermore, hybridization between divergent species may represent an underestimated driver of parasite evolution, with implications for transmission dynamics and disease emergence.

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SHIFT01-085

POSTER ON BOARD SHIFT 01
PARASITE ECOLOGY, BIODIVERSITY, AND TAXONOMY / EMERGING PARASITIC DISEASES

QUILL MITES OF THE FAMILY SYRINGOPHILIDAE (PROSTIGMATA: CHEYLETOIDEA) PARASITISING DOMESTIC CHICKENS (GALLUS GALLUS DOMESTICUS) AS AN UNEXPLORED LINK IN A POTENTIAL EPIDEMIOLOGICAL CHAIN

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Background and Aims: Domestic chickens (*Gallus gallus domesticus*) are one of the key pillars of food production. Their significant role in the global economy, impact on human, and the necessity to increase breeding efficiency make the protection of hens' health a matter of priority. One of the least studied representatives of acarofauna associated with chickens are Syringophilidae mites (Acariformes: Prostigmata). These permanent and highly specialised parasites inhabit the interior of feather quills, where they complete their entire life cycle. Syringophilidae pierce quill wall to feed on host-derived tissue fluids, giving them the potential to act as vectors.

Methods: Despite the detection of *Anaplasma phagocytophilum*, *Brucella,* and *Bartonella* in microbiota of quill mites associated with passerines, the composition of their microbial communities in the field of pathogen transmission has never been investigated. Furthermore, none of the available data concern the Syringophilidae-hens association.

Results: Chickens are parasitised by two quill mite species – *Syringophilus bipectinatus* (subfamily: Syringophilinae) and *Columbiphilus khushalkhani* (subfamily: Picobiinae). The two taxa differ markedly in morphology, reproductive strategies, and occupied microhabitats. Despite their widespread occurrence and high prevalence (reaching up to 75% in *S. bipectinatus*), the biological and ecological traits driving their success have never been investigated.

Conclusions: Given their significant potential role as vectors, the current state of knowledge on Syringophilidae–chicken interactions and the related threats will be outlined. Moreover, the associated challenges in assesing their epidemiological significance will be discussed. Addressing knowledge gaps in the biological and ecological factors driving Syringophilidae infestations is crucial for understanding the chains of pathogens transmission.

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SHIFT01-086

POSTER ON BOARD SHIFT 01
PARASITE ECOLOGY, BIODIVERSITY, AND TAXONOMY / EMERGING PARASITIC DISEASES

GENOME COMPARISON OF NEW BABESIA DIVERGENS AND BABESIA MICROTI ISOLATES FROM EUROPE

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Background and Aims: Human babesiosis is an emerging disease and *Babesia divergens* is responsible for most of the human cases in Europe. While *B. microti* is present in Europe, it seems to be less pathogenic in contrast to the situation in the US. Our aim was to identify species-specific genes that may be responsible for differences in pathogenicity between *B. divergens* and *B. microti* isolates of European origin.

Methods: Genomes of two B. *divergens* and two B. *microti* isolates found in Germany were sequenced using Oxford Nanopore Technology and compared using orthofinder (v 2.5.5). The genome comparison included the B. *microti* reference genome (RI), which was isolated in the US. The phylogeny of the new isolates was also determined.

Results: We will present the results from the genome comparisons showing specific genes in *B. microti* and the *B. divergens* isolates. All isolates were *sensu stricto*.

Conclusions: The higher pathogenicity of the US *B. microti* strains is likely to be conferred by a combination of genes present in RI that are absent in the two isolates from Europe.

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POSTER ON BOARD SHIFT 01
PARASITE ECOLOGY, BIODIVERSITY, AND TAXONOMY / EMERGING PARASITIC DISEASES

A BLOODY MESS: RETROSPECTIVE DATA FROM THE CANADIAN MARITIMES INDICATE DIROFILARIA IMMITIS IS ON THE RISE

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Background and Aims: *Dirofilaria immitis* (American heartworm) is an emerging parasitic threat in Atlantic Canada and can cause severe cardiopulmonary disease in dogs. Previously, this parasite was not endemic in the region, and positive cases were linked to travel or importation – this is no longer the case. We aimed to assess temporal trends in heartworm-positive test results and their demographic and provincial associations.

Methods: *Dirofilaria immitis* testing data from 1990 to 2025 were retrieved from the Atlantic Veterinary College's (AVC) Laboratory Management System. Data retrieved included test results from canine SNAP 4Dx, DiroCHEK® heartworm antigen test, and Knott's test, as well as demographic information. Retrieved data were analyzed using descriptive statistics and logistic regression to examine temporal trends and associations.

Results: Preliminary results indicated the proportion of *D. immitis* antigen-positive tests has increased over time, reaching approximately 50% in 2022 and 2023. The highest proportion of positives was observed in 2- to 4-year-old, mixed-breed, sterilized dogs. Age of animal and breed were statistically associated with a positive DiroCHEK® test result. Data analysis is ongoing.

Conclusions: Preliminary findings indicate an increase in the proportion of *D. immitis* antigen-positive test results at AVC. This increase may be partly attributable to imported animals, which were often young, sterilized, mixed-breed dogs – characteristics that were statistically associated with a positive test result in this study. These findings suggest that clinicians and pet owners should remain vigilant for *D. immitis* and consider regular testing and anthelmintic prophylaxis as preventive care to reduce the risk of severe disease.

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SHIFT01-088

POSTER ON BOARD SHIFT 01
PARASITE ECOLOGY, BIODIVERSITY, AND TAXONOMY / EMERGING PARASITIC DISEASES

PRELIMINARY STUDY ON INTESTINAL HELMINTHS OF WILD FELIDS IN POLAND - THE FIRST CASE OF ECHINOCOCCUS MULTILOCULARIS IN EUROPEAN WILDCAT IN POLAND

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Background and Aims: This study demonstrates for the first time in Poland the presence of *Echinococcus multilocularis* infection in wild felids. Felids can act as definitive hosts for numerous parasite species, including those with zoonotic potential. Among these, the most dangerous is *E. multilocularis*, whose larval stages cause alveolar echinococcosis in humans. The aim of this study was to preliminarily determine the occurrence of intestinal helminths in wild felids in Poland, with a specific focus on *Echinococcus* infections.

Methods: For molecular screening targeting *Echinococcus* species (and other selected tapeworms), fecal samples were collected after necropsy from the rectum of 7 European wildcats (*Felis silvestris*) and 14 Eurasian lynx (*Lynx lynx*). Following DNA isolation, the material was analyzed using a multiplex PCR method and a set of qPCR assays. Additionally, the sedimentation and counting technique (SCT) was used to examine the intestines of 5 wildcats and 12 lynxes.

Results: Both multiplex PCR and qPCR assays detected *E. multilocularis* DNA in one wildcat fecal sample (14%). Furthermore, 79 mature *E. multilocularis* tapeworms were recovered from the small intestine of the same animal, with species identification subsequently confirmed by sequencing. Other parasites identified in the wildcats included *Mesocestoides litteratus*, *Hydatigera* sp., hookworms, and *Toxocara cati*. In the lynx samples following parasites were detected: *Mesocestoides* sp., *Taenia* spp. (71%) and *T. cati*.

Conclusions: The finding of zoonotic parasites in wild felids (especially *E. multilocularis*) must be integrated into human infection risk assessments in Poland.

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POSTER ON BOARD SHIFT 01
PARASITE ECOLOGY, BIODIVERSITY, AND TAXONOMY / VETERINARY AND AQUATIC PARASITOLOGY (FISH, AMPHIBIANS, ETC.)

PHYLOGENETIC ANALYSIS OF COSMOCERCA SPECIES (NEMATODA: COSMOCERCIDAE) PARASITIZING JAPANESE RICE FROGS (FEJERVARYA KAWAMURAI) IN JAPAN

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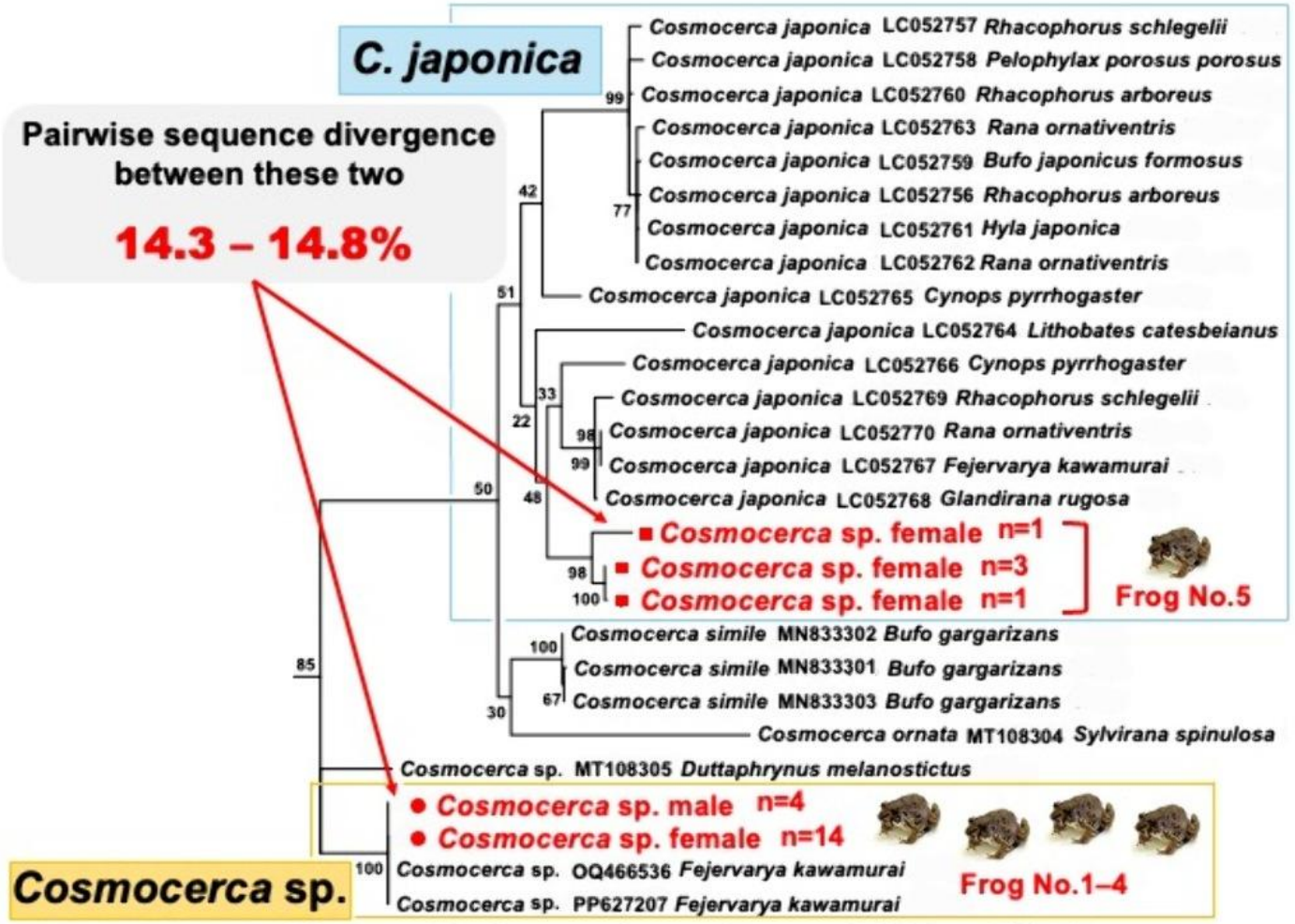
Background and Aims: *Cosmocerca japonica* Yamaguti, 1938, (Nematoda: Cosmocercidae) is reported from digestive tracts of 23 amphibian species in Japan. Morphology of male worm is used to identify *Cosmocerca* species. As morphological descriptions of *C. japonica* lack sufficient detail, the question remains whether *C. japonica* is the only valid species in Japan. We conducted a molecular phylogenetic analysis of *Cosmocerca* species found in *Fejervarya kawamurai* to clarify the taxonomy of Cosmocercidae nematodes in Japan.

Methods: A total of 23 worms (19 females and 4 males) were collected from the digestive tracts of five *F. kawamurai* caught in a paddy field in Hiroshima Prefecture, Japan. DNA was extracted, followed by PCR targeting partial COI mtDNA (384bp). Nematode morphologies were examined via light microscopy.

Results: Four male nematode specimens showing typical *Cosmocerca* morphology were collected from frogs 1–4. Their nucleotide sequences showed 100% homology with *Cosmocerca* sp. from Aichi and Kanagawa Prefectures. Fourteen female specimens were collected from frogs 1–3, which showed 100% homology with the males. No female specimen was present in frog 4. Five female specimens, but no males, were collected from frog 5. These females showed 14.3–14.8% pairwise sequence

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divergence from the other specimens in their COI mtDNA. (**Figure**)



(Figure: Phylogenetic analyses of COI mtDNA)

Conclusions: Two distinct *Cosmocerca* nematode species were collected from *F. kawamurai*. One species was found at three different locations in Japan, all in the same host species. The other clustered within *Cosmocerca japonica* but represented a distinct clade. These results suggest that *Cosmocerca* species in Japan are more diverse than previously reported.

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PARASITE ECOLOGY, BIODIVERSITY, AND TAXONOMY / VETERINARY AND AQUATIC PARASITOLOGY (FISH, AMPHIBIANS, ETC.)

DIVERSITY OF HELMINTHS IN SMALL RUMINANTS OF RURAL AREAS OF SWAT, PAKISTAN

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Background and Aims: Livestock plays a vital role in global agriculture, including Pakistan, where it contributes significantly to the national GDP and rural livelihoods. Small ruminants are well adapted to diverse agro-climatic conditions. However, changing environmental factors like climate variability, grazing practices, and altitudinal differences influence parasite transmission. Goats and sheep in Pakistan face a severe risk of gastrointestinal helminth infections, affecting productivity and animals health.

Methods: The present research was carried out to assess the prevalence of helminth parasites in small ruminants of rural areas of Swat, Pakistan. Faecal samples from the rectum of sheep and goats were collected randomly and put in plastic bottles containing 70% ethanol. The faecal specimens were transported to the Department of Zoology, for investigation of helminth parasites diversity.

Results: A total of 510 faecal samples were collected. The overall prevalence was observed as 88.6% including sheep 90.6% and goats 85.71%. The most prevalent species was *Haemonchus spp* 47%, *Trichuris spp* 21.6%, *Taenia spp* 14.4%, *Monezia spp* 1.38%, *Fasciola spp* 1.52%, *Dicrocoelium spp* 2.35%, *Paramphistomum spp* 1.94%, *Nematodirus spp* 1.52%. The association of helminth parasitic infection was noted statistically as ($P<0.05$ at 95%CI) with mode of nutrition, body condition, age, altitudes, and status of females. However, rate of prevalence was high in younger animals than older ones. The animals of higher altitudes were more infected than the animals of lower altitudes.

Conclusions: The current study evidenced higher helminth parasitic infection which can be prevented through ensuring anti-helminthic treatments of the small ruminants. Molecular studies of the reported helminths are suggested.

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PARASITE ECOLOGY, BIODIVERSITY, AND TAXONOMY / VETERINARY AND AQUATIC PARASITOLOGY (FISH, AMPHIBIANS, ETC.)

MOLECULAR AND PHYLOGENETIC INSIGHTS INTO POLYCHROMOPHILUS DIVERSITY IN BAT FLIES (DIPTERA: NYCTERIBIIDAE) FROM PORTUGAL

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Background and Aims: Haemosporidian parasites of the genus *Polychromophilus* are blood parasites infecting bats worldwide and transmitted by obligate hematophagous flies of the family Nycteribiidae. Despite increasing molecular data in Europe, their diversity, distribution, and host-vector associations remain poorly documented in parts of Western Europe. This study provides molecular evidence of *Polychromophilus melanipherus* in the nycteribiid bat fly *Penicillidia conspicua* parasitizing *Miniopterus schreibersii* (Miniopteridae) in Portugal

Methods: in July 2019, fieldwork was conducted at two caves in Portugal known to harbor bat colonies: one in Tomar, Santarém District and the other in Loulé, Faro. District. Sixty specimens of the bat fly *Penicillidia conspicua* were collected from *Miniopterus schreibersii* (Miniopteridae). All of which tested positive for amplification of the endogenous insect *cox1* gene.

Results: As a result, 25% (15/60) tested positive for *Polychromophilus* spp. based on amplification of the cytochrome b (*cytB*) gene. High-quality sequences of *cytB* and *clpC* genes were obtained and analyzed using Maximum-Likelihood phylogenetic inference. The sequences obtained herein clustered within *Polychromophilus melanipherus* and *Polychromophilus murinus* clades. Unfortunately, we did not recover *cytB* sequences from samples that clustered into the *P. murinus* clade in the *clpC*-based phylogenetic inference, precluding its positioning in both *cytB* and *cytB*+*clpC*-based phylogenetic inferences. Consequently, the discordant signal observed in the *clpC*-based phylogeny should be interpreted with caution.

Conclusions: These findings reinforce the role of nycteribiid flies as vectors facilitating parasite dispersal across bat populations and regions. This study provide new and important vector-based

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molecular data, extending existing host–parasite knowledge to nycteribiid bat flies and strengthening evidence for established transmission cycles in the studied region.

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PARASITE ECOLOGY, BIODIVERSITY, AND TAXONOMY / VETERINARY AND AQUATIC PARASITOLOGY (FISH, AMPHIBIANS, ETC.)

MOLECULAR IDENTIFICATION AND PATHOGENIC EFFECTS OF ANISAKIS LARVAE (NEMATODA: ANISAKIDAE) IN TWO SPECIES OF GROUPERS EPINEPHELUS AENEUS AND EPINEPHELUS MARGINATUS (SERRANIDAE) FROM NORTHERN TUNISIA

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Background and Aims: *Anisakis* larvae infect several fish organs and musculature. Humans are accidentally infected through the consumption of raw or undercooked infected fish, leading to severe abdominal pain, nausea, and allergic reactions. The aim of this study was to morphologically and genetically characterize *Anisakis* larvae collected from *Epinephelus aeneus* and *E. marginatus*, and to evaluate their prevalence and histopathological effects.

Methods: Collected *Anisakis* larvae were counted and preserved in 70% ethanol. The anterior and the posterior parts of selected larvae were used for morphological identification, while the middle part was stored in 100% ethanol for molecular analysis. For histopathological analysis, tissue from infected stomach were fixed in 10% neutral buffer formalin.

Results: Sequencing of cox2 gene of L3 collected from the two groupers identified *Anisakis simplex* s. s. and *Anisakis pegreffii*. Larvae were detected embedded in the stomach of *E. aeneus* and *E. marginatus*. Macroscopic comparison between non-infested and infested stomachs revealed hemorrhagic areas and gastric wall perforations at infection sites. Histological analysis showed encysted L3 larvae located in the submucosa and muscular layers, surrounded by a connective tissue capsule of variable thickness. Evidence of larval migration within the submucosa was observed. Infected tissues exhibited alterations, including fibrosis and necrosis. A pronounced infiltration of mononuclear inflammatory cells surrounding the larvae was also recorded.

Conclusions: In Tunisia, *grouper species* are not consumed raw. However, considering the high prevalence of infection 64.4% in *E. aeneus* and 41.5% in *E. marginatus*, cooking fish at temperatures above 70 °C, in accordance with WHO recommendations, would be advisable.

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PARASITE ECOLOGY, BIODIVERSITY, AND TAXONOMY / VETERINARY AND AQUATIC PARASITOLOGY (FISH, AMPHIBIANS, ETC.)

MORPHO-MOLECULAR CHARACTERIZATION AND SEASONALITY OF MYXIDIUM TUNISIENSIS (MYXOSPOREA: MYXIDIIDAE) IN RAJA RADULA (CHONDRICHTHYES: RAJIDAE) FROM BAY OF BIZERTE, TUNISIA

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Background and Aims: Myxosporea Bütschli, 1881 is a class of endoparasitic cnidarians recognized for their remarkable diversity and their significant impact on host health. Along the Tunisian coasts, Raja radula is abundantly caught throughout the year. However, the diversity of myxozoans infecting this species has not yet been established. The present study aimed to describe a new marine Myxidium species infecting the gallbladder of R. radula, based on detailed morphological characterization, and small subunit rDNA sequence analysis. In addition, seasonal variation in prevalence was assessed in relation to seawater temperature.

Methods: The bile of collected R. radula was extracted from each gallbladder. Infected biles were preserved in 96% ethanol for molecular analysis. DNA was extracted from the host bile. The SSU rDNA was amplified and PCR was performed.

Results: A new marine myxosporean, Myxidium tunisiensis, was found infecting the gallbladder of R. radula harvested from the Bay of Bizerte. Observed stages of the parasite were floating free in the bile. Plasmodia, disporic or polysporic, contain disporic pansporoblasts. Mature myxospores are elongated and typically fusiform. Myxospore valves are symmetrical with 9–10 longitudinal striations. Two polar capsules, were equal in size, with 5–6 polar filament turns. The infection was prevalent throughout the year with the highest prevalence in autumn (81.8%) and the lowest in winter (32.3%). The seasonal prevalence appeared to fluctuate according to seawater temperature.

Conclusions: Based on morphological and molecular differences with described species of Myxidium and considering differences in host and geographic locations, M. tunisiensis is a new species collected from R. radula off Tunisian waters.

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PARASITE ECOLOGY, BIODIVERSITY, AND TAXONOMY / VETERINARY AND AQUATIC PARASITOLOGY (FISH, AMPHIBIANS, ETC.)

CHARACTERIZATION OF DIATOMS ASSOCIATED WITH GASTROINTESTINAL COMPARTMENTS OF MARINE AND FRESHWATER FISH FROM VALLE DEL CAUCA, COLOMBIA

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Background and Aims: Diatoms are unicellular microalgae that play a key ecological role in aquatic ecosystems and are also recognized for their nutritional value and pharmacological potential, including antimicrobial and antiparasitic activities. This study aimed to identify, isolate, and characterize diatoms associated with the gastrointestinal compartments of marine and freshwater fish, and to compare these findings with diatom assemblages present in their surrounding aquatic environments.

Methods: A total of 42 gastrointestinal compartments from marine fish (*Mugil curema* and *Bagre panamensis*) were analyzed from the Puente del Piñal area (Buenaventura), along with 27 gastrointestinal compartments from freshwater fish (*Oncorhynchus mykiss*, *Oreochromis* spp., and *Colossoma macropomum*) collected from recreational fishing lakes in Palmira, Valle del Cauca. Sampling was conducted between March and May 2025. Diatoms were identified through taxonomic analysis of gastrointestinal samples and corresponding water samples from each site.

Results: Seven diatom genera were identified in the gastrointestinal compartments of marine fish: *Botrydiopsis* spp., *Cocconeis* spp., *Coscinodiscus* spp., *Cyclotella* spp., *Diploneis* spp., *Navicula* spp., and *Pinnularia* spp. In contrast, no diatoms were detected in the gastrointestinal compartments of freshwater fish, despite the presence of *Pinnularia* spp. in the water of fishing lakes. Water samples from marine environments revealed the presence of *Thalassiosira* spp.

Conclusions: The absence of diatoms in the gastrointestinal tract of freshwater fish, despite their environmental availability, suggests a digestive interaction potentially linked to dietary utilization. Preliminary observations further indicate that diatom biodiversity may be associated with parasitic loads in fish, supporting a possible role of diatoms in zoopharmacognosy.

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PARASITE ECOLOGY, BIODIVERSITY, AND TAXONOMY / VETERINARY AND AQUATIC PARASITOLOGY (FISH, AMPHIBIANS, ETC.)

THE MULTIFACETED NATURE OF CLINICAL TOXOPLASMOSIS IN ANIMALS: ABOUT ONE CASE OF ACUTE CHOLANGIO-HEPATITIS IN A IMMUNOCOMPETENT CAT

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Background and Aims: Cats have a central place in the life cycle of *Toxoplasma gondii*. Cats behave as definitive hosts with the development of the parasite in enterocytes and the subsequent production and release of oocysts through faeces. Cats may also behave as intermediate hosts with the development of *T. gondii* tachyzoites in different organs and tissues leading to a wide range of clinical signs. This presentation is about an unusual case of acute cholangio-hepatitis due to *T. gondii* in a young immunocompetent cat in France.

Methods: Case report

Results: A 1.5-year-old male cat was presented for vomiting and lethargy. On clinical examination, only hyperthermia of 40°C was noted. Bloodwork revealed elevated ALAT (> 1000 UI/L) and ASAT (180 UI/L). Abdominal ultrasound revealed signs of a cholangio-hepatitis. A cholecystocentesis revealed the presence of tachyzoites of *Toxoplasma gondii*. Serology was strongly positive for IgG and weakly positive for IgM. Coproscopy was negative. Clindamycine therapy for four weeks resulted in clinical cure.

Conclusions: Most *T. gondii* infections of cats are subclinical. Immunosuppressive therapy or co-infection (with feline immunodeficiency virus) may reactivate bradyzoite cysts and lead to fatal toxoplasmosis. However, clinical toxoplasmosis is also reported in cats with no predisposing factors. The zoonotic risk is very limited in the case of cats with clinical toxoplasmosis as they act as intermediate hosts and are not shedding oocysts.

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PARASITE ECOLOGY, BIODIVERSITY, AND TAXONOMY / VETERINARY AND AQUATIC PARASITOLOGY (FISH, AMPHIBIANS, ETC.)

FIRST REPORT OF AMBLYOMMA MIXTUM (ACARI: IXODIDAE) IN PERU

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Background and Aims: Hard ticks are ectoparasites of medical and veterinary importance due to parasitism *per se* and their role as vectors. Among them, the genus *Amblyomma* is of sanitary relevance because several of its species frequently parasitize humans. In Peru, 22 *Amblyomma* species have been reported, although various remain poorly studied. *Amblyomma mixtum* is a Neotropical generalist tick of medical and veterinary importance which is widely distributed from United States of America to Ecuador. This specie is one of the six species of the American continent belonging to the *Amblyomma cajennense* group. The aim of this study was to report the first record of *A. mixtum* in Peru.

Methods: Between August and September 2025, ticks were collected from visibly parasitized cattle in Suyo District, of Ayabaca, Piura, Peru. The ticks were morphological identified using morphological keys and for the case of *Amblyomma* confirmed by molecular analysis. Complete DNA was extracted and subjected to amplification of the 16S rRNA and COI genes.

Results: A total of 23 cattle parasitized by ticks were sampled. All of them showed parasitism by *Rhipicephalus microplus* (45 females, 21 males and 21 nymphs), while four were also co-parasitized with *A. mixtum* (1 female, 2 males and 1 larva). The Maximum-likelihood trees constructed with partial genes sequences resulted that the sequences obtained in this study are genetically compatible with other *A. mixtum* populations from America.

Conclusions: This is the first record of *A. mixtum* in Peru, so future studies are needed to determine its implications for public and animal health.

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PARASITE ECOLOGY, BIODIVERSITY, AND TAXONOMY / VETERINARY AND AQUATIC PARASITOLOGY (FISH, AMPHIBIANS, ETC.)

CLINOSTOMATIDAE SPECIES IN INTERMEDIATE AND DEFINITIVE HOSTS FROM TWO ETHIOPIA LAKES

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Background and Aims: Ethiopia has many lakes that are economically and ecologically important, especially in the Rift Valley and northern regions. Lakes Tana and Ziway support major commercial fisheries dominated by Nile tilapia and African catfish and lie along key migration routes for fish-eating birds. The study aims to identify Clinostomatidae parasites, including potentially zoonotic species, in both definitive and intermediate hosts in these two lakes.

Methods: Five Great White Pelicans (*Pelecanus onocrotalus*) (3 from Tana and 2 from Ziway lakes) and 186 Nile Tilapia (*Oreochromis niloticus*) (90 from Tana and 96 from Ziway lakes) were screened for the presence of Clinostomatidae trematodes in esophagus (birds), branchial and body cavities, kidney and muscle (fish). Morphological and molecular analyses, targeting the cox1 mtDNA, were used for the identification.

Results: Overall 125 specimens belonging to the genera *Clinostomum* (n=101) and *Euclinostomum* (n=24) were collected from 3 Great White Pelicans; four species (*C. phalacrocoracis*, *C. tilapiae, E. heterostomum* and *E. lauroi*) were detected in both lakes. Concerning tilapias, 299 metacercariae (257 *Clinostomum* spp. and 42 *E. heterostomum*) were collected from 27 fish (8 from Tana and 19 from Ziway lakes) and identified as *C. phalacrocoracis*, *C. tilapiae* and *E. heterostomum*.

Conclusions: Despite recent advancements, most of the identification data regarding Clinostomatidae species from fish in Africa remains limited at the genus level. An integrated taxonomic approach is essential to correctly identify parasites at the species level, which helps to unravel their specific life cycles, but also to identify potential zoonotic risks.

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POSTER ON BOARD SHIFT 01
PARASITE ECOLOGY, BIODIVERSITY, AND TAXONOMY / VETERINARY AND AQUATIC PARASITOLOGY (FISH, AMPHIBIANS, ETC.)

PREVALENCE AND SPATIAL DISTRIBUTION OF LAMELLODISCUS MONOGENEANS IN SPARID FISHES LANDED ALONG THE SENEGALESE COAST

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Background and Aims: This study investigates the prevalence of *Lamellodiscus* monogeneans in 552 specimens belonging to the genera *Dentex*, *Diplodus*, and *Pagrus* along the Senegalese coastline. Overall, 404 fish were infested, corresponding to a total prevalence of 73.19%. Marked differences were observed among host taxa: *Dentex* showed the lowest prevalence (49.79%), whereas *Diplodus* and *Pagrus* exhibited significantly higher values (88.32% and 94.18%, respectively). At the species level, *Pagrus caeruleostictus* emerged as the most heavily parasitized species, while *Dentex gibbosus* and *Dentex maroccanus* displayed the lowest infestation rates, suggesting potential interspecific differences in susceptibility or exposure. Spatial variation was also evident, with fish landed at Hann Bel-Air being more heavily infested than those from Soumbédioune, indicating that local environmental factors may influence parasite transmission dynamics.

Methods: Specimens were transported fresh to the Laboratory of General Parasitology at Cheikh Anta Diop University, identified following Bellemans et al. (1988), measured, and dissected. Gill arches were examined under a stereomicroscope, and Diplectanidae were isolated and mounted for microscopic observation according to the protocols of Malmberg (1957) and Bates (1997). Parasite structures were measured using an OPTIKA light microscope equipped with a digital camera, following the morphometric criteria proposed by Neifar et al. (2004).

Results: These findings confirm the widespread distribution of *Lamellodiscus* along the Senegalese coast and reveal clear variations linked to host taxonomy and sampling site. The results highlight the importance of ecological and environmental factors in shaping the epidemiology of these monogeneans.

Conclusions: Lamellodiscus shows widespread distribution, with host specificity and environmental factors strongly shaping infection patterns.

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PARASITE ECOLOGY, BIODIVERSITY, AND TAXONOMY / VETERINARY AND AQUATIC PARASITOLOGY (FISH, AMPHIBIANS, ETC.)

MUCH IS THERE BUT YET UNKNOWN: A LITERATURE REVIEW OF HELMINTHS OF AMPHIBIANS AND REPTILES IN MIDDLE AMERICA

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Background and Aims: Amphibians and reptiles play key ecological roles in terrestrial and freshwater ecosystems, yet their parasite diversity remains poorly documented compared to mammals and birds, particularly in biodiversity hotspots such as Middle America. Helminths associated with herpetofauna are relevant not only for wildlife health and conservation, but also within a One Health framework due to their zoonotic potential (e.g. *Spirometra*).

Methods: We conducted a comprehensive literature review of helminths reported from herpetofauna across Middle America, defined as the region extending from the Isthmus of Tehuantepec (Mexico) to the Uraba–Cupica region (Colombia). Extracted data included host and parasite taxonomy, geographic location, and collections. A total of 71 publications were analyzed, yielding 1,195 helminth records in Middle-American herpetofauna.

Results: Nematodes dominated the helminth records from Middle-American herpetofauna (64.1%), with *Aplectana incerta* and *A. itzocanensis* as the most frequently reported species, followed by Platyhelminthes (28.6%), with *Mesocestoides* sp. representing a zoonotic risk, as herpetofauna can act as intermediate hosts in complex transmission cycles. A single record of encysted plerocercoid larvae suggests involvement in zoonotic cestodes such as *Spirometra* sp. Studies were concentrated in Mexico and Costa Rica, while Honduras and El Salvador lacked records. Parasite diversity was highest in Anura and Squamata, with geographic and institutional biases evident.

Conclusions: Our review reveals substantial taxonomic, geographic and research-effort gaps in the study of helminths of Middle American herpetofauna. Addressing these gaps through increased endogenous research, expanded sampling in underrepresented regions, and integration of molecular tools is essential to better understand host–parasite dynamics, assess zoonotic risks, and support conservation.

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SHIFT01-100

POSTER ON BOARD SHIFT 01
PARASITE ECOLOGY, BIODIVERSITY, AND TAXONOMY / VETERINARY AND AQUATIC PARASITOLOGY (FISH, AMPHIBIANS, ETC.)

INTO COLD WATERS: DIVERSITY OF SKATES (RAJIFORMES: RAJIDAE) AND THEIR PARASITES IN ICELAND

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Background and Aims: Parasites comprise a significant part of the global biodiversity, although most remain undiscovered. Cartilaginous fishes (Chondrichthyes) host a multitude of parasitic organisms that are being negatively impacted along with their hosts due to increased anthropogenic stresses. Considering the ecological significance of both groups, conservation efforts are crucial. Skates, which exhibit high levels of endemism, emphasise this need. Previously, only 3 of 15 native skate species in Iceland have been assessed for parasites. Here we assess the parasites of skates obtained from surveys off Iceland and Northern Ireland and from historical collections in London's NHM.

Methods: Stomachs, spiral valves, gills, and cloacae were examined for helminths from 101 skates, including *Amblyraja radiata*, *A. hyperborea, Bathyraja spinicauda, Dipturus batis, D. intermedius*, *Malacoraja kreffti*, *M. spinacidermis*, *Rajella bigelowi*, *R. fyllae*, and *R. lintea*.

Results: For monogeneans, *Calicotyle* sp. was found in *A. radiata*, and *Rajonchocotyle* sp. in *R. fyllae* and *R. bigelowi*. The trematode *Otodistomum veliporum* was detected in *A. radiata*. For nematodes, *Anisakis* sp. was found in most host species. Seven cestode morphotypes were detected including the
genera *Echinobothrium* (1), *Rockacestus* (1), *Zyxibothrium* (1), *Echeneibothrium* (2), *Pseudanthobothrium* (1), and *Grillotia* (1). Future molecular work and parasite species (re-)descriptions will enable host specificity assessments.

Conclusions: Understanding the ecology of parasite species associated with threatened skate species will allow us to identify factors that contribute to the conservation of these host-parasite systems. This will ensure better maintenance of the integrity of the natural systems that support these symbiotic associations and ultimately their conservation.

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SHIFT01-101

POSTER ON BOARD SHIFT 01
PARASITE ECOLOGY, BIODIVERSITY, AND TAXONOMY / VETERINARY AND AQUATIC PARASITOLOGY (FISH, AMPHIBIANS, ETC.)

DETECTION OF CRYPTIC SPECIES DIVERSITY AND DEVELOPMENT OF A RAPID IDENTIFICATION APPROACH FOR THE TREMATODE GENUS SCAPHANOCEPHALUS FROM OFF ISHIGAKI-JIMA ISLAND, JAPAN

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Background and Aims: Encysted trematode metacercariae can produce conspicuous black/yellow/white spots on marine fishes and reduce market value in the Ryukyu Archipelago, Japan. Although Japanese material has so far been identified morphologically as *Scaphanocephalus adamsi*, molecular data suggest cryptic diversity. We report two co-occurring *Scaphanocephalus* lineages off Ishigaki Island and develop a rapid PCR–RFLP assay for identification.

Methods: Metacercariae were collected from landed parrotfishes and goatfishes around Ishigaki-jima Island. Larvae were provisionally assigned to *Scaphanocephalus* by external appearance and then measured (cyst diameter, body shape) to assess morphological separability. A 28S rDNA fragment (1218–1219 bp) was PCR-amplified, and restriction enzymes were screened to select diagnostic polymorphisms and an enzyme combination that distinguishes the two lineages and reference sequences in public databases, enabling a PCR–RFLP assay.

Results: Metacercarial morphology was highly variable (slim/round/intermediate) and did not match genotypes. In contrast, a two-enzyme PCR–RFLP scheme (BtsCI + MluCI) discriminated the two Ishigaki-jima lineages with 100% concordance (n=15), enabling rapid, low-cost identification.

Conclusions: Morphology alone could not reliably separate the cryptic lineages, whereas PCR–RFLP enables rapid, large-scale identification needed to compare ecological traits (e.g., microhabitat use, seasonality, host suitability, and site preference). The same enzyme set may also be applicable to American congeners (*S. expansus* and *S. robustus*), pending broader validation.

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SHIFT01-102

POSTER ON BOARD SHIFT 01
PARASITE ECOLOGY, BIODIVERSITY, AND TAXONOMY / VETERINARY AND AQUATIC PARASITOLOGY (FISH, AMPHIBIANS, ETC.)

TAXONOMIC REVISION AND PHYLOGENY OF EIGHT SPECIES IN THE COPEPOD GENERA CLAVELLA AND PRAECLAVELLA (SIPHONOSTOMATOIDA: LERNAEOPODIDAE) PARASITIZING MARINE DEMERSAL FISHES FROM JAPAN

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Background and Aims: Lernaeopodidae are fish-parasitic copepods whose adult females attach to gills or skin using a bulla, and their extensive segmental fusion limits diagnostic morphology. Among Japanese lernaeopodids, *Clavella* and closely related taxa lack modern redescriptions and linked DNA barcodes, hindering comparisons. We thus generated DNA barcodes and assessed genus boundaries using morphology and molecular phylogenetics.

Methods: Host fishes were obtained from fishers or markets. The body surface, fins, and oral cavity were examined; gills were removed, placed in artificial seawater, and screened under a stereomicroscope. Parasites were preserved in 99.5% ethanol for DNA analyses or 70% ethanol for morphology. Partial 28S rDNA and cox1 sequences were compared with public reference sequences.

Results: We recovered *Clavella collaris*, *C. diversia*, *C. gadomi*, *C. perfida*, one undescribed species of this genus, and three species currently assigned to *Praeclavella* (*P. irina*, *P. ovata*, and *P. scombropis*). *Clavella perfida*, *P. irina*, and the undescribed species were from gadids (gadiform), whereas others were from macrourids (gadiform), synagropids, and scombropids (both acropomatiforms). Phylogenetically, all formed a monophyletic clade, with the gadid-derived species clustering as a terminal subclade.

Conclusions: Our phylogeny does not support recent transfers of some *Clavella* species including several Japanese species to *Praeclavella* and indicates that boundaries between both genera require redefinition. Although the cosmopolitan gadiform parasite *C. adunca* was not recovered in this study, the gadid-derived species we sampled include an undescribed lineage, suggesting cryptic diversity within gadiform-associated congeners. Host-use patterns suggest shifts from acropomatiform hosts to gadiforms, informing reconstructions of historical host ranges and shallow-to-deep habitat expansion.

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SHIFT01-103

POSTER ON BOARD SHIFT 01
PARASITE ECOLOGY, BIODIVERSITY, AND TAXONOMY / VETERINARY AND AQUATIC PARASITOLOGY (FISH, AMPHIBIANS, ETC.)

MORPHOLOGICAL AND GENETIC CHARACTERIZATION OF THE NEMATODE CONTRACAECUM SP. LARVAE (ANISAKIDAE) REGISTERED IN CARP

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Background and Aims: Nematodes from the family Anisakidae are parasites that have a worldwide distribution

Methods: A total of 98 carp (*Cyprinus carpio*) of both sexes were collected from fisheries in the Surkhandarya and Jizzakh regions during 2025–2026.

Results: Among these, 11 individuals were found to be infected with *Contracaecum* sp. larvae, representing a prevalence of 9.1% and an infection intensity ranging from 1 to 3 specimens per fish. Morphological analysis revealed that the nematode samples were characteristically light brownish-yellow. The morphological features of the *C. quadripapillatum* L3 larvae obtained from carp (*n*=10) were as follows: body length 13.3–25.8 (17.6±0.26) mm, width 0.86–2.6 (1.24±0,3) mm. The mouth is surrounded by prominent papillae and three small lips, with a mouth length of 0.03–0.06 (0.03±0.01) mm. The length of the esophagus was 2.7–4.3 (3.6±0.03) mm, the length of the ventricular appendix was 0.74–1.00 (0.86±0.02) mm, and the intestinal cecum length was 1.76–3.8 (2.82±0.01) mm. The ratio of the ventricular appendix to the cecum length was 30.5%. Molecular genetic analysis yielded a nucleotide sequence of over 800 base pairs originating from the ribosomal DNA ITS1-5.8S-ITS2 region of the isolated *Contracaecum* sp. larvae. A comparison of this sequence with the *C. quadripapillatum* reference sequence (GenBank accession no. ON714982) in the NCBI database revealed only two nucleotide differences, representing a 0.2% genetic divergence. Furthermore, phylogenetic analysis based on ITS region sequences placed the studied larvae sample in the same clade as *C. quadripapillatum* with 98% bootstrap support.

Conclusions: This represents the first record of *C. quadripapillatum* in carp (*Cyprinus carpio*) within Uzbekistan.

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POSTER ON BOARD SHIFT 01
PARASITE ECOLOGY, BIODIVERSITY, AND TAXONOMY / VETERINARY AND AQUATIC PARASITOLOGY (FISH, AMPHIBIANS, ETC.)

HALOCERCUS PINGI: A HIGHLY LETHAL NEMATODE IN NEONATAL FINLESS PORPOISES

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Background and Aims: *Halocercus pingi* is a highly lethal pulmonary nematode repeatedly associated with fatal outcomes in neonatal toothed whales. The parasite has been detected exclusively in pre-feeding neonates less than one week of age and causes extremely heavy infections that obstruct the trachea and bronchi, leading to acute respiratory failure and death by asphyxiation. This strict age specificity strongly suggests vertical transmission, although the exact pathway remains unknown. This study aimed to characterize fatal *H. pingi* infection in neonatal toothed whales using im

Methods: Neonatal toothed whales recovered from the Korean coast were examined using an integrated postmortem approach. Whole-body computed tomography was performed prior to necropsy (virtopsy) to assess airway obstruction and pulmonary involvement. Conventional necropsy focused on the trachea, bronchi, and lungs. Recovered nematodes were identified by morphological examination and PCR-based molecular analysis. Respiratory tissues were examined histopathologically.

Results: *Halocercus pingi* infection was detected exclusively in neonates less than one week of age. All cases showed extremely heavy parasite burdens filling the tracheobronchial tree, resulting in complete or near-complete airway obstruction. CT imaging clearly demonstrated intraluminal airway occlusion prior to necropsy. Histopathological analysis revealed severe luminal obstruction with mucosal damage and inflammatory responses consistent with fatal respiratory failure. Molecular analyses confirmed all nematodes as *H. pingi*.

Conclusions: *Halocercus pingi* represents a major but underrecognized cause of early-life mortality in small toothed whales. The findings strongly support vertical transmission and highlight the need for systematic examination of fetuses and neonates from pregnant females to elucidate the parasite's transmission pathway and life cycle.

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POSTER ON BOARD SHIFT 01
PARASITE ECOLOGY, BIODIVERSITY, AND TAXONOMY / VETERINARY AND AQUATIC PARASITOLOGY (FISH, AMPHIBIANS, ETC.)

FROM WILD WATERS TO HUMAN HEALTH: A PILOT STUDY TO ADDRESS THE KNOWLEDGE GAP ON ZOONOTIC HELMINTHS IN FRESHWATER FISH (EURO-PAR-FISH)

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Background and Aims: In 2016 a consortium consisting of Poland (as a leader), Italy, France and Germany as well as Serbia and Latvia as observers began work on implementing the Tailor Made (TM) project announced by European Food Safety Agency (EFSA). In line with EFSA 2024 opinions, this project aims to fill knowledge gaps on zoonotic foodborne parasites in wild freshwater fish. This initiative underscores collaboration among partners to foster long-term partnerships in researching zoonotic helminths in wild freshwater fish.

Methods: The partners will collect the samples and perform the analyses to detect fishborne zoonotic parasites in the chosen species of freshwater fishes. The detected parasites will be identified on a morphological basis, followed by molecular biology tools with use of sequencing to confirm their species identification. After completing the analytical phase a final database will be created which will serve as a foundation for creating a spatial database—map and will facilitate risk assessment efforts.

Results: The project is currently in the initial implementation phase. Results obtained during the first 6 months of the project will be presented at the ICOPA conference.

Conclusions: This TM activity will benefit both EFSA and each partner country, as well as the entire community of freshwater fish consumers at risk from these FBZH. The project will deliver benefits, including the establishment of standard operating procedures (SOP) for the detection and molecular characterization of parasites, training sessions for partners, creation of database with epidemiological data) and genetic data from molecular characterization, including a map of FBZH occurrence and risk assessment.

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SHIFT01-106

POSTER ON BOARD SHIFT 01
PARASITE ECOLOGY, BIODIVERSITY, AND TAXONOMY / VETERINARY AND AQUATIC PARASITOLOGY (FISH, AMPHIBIANS, ETC.)

NEMATODE PARASITES OF TWO SPECIES OF CAMALLANUS (CAMALLANIDAE) FROM PIKE-PERCH FISHES IN THE UZBEKISTAN

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Background and Aims: Nematodes of the family Camallanidae Railliet et Henry, 1915, are mostly gastrointestinal parasites of marine, brackish-water and freshwater fishes.The aim of the work is to study prevalence and morphology of adult individuals of two nematode species belonging to the genus Camallanus, recovered from the fish Sander lucioperca (pike-perch) distributed in the water bodies of Uzbekistan.

Methods: Nematodes were collected on June 12 and August 14, 2025, from pike-perch in the Shurkul and Jizzakh reservoirs and subjected to helminthological examination.

Results: The species Camallanus truncatus was identified from the stomach of pike-perch from the Jizzakh reservoir, and the species C. lacustris from the the Shurkul reservoir. The prevalence of C. truncatus was 14.3 % and the infection intensity was 2-13 copies. The length of the body of male C. truncatus 4.7-7.0 mm, body width 0.12-0.18 mm. The length body of a female 4.8-12.0 mm and body width 0.19-0.25 mm. The chitinous tridents of the buccal capsule are long and extend beyond half the distance between the buccal capsule and the nerve ring. The prevalence C. lacustris 7.7 % and the infection intensity 1-16 copies. T The body of length of male C. lacustris 2.29-6.23 mm, the width 0.16-0.22 mm. The length of the female 6.5 -12.0 mm and the body width 0.21-0.29 mm. he chitinous tridents of the buccal capsule are short.

Conclusions: These morphometric parameters are generally consistent with the data presented in the previous literature and we propose conducting molecular research.

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SHIFT01-107

POSTER ON BOARD SHIFT 01
PARASITE ECOLOGY, BIODIVERSITY, AND TAXONOMY / VETERINARY AND AQUATIC PARASITOLOGY (FISH, AMPHIBIANS, ETC.)

FIRST RECORD OF PARADILEPIS SCOLECINA (RUDOLPHI, 1819) HSÜ, 1935 (CESTODA: GRYPORHYNCHIDAE) IN REPUBLIC OF KOREA WITH NOTES ON ULTRASTRUCTURE AND ECOLOGICAL RISK IN GREAT CORMORANTS, PHALACROCORAX CARBO SINENSIS (STAUNTON, 1796) (AVES: PHALACROCORACIDAE)

Heon Woo Lee[1], Ha-Eun Lee[1], Changhyeon Lee[1], Hyunmin Oh[1], Emmanuel Opara[1], Mohammed Mebarek Bia[2], Sunmin Kim[3], Subin Lee[4], Seung-Hun Lee[4], Seongjun Choe[1,5]
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Background and Aims: *Paradilepis scolecina* is a widely distributed gryporhynchid cestode that uses piscivorous birds as definitive hosts, but it has not previously been recorded in the Republic of Korea. The great cormorant (*Phalacrocorax carbo sinensis*) has recently shown an increasing tendency toward year-round residency in Korea, potentially facilitating the establishment of trophically transmitted parasites. This study aimed to document the first occurrence of *P. scolecina* in Korea and to characterize the species using integrative morphological, molecular, and ultrastructural approaches.

Methods: Adult cestodes were collected from the intestines of resident great cormorants in Gangwon Province, Korea. Species identification was conducted using light microscopy based on established taxonomic keys, scanning electron microscopy (SEM) for tegumental ultrastructure, and molecular analyses targeting LSU and SSU rRNA genes. Phylogenetic analyses were performed using maximum likelihood and Bayesian inference methods.

Results: The recovered specimens exhibited 20 parvoid rostellar hooks arranged in a double row, conforming to the scolecina-type hook pattern. LSU rRNA sequences showed 99.9% identity with *P. scolecina* isolates from South Africa, and SSU rRNA sequences clustered within the *Paradilepis* clade, supporting species-level identification. SEM revealed a previously undescribed cirral ultrastructure characterized by rose-thorn–shaped spines interspersed with capilliform filitriches. The parasite showed high prevalence and infection intensity in examined hosts.

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Conclusions: This study represents the first confirmed record of *P. scolecina* in Korea and provides novel ultrastructural data for the family Gryporhynchidae. The occurrence of this cestode in increasingly resident cormorant populations highlights the need for further studies on transmission dynamics and potential ecological implications in Korean freshwater ecosystems.

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POSTER ON BOARD SHIFT 01
PARASITE ECOLOGY, BIODIVERSITY, AND TAXONOMY / VETERINARY AND AQUATIC PARASITOLOGY (FISH, AMPHIBIANS, ETC.)

FIRST RECORD OF POMPHORHYNCHUS SEBASTICHTHYDIS YAMAGUTI, 1939 (ACANTHOCEPHALA: POMPHORHYNCHIDAE) IN THE KOREAN ROCKFISH, SEBASTES SCHLEGELII (HILGENDORF, 1880), FROM KOREA.

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Background and Aims: Acanthocephalans of the genus *Pomphorhynchus* are intestinal parasites of freshwater and marine fishes distributed across Eurasia. *Pomphorhynchus sebastichthydis* Yamaguti, 1939 was originally described from oblong rockfish in Japan but has not previously been recorded from Korean waters. The aim of this study was to document the first confirmed occurrence of *P. sebastichthydis* in Korea based on morphological and molecular evidence and to clarify the regional occurrence of marine acanthocephalans in East Asian coastal waters.

Methods: Seven Korean rockfish (*Sebastes schlegelii*) collected from the Yellow Sea off Taean-gun, Korea, were examined for intestinal parasites. Recovered acanthocephalans were examined under light microscopy following staining with Semichon's acetocarmine and standard mounting procedures. Genomic DNA was extracted from adult specimens, and the small subunit ribosomal RNA (SSU rRNA) gene was amplified and sequenced. Phylogenetic analyses were conducted using maximum likelihood and Bayesian inference methods.

Results: Seven adult acanthocephalans were recovered from a single host specimen (prevalence: 1/7). The specimens exhibited diagnostic morphological characters of *P. sebastichthydis*, including a proboscis bearing 12 longitudinal rows of 11–12 hooks and the presence of a characteristic neck bulb. SSU rRNA sequence analysis placed the specimens within the family Pomphorhynchidae, supporting the morphological identification.

Conclusions: This study represents the first confirmed record of *Pomphorhynchus sebastichthydis* from Korean marine waters and from *Sebastes schlegelii*. The findings provide baseline morphological and molecular data for this poorly documented species and highlight the need for further surveys and molecular characterization of marine acanthocephalans in the region.

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POSTER ON BOARD SHIFT 01
PARASITE ECOLOGY, BIODIVERSITY, AND TAXONOMY / VETERINARY AND AQUATIC PARASITOLOGY (FISH, AMPHIBIANS, ETC.)

MOLECULAR CHARACTERIZATION OF OSTERTAGIA ANTIPINI (NEMATODA: OSTERTAGIINAE) AND ITS ALTERNATIVE MORPHOTYPE, OSTERTAGIA LYRATAEFORMIS, IN ALCES ALCES

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Background and Aims: Accurate identification of parasite species is essential for wildlife surveillance, where interspecific transmission of parasites between domestic animals and wildlife is possible. Male polymorphisms within the same species are frequently observed in the subfamily Ostertagiinae, which hinders our understanding of parasite composition in wild cervids. *Ostertagia antipini* Matschulski, 1950 is a major gastrointestinal nematode of *Alces alces*, and *Ostertagia lyrataeformis* Dróżdż, 1965 is reported as its alternative morphotype. We conducted a phylogenetic analysis to clarify their species status as molecular information was not available for either *O. antipini* or *O. lyrataeformis*.

Methods: Male worms were collected from the intestines of culled *A. alces* calves in Norway. The posterior parts of the worms were cleared with lactophenol solution and subjected to morphological identification. DNA was extracted from the anterior parts, followed by PCR targeting the ~300-bp ITS2 region.

Results: *Ostertagia antipini* and *O. lyrataeformis* were distinguished by their spicule morphology. Three sequences were obtained for *O. antipini* and one for *O. lyrataeformis*. All four sequences showed 100% homology but showed 2.1–5.9% pairwise sequence divergence from *O. antipini* collected from *A. alces* in Russia. Instead, the phylogenetic relationships inferred using the maximum likelihood placed the sequences within *Ostertagia*

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leptospicularis (Figure).

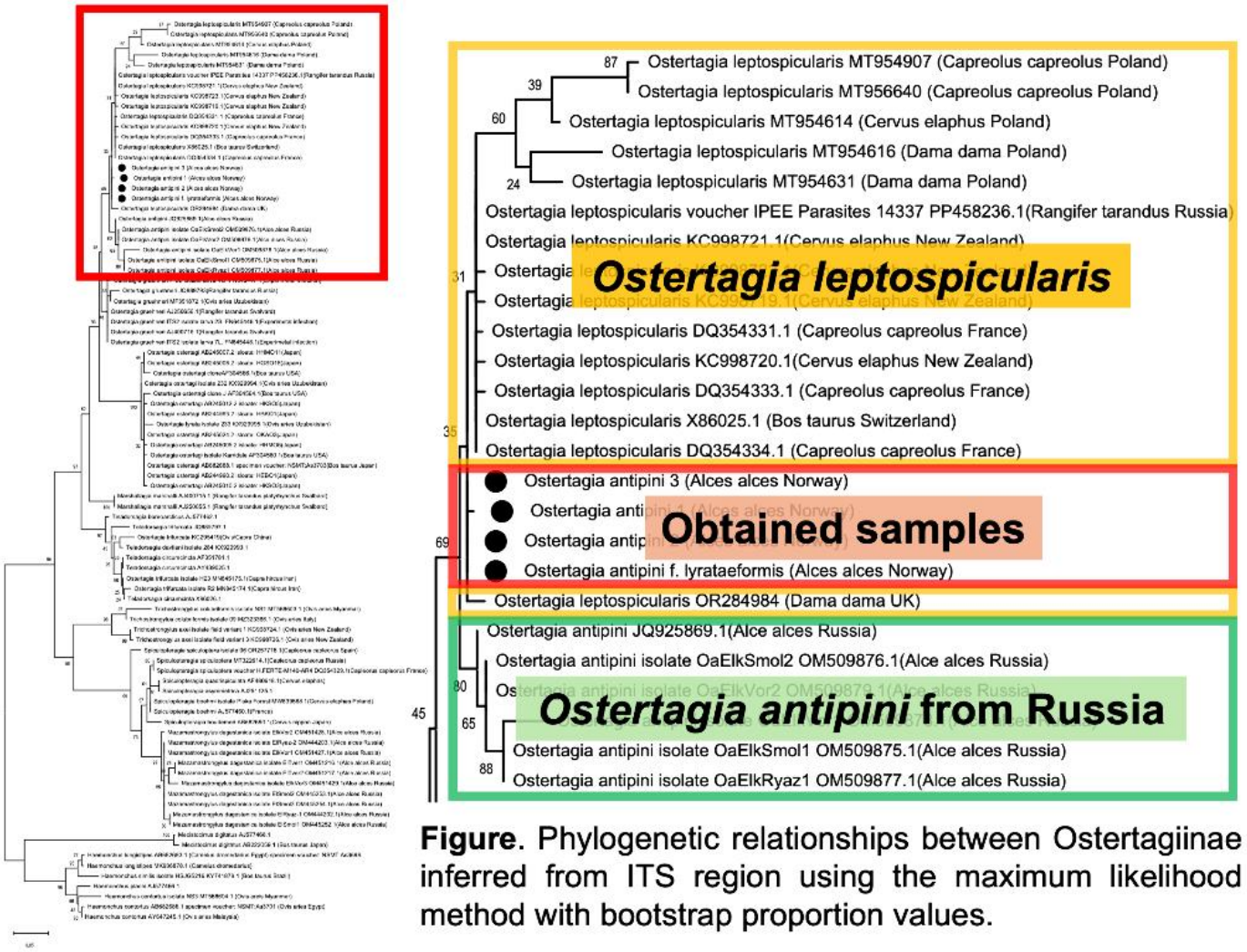


Figure. Phylogenetic relationships between Ostertagiinae inferred from ITS region using the maximum likelihood method with bootstrap proportion values.

Conclusions: Here we demonstrated that *O. lyrataeformis* is an alternative morphotype of *O. antipini*. However, our sequences clustered with *O. leptospicularis*, a species commonly found in *Capreolus capreolus*. Based on preliminary results, these two species may form a species complex, however further analysis is currently in progress.

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POSTER ON BOARD SHIFT 01
PARASITE ECOLOGY, BIODIVERSITY, AND TAXONOMY / VETERINARY AND AQUATIC PARASITOLOGY (FISH, AMPHIBIANS, ETC.)

EFFECTS OF EXPERIMENTAL INFECTIONS ON HOST METABOLISM AND OXIDATIVE DAMAGE IN NAÏVE AND EXPERIENCED POPULATIONS

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Background and Aims: Populations can experience differences in their biotic and abiotic environment, which can lead to divergence in a number of important morphological, behavioural and physiological traits. Exposure to parasites, in particular, can lead to population-level differences in energy allocation, which may result in altered metabolic activities among populations experiencing different levels of infection risk. The degree to which parasite exposure drives differences in whole organisms and sub-cellular metabolic rates across populations has rarely been explored. We aim to explore why differences at a whole-organism level often occurs through sub-cellular mechanisms.

Methods: Using field mesocosms in natural lakes, we investigate cellular mechanisms underlying changes in oxygen uptake following parasite infection in a parasite-naïve and a parasite-experienced population of pumpkinseed sunfish. Oxygen uptake in respirometers was measured in deparasitised fish before and after exposure to parasite infections in lake mesocosms. These whole-organisms aerobic performance metrics were then linked to cellular responses associated with immune stimulation, including oxidative damage, maximal mitochondrial enzyme activity, and antioxidant activity.

Results: Susceptibility to infection differs between populations, with the highest experimentally induced parasite infections found in the naïve population. We also found differences in both whole-organism and sub-cellular estimates of aerobic metabolism between populations depending on infection treatment.

Conclusions: Population-level differences in infection susceptibility may reflect metabolic adaptations in the parasitized population that reduce the costs or damages of infection, resulting in lower oxidative damage and smaller increases in mitochondrial activity. This study provides insights into why populations and species exhibit divergent responses to parasite infections based on their environmental conditions.

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POSTER ON BOARD SHIFT 01
PARASITE ECOLOGY, BIODIVERSITY, AND TAXONOMY / VETERINARY AND AQUATIC PARASITOLOGY (FISH, AMPHIBIANS, ETC.)

MOLECULAR DIVERSITY OF EQUINE STRONGYLE PARASITES IN ALBERTA HORSES REVEALED BY NEMABIOME METABARCODING

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Background and Aims: Strongyles are the most prevalent parasites of equids worldwide and occur as complex mixed-species infections associated with varied pathological outcomes. Because disease expression depends on the species involved, accurate species-level diagnosis is essential for effective parasite control. However, routine diagnostics such as fecal egg counts(FEC) cannot differentiate strongyle species. Recent advances in molecular diagnostics, particularly equine ITS2 nemabiome metabarcoding, enable species-level profiling of strongyle communities directly from fecal samples. Despite the economic importance of the horse industry in Alberta, province-wide molecular data on strongyle diversity are lacking. This study aims to characterize strongyle community composition in Alberta horses using ITS2 metabarcoding, providing a baseline for regional surveillance and evidence-based parasite control.

Methods: From May-October 2023, fecal samples were collected from 474 domestic horses and 12 donkeys in Alberta. Metadata, including age, sex, farm type, and treatment hisrory were recorded. Strongyle infection was confirmed using McMaster FEC method. Larval coprocultures were prepared from positive samples, and third-stage larvae were harvested for molecular analysis. DNA was extracted from pooled third-stage larvae and ITS2 region was amplified and sequenced using Illumina MiSeq metabarcoding to characterize strongyle community composition at the species level.

Results: Strongyle eggs were detected in approximately 70% of sampled equids, confirming widespread infection across the study population. ITS2 nemabiome sequencing and bioinformatic analyses are ongoing and will characterize strongyle species composition, and community structure across Alberta equid populations.

Conclusions: By applying ITS2 nemabiome metabarcoding, this study advances species-level surveillance of equine strongyles in Alberta and supports improved detection of pathogenic and potentially re-emerging species.

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SHIFT01-112

POSTER ON BOARD SHIFT 01
PARASITE ECOLOGY, BIODIVERSITY, AND TAXONOMY / VETERINARY AND AQUATIC PARASITOLOGY (FISH, AMPHIBIANS, ETC.)

PARASITES OF THE SALMON (SALMO SALAR) FROM THE SOUTHERN BALTIC SEA

Katarzyna Nadolna-Ałtyn[1], Joanna Pawlak[1], Magdalena Podolska[1], Adam Lejk[2]
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Background and Aims: Salmon *Salmo salar* is an important commercially fished species. Despide of that, little is known about parasite fauna of this fish in the southern Baltic Sea. The dispersion of the Anisakidae nematodes in this area has been observed in recent years. The parasites may affect the condition of the fish. The aim of our studies was to analyse the presence of parasites and its impact on condition of salmon sampled in Polish marine waters.

Methods: The condition of salmons sampled in 2005-2025 was analysed using Generalized Linear Models. The presence of liver and intestinal parasites in fish collected in 2020-2025 was analysed.

Results: The GLM showed a statistically significant decreasing linear trend in salmon condition between 2005 and 2017, promising rise between 2018 and 2023 and sharp decline in 2024. All analysed fish were infected with intestinal parasite *Eubothrium* sp. Anisakidae nematodes such as *Contracaecum* sp. and *Pseudoterranova* sp. were detected in the livers of salmon. The prevalence of infection with Anisakids differs among years, reaching up to 24% in 2024.

Conclusions: The presence of *Eubothrium* sp. may significantly affect the intake of nutrients. Moreover, parasitic infections may negatively influence salmon condition. The occurrence of zoonotic nematodes in fish may also pose a risk to human health.

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POSTER ON BOARD SHIFT 01
PARASITE ECOLOGY, BIODIVERSITY, AND TAXONOMY / VETERINARY AND AQUATIC PARASITOLOGY (FISH, AMPHIBIANS, ETC.)

DIET COMPOSITION OF ATLANTIC SALMON (SALMO SALAR) AND ITS ROLE IN THE TRANSMISSION OF ANISAKIDAE NEMATODES IN THE BALTIC SEA

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Background and Aims: Atlantic salmon (*Salmo salar*) is one of the top predators in the Baltic Sea, alongside marine mammals. Fish diet provides valuable information on trophic relationships and potential parasite transmission. In recent years, increasing dispersion of Anisakidae nematodes (*Contracaecum* sp., *Anisakis* sp., *Pseudoterranova* sp.) has been reported in Baltic marine organisms. This study aimed to determine the potential transmitters of salmon infection with Anisakidae nematodes based on diet composition.

Methods: Salmon stomachs collected from the Baltic Sea between 2020 and 2025 were analysed. Prey items were identified to the lowest possible taxonomic level. Based on the frequency of occurrence of food items, potential transmitters of infection were assessed.

Results: Among the examined years, the most common food component in salmon stomach was sprat *Sprattus sprattus*. Other fish, such as herring *Clupea harengus*, three-spined stickleback *Gasterosteus aculeatus*, and great sandeel *Hyperoplus lanceolatus*, were also recorded. The frequency of occurrence of invertebrates in the diet was low; however, crustaceans such as *Gammarus* spp. and *Mysis mixta* were found in stomachs.

Conclusions: Fish species that occur in the diet of salmon could also be a source of infection of parasite, which our previous studies confirmed for sprat or great sandeel. Due to the fact that the most frequent food item in salmon stomachs was sprat, it may play an important role as a transmitter of Anisakidae nematodes. The presence of zoonotic nematodes in fish may pose a risk not only to the condition of fish but also to human health.

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POSTER ON BOARD SHIFT 01
PARASITE ECOLOGY, BIODIVERSITY, AND TAXONOMY / VETERINARY AND AQUATIC PARASITOLOGY (FISH, AMPHIBIANS, ETC.)

PARASITOLOGICAL MONITORING OF EUROPEAN BISON (BISON BONASUS) IN A LARGE DANISH NATURE RESERVE

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Background and Aims: Large grazers are increasingly used in nature conservation areas in Denmark to promote open, diverse landscapes. It is well known that certain parasites pose a health risk for grazing ruminants, including European bison. The aim was to conduct a longitudinal survey of endoparasites in European bison.

Methods: The study included a herd of 31 European bison kept in a 4,000-hectare nature area with bogs, meadows, and forests. Since 2022, faecal samples were collected 3-5 times annually and analysed using traditional parasitological methods. In 2024, 10 bison were immobilised, and faecal samples were collected for ITS-2 sequencing to identify nematode species. Blood samples were collected for molecular analysis of blood-borne pathogens.

Results: Mean strongyle faecal egg count (FEC) ranged between 28-112 eggs/g (EPG), with a prevalence of 61-100%. Liver fluke ranged between 0-52 EPG with prevalences of 0-100%, while rumen fluke ranged between 0-13 EPG with prevalences of 44-100%. Liver fluke FEC increased in fall 2022, and the flock was treated with triclabendazole. This pattern was observed again in 2023 and 2024, peaking with high FEC in January 2025. ITS-2 sequencing showed *Trichostrongylus, Haemonchus, Ostertagia*, and *Cooperia* as the most frequent strongyles. Blood microbiome analysis revealed that bisons were positive for *Babesia*, *Anaplasma*, and *Trypanosoma*.

Conclusions: The strongyle FEC were at low levels. ITS-2 sequencing revealed a proportion of *H. contortus* which was unsuspected due to low FEC. The liver fluke FEC gradually increased, despite regular deworming. Our results support the need for regular monitoring due to large seasonal variations. The study is ongoing.

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POSTER ON BOARD SHIFT 01
PARASITE ECOLOGY, BIODIVERSITY, AND TAXONOMY / VETERINARY AND AQUATIC PARASITOLOGY (FISH, AMPHIBIANS, ETC.)

INTEGRATIVE MORPHOLOGICAL AND MOLECULAR ANALYSES REVEAL NEW PROCAMALLANUS SPECIES (NEMATODA: CAMALLANIDAE) FROM FRESHWATER FISHES OF THE SOUTHERN PERUVIAN

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Background and Aims: Parasitic nematodes of the family Camallanidae are diverse and widely distributed in freshwater and marine fishes, but their taxonomy is often complicated by strong morphological similarity among species. As part of a broader program investigating the diversity of parasitic nematodes infecting freshwater fishes from the Peruvian Amazon, this study aimed to assess the taxonomic status of camallanid nematodes parasitizing two fish species using an integrative morphological and molecular approach.

Methods: Between August and September 2025, specimens of *Pimelodina flavipinnis* and *Calophysus macropterus* were collected by artisanal fishermen from the Madre de Dios River (Puerto Maldonado, Peru). Nematodes were examined morphologically and analyzed molecularly using 18S rRNA sequences and phylogenetic analysis.

Results: All specimens were assigned to *Procamallanus* (*Spirocamallanus*) based on the presence of spiral thickenings in the buccal capsule. Specimens from *C. macropterus* showed morphological similarities to *P. rarus*; however, phylogenetic analyses revealed clear genetic divergence, and males differed in the morphology of the major spicule, supporting species-level distinction. Specimens from *P. flavipinnis* differed from *P. rarus* mainly in tail morphology and formed a closely related but distinct lineage in the phylogenetic tree. Together, morphological and molecular evidence supports the presence of two distinct, previously undescribed species.

Conclusions: Our findings highlight the limitations of morphology-based taxonomy in *Procamallanus* and emphasize the importance of integrative approaches to uncover hidden diversity in Amazonian freshwater parasites.

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POSTER ON BOARD SHIFT 01
PARASITE ECOLOGY, BIODIVERSITY, AND TAXONOMY / VETERINARY AND AQUATIC PARASITOLOGY (FISH, AMPHIBIANS, ETC.)

FIRST INTEGRATIVE RECORD OF A SPIRUROMORPH NEMATODE LARVA PARASITIZING BRACHYPLATYSTOMA PLATYNEMA IN THE PERUVIAN AMAZON

Nicolas Tarmeño Rojas, Sergio Santillan Quiñones, David Lopez Godoy, Ruperto Severino, Jhon Chero
Universidad Nacional Mayor de San Marcos, Facultad De Ciencias Biológicas, Lima, Peru

Background and Aims: *Brachyplatystoma platynema* is a large Amazonian catfish with ecological importance in riverine ecosystems, yet its parasitological diversity remains virtually unknown. Despite reports of spiruromorph nematodes infecting freshwater fishes worldwide, larval forms are rarely documented in the Neotropical region and often remain unidentified due to limited morphological resolution and scarce molecular data. This study reports the first record of a nematode larva infecting *B. platynema* in the southern Peruvian Amazon and aims to clarify its taxonomic identity and phylogenetic position using an integrative morphological and molecular approach.

Methods: In August 2024, two specimens of *B. platynema* were collected from the Madre de Dios River, Peru. Nematode larvae recovered from the digestive tract were morphologically examined using light microscopy and scanning electron microscopy, and molecularly characterized through sequencing of the 18S rRNA gene followed by phylogenetic analysis.

Results: A total of 135 nematode larvae were recovered. The specimens exhibited a well-developed vestibule, consistent with third-stage spiruromorph larvae, but differed from previously described taxa in tail morphology and in the relative proportions of the muscular and glandular oesophagus. Phylogenetic analyses placed the sequences within a well-supported clade of Spiruromorpha, forming a sister relationship with species of *Cyrnea* and an unidentified spiruromorph lineage.

Conclusions: This study provides the first integrative morphological and molecular characterization of a spiruromorph nematode infecting *B. platynema* and expands current knowledge of parasite diversity in Amazonian freshwater fishes. The findings highlight the importance of combining morphology and molecular data to uncover overlooked parasite diversity in poorly studied host species.

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POSTER ON BOARD SHIFT 01
PARASITE ECOLOGY, BIODIVERSITY, AND TAXONOMY / VETERINARY AND AQUATIC PARASITOLOGY (FISH, AMPHIBIANS, ETC.)

IN VITRO EFFICACY OF DIFFERENT FORMULATIONS AGAINST THE FISH PATHOGEN SAPROLEGNIA PARASITICA

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[1]University of Bologna, Department Of Veterinary Medical Sciences, Ozzano dell'Emilia, Italy, [2]Adisseo S.A.S., Antony, France

Background and Aims: *Saprolegnia parasitica* is the major oomycete pathogen in freshwater aquaculture, causing saprolegniosis in fish and fish eggs and leading to significant economic losses worldwide, particularly in intensive farming systems, where stress and high stocking densities favor outbreaks. Despite its impact, effective and environmentally safe treatments are limited. The objective of this study was to evaluate *in vitro* the efficacy of seven formulations developed by Adisseo S.A.S. (France) against a reference strain of *S. parasitica*.

Methods: Each product was tested at five concentrations and tests were performed in triplicate, following a protocol in agar to establish the minimum inhibitory concentration (MIC), a protocol to determine the minimum lethal concentration (MLC) after one hour contact in water, and a third protocol based on continuously agitated broth technique for seven days, to establish MIC and MLC.

Results: With the first two protocols, five products exhibited inhibitory activity at different concentrations, with MLC values lower than the MIC values. Continuous-agitation broth assays performed for these five products revealed concordance between MIC and MLC values for all tested products at even lower concentrations: based on the results obtained from the latter protocol, three products showed the highest effectiveness, with MIC/MLC values of 312.5 ppm.

Conclusions: Our results highlight the effectiveness of several of the tested products against *S. parasitica*, particularly in water and liquid media. Further studies are required to determine the optimal contact time for the most effective treatments.

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POSTER ON BOARD SHIFT 01
PARASITE ECOLOGY, BIODIVERSITY, AND TAXONOMY / VETERINARY AND AQUATIC PARASITOLOGY (FISH, AMPHIBIANS, ETC.)

MYSTERY OF MESOCOELIUM IN AMPHIBIANS FROM JAPANESE ISLANDS

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[1]Kyoto University, Graduate School Of Global Environmental Studies, Kyoto, Japan, [2]The University of Shiga Prefecture, Faculty Of Environmental Science, Shiga, Japan, [3]Kyoto University, Graduate School Of Human And Environmental Studies, Kyoto, Japan

Background and Aims: The genus *Mesocoelium* (Digenea: Mesocoeliidae) parasitizes in the digestive tract of amphibians and reptiles. Members of this genus have two-host life cycle consisting of terrestrial gastropods and vertebrates. At least three congeneric species are distributed in Japan: *M. brevicaecum*, *M. japonicum*, and an undescribed species, *Mesocoelium* sp. 1 (sensu Waki et al., 2022). *Mesocoelium brevicaecum* is the most widespread species among Japanese amphibians, while previous studies indicated that *M. japonicum* and *Mesocoelium* sp. 1 are scattered throughout Japan regardless of the range of their intermediate hosts. Therefore, this study aims to reveal such a mysterious distribution of *Mesocoelium* in Japan.

Methods: We collected *Mesocoelium* specimens from amphibians from Honshu, Shikoku, Kyushu, and Okinawa islands (Japan). The specimens were identified morphologically and molecularly, and their 28S rDNA and *cox1* sequences were used for the phylogenetic analysis.

Results: *Mesocoelium japonicum* was detected in amphibians from Honshu, Shikoku, and Kyushu islands, overlapping the range of *M. brevicaecum*. *Mesocoelium* sp. 1 was found in frogs from southern Kyushu: Satsuma Peninsula and Yakushima Island.

Conclusions: This result suggests that abiotic factors may contribute to the formation of the distribution of *Mesocoelium* species in Japan rather than biotic factors.

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POSTER ON BOARD SHIFT 01
PARASITE ECOLOGY, BIODIVERSITY, AND TAXONOMY / VETERINARY AND AQUATIC PARASITOLOGY (FISH, AMPHIBIANS, ETC.)

COMPLETE MITOGENOME OF EUDIPLOZOON NIPPONICUM SENSU STRICTO (POLYOPISTHOCOTYLEA: DIPLOZOIDAE)

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[1]The University of Shiga Prefecture, Faculty Of Environmental Science, Shiga, Japan, [2]Kochi University, Faculty Of Agriculture And Marine Science, Nankoku, Japan

Background and Aims: The genus *Eudiplozoon* comprises two species *E. nipponicum* (Goto, 1891) and *E. kamegaii* Nishihira & Urabe, 2020. They are distinguishable by host taxa, clamp morphology and the ITS-2 genetic maker, but could not be differentiated by the mt-COI gene sequences, leaving their status as independent species unsolved. Therefore, the complete mitogenome sequences of *E. nipponicum sensu stricto* of two worm pairs from crucian carp were compared with those of *Eudiplozoon* from common carp registered in INSDC.

Methods: The whole mitogenomes of *Eudiplozoon* were sequenced on the DNBSEQ-G400 platform and were assembled using GetOrganelle with the COI gene of *E. nipponicum* as a seed sequence.

Results: The new sequences showed 99.7% identity. The mitogenome sizes were 15,338–15,227bp, which were smaller than those of *Eudiplozoon* from common carp (17,038–17,328bp), due to the size difference of the long non-coding region. The arrangements of the PCG and tRNA were identical between the new *Eudiplozoon* mitogenomes and those from common carp. The complete mt-COI gene of *E. nipponicum sensu stricto* was different from that of *Eudiplozoon* from common carp by 17.0% at the nucleotide level and 9.7% at the amino acid level. The phylogenetic analysis based on the COI sequences obtained by the PCR using new primers based on the complete mitogenome data sequences revealed that the worms from crucian carp were separated from those from common carp.

Conclusions: These results indicate that *E. nipponicum* and *E. kamegaii* are separate species, and that the CO1 sequence results in Nishihira & Urabe (2020) were likely due to some experimental errors.

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POSTER ON BOARD SHIFT 01
PARASITE ECOLOGY, BIODIVERSITY, AND TAXONOMY / VETERINARY AND AQUATIC PARASITOLOGY (FISH, AMPHIBIANS, ETC.)

INTEGRATING EDNA AND OLIGOCHAETE HOST SURVEILLANCE TO IMPROVE EARLY DETECTION OF MYXOBOLUS CEREBRALIS IN ALBERTA

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[1]University of Alberta, Edmonton, Canada, [2]University of Alberta, School Of Public Health, Edmonton, Canada

Background and Aims: Whirling Disease (WD), caused by *Myxobolus cerebralis*, poses a significant threat to aquatic ecosystems and fisheries across North America. This invasive parasite uses a complex two-host lifecycle exploiting the freshwater oligochaete, *Tubifex tubifex*, and various salmonids with infection often leading to severe physical deformities and high mortality rates. Since its initial detection in Johnson Lake, Alberta, Canada in 2016, WD has been primarily monitored in central and southern Alberta watersheds. However, northern Alberta watersheds remain understudied despite their strong social, ecological, and cultural importance, in addition to being a critical habitat for the endangered Athabasca River Rainbow trout.

Methods: This study assessed the distribution and prevalence of *M. cerebralis* across Albertan watersheds using multiple sampling approaches (*i.e.,* environmental DNA, wild fish collections, triactinomyxon filtrations, and oligochaete sampling). Parasite presence and infection intensity were confirmed using molecular and histological analyses. Additionally, the identification of five genetically distinct lineages of *Tubifex tubifex* prompted an oligochaete WD challenge study to evaluate lineage-specific contributions to *M. cerebralis* transmission and inform host surveillance strategies.

Results: Our results demonstrate that eDNA detection provides a measurement of *M. cerebralis* that is comparable to traditional methodologies and can detect *M. cerebralis* prior to observable salmonid mortality. Furthermore, the identification of the T3 *T. tubifex* lineage as the primary driver of WD transmission in Alberta provides critical insight for targeted early detection and surveillance strategies.

Conclusions: Together, these findings support the integration of eDNA and T3 lineage host monitoring to improve WD early detection in Alberta and limit further spread.

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POSTER ON BOARD SHIFT 01
PARASITE ECOLOGY, BIODIVERSITY, AND TAXONOMY / VETERINARY AND AQUATIC PARASITOLOGY (FISH, AMPHIBIANS, ETC.)

BIODIVERSITY AND HOST ASSOCIATIONS OF MYXOZOANS PARASITES FROM FRESHWATER FISHES IN THE PARDO RIVER, SOUTHEASTERN BRAZIL

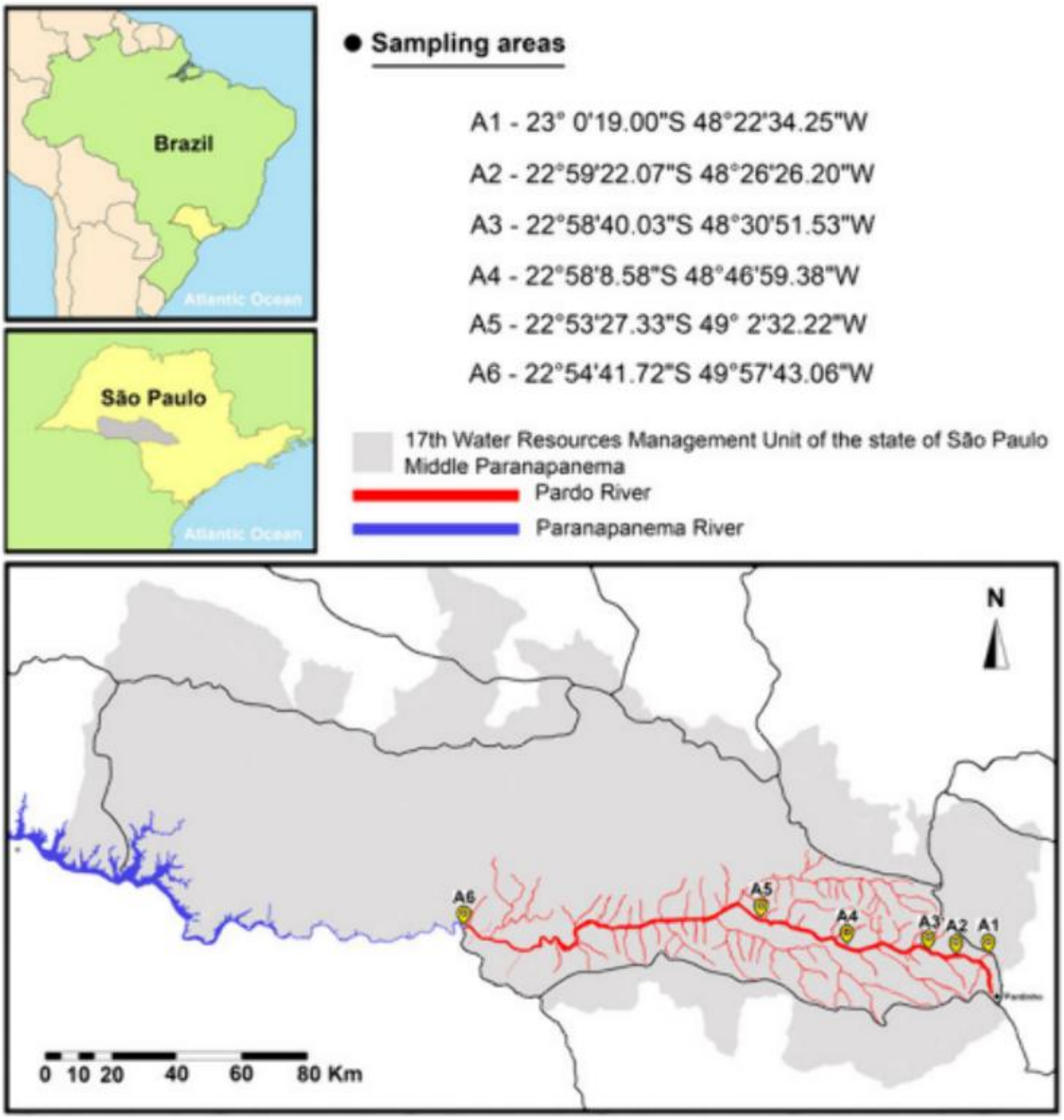
Diego Vieira, Reinaldo Da Silva
Universidade Estadual Paulista - UNESP, Botucatu, Brazil

Background and Aims: Myxozoans are highly diverse cnidarian parasites of freshwater fishes, yet many Neotropical river basins remain insufficiently surveyed. This study investigated the biodiversity, host associations and tissue tropism of myxozoan parasites infecting fishes from the Pardo River basin, São Paulo State, Brazil.

Methods: Between 2020 and 2023, freshwater fishes were collected at six sampling sites along the Pardo River basin, encompassing distinct environmental characteristics. Whenever possible, the same host species were sampled at different sites to allow spatial comparisons. All organs were examined for myxozoan infections. Parasites were identified based on spore morphology, host identity and infection site, and diversity patterns were analyzed according to infected organs, host families and host

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orders.

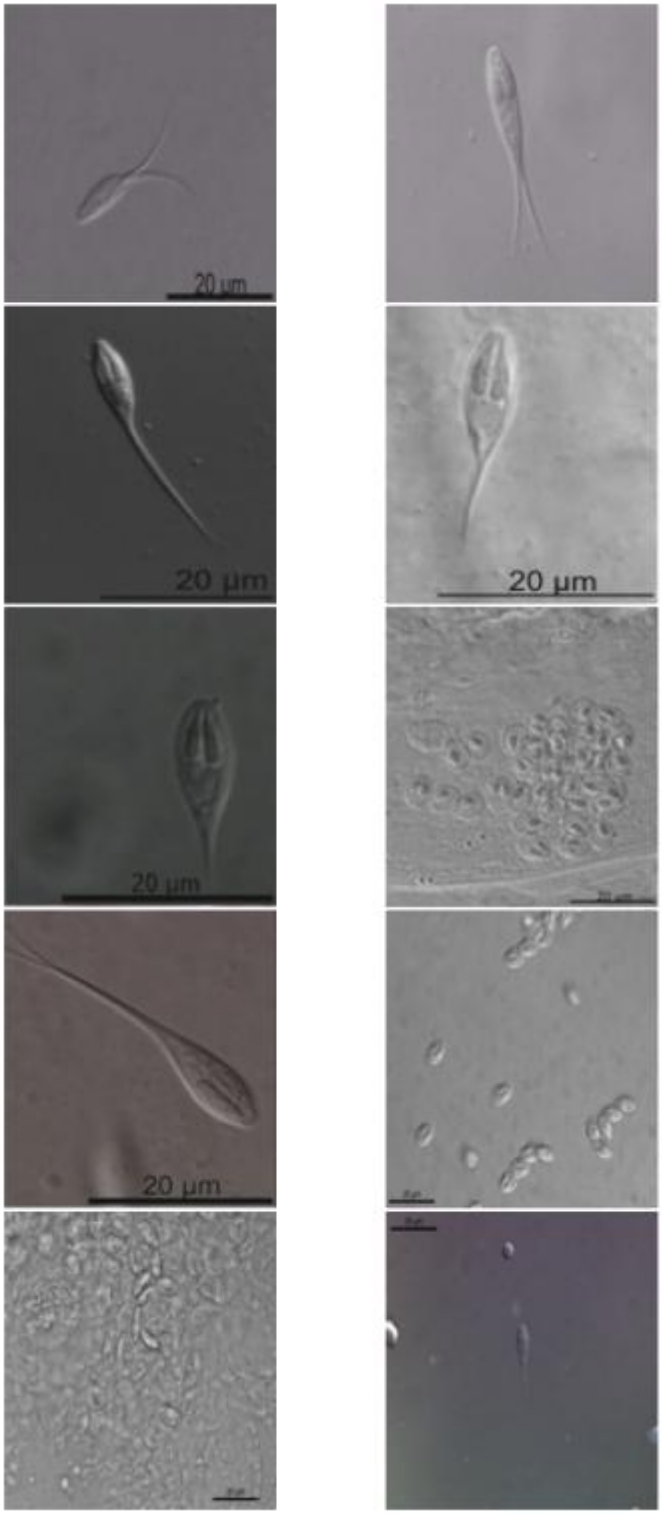


Results: A total of 20 myxozoan taxa were recorded, including 15 *Henneguya* spp. (75%) and 5 *Myxobolus* spp. (25%). Although all organs were examined, infections were restricted to the gills (18 taxa, 90%), swim bladder (1 taxon, 5%) and skin (1 taxon, 5%). Hosts belonged to three fish orders, with Characiformes showing the highest parasite richness (12 taxa, 60%), followed by Siluriformes (7 taxa, 35%). At the family level, Characidae presented the greatest diversity (7 taxa), followed by

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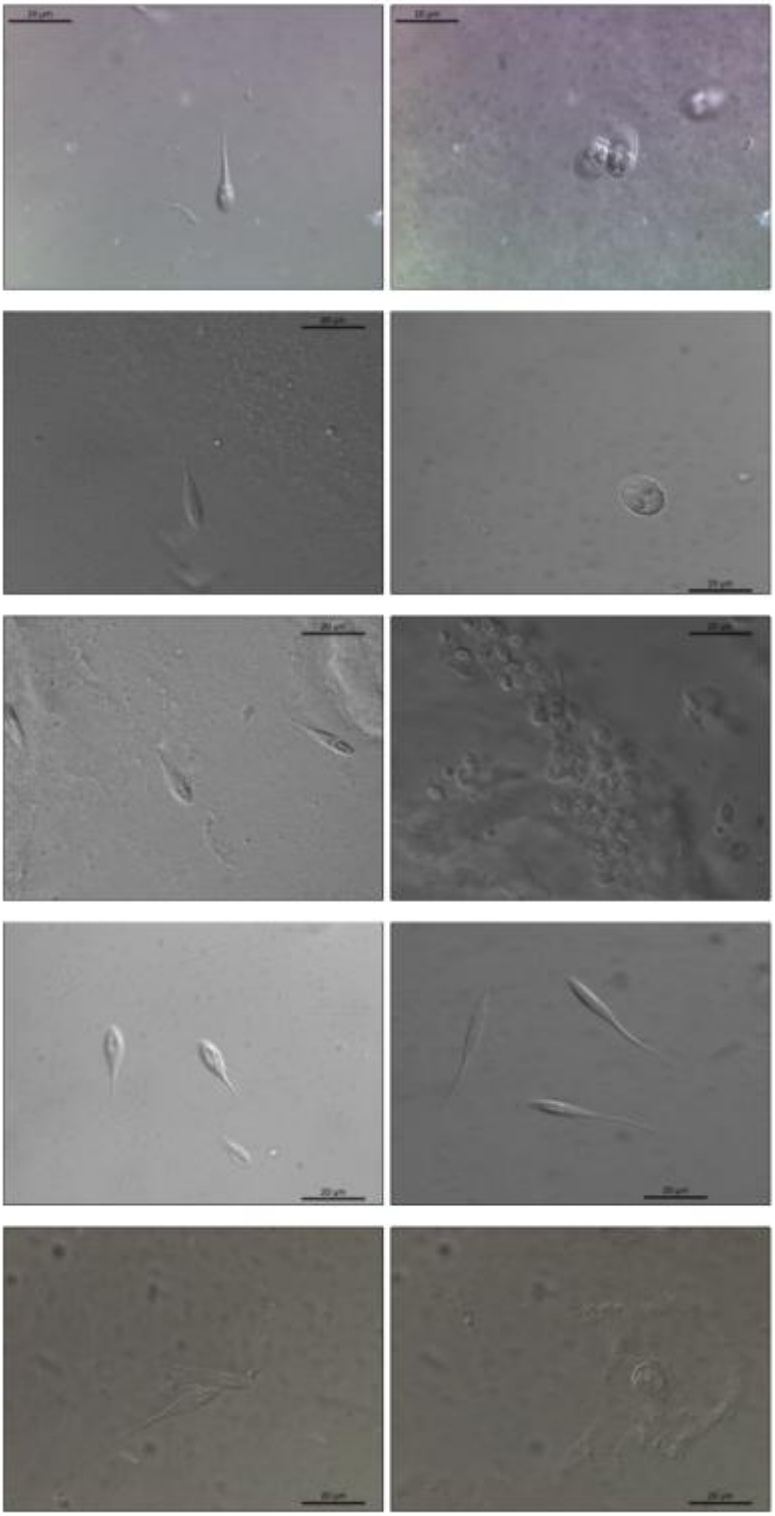
Heptapteridae (4 taxa). Several taxa exhibited distinct morphological traits, strict host and tissue specificity, and restricted spatial distributions, suggesting putative new

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species.

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Conclusions: The Pardo River basin harbors a high and largely undocumented diversity of myxozoan parasites. Integrative morphological and molecular approaches are expected to further resolve species boundaries and host-associated phylogenetic patterns in this Neotropical system.

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POSTER ON BOARD SHIFT 01
PARASITE ECOLOGY, BIODIVERSITY, AND TAXONOMY / VETERINARY AND AQUATIC PARASITOLOGY (FISH, AMPHIBIANS, ETC.)

INVESTIGATING THERAPEUTIC FOLLOW-UP IN CANINE VISCERAL LEISHMANIASIS: ROLE OF COINFECTIONS – PRELIMINARY DATA

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Background and Aims: In Brazil, miltefosine is the only drug approved for the treatment of canine visceral leishmaniasis (CVL). Therapeutic success may be influenced by coinfections with other vector-borne pathogens (VBPs). This study investigated VBP-coinfections that may influence the response to infection and treatment of CVL in dogs naturally infected with *L. infantum*.

Methods: Thirty-three *Leishmania*-seropositive dogs from Florianópolis (Santa Catarina, Brazil) were evaluated for clinical score and parasite load at T0 (pre-treatment), T1 (post-treatment), and T2 (six months later). *Leishmania* load was quantified in skin samples, and blood samples were screened for selected VBPs by PCR/qPCR at T0.

Results: Eleven dogs (33%) were coinfected with at least one VBP, including *Anaplasma* spp./*Ehrlichia canis*, *E. canis*, '*Candidatus* Mycoplasma haematoparvum', *Hepatozoon canis*, and *Acanthocheilonema reconditum*/*Dirofilaria immitis*. Seven dogs (21.2%) died during the follow-up, including two coinfected dogs that did not receive treatment for secondary infections. Parasite load and clinical scores significantly decreased in CVL dogs (T0 to T1, $p = 0.0001$; T0 to T2, $p = 0.0017$), whereas coinfected CVL dogs showed persistently high parasite burdens and limited clinical improvement. Principal component analysis (PCA) explained 52.6% of variance, with clustering by treatment protocol and coinfection status. Dogs receiving the full treatment protocol showed more homogeneous responses, while partial or single-drug treatments displayed distinct profiles. Coinfected dogs clustered separately from CVL dogs, with vectors indicating positive correlations among coinfection status, parasite load, and clinical score.

Conclusions: These findings highlight the importance of screening for secondary infections to improve therapeutic outcomes and prognosis in endemic areas.

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POSTER ON BOARD SHIFT 01
PARASITE ECOLOGY, BIODIVERSITY, AND TAXONOMY / VETERINARY AND AQUATIC PARASITOLOGY (FISH, AMPHIBIANS, ETC.)

PHYLOGENETIC POSITION OF TRYPANOSOME SPECIES FROM JAPANESE RICE FROG (FEJERVARYA KAWAMURAI) IN JAPAN INFERRED FROM 18S RRNA GENE SEQUENCES

Hiromu Yamada, Junnosuke Abe, Aya Masuda, Jun Matsumoto
College of Bioresource Sciences, Nihon University, Veterinary Medicine, Kanagawa, Japan

Background and Aims: Anuran trypanosomes are globally distributed, encompass approximately 90 described species (Votýpka *et al*., 2025). They are reportedly highly polymorphic, which have hindered accurate taxonomy based solely on morphology. Recent studies utilizing molecular data have revealed extensive anuran trypanosome diversity, particularly in neotropical regions. In Japan, although 11 anuran trypanosomes have been described through morphological identification, no molecular information is available. Here, we conducted a molecular phylogenetic analysis of trypanosomes found in the Japanese rice frog (*Fejervarya kawamurai*) to reevaluate the current anuran trypanosome systematics in Japan.

Methods: A total of 71 *F. kawamurai* were collected from paddy fields in Kanagawa and Hiroshima Prefectures, Japan. DNA was extracted from the blood, followed by PCR targeting a 1500-bp region of the 18S rRNA gene. Blood smears from PCR-positive frogs were examined under a light microscope.

Results: Trypanosome infections were detected in seven frogs (9.9%). Two sequences displayed signs of co-infection. Of the remaining five sequences, all clustered within the "Frog 2" clade of aquatic trypanosomes categorized by Bernal and Pinto (2016) (**Figure**). Pairwise sequence similarity between the two lineages was 94.61%. High polymorphism was observed within the same

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sequence.

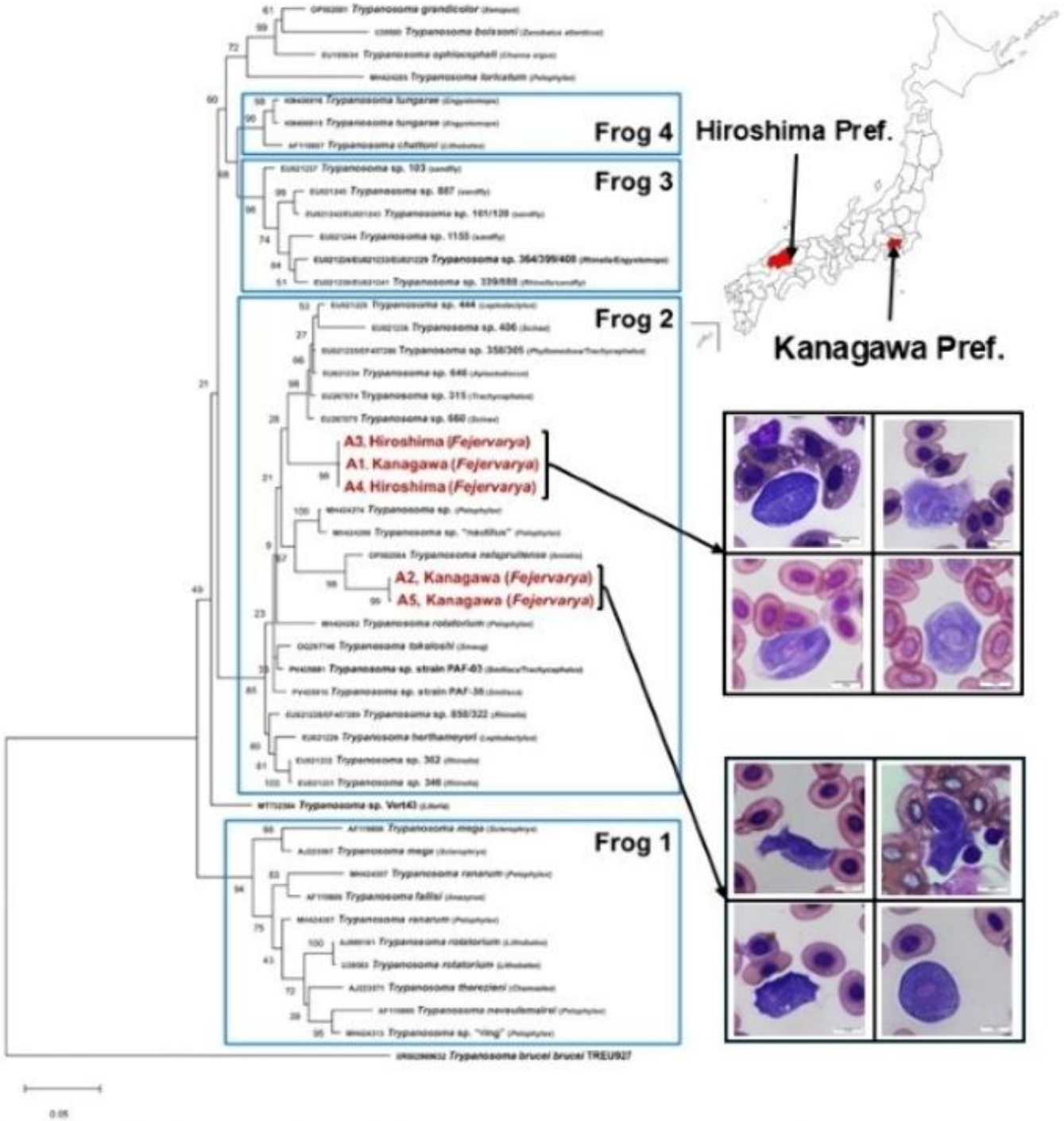


Figure. Phylogenetic relationships among anuran trypanosomes.

Conclusions: We confirmed the existence of at least two distinct trypanosome species from *F. kawamurai* in Japan. Whether these trypanosomes are specific to *F. kawamurai* is yet to be elucidated. The sequences obtained here did not reflect morphology-based classification, suggesting the need for molecular data to reevaluate anuran trypanosome systematics in Japan.

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POSTER ON BOARD SHIFT 01
PARASITE ECOLOGY, BIODIVERSITY, AND TAXONOMY / PARASITES IN CONSERVATION BIOLOGY AND BIODIVERSITY MONITORING

ASSESSMENT OF NOSEMA SPP. INFECTION INTENSITY IN APIS MELLIFERA AND MELIPONINI COLONIES FROM VALLE DEL CAUCA, COLOMBIA

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Background and Aims: Nosemosis is an infectious disease caused by microsporidia of the genus *Nosema* that affects the health and productivity of bees. In this study, the presence of *Nosema* spp. was evaluated in 12 colonies of *Apis mellifera* and 16 colonies of the tribe Meliponini in the municipalities of Palmira and Cali, Valle del Cauca.

Methods: Samples of 60–120 bees were collected, and the abdominal segment of adult worker bees was removed for subsequent observation by bright-field optical microscopy and spore quantification using a Neubauer chamber to determine the level of infection (mean spore abundance per bee).

Results: In *A. mellifera*, propagules compatible with *Nosema* spp. were detected in 100% of the colonies analyzed. Infection levels were classified as: low (4/12 colonies, 33.3%) with an average of 4×10^5 spores per colony (range: $2–6 \times 10^5$); medium (3/12 colonies, 25%) with an average of 1.9×10^6 spores (range: $1–2 \times 10^6$); and high (5/12 colonies, 41.67%) with an average of 8×10^6 spores (range: $4–12 \times 10^6$). Statistically significant differences ($p < 0.05$) were found among groups using one-way analysis of variance (one-factor ANOVA). In contrast, all Meliponini species (*Nannotrigona camargoi*, *Nannotrigona mellaria*, *Tetragonisca angustula*, *Scaptotrigona ederi*, and *Partamona peckolti*) were negative for the presence of *Nosema* spp. spores.

Conclusions: These findings provide valuable information for the sanitary monitoring of bee populations in the region and highlight the importance of implementing preventive health management strategies to mitigate the impact of nosemosis in apicultural production systems.

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PARASITE ECOLOGY, BIODIVERSITY, AND TAXONOMY / PARASITES IN CONSERVATION BIOLOGY AND BIODIVERSITY MONITORING

COMPARATIVE POPULATION GENETICS OF PARELAPHOSTRONGYLID NEMATODES IN BOREAL CARIBOU AND WHITE-TAILED DEER IN WESTERN MANITOBA, CANADA

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Background and Aims: Up-to-date information on the distributions of parasites that affect vulnerable wildlife populations is critical for informing management strategies, especially in the case of Manitoba's imperiled boreal caribou (*Rangifer tarandus caribou*). Boreal caribou face many threats, including northward expansion of white-tailed deer into caribou habitat, enabled by climate change and habitat alteration. White-tailed deer spread parasites which can infect caribou, such as brainworm and muscleworm. However, little is known about the distribution of these nematode parasites in Manitoba. This study aims to assess the prevalence and phylogeography of muscleworm and brainworm in western Manitoba, a region with limited sampling historically.

Methods: To achieve this, caribou and white-tailed deer fecal pellets will be collected from several caribou management units in western Manitoba spanning from north to south. Nematode larvae will be isolated from the pellets and DNA sequencing used to confirm the identity of the parasites.

Results: Network analyses will be performed to identify subpopulations of parasites, and the fixation index (FST) will be calculated to estimate genetic differentiation among subpopulations. An AMOVA will be performed to predict genetic variance due to location and host species.

Conclusions: This work will clarify the phylogeography and the evolutionary history of muscleworm in Manitoba, as well as provide updated information on brainworm distribution. Additionally, although brainworm is rare in caribou, recent cases in Alberta emphasize the importance of routine monitoring, especially in areas where white-tailed deer and caribou ranges overlap.

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PARASITE ECOLOGY, BIODIVERSITY, AND TAXONOMY / PARASITES IN CONSERVATION BIOLOGY AND BIODIVERSITY MONITORING

A NEW MICROPYGOMYIA (SAUROMYIA) SAND FLY FROM CHIHUAHUA, NORTHERN MEXICO: INTEGRATIVE TAXONOMY AND IMPLICATIONS FOR LEISHMANIASIS SURVEILLANCE

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Background and Aims: Accurate identification of phlebotomine sand flies is essential for leishmaniasis surveillance and vector control. However, species diversity remains under-documented in northern Mexico. We describe *Micropygomyia (Sauromyia) batopilensis* sp. nov. and evaluate its series placement using morphology and the cytochrome c oxidase subunit I (COI) marker. We also discuss the implications of the presence of *M. batopilensis* for local vector surveillance in Batopilas, a locality with suspected cutaneous leishmaniasis.

Methods: Sand flies were collected during the rainy seasons of 2024 and 2025 in caves and peridomestic settings around San Jose de Valenzuela, Batopilas, Chihuahua, Mexico, using CDC light traps and aspiration. Adults were slide-mounted, measured, and compared with published keys. COI barcodes (about 650 base pairs) were assembled from two overlapping fragments; maximum-likelihood phylogeny and uncorrected pairwise distances were estimated with GenBank *Micropygomyia (Sauromyia)* sequences. Selected specimens were screened for *Leishmania* DNA.

Results: *Micropygomyia (Sauromyia) batopilensis* sp. nov. is diagnosable by characters in both sexes, particularly male terminalia and female spermathecae. COI distances to other *Micropygomyia (Sauromyia)* species ranged from 12.0 to 22.9 percent, with low intraspecific variation. In the COI

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tree, *Mi.* batopilensis grouped with *Mi.* ferreirana and *Mi.* capixaba (Oswaldoi series), whereas Atroclavata-series taxa were more distant; *Leishmania* DNA was not detected in screened material.

Conclusions: This is the first formally described *Micropygomyia (Sauromyia)* species from Chihuahua and adds a second phlebotomine species to the state fauna. Integrative taxonomy highlights discordance between morphology-based series classification and mitochondrial phylogeny, providing baseline data for leishmaniasis vector surveillance in Sierra Tarahumara canyon habitats.

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PARASITE ECOLOGY, BIODIVERSITY, AND TAXONOMY / PARASITES IN CONSERVATION BIOLOGY AND BIODIVERSITY MONITORING

THE USE OF PARASITES IN AN INTERDISCIPLINARY APPROACH TO STOCK DISCRIMINATION OF ATLANTIC HERRING IN ICELANDIC WATERS

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Background and Aims: Atlantic herring (*Clupea harengus*) exhibits a complex population structure, with morphologically similar stocks of different origins migrating to Icelandic feeding grounds in April to October. To ensure sustainable harvesting practices, mixed fisheries need to consider each stock's contribution to the total catch and employ reliable discrimination methods. In this case, Icelandic management relies on a single method, assigning individuals to their stock based on maturity stage.

Methods: We focused on an integrated approach, using otolith shape analysis and parasite composition to differentiate Norwegian spring spawning herring (NSSH) from Icelandic summer spawning herring (ISSH). Both methods inform stock origin: otolith shape reflects genetics and early-life environmental conditions, while parasite composition reflects environmental conditions. We examined 209 adult and 42 juvenile individuals caught in Icelandic waters between March and October 2025. The ShapeR package was used to distinguish shape patterns in the otoliths between stocks. Parasite composition was analyzed with linear modelling for each organ, with parasites barcoded (Sanger sequencing) to confirm identification.

Results: The results indicated differences in otolith shape, but also in organ-specific parasite composition, primarily driven by larvae of the nematode *Anisakis simplex* in the outer stomach, pyloric caeca, and intestine. The NSSH stock had a higher abundance of nematodes in the outer stomach, whereas the ISSH had higher abundance in the outer pyloric caeca and intestine.

Conclusions: The addition of parasites to integrated approaches should therefore not be overlooked. Their inclusion in stock discrimination methods could improve accuracy, thereby limiting the risk of overexploitation, and improving sustainable management in Icelandic waters.

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PARASITE ECOLOGY, BIODIVERSITY, AND TAXONOMY / PARASITES IN CONSERVATION BIOLOGY AND BIODIVERSITY MONITORING

META-ANALYSIS REVEALS TEMPERATURE- AND LIFE-CYCLE DEPENDENT LONG-TERM TRENDS IN WILDLIFE PARASITE POPULATIONS

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Background and Aims: Parasites occur in every ecosystem across the globe and affect nearly all organisms. Notwithstanding the important medical and economic burdens imposed by a few species, parasites strongly contribute to biodiversity and support ecosystem stability by performing essential ecological functions. Despite their ecological significance, our understanding of how parasitic organisms are affected by anthropogenic changes remains poor and overarching long-term trends in parasite abundance or diversity have yet to be identified.

Methods: We performed a quantitative synthesis of long-term changes in eukaryotic parasite populations of wild animals. After compiling a dataset of 1600 time series of infection level changes in parasite populations from 115 studies, we used Bayesian hierarchical modelling to investigate whether temporal changes in prevalence were linked to parasite life cycle complexity and temperature changes.

Results: Our analyses show that temporal trends in parasite prevalence were linked to both life cycle complexity and temperature. More specifically, we found evidence that parasites with 3+-hosts life cycles decline more frequently than the 1- and 2-hosts life cycles parasites. We also found evidence of stronger temperature sensitivity in 3+-hosts parasites compared to the other groups, suggesting that 3+-hosts parasites tend to decline with increasing temperatures.

Conclusions: We performed the first global quantitative synthesis of long-term infection changes in wildlife parasites. Our study corroborates the hypothesis that environmental changes disproportionally impact parasites with highly complex life cycles, which suggests that these parasites may be more prone to co-extinctions. Additionally, we show that prevalence changes are linked to temperature changes, suggesting that climate change likely affects parasite transmission.

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DID YOUR COUSIN GIVE YOU WORMS? THE CASE OF THE FRENCH AQUITANIAN PIKE, ESOX AQUITANICUS

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Background and Aims: Introducing non-native freshwater fish species impact the ecosystems they colonise. Such impacts include hybridisation with native species, biological invasions, and the introduction of parasites, potentially resulting in parasite spillover. In 2014, the endemic Aquitanian pike, *Esox aquitanicus*, was identified in southwestern France after long being confused with the Northern pike, *Esox lucius*, introduced in this region in the 1950s. Today, *E. aquitanicus* is threatened and classified as Vulnerable on the IUCN Red List. In addition to hybridisation and competition between these two closely related species, *E. lucius* may have introduced non-native parasites to *E. aquitanicus*. Studying the parasitofauna of this endemic species is therefore essential, as it can reveal additional threats to its conservation, while also improving our knowledge of freshwater biodiversity.

Methods: This study is the first inventory of the parasitofauna of *E. aquitanicus*. We compared it with existing data on *E. lucius* obtained from literature and from field studies. Over a three-year period, we collected pike specimens in southwestern France. Parasites found in dissected, preserved organs were identified using both morphological and molecular approaches.

Results: Apart from generalist parasites, species common to both pike species included one monogenea initially thought to be specific to *E. lucius*. In addition, an unexpected cestode was also found in *E. aquitanicus*, suggesting a newly recognised parasitic relationship.

Conclusions: This study provides new data on the parasitofauna of a poorly characterised endemic species and contributes to a better understanding of parasite life cycles and hosts in French freshwater ecosystems.

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PARASITE ECOLOGY, BIODIVERSITY, AND TAXONOMY / PARASITES IN CONSERVATION BIOLOGY AND BIODIVERSITY MONITORING

ASSESSMENT OF ANTHROPOGENIC IMPACTS ON THE AQUATIC ENVIRONMENT AND BIODIVERSITY IN THE BANGWEULU–MWERU ECOREGION (DR CONGO): AN ICHTHYOPARASITOLOGICAL PERSPECTIVE

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Background and Aims: The Democratic Republic of Congo is drained by an extensive hydrographic network that supports agriculture and domestic activities. However, many aquatic ecosystems are increasingly threatened by anthropogenic pressures, particularly mining activities which have resulted the widespread discharge of untreated effluents enriched with trace metal elements (TMEs) into rivers and lakes. Although some studies have reported elevated TME concentrations in water, sediment, and fish tissues, information remains limited and the ecological consequences are still poorly understood. The study aimed to assess the impacts of mining-related pollution on aquatic ecosystems in the Bangweulu–Mweru Ecoregion, by integrating chemical and biological approaches.

Methods: The Lubumbashi River was selected because it experiences high mining pressures. Pollution levels were assessed by quantifying TMEs in water and sediment samples using inductively coupled plasma-based analysis. Fish stress responses were evaluated using stress biomarkers, particularly histological alterations in gill tissues. In parallel, the parasitological status of fish was examined to document parasite diversity and to assess the suitability of fish parasites as bioindicators of environmental degradation.

Results: Trace metal elements showed elevated concentrations in water and sediment, indicating significant contamination of the Lubumbashi River. Fish exhibited marked gill lesions, including lamellar fusion, telangiectasia, and epithelial lifting, consistent with metal-induced stress. Parasitological analyses revealed high parasite diversity across multiple taxa, including monopisthocotylans, trematodes, nematodes, cestodes, and acanthocephalans.

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Conclusions: By combining TME analysis, stress biomarker responses and parasitological indicators, this research provides a comprehensive evaluation of mining-related pollution impacts on aquatic health, supporting environmental monitoring strategies and informing sustainable management of aquatic resources.

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PARASITE ECOLOGY, BIODIVERSITY, AND TAXONOMY / PARASITES IN CONSERVATION BIOLOGY AND BIODIVERSITY MONITORING

ENDOPARASITE ASSEMBLAGES IMPLY TROPHIC-LINK VARIATION AMONG ECOPHENOTYPES OF JAPANESE EELS

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Background and Aims: Conservation programs for habitat-spanning species require tools to assess trophic interactions across ecosystems, but comprehensive monitoring is often limited by cost and complexity. We tested whether trophically transmitted endoparasites can serve as surrogates for trophic-link diversity in the endangered Japanese eel *Anguilla japonica* by distinguishing ecophenotypes in a cove–river system.

Methods: We sampled 174 wild eels from a cove–river system in Ehime Prefecture, Japan, from April 2008 to October 2009. Otolith Sr:Ca profiles classified individuals as freshwater residents, brackish-water residents, marine residents, or habitat shifters. Gastrointestinal tracts were screened for endoparasites, which were identified to the lowest feasible taxonomic level. Parasite richness and assemblage composition were compared among ecophenotypes using regression and multivariate community analyses.

Results: We detected 17 endoparasite taxa (8 Digenea, 4 Cestoda, 2 Nematoda, 3 Acanthocephala), and assemblage structure differed among host ecophenotypes. Marine residents had higher richness than freshwater ones and included taxa using planktonic copepods and benthic decapods as intermediate hosts, as well as taxa with gastropod–fish life cycles. Freshwater residents harbored fewer taxa, dominated by parasites using isopods and copepods as intermediate hosts. Brackish-water residents and habitat shifters showed intermediate or mixed assemblages.

Conclusions: Endoparasite assemblages capture variation in transmission pathways and may provide a cost-effective proxy for trophic-link variation among eel ecophenotypes. The presence of habitat shifters suggests that conserving river habitats alone is insufficient; trophic networks in estuarine and coastal zones should also be protected. Parasite community data, when available, can offer actionable insights into food-web dependence in conservation planning.

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SPECIES OF AEQUIYOLDIA (BIVALVIA: SAREPTIDAE)) FROM THE ANTARCTIC AND SUBANTARCTIC ARE PARASITIZED BY THE SAME LARVAL PARASITE (DIGENEA: GYMNOPHALLIDAE) REVEALING PRESENT CONNECTIVITY THROUGHOUT THE DRAKE PASSAGE

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Background and Aims: Parasites from Southern Ocean, have been scarcely studied, due to the challenges associated with obtaining biological material from Antarctic. *Aequiyoldia eightsii* is a common bivalve in the Antarctic Peninsula but an undescribed speciers of *Aequiyoldia* is found in subantarctic region. Based on the identification assisted by molecular markers,we evaluate if two congeneric species of *Aequiyoldia* inhabiting Antarctic and subantarctic environments are host for the same parasite species.

Methods: A total of 210 individuals of *A. eightsii* were collectedat three sites in Antarctic Peninsula. Each specimen was sexed, and its maximum valvar length measured. The valves, mantle, gills, gonad, and digestive gland were examined. DNA extraction of each parasite sample was performed using the commercial kit Zymo Research following the manufacture recommendations. To maximize the chances of correct species identification, we used two phylogeny-based approaches. The first consisted of phylogenetic analysis for each gene separately and the second approach included concatenated genes.The best models for each phylogenetic tree were chosen according to the Akaike information criterion values supplied in Mega v6.

Results: The phylogenetic reconstruction based in two approach, confirm that the same species of *Gymnophalloides* sp. is found in two congeneric bivalves in the Antartic and subantarctic separated by the Drake Passage

Conclusions: Distribution of trematodes in marine birds in Antarctica is drived by suitable intermediate hosts and foraging behavior of the potential final hosts. According to our results the migratory pattern of the definitive host play a critical role explaining the diversity of parasites living in the Antarctic Peninsula and Subantarctic region.

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REMOVING BARRIERS TO THE CONSERVATION OF METAZOAN PARASITES OF EUROPEAN FRESHWATER FISH

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Background and Aims: Freshwater biodiversity is in rapid decline and freshwater fish, and their parasites, are highly vulnerable. Despite the long tradition of freshwater fish-parasite research, our knowledge of the diversity and vulnerability of freshwater fish parasites remains limited, and their conservation faces substantial barriers. Here we identify knowledge gaps and propose solutions for advancing the integration of freshwater fish parasites into biodiversity monitoring and environmental governance.

Methods: We reviewed existing data on European freshwater fish parasite diversity and identified key challenges and potential solutions to address scientific, societal, policy, and management barriers to parasite conservation.

Results: Current understanding of long-term changes in European freshwater fish parasites is largely limited to three hosts, European eel, Arctic charr and brown trout, and two regions, Britain and

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Norway. We identified four key solutions to improve the detection, identification, and integration of parasites into monitoring and governance; (1) data sharing, (2) curated reference libraries of molecular barcodes and morphological voucher specimens in museums, (3) non-invasive parasite detection through eDNA, and (4) integration with social science approaches.

Conclusions: By highlighting current scientific, societal, and management barriers and potential solutions to the conservation of European freshwater fish parasites, our study paves the way for the first comprehensive and concerted effort to place parasites on the map of biodiversity monitoring and conservation policy, both locally and internationally. Achieving a paradigm shift of taxonomically unbiased conservation goals will build a foundation for improved transnational monitoring of biodiversity and ecosystem change for policy and society.

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PARASITE ECOLOGY, BIODIVERSITY, AND TAXONOMY / PARASITES IN CONSERVATION BIOLOGY AND BIODIVERSITY MONITORING

PARASITE MANIPULATION IN BRAINWORM TRANSMISSION: NO EVIDENCE FOR CHANGES IN VERTICAL CLIMBING BEHAVIOUR OF THE TERRESTRIAL SNAIL ZONITOIDES ARBOREUS

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Background and Aims: Parasite-mediated behavioural modification of intermediate hosts is frequently proposed as a mechanism influencing parasite transmission, but its occurrence may depend on host behaviour and transmission mode. *Parelaphostrongylus tenuis* (brainworm) is a parasitic nematode of white-tailed deer (WTD) and other ungulates distributed across central and eastern North America, where transmission likely occurs through incidental ingestion of infected gastropods. Although many gastropod hosts are ground-dwelling and exhibit low infection prevalence, ungulates may become infected by consuming gastropods that have climbed vegetation. We tested whether infection with *P. tenuis* alters the climbing behaviour of *Zonitoides arboreus*, a typically ground-dwelling snail host species. We hypothesized that behavioural effects would depend on parasite developmental stage, with no change in climbing in snails harbouring second-stage larvae (L2) and altered climbing once infective third-stage larvae (L3) developed.

Methods: Laboratory-raised snails were exposed to *P. tenuis* larvae or sham-exposed, and climbing height was measured four times daily over two days at 14 (L2) and 28 (L3) days post-exposure.

Results: Generalized linear mixed models indicated that climbing was not influenced by infection status at either developmental stage, though snails consistently climbed highest after 7:00 am.

Conclusions: Our results suggest that *P. tenuis* does not manipulate the climbing behaviour of *Z. arboreus*. Given the diversity of gastropod intermediate hosts, future studies should examine species with differing arboreal tendencies and evaluate how environmental factors, such as light and temperature, influence host behaviour and the degree of parasite manipulation. This work informs our understanding of brainworm transmission dynamics and cervid conservation in Manitoba and beyond.

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SEASONALITY MATTERS: BLOOD PARASITE INFECTIONS IN REPTILES FROM THE COLOMBIAN ORINOQUÍA

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Background and Aims: Colombia is a country with high reptile species richness; however, information on their health status and parasite ecology remains limited, particularly in the Orinoquía region. Hemoparasites can affect host physiology, behavior, and survival. This study aimed to characterize the diversity and prevalence of hemoparasites in reptiles from two regions of the Colombian Orinoquía (Paz de Ariporo and Yopal, Casanare), and to evaluate differences under hydroclimatic conditions (wet and dry seasons).

Methods: Individuals were captured using manual capture, nets, nooses, and Tomahawk traps. Blood samples were collected and analyzed using Giemsa staining and light microscopy for hemoparasite detection. Prevalence was calculated by species, locality, habitat type, and season.

Results: A total of 323 reptiles belonging to eight species were sampled across three orders and five families.Overall hemoparasite prevalence was 25.7% (83/323). *Podocnemis vogli* showed the highest infection frequency (40%; 54/135), followed by *Caiman crocodilus* (22.02%; 24/109). No infections were detected in *Chelonoidis carbonaria* (n = 45) or *Iguana iguana* (n = 22). In 3/9 individuals of *Tupinambis teguixin* were detected parasites, while single individuals of *Chelus orinocensis* and *Eunectes murinus* were infected. Infection prevalence was higher during the wet season (28.5%) than during the dry season (23.3%), and aquatic-associated species showed higher infection levels than terrestrial taxa.

Conclusions: Hemoparasite transmission in reptiles from Casanare appears to be influenced by hydroclimatic seasonality, habitat use, vector ecology, and host biological traits. These findings provide valuable insights into hemoparasite ecology and contribute baseline information relevant for wildlife health assessment and conservation strategies in the Orinoquía.

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IT'S ONLY WORDS: HOW DOES INFECTION TERMINOLOGY VARY ACROSS DISCIPLINES?

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Background and Aims: Sharing ideas across disciplines can foster scientific innovation and help resolve pressing issues in human and animal heath. Indeed, many issues currently facing wildlife and society are complex and require inter-disciplinary knowledge. However, different disciplines use distinct terms to describe similar concepts, leading to discrepancies in language use in the literature. These differences can generate misunderstandings and confusion, hampering synthetic research studies. This problem parallels the issue of publication siloes which has been well described in the One Health literature.

Methods: Here, we study how terminology use (related to infection, parasites and disease ecology) and publication venue varies across academics in different disciplines (parasitology, wildlife disease ecology, animal ecology). We invite attendees of ICOPA to take our survey to help us understand and quantify these differences. The same poster will be presented at other conferences focused on wildlife disease ecology and animal ecology to collect matching survey data, and the results will be compared across attendees' self-identified research fields.

Results: The results from this survey, in combination with a systematic literature review, will be the basis of a synthesis paper aimed at bridging the terminology gap and proposing a more cohesive way forward across disciplines.

Conclusions: Our findings will identify the extent to which there is a terminology gap among parasitology, wildlife disease ecology and animal ecology when thinking about animal health problems. Identifying gaps is the first step toward bridging them, enhancing understanding of field-specific terminology by non-specialists and paving the way for greater clarity in term and concept use moving forward.

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SHIFT01-137

POSTER ON BOARD SHIFT 01
PARASITE ECOLOGY, BIODIVERSITY, AND TAXONOMY / PARASITES IN CONSERVATION BIOLOGY AND BIODIVERSITY MONITORING

ENDOPARASITES, ACORN CROPS, AND CORTISOL IN NORTH AMERICAN FLYING SQUIRRELS (GLAUCOMYS SPP.)

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Background and Aims: Coinciding with ongoing climate change, southern flying squirrels (*Glaucomys volans*) have expanded their northern range edge, leading to the apparent displacement of northern flying squirrels (*G. sabrinus*) in the contact zone. Introduction of the intestinal worm *Strongyloides robustus* by its reservoir host, the southern flying squirrel, is proposed to drive the displacement of the naïve northern species. Here, we aim to identify whether *S. robustus* infection is differentially associated with host metabolic demands and food availability in northern and southern flying squirrels.

Methods: In the Kawartha Highlands, Ontario, Canada from 2021-2025, acorn abundance, squirrel abundance, and fecal samples for parasite and cortisol analysis were collected. Fecal flotations were conducted to evaluate infections, and an enzyme immunoassay was used to quantify fecal cortisol.

Results: Northern flying squirrels had higher infection frequencies, and higher cortisol metabolite levels associated with *S. robustus* detection during acorn scarcity than southern flying squirrels. Southern flying squirrel abundance varied in response to acorn crops, and infection frequency among both species appeared to vary in response to population dynamics.

Conclusions: These findings suggest that northern and southern flying squirrels have different immune and physiological responses to *S. robustus* infection. Northern flying squirrels appear to be more susceptible to infection and face higher metabolic demands during infection than southern flying squirrels, particularly during periods of food scarcity. These differences in susceptibility and energetic costs between the host species may contribute to the observed decline of northern flying squirrels in the expanding contact zone.

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SHIFT01-138

POSTER ON BOARD SHIFT 01
PARASITE ECOLOGY, BIODIVERSITY, AND TAXONOMY / PARASITES IN CONSERVATION BIOLOGY AND BIODIVERSITY MONITORING

INTEGRATED APPROACHES TO BAT HEALTH: PARASITES, VECTORS, AND FUNGAL INTERACTIONS

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Background and Aims: Bats are important reservoirs for diverse parasites, including haemoparasites, fungal pathogens, and vector-borne organisms. Parasitic infections can disrupt immune function and energy balance, potentially increasing host susceptibility to secondary infections and altering pathogen transmission dynamics. This study aims to (i) establish baseline prevalence and diversity of bat haemoparasites, haematophagous ectoparasites, and associated Laboulbeniales fungi; (ii) assess co-infection patterns relevant to host susceptibility and vector competence; and (iii) evaluate bat ectoparasites as minimally invasive sentinel tools for bat health surveillance.

Methods: Fieldwork is conducted in Parque Nacional Cusuco, Honduras, across multiple habitats and elevations. Free-ranging bats are captured using mist nets and undergo standardised biometric assessment, ectoparasite quantification, and non-lethal blood sampling following animal welfare guidelines. Bat flies are removed and examined microscopically for Laboulbeniales fungal colonisation. Molecular detection of haemoparasites and fungal taxa from bat blood and ectoparasite tissue is performed using nanopore sequencing to characterise pathogen diversity, prevalence, and co-occurrence.

Results: Field sampling and laboratory analyses are ongoing. The study is expected to generate baseline data on haemoparasite diversity in bats and their ectoparasitic vectors, the prevalence of Laboulbeniales fungi on bat flies, and the infection status of Honduras for *Pseudogymnoascus destructans*. Associations among fungal infection, ectoparasite burden, and haemoparasitic infection will be explored at host and vector levels.

Conclusions: This integrated framework will provide foundational insight into host–vector–pathogen relationships in an understudied Neotropical bat community and establish a reference for longitudinal surveillance. The results will inform future studies on fungal hyperparasites, vector competence, and wildlife disease dynamics within a One Health context.

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SHIFT01-139

POSTER ON BOARD SHIFT 01
TRANSMISSION DYNAMICS, EPIDEMIOLOGY, AND GLOBAL HEALTH / VECTOR BIOLOGY, PARASITE TRANSMISSION AND CONTROL STRATEGIES

REDUCTION IN ASYMPTOMATIC MALARIA INFECTION AMONGST SCHOOL CHILDREN IN THREE ECOLOGICAL ZONES OF GHANA BETWEEN 2017 AND 2023

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Background and Aims: Eliminating asymptomatic malaria remains a challenge in Ghana, despite the implementation of several intervention measures. Changes in malaria prevalence are important indicators of the success of intervention programmes. This study aimed to determine the temporal dynamics and spatial distribution of asymptomatic malaria amongst school children, by comparing infection rates from a cross-sectional study conducted in 2023 with a previously published data in 2017 in the same sites in Ghana.

Methods: A cross-sectional study was conducted in 2023 with 1,154 school children between the ages of 3 to 12 years in three ecological zones of Ghana. Finger prick blood was obtained and used to make dried blood spots (DBS) and thick and thin smears. Genomic DNA was extracted from the DBS and used to amplify the *P. falciparum* mitochondria cytochrome oxidase III gene using species-specific primers. Previously published *P. falciparum* prevalence data obtained using the same study design in 2017 was used as a comparator to help in understanding the changing parasite prevalence.

Results: Asymptomatic *P. falciparum* infection by microscopy decreased from 16.05 % to 1.21 %, and by PCR from 39.95 % in 2017 to 17.33 % in 2023 respectively. Significantly, infection rates in the Sahel zone decreased by 60 % in 2023 compared to 2017. Submicroscopic infections were lower in 2023 (16.46 %) than in 2017 (24.35 %) with the forest zone having the highest submicroscopic infections at both timepoints (51.28 % in 2017 and 28.28 % in 2023).

Conclusions: This study highlights a reduction in the prevalence of asymptomatic malaria among schoolchildren.

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POSTER ON BOARD SHIFT 01
TRANSMISSION DYNAMICS, EPIDEMIOLOGY, AND GLOBAL HEALTH / VECTOR BIOLOGY, PARASITE TRANSMISSION AND CONTROL STRATEGIES

IMPACTS OF HOST HIBERNATION: SNAIL–TREMATODE INTERACTION

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Background and Aims: Many gastropods can drastically reduce their metabolic activity when faced with unsuitable environmental conditions, also known as aestivation. Gastropods also serve as vital intermediate hosts for a diverse array of parasitic trematode species, and thus the epidemiology of trematode infections is in part influenced by changes in snail host biology. Despite there being a significant focus on snail-trematode transmission cycles at an ecosystem level, how snail dormancy affects development, survival, and infectivity of trematodes specifically at the host-parasite level still remains poorly understood.

Methods: To investigate how host dormancy affects the development, survival, and infectivity of parasites, we induced dormancy in multiple snail-parasite systems during controlled infection experiments. Structural alterations in host tissues and parasite development during dormancy were assessed by histology. We further conducted an epigenetic analysis using DNA methylation as a marker for metabolic activity within the host, and parasite release time and load as an indicator for parasite development.

Results: We established husbandry guidelines for *Oreohelix subrudis* snails, allowing us to conduct controlled experiments within a laboratory setting. We also found that proteins sourced from *O. subrudis* snail plasma trigger aestivation in other gastropods via evolutionarily conserved pathways.

Conclusions: Since snail dormancy relies heavily on environmental conditions, understanding the dynamics between host dormancy and parasitization is invaluable for predicting patterns of infection prevalence in response to environmental changes within an ecosystem, thus allowing for more effective and targeted disease control.

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POSTER ON BOARD SHIFT 01
TRANSMISSION DYNAMICS, EPIDEMIOLOGY, AND GLOBAL HEALTH / VECTOR BIOLOGY, PARASITE TRANSMISSION AND CONTROL STRATEGIES

PARASITIC INFECTIONS OF RODENTS CAPTURED FROM SELECTED AREAS OF SOKOTO METROPOLIS, SOKOTO STATE, NIGERIA.

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Background and Aims: Rodents are widely distributed mammals that frequently live in close association with humans making them important reservoirs of parasites of public health and veterinary importance. In urban environments like Sokoto Metropolis, rodents commonly inhabit homes, markets, hostels,abattoirs,refuse dumps and food storage areas increasing the risk of parasite transmission. Rodents harbour haemoparasites, gastrointestinal helminths and ectoparasites, many of which are zoonotic. Haemoparasites such as *Plasmodium* spp. and *Leishmania* spp. are transmitted by arthropod vectors, while gastrointestinal helminths like *Hymenolepis* spp. are spread through faeco-oral contamination. Ectoparasites including fleas, ticks, mites, and lice further enhance disease transmission. Due to limited integrated data on rodent parasites in Sokoto Metropolis, this study investigated these parasite groups to assess potential zoonotic risks.

Methods: A cross-sectional study was conducted in selected areas of Sokoto Metropolis. Rodents were trapped using baited wire cages, identified morphologically, and examined for parasites. Blood samples were collected and Giemsa-stained smears examined for haemoparasites. Gastrointestinal tracts were analysed for helminths using standard techniques, while ectoparasites were collected, preserved, and identified microscopically. Data were analyzed descriptively.

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PLATE 1: *Hymenolepis nana egg* recovered from infected rodent viewed under light microscope at magnification ×100

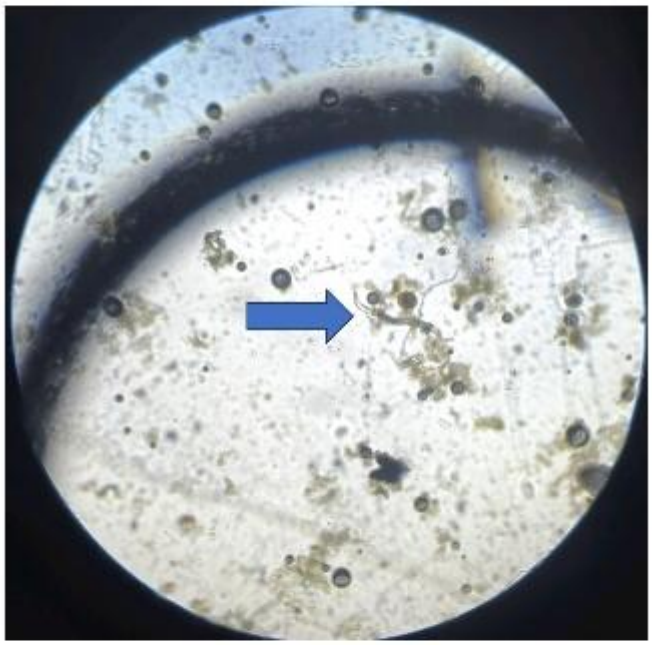


PLATE 2: *Hymenolepis nana larva* recovered from infected rodent viewed under light microscope at magnification ×100

Results:

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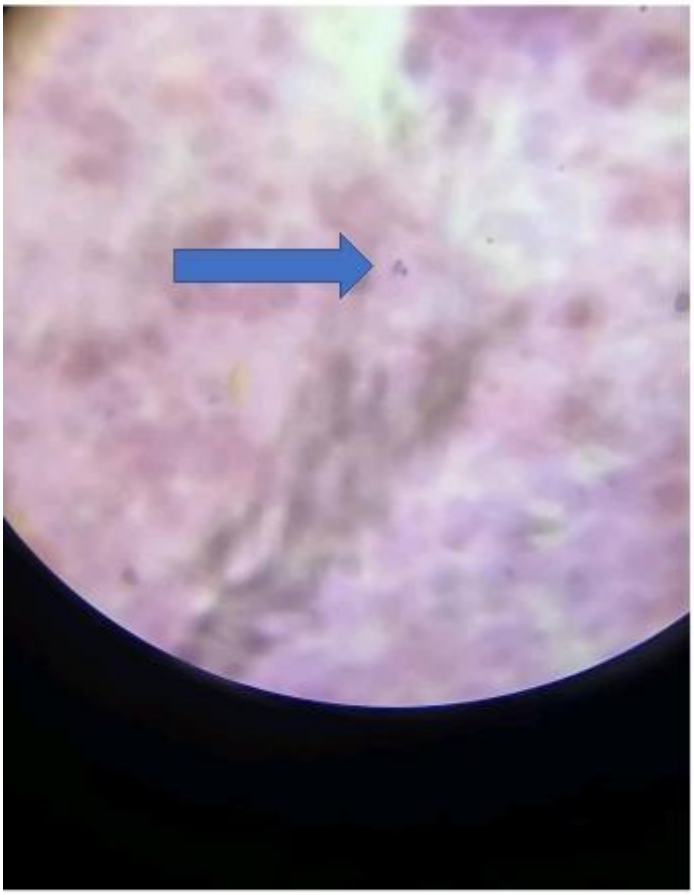


Plate 1: Amastigote form of Leishmania spp viewed under microscope x 100 magnification oil immersion using thin blood smear . Thirteen rodents belonging to four species were examined. *Plasmodium* spp. and *Leishmania* spp. were detected as haemoparasites. Only *Hymenolepis nana* was identified among gastrointestinal helminths, with low occurrence. Ectoparasites were common, with a 69.2% infestation rate, especially in poorly sanitized areas.

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Conclusions: Rodents in Sokoto Metropolis harbour parasites of zoonotic significance, highlighting the need for improved sanitation, rodent control, and continuous surveillance.

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SHIFT01-143

POSTER ON BOARD SHIFT 01
TRANSMISSION DYNAMICS, EPIDEMIOLOGY, AND GLOBAL HEALTH / VECTOR BIOLOGY, PARASITE TRANSMISSION AND CONTROL STRATEGIES

MOLECULAR IDENTIFICATION AND CHARACTERIZATION OF HEPATOZOON CANIS FROM DOGS AND TICKS IN NORTHERN MEXICO

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Background and Aims: Hepatozoonosis is a tick-borne disease (TBD) of dogs and other carnivorous caused by apicomplexan protozoans from the genus *Hepatozoon,* which affect mammals, birds, reptiles and amphibians acting as intermediate hosts. The definitive hosts are hematophagous arthropods, mainly ticks, in which the infective oocysts develop. Dogs and other carnivorous are infected by ingestion of ticks containing *Hepatozoon's* oocysts. In North America, the causative agent of hepatozoonosis in dogs is *H. americanum* with a few reports attributed to *H. canis*. In Mexico, hepatozoonosis has been documented since 2012. However, the *Hepatozoon* species and the tick vector remain unidentified. Herein the molecular identification and characterization of *Hepatozoon* circulating in dogs and ticks in Northern Mexico were investigated.

Methods: *Hepatozon*'s meronts were detected in blood smears from four naturally infected dogs that presented to two veterinary clinics in Victoria, Tamaulipas. From these, only one dog was infested with ticks identified as *Amblyomma mixtum* and *A. imitator.* PCR for 18S rRNA of *Hepatozoon* was performed from both, blood and ticks.

Results: of PCR and sequencing analysis determined that dogs were infected with *H. canis*. In addition, *H. canis* was also determined in both *A. mixtum* and *A. imitator* ticks.

Conclusions: In conclusion, *H. canis* is the causative agent of hepatozoonosis in Northern Mexico. However, further studies are required in order to dilucidate the role of *A. mixtum* and *A. imitator*, and other ticks as competent vectors of this pathogen.

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SHIFT01-144

POSTER ON BOARD SHIFT 01
TRANSMISSION DYNAMICS, EPIDEMIOLOGY, AND GLOBAL HEALTH / VECTOR BIOLOGY, PARASITE TRANSMISSION AND CONTROL STRATEGIES

IMPACT OF CLIMATE CHANGE ON THE DISTRIBUTION OF INSECT VECTORS AND VECTOR-BORNE DISEASES IN OWERRI WEST LGA, IMO STATE , NIGERIA.

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Background and Aims: This study examines the influence of climate variability on the distribution of insect vectors and the dynamics of vector-borne diseases in Owerri West Local Government Area, Imo State, Nigeria.

Methods: Using a descriptive cross-sectional design, data were collected from 234 respondents comprising residents, health personnel, and environmental officers through a multi-stage sampling approach. Primary data were obtained using a structured questionnaire (CCIVDVBDQ) based on a four-point Likert scale, while secondary data on disease incidence and climatic variables (temperature, rainfall, and relative humidity) were sourced from relevant agencies. Instrument validity was established through expert review, and reliability testing yielded a Cronbach's alpha coefficient of 0.78.

Results: Descriptive statistical analysis (mean and standard deviation; criterion mean = 2.50) showed that climate change significantly influences vector distribution, seasonal activity, and disease transmission. Temperature, rainfall, and humidity strongly affected insect vectors (Grand mean = 4.10, SD = 0.836), while climate change also promoted their spread into non-endemic areas (Grand mean = 4.19, SD = 0.812). It further increased transmission and infection rates among vulnerable populations (Grand mean = 4.26, SD = 0.773), especially in poor communities.

Conclusions: The study established that climate change significantly influences insect vector ecology and disease transmission. Changes in temperature, rainfall, and humidity enhance vector survival, spread, and transmission, increasing the burden of vector-borne diseases, especially among vulnerable populations.

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POSTER ON BOARD SHIFT 01
TRANSMISSION DYNAMICS, EPIDEMIOLOGY, AND GLOBAL HEALTH / VECTOR BIOLOGY, PARASITE TRANSMISSION AND CONTROL STRATEGIES

THE ROLE OF RHIPICEPHALUS SANGUINEUS TICK IN THE TRANSMISSION OF LEISHMANIA INFANTUM IN RESERVOIR HOSTS

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Background and Aims: Canine visceral leishmaniasis (CVL), caused primarily by *Leishmania infantum*, is a major zoonotic disease in the Mediterranean region, the Middle East, and parts of Latin America. Dogs act as the main reservoir hosts and are central to maintaining the parasite's lifecycle. Transmission traditionally occurs through the bite of infected female phlebotomine sandflies. Recently, attention has turned toward whether ticks could also participate in transmission, given the frequent detection of *Leishmania* DNA in various tick species.

Methods: A review of studies from 1990–2024 on *Leishmania infantum* transmission, vector competence, and reservoir hosts was conducted. Parasite DNA was extracted from ticks and confirmed through PCR analysis.

Results: *Leishmania* DNA has been repeatedly detected in *Rhipicephalus sanguineus* ticks, and dogs, as the main reservoir hosts, carrying these ticks, exhibited the highest rates of infection.

Conclusions: Canine leishmaniasis relies primarily on sandfly transmission, with dogs serving as the principal reservoir. Current evidence does not support ticks as biological vectors. It appears that the biological transmission of *Leishmania* parasite by ticks to dogs, as the reservoir hosts, requires further investigation.

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SHIFT01-146

POSTER ON BOARD SHIFT 01
TRANSMISSION DYNAMICS, EPIDEMIOLOGY, AND GLOBAL HEALTH / VECTOR BIOLOGY, PARASITE TRANSMISSION AND CONTROL STRATEGIES

ODOURS FROM LEISHMANIA INFANTUM–INFECTED DOGS AND FOXES ENHANCE THE ATTRACTION OF PHLEBOTOMUS PERNICIOSUS VECTOR

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Background and Aims: Sand flies are vectors of *Leishmania infantum*, which causes infection in several host species, including dogs, foxes and humans. Growing evidence suggests that this protozoan might induce changes in the composition of vertebrate host odours, eventually modulating the host seeking behavior of the vector. Here, we investigated whether volatile organic compounds (VOCs) profile from dogs and foxes are modified in the presence of *L. infantum* infection, and affect the attraction of *Phlebotomus perniciosus* vector.

Methods: Odors of *Leishmania infantum*-infected and non-infected dogs and foxes were sampled on standardized sterile filter-papers. VOCs were profiled by GC-MS and active compounds selected based on electroantennography (EAG). Behavioral responses of female *P. perniciosus* (*n*=50 specimens per replicate) were assessed for EAG active compounds and host odour using Y-tube olfactometer assays.

Results: VOCs derived from *L. infantum*-infected dogs (*n*=3) and fox (*n*=1) where characterized by the presence of 3-methylbutanoic acid, which was absent in negative animals (i.e., 3 dogs and 2 foxes). Nonanal was present in higher abundance in infected individuals when compared with non-infected ones. Moreover, in behavioral assays, the above compounds as well as filter papers from the lesions of infected animals were highly attractive ($p<0.05$) to *P. perniciosus* females.

Conclusions: Data indicate that *L. infantum* infection alters the VOCs profile in dogs and foxes, increasing *P. perniciosus* attraction to infected hosts. Therefore, infection-driven odour modulation may contribute to transmission ecology by increasing vector contact with infected reservoirs. Moreover, the identification these VOCs highlights their potential as non-invasive biomarkers for future diagnostic and surveillance tools.

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SHIFT01-147

POSTER ON BOARD SHIFT 01
TRANSMISSION DYNAMICS, EPIDEMIOLOGY, AND GLOBAL HEALTH / VECTOR BIOLOGY, PARASITE TRANSMISSION AND CONTROL STRATEGIES

DOGS AS POTENTIAL LEISHMANIA INFANTUM RESERVOIRS IN TIRADENTES CITY, MINAS GERAIS, BRAZIL

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Background and Aims: In the Americas, the etiological agent of visceral leishmaniasis(VL) is L*eishmania infantum*.This disease can be fatal if specific treatment is not administered.The domestic dog is considered the primary reservoir host for *L. infantum*; therefore, early detection, close surveillance, and treatment of infected dogs are essential to control the spread of the parasite. Approximately 90% of human VL cases occur in India, Brazil, and Sudan. In Brazil, the state of Minas Gerais(MG) is among the ten states with the highest incidence and serves as a significant region for tourism.This study aimed to characterize the population of free-roaming dogs in the tourist city of Tiradentes/MG/Brazil and explored the implications of their presence on the streets for both animal and public health.

Methods: Our investigation involved photographing free-roaming dogs throughout Tiradentes.Data collection took place during a holiday period(July 2024) when the city's population was significantly increased by tourists.

Results: Figure1 provide visual documentation of the conditions faced by free-roaming dogs in Tiradentes.The images capture these dogs foraging in garbage, scavenging for sustenance, and resting in public streets and squares. Several dogs exhibited signs of weakness, suggesting potential health issues. Figure 1 - Free-roaming dogs in the streets of Tiradentes city, Minas Gerais,

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Brazil



Conclusions: The risk of *L.infantum* transmission from free-roaming dogs in the streets of Tiradentes is a significant concern.Our observations suggest a potential risk of transmitting infectious diseases, such as VL, to the visiting tourist population.We urge public authorities to address this issue by implementing effective measures for dog population management.

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SHIFT01-148

POSTER ON BOARD SHIFT 01
TRANSMISSION DYNAMICS, EPIDEMIOLOGY, AND GLOBAL HEALTH / VECTOR BIOLOGY, PARASITE TRANSMISSION AND CONTROL STRATEGIES

GENOME ARCHITECTURE AND CHROMOSOMAL ORGANIZATION OF RHODNIUS PROLIXUS IN COLOMBIA

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Background and Aims: Chagas disease remains a major public health challenge in Latin America and worldwide, with vector-borne transmission playing a central role in endemic regions such as Colombia. *Rhodnius prolixus* is one of the principal domiciliated vectors involved in the transmission of the protozoan parasite *Trypanosoma cruzi*. Despite its epidemiological relevance and long-standing use as a model organism in vector biology, genomic resources available for *R. prolixus* have been limited. The existing genome corresponds to a draft assembly with low contiguity (low N50), deficiencies in structural resolution, and limited functional annotation, constraining comprehensive genomic analyses.

Methods: In this study, a chromosome-level reference genome of *R. prolixus* was generated using long-read sequencing technologies (PacBio) combined with a specialized bioinformatic workflow for de novo assembly, genome polishing, and quality assessment. Structural annotation was supported by transcriptomic data generated through RNA sequencing (RNA-seq), which were used to refine gene models, delineate coding regions, and improve annotation accuracy.

Results: The resulting genome assembly shows a substantial improvement in continuity and structural organization compared to previous versions, with reduced fragmentation and improved representation of repetitive and complex genomic regions. The reconstructed chromosomal architecture reveals coherent patterns of genome organization and provides a robust framework for downstream genomic analyses.

Conclusions: Integration of RNA-seq data strengthened the annotation process, enabling more precise definition and validation of gene models. Overall, this chromosome-level genome assembly and its associated functional annotation represent a high-quality genomic resource for *R. prolixus*, overcoming key limitations of earlier assemblies and expanding the resources available for molecular and genomic studies of this important vector of Chagas disease.

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POSTER ON BOARD SHIFT 01
TRANSMISSION DYNAMICS, EPIDEMIOLOGY, AND GLOBAL HEALTH / VECTOR BIOLOGY, PARASITE TRANSMISSION AND CONTROL STRATEGIES

MOLECULAR DETECTION OF PATHOGENS IN BLACK FLIES (DIPTERA: SIMULIIDAE) IN NORTHERN AND WESTERN THAILAND

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Background and Aims: Black flies (Diptera: Simuliidae) are important neglected vectors of diverse pathogens at the wildlife–livestock–human interface. *Simulium nodosum* and *Simulium nigrogilvum* are mammal-biting species in Thailand and potential vectors of zoonotic *Onchocerca* spp. This study aims to detect filarial nematodes and blood protozoa in these species using PCR-based xenomonitoring.

Methods: Adult female black flies were collected by sweep netting in Tak and Chiang Mai provinces. Heads and thoraces of 10 individuals of each species were pooled and molecularly screened for three pathogen groups; targeting filarial 18S ribosomal RNA for screening and cytochrome *c* oxidase subunit I (*COI*) for confirmation, *Leucocytozoon* cytochrome *b*, and trypanosomatid 18S ribosomal RNA. Positive amplicons were sequenced, compared with references by BLAST and analysed phylogenetically.

Results: Filarial *COI* DNA were detected in 17/31 *S. nigrogilvum* pools and 1/111 *S. nodosum* pool, corresponding to minimum infection rates of ~5.5% and ~0.1%, respectively. *COI* sequences showed high similarity to bovine-associated *Onchocerca* sp. type I, providing the first record of this lineage in *S. nodosum*. Trypanosomatid DNA occurred in 4/31 *S. nigrogilvum* and 3/111 *S. nodosum* pools; sequencing confirmed *Trypanosomatidae* spp. and *Trypanosoma avium* in two *S. nodosum* pools. All pools were negative for *Leucocytozoon*.

Conclusions: These findings demonstrate infection of mammal-biting black flies with *Onchocerca* sp. type I and provide the first evidence of trypanosomatid DNA in *S. nodosum* and *S. nigrogilvum* in Thailand. They support the role of these species as potential bridge vectors of zoonotic parasites and highlight molecular xenomonitoring for early detection of emerging vector-borne diseases.

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SHIFT01-150

POSTER ON BOARD SHIFT 01
TRANSMISSION DYNAMICS, EPIDEMIOLOGY, AND GLOBAL HEALTH / VECTOR BIOLOGY, PARASITE TRANSMISSION AND CONTROL STRATEGIES

DEVELOPMENT AND EFFECTIVENESS TEST OF AN INTERNET-BASED REAL-TIME MONITORING DEVICE FOR MALARIA MOSQUITOES

Xiao-Guang Chen
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Background and Aims: Malaria, transmitted by Anopheles mosquitoes, endangers billions of people globally and imposes substantial economic burdens. Traditional vector control methods are increasingly ineffective due to insecticide resistance and behavioral shifts of Anopheles from the endophilic to the exophilic. Conventional monitoring methods are labor-intensive, inefficient, and lack real-time data transmission. Existing internet-based monitoring devices have limitations in sensitivity and specificity for Anopheles detection.

Methods: An internet-based real-time monitoring device named Black Box was designed and 3D-printed, which integrates multiple mosquito-attracting factors (specific wavelength light, temperature simulation, chemical lures, photocatalytic materials) with automatic identification and wireless transmission modules. The efficacy of Black Box was validated in Laboratory, Semi-field and Field.

Results: In laboratory tests, the Black Box achieved trapping rates of 94.00% (*An. sinensis*), 95.00% (*An. stephensi*), and 95.00% (*An. anthropophagus*), with data consistency rates (cloud vs. actual capture) exceeding 97%. In semi-field settings, trapping rates were 73.60% (*An. sinensis*), 90.80% (*An. stephensi*), and 83.00% (*An. anthropophagus*), with consistency rates above 92%. Field tests (30 days) captured 5109 mosquitoes including 222 *An. sinensis*, with an overall data consistency rate of 94.2%. The device outperformed traditional traps in capturing Anopheles (222 vs. 8 by light traps). Real-time monitoring revealed bimodal mosquito activity peaks (sunrise and sunset), and 18.75% of field-captured *An. sinensis* were blood-fed, indicating outdoor feeding behavior.

Conclusions: These findings indicated that Black Box can efficiently trap and real-time monitor the vector mosquitoes including anopheles and offer a scientific basis for dynamic malaria risk assessment and targeted control strategies.

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POSTER ON BOARD SHIFT 01
TRANSMISSION DYNAMICS, EPIDEMIOLOGY, AND GLOBAL HEALTH / VECTOR BIOLOGY, PARASITE TRANSMISSION AND CONTROL STRATEGIES

A SURVEY OF SAND FLIES (DIPTERA: PSYCHODIDAE) COLLECTED WITH DIFFERENT COLORS OF LIGHT-EMITTING DIODES (LEDS), ACRE STATE, BRAZILIAN AMAZON

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Background and Aims: Sand flies are dipterans of epidemiological importance because they transmit parasites responsible for causing leishmaniasis. These insects can be attracted by different stimuli, odors, and light sources. This study aims to verify the diversity of phlebotomine fauna collected with different LED colors in a preserved forest area in the Brazilian Amazon.

Methods: The collection was carried out using CDC-type light traps, adapted with lights of the following colors: incandescent (control) and LEDs in ultraviolet, white, blue, and green colors. The collections were conducted for five consecutive days, according to the Latin square design.

Results: A total of 24,126 sandflies were collected, distributed across 13 genera and 37 species, with the *Trichophoromyia ubiquitalis* (15,369) species being the highest number collected in all colors. Incandescent light proved to be more efficient in terms of the abundance of insects collected, compared to other types of light. The greatest species richness was observed under ultraviolet and green lights.

Conclusions: Our results indicate that certain light sources, like ultraviolet, can be effective in increasing species richness.

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TRANSMISSION DYNAMICS, EPIDEMIOLOGY, AND GLOBAL HEALTH / VECTOR BIOLOGY, PARASITE TRANSMISSION AND CONTROL STRATEGIES

FLEA SPECIES DIVERSITY AND HOST ASSOCIATIONS IN DOMESTIC AND WILD CANIDS AND FELIDS FROM ROMANIA

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Background and Aims: Fleas (Siphonaptera) are important ectoparasites of carnivores, acting as both direct parasites and vectors of pathogens, including zoonotic agents. This study aimed to investigate flea species diversity, host associations, and seasonal patterns in domestic and wild canids and felids from Romania.

Methods: Fleas were collected opportunistically using fine needle combs from domestic dogs and cats and from wild canid and felids carcasses, and identified morphologically. Prevalence was calculated for each flea species, and comparisons were made between domestic and wild host groups and across seasons.

Results: Domestic cats were infested by *Ctenocephalides felis* (87.5%) and *C. canis* (12.5%), while dogs were infested by *C. canis* (55.93%), *C. felis* (44.06%), *Pulex irritans* (35.59%), and *Ctenophthalmus* spp. (1.69%). In wild carnivores, the most prevalent species was *P. irritans* (32.1%), followed by *Chaetopsylla* spp. (15.3%), *C. canis* (11.7%), *Ch. trichosa* (11.7%), and *C. felis* (7.3%), *Ch. homoea* (5.8%), *Ch. globiceps* (5.8%), *Paraceras melis* (3.6%), *Monopsyllus sciurorum* (0.7%), *Ch. rothschildi* (0.7%), and *Echidnophaga gallinacea* (0.7%). Flea infestations were recorded in all sampled seasons with no strong seasonal restriction evident for the dominant flea species, suggesting year-round transmission potential in both domestic and sylvatic cycles.

Conclusions: These findings provide preliminary data on flea diversity and host associations in Romanian domestic and wild carnivores. Flea communities differed markedly between domestic and wild carnivores, with higher species diversity in wildlife and evidence of spillover at the wildlife–domestic interface. The lack of strong seasonality suggests continuous circulation and sustained zoonotic risk.

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TRANSMISSION DYNAMICS, EPIDEMIOLOGY, AND GLOBAL HEALTH / VECTOR BIOLOGY, PARASITE TRANSMISSION AND CONTROL STRATEGIES

EVALUATION OF SKIN AND TEXTILE REPELLENTS FOR THEIR PROTECTIVE ACTIVITY AGAINST THE LOIASIS VECTORS CHRYSOPS SILACEA AND CHRYSOPS DIMIDIATA

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Background and Aims: Loiasis is a vector-borne disease in the Afrotropics, caused by *Loa loa* and transmitted by *Chrysops* flies. Nearly 42 million live in moderate to high transmission areas and over 23 million people are infected. There are no established control tools available to reduce transmission risk. Whereas repellents protect against bites of other hematophagous arthropods, data on their protective efficacy against *Chrysops* in humans is lacking. Therefore, we decided conduct a randomized controlled clinical trial assessing four commercial skin-applied and two textile-applied repellents against *Chrysops* bites was conducted in a hyperendemic loiasis region in Gabon.

Methods: In the first clinical trial, volunteers were randomly assigned to one of the skin repellents DEET, Icaridin, Citriodiol, IR3535, or an inactive control. Primary endpoint was the number of blood meal attempts by *Chrysops*, while frequencies and duration of *Chrysops* landings on clothing and exposed skin were secondary outcomes. The second clinical trial applied the same setup with the textile repellents Icaridin and Permethrin/*Chrysanthemum* alone and in combination with two of the most active skin repellents of the first trial.

Results: of tested repellents showed a statistically significant, but not complete reduction of blood meal attempts. Combination of textile repellents with one of the active skin repellents showed the highest reduction of blood meal attempts and are therefore the best available options for reduction of exposure to infective bites.

Conclusions: Studies showed a substantial but not complete protective efficacy of skin repellents against *Chrysops* bites, the combination is the best option to reduce exposure to infective bites.

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TRANSMISSION DYNAMICS, EPIDEMIOLOGY, AND GLOBAL HEALTH / VECTOR BIOLOGY, PARASITE TRANSMISSION AND CONTROL STRATEGIES

THE PHILIPPINE HEARTWORM PROTOCOL INITIATIVE: A DATA-DRIVEN APPROACH TO IMPROVING CANINE HEARTWORM MANAGEMENT IN THE PHILIPPINES

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Background and Aims: The Philippines is among the countries with the highest dog population. Among the many parasitic diseases, canine heartworm disease (CHD) due to *Dirofilaria immitis* is undeniably among the most devastating. In the Philippines, where the parasite is endemic and mosquito vectors are widespread, the risk of transmission persists. With limited epidemiologic data and no standardized local management protocol, the impact of CHD is uncertain. Moreover, compliance among dog owners is critical to successful prevention and control. The Philippine Heartworm Protocol Initiative (PHPI) was established to develop feasible recommendations grounded in scientific evidence to significantly improve CHD control in the country.

Methods: PHPI conducted an epidemiological study utilizing antigen detection in 700 dogs from several areas in the country to estimate the latest prevalence. Surveys assessing the current practices of almost 700 Filipino veterinarians in CHD management, as well as the knowledge, attitudes, and practices (KAP) of more than 1,000 Filipino dog owners, were also conducted to identify critical gaps.

Results: An overall prevalence of 5.3% was obtained, with cities in the National Capital Region having the greatest number of heartworm antigen-positive dogs. The survey on management practices revealed deviations from recommended international standards in the timing of prevention, diagnostic testing, and treatment protocol. Meanwhile, the KAP survey of dog owners indicated varying levels of understanding and attitudes across regions, with notable gaps in knowledge and preventive practices.

Conclusions: Despite the low prevalence, the study revealed gaps in veterinarians' practices and dog owners' KAP that must be addressed to more effectively control CHD.

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TRANSMISSION DYNAMICS, EPIDEMIOLOGY, AND GLOBAL HEALTH / VECTOR BIOLOGY, PARASITE TRANSMISSION AND CONTROL STRATEGIES

A HOST-FREE IN VITRO MEMBRANE FEEDING SYSTEM FOR AN EMERGING TICK VECTOR, HAEMAPHYSALIS LONGICORNIS

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Background and Aims: *Haemaphysalis longicornis* is a parthenogenetic three-host tick that has expanded from East Asia into Australasia and the Americas, posing increasing veterinary and public health concerns. Experimental research on this species has been constrained by reliance on live vertebrate hosts, limiting scalability and ethical feasibility. This study aimed to establish a reproducible, host-free in vitro feeding system for adult and nymphal stages of *H. longicornis*.

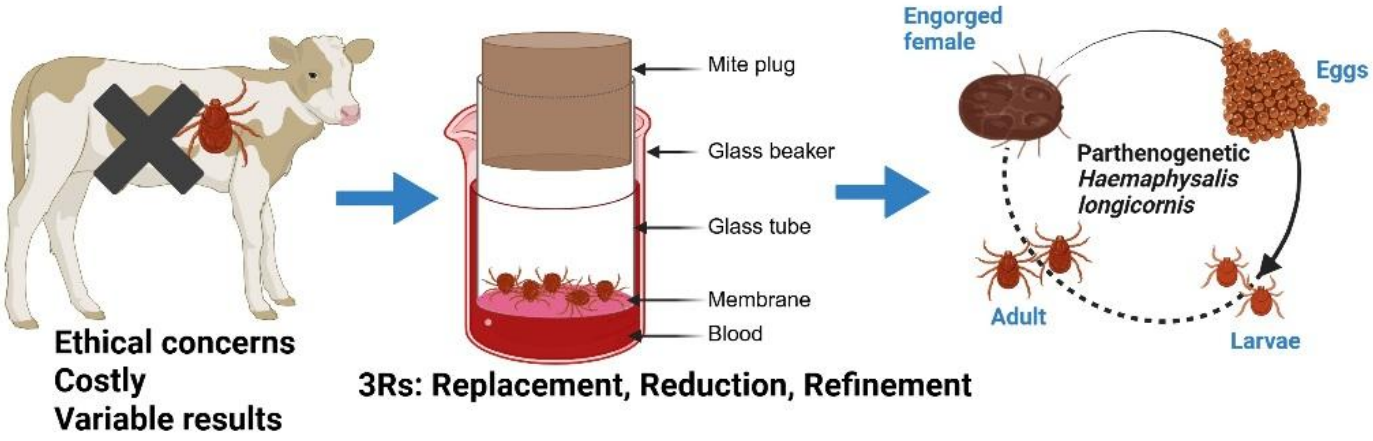
Methods: Adult and nymphal *H. longicornis* were fed using a silicone membrane-based artificial feeding system. Field-collected parthenogenetic adult females from Australia were evaluated across six independent experiments for attachment, engorgement, oviposition, egg hatchability and bloodmeal-to-egg conversion efficiency. Nymphal feeding performance was assessed across four experiments based on attachment, engorgement, detachment and moulting.

Results: Adult feeding performance was robust, with 67% (35/52) of ticks attaching and 74.3% (26/35) of attached ticks engorged. Engorged females achieved a mean weight of 161 mg, produced a mean egg mass of 67 mg, demonstrating a bloodmeal-to-egg conversion efficiency of 40%. All engorged females oviposited and produced viable larvae, with egg hatchability exceeding 92%. Nymphal feeding performance was similarly strong: with 91.5% (65/71) attached within 2–5 days; 98.5% (64/65) of attached nymphs engorged; and detached within 5–10 days. Following detachment, 89.1% (57/64) successfully moulted within 2–3 weeks.

Conclusions: This study establishes a reproducible, host-free, multi-life-stage in vitro feeding platform for *H. longicornis* that supports adult reproduction and nymphal development. The system provides an ethical and scalable studies of tick biology, control strategies and pathogen–vector

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interactions.



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TRANSMISSION DYNAMICS, EPIDEMIOLOGY, AND GLOBAL HEALTH / VECTOR BIOLOGY, PARASITE TRANSMISSION AND CONTROL STRATEGIES

THE EFFECTS OF HABITAT ON PALEARCTIC CULICOIDES SPP. ABUNDANCE AND COMMUNITY COMPOSITION ACROSS AN AGRO-ECOLOGICAL GRADIENT

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Background and Aims: Biting midges (genus *Culicoides* Latreille) are important vectors of livestock arboviruses, including Bluetongue virus (BTV). While their distribution has been widely studied within farm environments, less is known about their occurrence across surrounding landscapes. This study assesses how habitat type and distance from farm influence *Culicoides* abundance along an agro-ecological gradient on a sheep farm in eastern England. The site was selected after BTV-associated mortalities in 2024 were observed exclusively in a pasture distant from the main farm, suggesting the potential role of local habitat features in maintenance and transmission risk.

Methods: Adult *Culicoides* were samped May-September 2025 using twelve CO2-baited CDC traps deployed along a transect spanning pasture, woodland and cropland habitats. Meteorological data was recorded. Specimens were identified morphologically to species level, with molecular methods used to resolve cryptic species. Abundance was analysed using negative binomial generalised linear mixed models, with additional pasture-only models assessing within-habitat variation.

Results: A total of 9,219 *Culicoides* were collected from 242 trap catches over 22 sampling nights. Abundance differed significantly among habitats, with highest counts in pasture, lowest in cropland and intermediate levels in woodland. Distance from farm and meteorological variables showed no significant effects. Marked trap-to-trap variation indicated fine-scale spatial heterogeneity in midge activity. Differences in species composition were observed across habitats.

Conclusions: Habitat was the strongest predictor of *Culicoides* abundance. Fine-scale spatial heterogeneity highlights the importance of local habitat features when assessing vector and disease transmission risk. Ongoing analyses will further clarify how agro-ecological gradients shape vector communities.

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TRANSMISSION DYNAMICS, EPIDEMIOLOGY, AND GLOBAL HEALTH / VECTOR BIOLOGY, PARASITE TRANSMISSION AND CONTROL STRATEGIES

FEASIBILITY, ACCEPTABILITY AND HEALTH IMPACT OF LARGE-SCALE IVERMECTIN–ALBENDAZOLE CO-ADMINISTRATION IN SCHOOL-BASED MASS DRUG ADMINISTRATION IN KISORO, UGANDA

Jennifer Keiser
Swiss Tropical and Public Health Institute, Allschwil, Switzerland

Background and Aims: Soil-transmitted helminth (STH) infections remain highly prevalent among school-aged children in endemic settings. Although co-administration of ivermectin-albendazole has been shown to be more efficient than albendazole monotherapy, there is limited research on its feasibility, acceptability, safety, and health impact under routine programmatic conditions. This study provides evidence to inform integration of co-administration therapy into national deworming programmes.

Methods: A large-scale implementation study was embedded within a routine school-based mass drug administration (MDA) campaign in Kisoro District, Uganda. For MDA, all 283 primary schools were included, with sub-counties assigned to receive either albendazole alone or ivermectin-albendazole co-administration with a ratio of 3:1. Health impact was assessed in a subsample of 60 children in each of 20 sentinel schools examined in parasitological surveys prior and three months post-MDA. Feasibility and acceptability were assessed through interviews with health workers and village health teams (VHTs). Implementation-related costs, performance indicators and passive pharmacovigilance data were collected using adapted programme documentation tools.

Results: We observed high average treatment coverage (>90%) in the combination arm, with 29,157 children treated. The parasitological data from 10 sentinel schools in each of the two treatment arms will be presented and compared. The perception of involved health workers and VHTs on feasibility and acceptability of the co-administration and estimated incremental cost per child treated will be shown.

Conclusions: This study provides guidance on uptake of improved deworming strategies into existing routine control activities to STH endemic countries by estimating the logistics and additional efforts needed.

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ENVIRONMENTAL AND CLIMATIC DRIVERS OF INTESTINAL PARASITE CARRIAGE IN HOUSEFLIES (MUSCA DOMESTICA) ACROSS GHANAIAN AGROECOLOGICAL ZONES

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Background and Aims: Houseflies (*Musca domestica*) are common synanthropic insects that frequently interact with human waste, animal feces, and decomposing organic matter, making them potential mechanical carriers of intestinal parasites. However, limited information exists on how environmental and climatic conditions influence parasite carriage in tropical urban ecosystems. This study investigated the ecological and environmental drivers of parasite carriage in houseflies across Ghana's major agroecological zones.

Methods: Adult houseflies were collected from garbage dumps, abattoirs, homes, markets, and hospitals within the coastal savanna, forest, and guinea savanna zones of Ghana during wet and dry seasons in 2023–2024. Fly abundance was assessed using Scudder grill. Parasites carried externally on flies were isolated using phosphate-buffered saline washing, centrifugation, and microscopic examination, including Ziehl–Neelsen staining. Statistical analyses were performed using generalized linear mixed models to assess associations between parasite prevalence and environmental variables.

Results: Overall parasite prevalence was 17.5% (105/600 pooled samples). Nine parasite taxa were identified, with *Ascaris* spp. showing the highest prevalence (7.83%). Parasite prevalence was highest in the coastal savanna and forest zones and increased significantly during the wet season. Season, temperature, and fly abundance were significant predictors of parasite carriage.

Conclusions: Houseflies may serve as practical indicators of environmental contamination and potential dissemination pathways for intestinal parasites in urban tropical settings. Improved sanitation and waste management may reduce fly-associated parasitic contamination risks.

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BASELINE EPIDEMIOLOGY AND MICRO GEOGRAPHIC DISTRIBUTION OF FEMALE GENITAL SCHISTOSOMIASIS IN THE FEDERAL CAPITAL TERRITORY, NIGERIA

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Background and Aims: Female genital schistosomiasis (FGS), a chronic outcome of *Schistosoma haematobium* infection, remains underdiagnosed and insufficiently integrated into schistosomiasis control programs that largely prioritize urinary schistosomiasis in school-aged children. Evidence on confirmed FGS among women in the Federal Capital Territory (FCT) is limited. This study aimed to generate baseline epidemiological data on FGS in the FCT to guide targeted interventions and support policy integration.

Methods: A cross-sectional survey was implemented in 20 purposively selected communities across AMAC and Gwagwalada Area Councils. Households were systematically enumerated for participant recruitment. Urinary schistosomiasis was assessed using urine filtration microscopy among individuals aged 10–59 years. Sexually active women underwent colposcopy, and only colposcopy confirmed FGS lesions were analyzed. Prevalence differences across councils, communities, and age groups were examined using chi square tests, Fisher's exact test, Cochran–Mantel–Haenszel methods, and binomial logistic regression.

Results: FGS prevalence was significantly higher in Gwagwalada (28.9%; 83/287) than AMAC (14.5%; 43/296) ($p<0.001$). Women in Gwagwalada were nearly twice as likely to have confirmed FGS

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(RR=1.99; 95% CI: 1.43–2.77). Urinary schistosomiasis prevalence was similar across councils (AMAC: 7.7%; Gwagwalada: 6.4%; p=0.47). Significant community level clustering was observed for FGS in Gwagwalada (p=0.005) and urinary egg positivity in AMAC (p<0.001). FGS prevalence increased with age, while urinary egg positivity declined.

Conclusions: FGS shows a distinct epidemiological pattern, disproportionately affecting adult women and demonstrating microgeographic clustering. Integrating FGS screening into reproductive health services, expanding preventive chemotherapy beyond school aged children, and prioritizing high burden communities are critical for equitable schistosomiasis elimination.

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TRANSMISSION DYNAMICS, EPIDEMIOLOGY, AND GLOBAL HEALTH / VECTOR BIOLOGY, PARASITE TRANSMISSION AND CONTROL STRATEGIES

NOMENCLATURE AND TAXONOMIC DRIFT IN MEDICAL MALACOLOGY AND ITS POTENTIAL CONSEQUENCES

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Background and Aims: Taxonomic stability and accuracy are fundamental to effective disease control, yet recent proposed nomenclature changes and persistent misidentifications of schistosomiasis snail vectors cloud decades of medical malacology progress. Critical issues center on African species *Biomphalaria sudanica*, *B. choanomphala*, and *B. pfeifferi*. Despite genomic evidence suggesting *B. sudanica* and *B. choanomphala* represent ecophenotypes with minimal genetic divergence, recent publications universally apply *B. choanomphala* to all Lake Victoria populations, including shallow-water snails traditionally identified as *B. sudanica*. This has public health implications as *B. choanomphala* exhibits higher compatibility with *Schistosoma mansoni* than *B. sudanica*, confusing risk assessment.

Methods: Recent publications incorrectly report single species (*B. pfeifferi*) encompassing broader range than genomic studies support, instead of reporting mixed species assemblages. This creates misleading transmission risk assessments because *B. pfeifferi* is the most compatible African species. Problems extend beyond scientific literature as high-profile media outlets publish images of non-host species while reporting on schistosomiasis, potentially misleading communities. Taxonomic databases like MolluscaBase exhibit incomplete entries, with medically important species absent despite established transmission roles.

Results: Nomenclature inconsistency makes literature difficult to track, prevents reliable field identification, and disconnects compatibility data from species names. For medically important snails, nomenclature changes should require particular molecular evidence and community consensus. We propose formal guidelines including: 1) retention of established names until population genomics definitively resolve species boundaries, 2) standardized molecular identification protocols, 3) database corrections, and 4) engagement balancing taxonomic precision with public health pragmatism.

Conclusions: Without coordinated action, proliferating synonyms and misidentifications will undermine snail-based schistosomiasis elimination strategies.

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TRANSMISSION DYNAMICS, EPIDEMIOLOGY, AND GLOBAL HEALTH / VECTOR BIOLOGY, PARASITE TRANSMISSION AND CONTROL STRATEGIES

NATURALLY ACQUIRED IMMUNE RESPONSES TO ANTIGENS OF THE SEXUAL AND ASEXUAL OF PLASMODIUM FALCIPARUM IN THE SOUTHERN AND CENTRAL REGIONS OF SENEGAL

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Background and Aims: Despite sustained malaria control interventions in Senegal, transmission remains seasonal. Malaria transmission depends on the presence of infectious sexual-stage parasites in human peripheral blood. Naturally acquired immune responses to both sexual and asexual stages can modulate transmission, contribute to protection, and support the development of transmission-blocking vaccines. This study evaluated antibody response profiles to asexual blood-stage antigens (PfAMA1, $PfMSP1_{19}$, GLURP) and sexual-stage antigens (Pfs230C1, Pfs48/45.6C) in populations naturally exposed to different malaria transmission intensities in Senegal.

Methods: A cross-sectional survey was conducted at the end of the transmission season in southern (Saraya) and central (Keur Socé) Senegal. A total of 1,106 asymptomatic individuals aged ≥5 years were enrolled. Capillary blood samples were collected for rapid diagnostic testing, microscopy, and dried blood spots for immunological assays. Antibody responses were measured using a Luminex multiplex assay.

Results: The study population was predominantly young (median age: 12 years). *Plasmodium falciparum* prevalence by microscopy was 22.1% in Saraya and 2.2% in Keur Socé. In Saraya, *P. malariae* (1.61%) and *P. ovale* (0.18%) were also detected. Seroprevalence of antibodies to all five antigens was significantly higher in Saraya. Antibody responses varied with endemicity and increased with age for all antigens except Pfs48/45. $PfMSP1_{19}$ showed the highest seroprevalence in Saraya (49.9%).

Conclusions: These findings indicate that antibody prevalence and levels increase with exposure intensity, suggesting a role in naturally acquired immunity. Monitoring children receiving seasonal malaria chemoprevention is important, as reduced antibody responses were observed in this group.

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TRANSMISSION DYNAMICS, EPIDEMIOLOGY, AND GLOBAL HEALTH / VECTOR BIOLOGY, PARASITE TRANSMISSION AND CONTROL STRATEGIES

RESIDUAL EFFICACY AND KNOCKDOWN PERFORMANCE OF DELTAMETHRIN-TREATED SURFACES AGAINST ANOPHELES MACULATUS

Koon Weng Lau[1,2], Chee Dhang Chen[3,4], Han Lim Lee[5]
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Background and Aims: *Anopheles maculatus* is a key malaria vector in Peninsular Malaysia, particularly in forested and hilly regions. This study evaluated the residual efficacy of deltamethrin-based indoor residual spraying (IRS) applied using an Agrofog Stainless Steel Sprayer (Model 108 WS) against *An. maculatus* under controlled laboratory conditions.

Methods: Laboratory-reared, unfed female *An. maculatus* were exposed for 30 minutes to treated plywood and concrete surfaces sprayed with deltamethrin 25% w/w at a target dosage of 25 mg active ingredient/m^2. Treated surfaces were positioned under both indoor and outdoor (keep under roof's eaves protected from direct sunlight and rain) conditions, and knockdown and mortality responses were monitored over a 67-week period. The 50% (KT_{50}) and 90% (KT_{90}) knockdown times were determined using probit analysis.

Results: Indoor-treated surfaces demonstrated consistently high bioefficacy, achieving 90–100% mortality throughout the 67-week evaluation period. KT_{50} values ranged from 6.68 to 26.49 minutes for plywood and 10.07 to 24.18 minutes for concrete, indicating rapid knockdown across substrate types. In contrast, outdoor-treated surfaces exhibited reduced persistence, maintaining >80% mortality for up to 3 weeks and >50% mortality for up to 9 weeks, likely due to environmental degradation, particularly photolysis of deltamethrin.

Conclusions: These findings highlight the prolonged residual activity and operational effectiveness of deltamethrin IRS on common indoor surfaces, supporting its continued use in malaria vector control programmes. However, its limited outdoor persistence underscores the need for protective strategies or alternative formulations for outdoor applications. Overall, this study reinforces the role of IRS as a cornerstone intervention for sustained indoor malaria vector control in endemic settings.

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TRANSMISSION DYNAMICS, EPIDEMIOLOGY, AND GLOBAL HEALTH / VECTOR BIOLOGY, PARASITE TRANSMISSION AND CONTROL STRATEGIES

SEROPREVALENCE OF HUMAN TOXOPLASMOSIS AT PIKINE HOSPITAL, SUBURBAN DAKAR, SENEGAL: A RETROSPECTIVE STUDY (2020–2024)

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Background and Aims: Toxoplasmosis is a zoonotic disease of major public health concern, particularly in developing countries where data remain limited. In Senegal, information on the seroprevalence of human toxoplasmosis is scarce, especially in suburban areas where environmental and socioeconomic factors may influence transmission. This study aimed to determine the seroprevalence of human toxoplasmosis and describe its epidemiological characteristics at Pikine Hospital, suburban Dakar, Senegal.

Methods: A retrospective descriptive study was conducted using laboratory records of patients tested for *Toxoplasma gondii*antibodies at Pikine Hospital between January 2020 and December 2024. Anti-*T. gondii* IgG and IgM antibodies were detected using ELISA Platelia kits (Bio-Rad), and results were interpreted according to the manufacturer's recommended cut-off values. Sociodemographic and clinical data were analyzed when available using appropriate statistical methods.

Results: A total of 1,601 patients were included. The overall seroprevalence of anti-*T. gondii* IgG was 34.97%, indicating prior exposure to the parasite. Anti-*T. gondii* IgM antibodies were detected in 0.81% of patients, suggesting possible recent infection, while 0.5% were positive for both IgG and IgM. Seroprevalence varied by age, sex, and year of testing, with higher rates observed among individuals aged 15–45 years.

Conclusions: This study reveals a moderate seroprevalence of toxoplasmosis among patients attending Pikine Hospital, reflecting substantial exposure to *T. gondii* in this suburban area of Dakar. These findings emphasize the need for strengthened preventive measures and routine screening, particularly among high-risk populations.

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TRANSMISSION DYNAMICS, EPIDEMIOLOGY, AND GLOBAL HEALTH / VECTOR BIOLOGY, PARASITE TRANSMISSION AND CONTROL STRATEGIES

VECTOR CONTROL POTENTIAL OF NATURAL SUBSTANCES: EVIDENCE FROM ANOPHELES SPP. ASSAYS

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Background and Aims: Malaria, an endemic disease caused by *Plasmodium* spp. and transmitted by *Anopheles* spp., remains a public health concern in Brazil, particularly in the Amazon region, where it disproportionately affects peri-urban and rural populations, causing significant social and economic impacts. As a result, vector control strategies based on natural substances with insecticidal potential have gained increasing attention. This study aimed to evaluate the larvicidal and adulticidal activity of natural compounds against *Anopheles* spp.

Methods: Larvicidal assays were performed according to World Health Organization guidelines (2005) using third-instar *An. aquasalis* larvae. Abutasterone, vitexin, and oosporin were diluted in DMSO and tested at eight concentrations (0.2 to 2 PPM); control groups received solvent only. Larval mortality was assessed at 24, 48, and 72 hours. Adulticidal assays were conducted with *An. darlingi* female exposed for 60 minutes to plates impregnated with the substances at concentrations of 200, 20, and 2 µmol/m^2. Survival was monitored for up to 15 days. Data were analyzed using the Log-rank test.

Results: Abutasterone exhibited significant larvicidal activity at 1.2 PPM ($p < 0.0006$) compared to the control, while vitexin showed larvicidal effects across five concentrations (0.4 to 1.4 PPM; $p < 0.001$). In adulticidal assays, abutasterone significantly reduced mosquito survival at 200 µmol/m^2 ($p = 0.032$).

Conclusions: These preliminary findings highlight the larvicidal and adulticidal potential of the tested compounds, particularly abutasterone and vitexin. Further studies with expanded replication are required to confirm and validate these results.

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WILD-CAUGHT NON-ANOPHELINE MOSQUITOES CARRYING PLASMODIUM FALCIPARUM SPOROZOITES IN EASTERN ZIMBABWE

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Background and Aims: During field evaluation of a focused case-response malaria entomological surveillance approach, adult mosquitoes from several non-anopheline genera were caught, alongside anophelines, associated with villages that had the prevailing highest numbers of malaria cases for the week in sampled health centre catchments. In the current study the non-anopheline mosquito samples were subjected to analysis for malaria infection and blood meal source.

Methods: The focused case-response approach and standard routine entomological surveillance were conducted simultaneously every month in 24 health centre catchments of Mutasa district from 2022 – 2023, using prokopack aspiration and CDC light traps. Captured adult mosquitoes were analyzed by *Plasmodium falciparum* circumsporozoite ELISA and PCR for detection of malaria sporozoites and blood meal host.

Results: A total of 781 adult *Culex* (735, 94.1%), *Mansonia* (11, 1.4%) and *Aedes* (35, 4.5%) were captured. Unlike their anopheline counterparts hardly found indoors (6.3%), the non-anophelines were caught indoors at much higher frequency (52.1%, $P < 0.001$, $N = 4,975$). One *Culex pipiens* (0.4%) was *P. falciparum* sporozoite-positive by ELISA with PCR confirmation, while a further 17 (6.3%) were positive by PCR, rivalling corresponding proportions for *Anopheles gambiae* (0.4% and 4.0%, respectively). Of 51 *Cx pipiens* caught blood-fed, 19 were gorged on human blood (HBI, 0.33).

Conclusions: The findings are a first demonstration of natural sporozoite carriage in non-anopheline mosquitoes. Caught indoors and outside in areas with active malaria cases despite IRS, these culicines merit follow-up studies to ascertain whether they may play a veiled role in sustaining malaria transmission.

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EFFECTIVENESS OF IMPREGNATED COLLARS IN CONTROLLING CANINE TEGUMENTARY LEISHMANIASIS IN AN ENDEMIC AREA OF BRAZIL

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Background and Aims: Canine tegumentary leishmaniasis (CTL) is a vector-borne zoonotic disease caused by *Leishmania braziliensis* in northeastern Brazil. This study aimed to evaluate the effectiveness of 4% deltamethrin-impregnated collars as a control and preventive measure for CTL in an endemic area of Bahia.

Methods: An experimental longitudinal study was conducted between September 2024 and June 2025 in rural communities with intense transmission. A total of 526 dogs were enrolled and allocated to control and intervention groups according to the use of impregnated collars. All animals underwent clinical examination and serological testing using an in-house ELISA based on soluble *Leishmania* antigen. Dogs were classified as seropositive when presenting positive or borderline results, and CTL cases were defined as seropositive animals with at least one compatible skin lesion.

Results: In the control group, seroprevalence and incidence were 44.5% and 68.9%, respectively, while in the intervention group these values were 43.7% and 67.0%. The prevalence and incidence of clinical disease were 10.6% and 7.7% in the control group and 11.5% and 2.8% in the intervention group. Multivariable analysis identified belonging to the intervention group and a higher number of household residents as protective factors against disease development.

Conclusions: These results indicate that deltamethrin-impregnated collars reduce the incidence of clinical CTL and reinforce their relevance as a complementary control strategy. The findings provide epidemiological evidence to support public health decision-making and integrated control measures in areas of intense transmission, targeting canine reservoirs and reducing human exposure in endemic rural settings through sustainable vector control interventions and community-based prevention programs.

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IMPACT OF A COMMUNITY-DIRECTED IVERMECTIN TREATMENT ON ONCHOCERCIASIS TRANSMISSION IN RURAL TANZANIA: A PARASITOLOGICAL AND ENTOMOLOGICAL SURVEY

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Background and Aims: Despite over 25 years of community-directed treatment with ivermectin (CDTI), onchocerciasis remains a major public health challenge in Mahenge, Tanzania, where its association with high epilepsy prevalence prompted a shift from annual to biannual CDTI. There is limited data on the impact of biannual CDTI. Therefore, the effect of biannual CDTI on reducing onchocerciasis transmission in the region was assessed.

Methods: To assess the impact of this strategy, parasitological surveys were conducted in 2017/18, 2021, and 2024 across low, medium, and high-altitude villages, alongside a 2024 entomological survey. Children aged 6–10 years were tested for *Onchocerca volvulus* exposure using the Ov16 RDT test and interviewed about ivermectin use. *Simulium* flies were collected via Human Landing Catch and analyzed using O-150 PCR.

Results: In total, 1,341 children (52.4% female) were screened in 2024. Following eight rounds of biannual CDTI, onchocerciasis prevalence decreased from 17.2% in 2017 to 13.3% in 2021 and 10.7% in 2024. However, non-uptake of ivermectin increased to 21.6% in 2024. Anti-Ov16 seropositivity was significantly lower in 2024 (OR = 0.61, p = 0.001) compared to 2017/18, with higher risk among older children and those in medium-altitude villages. Of 21,152 *Simulium* flies collected, 20,321 were identified as *S. damnosum s.l.*; 43.7% of pooled samples tested positive for *O. volvulus*, corresponding to an infectivity rate of 0.58%.

Conclusions: Although biannual CDTI has reduced transmission, persistent vector infectivity poses a major barrier to achieving WHO's 2030 elimination target. Strengthening CDTI coverage and integrating vector control interventions are essential, particularly in medium- and high-altitude areas.

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PLASMODIUM FALCIPARUM MSP1 GENE POLYMORPHISM IN A HIGH TRANSMISSION AREA: A CROSS-SECTIONAL STUDY IN KOUNKANE, SOUTHERN SENEGAL

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Background and Aims: Despite ongoing control efforts, the persistence of malaria in certain areas may be associated with the genetic diversity of the parasite. This study aimed to assess the genetic polymorphism of the *msp1* gene of *Plasmodium falciparum* in a high-transmission area in Senegal

Methods: A cross-sectional descriptive study was conducted in Kounkané, southern Senegal, from September 14 to December 14, 2024, among patients with microscopically confirmed malaria. Parasite DNA was extracted from dried blood spots using the Chelex method. Real-time PCR targeting the *Pf18S* gene was used to confirm *Plasmodium falciparum* infection. Genotyping of block 2 of the *msp1* gene was performed using nested PCR

Results: A total of 89 patients were included, with a predominance of children under 10 years of age. The K1 allelic family was predominant, with a frequency of 65.9%. Overall, 32 alleles were identified, showing polymorphism in the K1 and Mad20 families, while Ro33 was monomorphic. The multiplicity of infection (MOI) was 1.64. A negative correlation was observed between MOI and age, whereas a positive correlation was found between MOI and parasite density

Conclusions: These findings highlight a high genetic diversity of *Plasmodium falciparum* in the study area and emphasize the need for strengthened molecular surveillance to better inform malaria control strategies

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MALARIA PARASITAEMIA AND IPTP COMPLIANCE RATE AMONG PREGNANT WOMEN IN DELTA STATE, NIGERIA.

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Background and Aims: Background: Malaria during pregnancy has significant public health implications. The National Strategic Plan on malaria proposes the use of Intermittent Preventive Treatment (IPTp) with Sulphadoxine-Pyrimethamine (SP) as direct observed treatment (DOT) in at least two doses during pregnancy.

Methods: Method: This study investigated malaria parasitaemia and the compliance level with IPTp in pregnant women attending antenatal care in selected parts of Delta State, Nigeria. One thousand consenting pregnant women had their peripheral blood taken and examined for malaria parasites using thin and thick blood films. IPTp compliance was obtained from structured questionnaires and the hospital records.

Results: A prevalence of 62.4% was observed, with 25.1% IPTp compliance, and 35.5% infection in this group. Of the 251 compliant, 126(50.2%) took the SP drug once with 77(61.1%) infected, 87(34.7%) took twice with 47(54.0%) infected, 27(10.8%) took thrice and 12(44.4%) infected while 11(4.4%) with 3 (27.3%) infected took more than three times. Some 226 (90.0%) did not take the DOT as directed by WHO.

Conclusions: . Conclusion: The study showed that pregnant women in the study area were aware of the benefits of IPTp with SP during pregnancy; however, variable factors such as cultural, economic, and educational background influenced the compliance level in the study area.

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EFFECT OF AQUEOUS NIGELLA SATIVA SEED EXTRACT ON LARVAL STAGES OF SCHISTOSOMA HEMATOBIUM

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Background and Aims: To determine the lethal effect of *Nigella sativa* seed extract concentrations on the egg viability and cercaria infectivity of *Shistosoma haematobium* in Nkalagu, Ishielu Local Government Area of Ebonyi State, Nigeria.

Methods: The study was carried out using standard parasitological and malacological techniques. Snail hosts were picked from waterbodies in the study sites for cercaria shedding, viability and potency for transmission while urine samples were collected from pupils in sampled schools closely located near the water bodies and screened for the eggs of *Schistosoma haematobium*

Results: The egg viability test for *Schistosoma haematobium* showed that there was a statistical difference in the number of viable, calcified and resistant eggs tested ($p < 0.05$) but recorded a significance difference at different time duration ($p > 0.05$). High morphological and tegumental changes in eggs of *S. haematobium* occurred at high concentrations and longer periods of exposure to *N. sativa* aqueous seed extract. The reverse was observed at lower concentrations and shorter period of exposure to the extract. This was the same case with cercaria upon exposure to *N. sativa* aqueous seed extract.

Conclusions: The current result on the cercaricidal activity of *N. sativa* suggest that this plant has a potential inhibitory effect on Schistosoma parasites and intermediate snail hosts. Therefore, there is a need for public awareness programs on the use of *N. sativa* seed due to its antiparasitic properties and its relevance against urinary schistosomiasis infection in endemic communities

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HISTOLOGICAL ANALYSIS OF SCHISTOSOME IN THE INTERMEDIATE HOST

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Background and Aims: Schistosomiasis is one of the most significant and prevalent waterborne parasitic diseases. Even though many studies have been reported about schistosomiasis, the dynamics of schistosome in intermediate host snails is little known. In the present study, the dynamics of Schistosoma larvae in infected snails was histologically investigated.

Methods: To examine the localization of *Schistosoma mansoni* (*S. mansoni*) parasites in the snails, *Biomphalaria glabrata* snails infected with miracidia were harvested and examined by stereoscopic observation. Then, frozen sections were prepared and stained with H&E. Furthermore, immunohistochemical detection of parasites was performed using anti-*S. mansoni* antibody, and their localization in the snails was analyzed.

Results: The dynamics of Schistosoma larvae in infected snails was histologically visualized and we revealed that the larvae initially invade the surface of the head-foot area of the snail and localize there in the early period of their development, then locate to the hepatopancreas and ovotestis areas in the late period of the infection.

Conclusions: The present study revealed the dynamics of Schistosoma larvae in intermediate snails, shown as the differential localization of *S. mansoni* larvae at early and late infection stages.

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CHANGES IN GAMETOCYTE COUNTS FOLLOWING ARTESUNATE-LUMEFANTRINE TREATMENT IN NORTHERN BOTSWANA

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Background and Aims: Gametocytes are the asexual parasite forms that are critical in the transmission of malaria parasites to mosquitoes. Recently, the importance of gametocyte densities and prevalence following treatment with Artemisinin Combination Therapy (ACTs) in transmission has been debated. Botswana uses Artemether-Lumefantrine (AL) for treatment. We examined gametocyte counts following treatment in the northern endemic districts for differences.

Methods: Methods: Data on treatment outcomes from 6 hospitals and clinics in the northern districts from 2022 to 2025 were collated. Subjects reporting to the outpatient department with fever and treated with AL were followed. Gametocyte positivity following treatments on days 3, 7, 14, and 21 was recorded.

Results: Results The results revealed that only Chobe district had zero gametocytes throughout the follow-up period. Ghanzi and Tutume had zero parasites on day 7. Very low levels were recorded in Okavango. Boteti had stable prevalence from days 7 to 21. Okavango maintained low prevalence throughout the follow-up period. All other districts showed notable increases on days 14 and 21. Districts with higher parasite prevalence at an earlier follow-up day tended to also have higher parasite prevalence at subsequent follow-up days (r=0.51).

Conclusions: Chobe stands out as a district recording zero gametocytes. All other districts recorded variable levels of gametocytes with increases towards day 21. How these affect transmission is unknown.

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COMPARATIVE SPEED OF IXODES SCAPULARIS KILL AND PREVENTION OF PATHOGEN TRANSMISSION OF DOGS TREATED WITH ENDECTOSIDE PRODUCTS CONTAINING EITHER LOTILANER, SAROLANER, OR AFOXOLANER

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Background and Aims: *Ixodes scapularis* commonly infests dogs in North America and transmits pathogens of veterinary and public health importance. Because isoxazoline-containing canine endectocides require ticks to attach and begin feeding for drug exposure, speed-of-tick-kill is a key determinant of pathogen transmission prevention. This study compared three monthly-dosed canine endectocides for speed-of-kill against *I. scapularis* infestations and prevention of *Borrelia burgdorferi* and *Anaplasma phagocytophilum* transmission.

Methods: Dogs were blocked by pre-treatment tick counts and randomized to four groups (8 dogs/group): untreated or treated (Day 0) with a sarolaner-, afoxolaner-, or lotilaner-containing endectocide. Dogs were infested with 50 lab-reared adult *I. scapularis* on Days -2, 21, and 28. Tick counts were completed 4, 8, 12, 24, and 48-h post-treatment or post-reinfestation. Acaricidal efficacy was compared using geometric mean live tick counts. On Day 30, dogs were challenged for five days with 50 field-collected *I. scapularis*. Pathogen transmission was evaluated using a patient-side lateral flow assay.

Results: At 8-h post-treatment, only lotilaner achieved significantly lower mean tick counts than untreated controls. After Day 28 reinfestation, lotilaner produced significantly lower mean ticks counts than untreated controls at 8-48 h, sarolaner at 8 and 12-h, and afoxolaner at 8-24 h. Speed-of-tick-kill from Day 0 to Day 28 was maintained only by lotilaner. Upon challenge with field-collected ticks, all untreated dogs seroconverted to both pathogens. All isoxazolines prevented *B. burgdorferi* transmission, but only lotilaner significantly reduced *A. phagocytophilum* transmission.

Conclusions: The sustained rapid speed-of-tick-kill of lotilaner is a desirable characteristic to quickly reduce live ticks on dogs and pathogen transmission risk.

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EVALUATION OF DIFFERENT COMPOSTING TECHNIQUES FOR THE CONTROL OF PARASITES IN SLAUGHTERHOUSE WASTE

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Background and Aims: Slaughterhouse waste is often contaminated with protozoan oocysts, and helminth eggs. Inadequate management of these organic wastes is responsible for environmental degradation and health risks to public and animals. It highlights the necessity of adopting sustainable and environmentally friendly methods for the treatment and potential recycling of slaughterhouse waste.

Methods: The static and semi open pile composting techniques were used to treat the slaughterhouse waste and control of parasites. Biological decomposer solution was prepared in the lab and mixed with slaughterhouse waste to speed up the process of composting. Key composting parameters such as temperature, humidity, pH, quantification of parasitic eggs, and viability of parasitic eggs were closely monitored after every fifth day.

Results: For the both composting methods, the initial pH was 6.6 on day first. It peaked (8.5) on day five, then gradually decreased, reaching a value of 7.3 by day 40 to 50 in open pile method. In semi open and static pile method, the thermophilic phase was maintained till day 35 and 25, respectively. Throughout the composting process, the level of humidity in static pile was higher as compared to semi open pile. In the semi open pile method, the egg per gram (EPG) values decreased rapidly from 1900 to 100 by day 20 however, in static pile the EPG was stabilized at 300 from day 35 onwards.

Conclusions: Our findings suggested that the semi open pile method is a better technique for the reduction of parasitic eggs in the slaughterhouses wastes as compared to the static pile method.

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SAND FLY SURVEY TO ELUCIDATE THE TRANSMISSION CYCLE OF CANINE LEISHMANIASIS IN ZAMBIA

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Background and Aims: Leishmaniasis has not been recognized in Zambia for at least the past two decades. We recently confirmed the first cases of canine leishmaniasis caused by *Leishmania infantum* in Livingstone, Southern Province, and subsequent surveys revealed high seroprevalence in both Livingstone and Lusaka. Seropositive dogs may serve as important reservoirs of *Leishmania* parasites for vector sand flies. Therefore, an entomological survey of sand fly fauna was conducted to investigate the potential vectors in Zambia.

Methods: An entomological survey of sand flies was conducted from 2022 to 2025 in Livingstone, Lusaka, and Mfuwe using CDC light traps. The head and abdominal segments of the collected specimens were used for morphological identification, and the remaining body was used for identification based on partial mitochondrial cytochrome *c* oxidase subunit I sequences.

Results: Five Sergentomyia species—*S. congolensis*, *S. africana africana*, *S. saliburiensis*, *S. bedfordi*, and *S. antennata*—and one *Phlebotomus* species were detected in Livingstone. *S. congolensis* was the predominant species, while *Phlebotomus* was rare. Additional surveys in Lusaka and in Mfuwe (Northeastern Zambia) yielded only *Sergentomyia* species. Analysis of collection sites showed that approximately 40% of *Sergentomyia* specimens were captured in chicken houses in both Livingstone and Mfuwe, followed by dog kennels (≈20%) in Mfuwe.

Conclusions: *Sergentomyia* species are generally considered non-mammalian-feeding; however, our findings, together with accumulating reports of *Leishmania* DNA and/or parasites detected in several *Sergentomyia* species across the Old World, underscore the need to assess the vector competence of *Sergentomyia* for mammalian and canine leishmaniases in Zambia.

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PUBLIC HEALTH SURVEILLANCE FOR SFTS PREPAREDNESS: LONG-TERM TICK MONITORING AND SFTSV SCREENING IN DANGJIN, REPUBLIC OF KOREA (2018 TO 2024)

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Background and Aims: Hard ticks (Ixodidae) transmit Severe fever with thrombocytopenia syndrome virus (SFTSV), an emerging public health concern in East Asia; we conducted multi-year ecological and molecular surveillance of ticks and SFTSV in Dangjin-City, Chungcheongnam Province, Republic of Korea, where human cases occur but tick viral prevalence is unclear.

Methods: From 2018–2024, ticks were collected monthly (April–November) across four habitats (grassland, mountain road, mixed forest, cemetery) using standardized 24-h CO2-baited traps. Specimens were morphologically identified and pooled by species, stage, sex, and habitat (≤50 larvae, 30 nymphs, or 5 sex-separated adults per pool). In total, 36,478 ticks were grouped into 3,106 pools, and all pools (including *Haemaphysalis longicornis*, *H. flava*, and *Ixodes nipponensis*) were screened for SFTSV. Total RNA was extracted and tested by nested RT-PCR, with amplicons confirmed by agarose gel electrophoresis.

Results: A total of 72,956 ticks belonging to *Haemaphysalis longicornis* (46,269), *H. flava* (1,143), and *Ixodes nipponensis* (655)were collected. *Haemaphysalis longicornis* was the most frequently collected, representing 63.42% of all adult and nymphal ticks. Tick abundance peaked during 2018-2019 and was highest in grassland habitats. None of the 3,106 pools tested positive for SFTSV.

Conclusions: Although SFTSV was not detected, the persistently high abundance and broad ecological distribution of *Haemaphysalis* species suggest that Dangjin-City maintains environmental conditions that could support pathogen introduction or amplification. These long-term data provide a valuable baseline for early-warning systems and support targeted surveillance in nearby high-

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incidence regions. Integrating ecological, climatic, and epidemiological data with multi-pathogen screening is essential to strengthen One Health–based risk assessment in low-prevalence areas.

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TRANSMISSION DYNAMICS, EPIDEMIOLOGY, AND GLOBAL HEALTH / VECTOR BIOLOGY, PARASITE TRANSMISSION AND CONTROL STRATEGIES

BASELINE PARASITOLOGICAL SURVEY FINDINGS AND INITIAL MDA COVERAGE FROM THE EKAIO CLUSTER-RANDOMISED TRIAL OF MOXIDECTIN VERSUS IVERMECTIN IN ANGOLA

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Background and Aims: Onchocerciasis remains a public health problem in sub-Saharan Africa. Despite decades of ivermectin mass drug administration (MDA), transmission persists in several areas, highlighting the need for alternative strategies. Moxidectin is an anti-parasitic drug with a longer half-life than ivermectin and superior efficacy. EKAIO is a cluster-randomised trial evaluating the impact of community-wide moxidectin versus ivermectin MDA on onchocerciasis and other diseases in Bié Province, Angola. A baseline parasitological survey was conducted to characterise infection and transmission levels prior to the first round of MDA, that was subsequently implemented in an area of 30,000 people.

Methods: Baseline surveys were conducted in 60 villages across 20 clusters. Parasitological assessments included collection of: skin snips for detection of *Onchocerca volvulus* microfilariae by microscopy and molecular diagnostics; a finger prick blood sample to prepare dried blood spots for serological analyses of onchocerciasis and thick blood smears for *Loa loa* detection by microscopy; and stool samples for molecular detection of soil-transmitted helminths. Clinical exams were conducted for onchocerciasis and scabies.

Results: A total of approximately 2,600 individuals aged ≥1 year participated in the parasitological survey. Microfilariae were detected in 29.5% (496/1682) of the participants and 35.7% (923/ 2591)

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were seropositive. Additional analyses of the remaining parasitological samples are underway. MDA had 86.5% coverage in the moxidectin arm and 85.9% in the ivermectin arm.

Conclusions: These findings confirm high endemicity and active transmission of onchocerciasis in the EKAIO study area, and provide critical data for evaluating the impact of moxidectin versus ivermectin MDA.

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MITOGENOMIC CHARACTERISATION OF NON-NATIVE FRESHWATER SNAILS IN AUSTRALIA: IMPLICATIONS FOR BIOSECURITY AND TREMATODE VECTOR SURVEILLANCE

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Background and Aims: Freshwater snails play essential roles in the transmission of trematode parasites that affect humans, livestock and wildlife. Australia's freshwater ecosystems are increasingly influenced by non-native and potentially invasive snail species that may pose biosecurity challenges and alter parasite transmission dynamics.

Methods: This study characterised the complete mitochondrial genomes for four non-native snail taxa established in Australia (*Pseudosuccinea columella*, *Orientogalba viridis*, *Physa acuta* and *Planorbella* sp.) and for *Galba truncatula*, a high-priority exotic species considered a potential invader. Using long-read sequencing and comparative analyses, 15 complete mitogenomes were assembled, annotated and compared across three families (Lymnaeidae, Physidae and Planorbidae).

Results: Genome sizes ranged from 13.7 to 14.3 kb and exhibited conserved gene organisation and marked A+T bias. Australian populations of *P. columella*, *O. viridis* and *Ph. acuta* showed very limited mitochondrial nucleotide variability, consistent with founder effects, demographic bottlenecks and self-fertilisation, in contrast to the marked divergence observed among lineages within *Galba* from European laboratory strains. Phylogenetic inference based on concatenated and single-gene (cytochrome *c* oxidase subunit 1, *cox*1) datasets confirmed well-resolved family-level relationships and revealed cryptic diversity within *Galba*.

Conclusions: The mitogenomes defined here provide molecular references for future taxonomic studies, diagnostic assay development and environmental (e)DNA monitoring of freshwater snails. These genomic resources establish a basis for biosecurity preparedness and vector surveillance in Australia and contribute to a broader One Health approach by supporting early detection, accurate identification and risk assessment of trematode-transmitting snails in freshwater ecosystems.

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TRANSMISSION DYNAMICS, EPIDEMIOLOGY, AND GLOBAL HEALTH / VECTOR BIOLOGY, PARASITE TRANSMISSION AND CONTROL STRATEGIES

MOLECULAR DETECTION OF WUCHERERIA BANCROFTI IN MOSQUITO VECTORS IN OMUN-OWODE, A NON-HOTSPOT AREA OF OSOGBO, OSUN STATE, NIGERIA

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Background and Aims: Lymphatic filariasis, caused by *Wuchereria bancrofti*, remains a significant public health concern in many rural communities. This study aimed to detect the presence of *W. bancrofti* sporozoites in mosquito vectors using molecular techniques and assess the potential for ongoing transmission in Omun-Owode, Osogbo LGA.

Methods: Mosquitoes were collected using pyrethrum spray catches (PSC) and CDC light traps (Model 512, John W. Hock), then identified morphologically using standard taxonomic keys. Blood meal analysis and molecular detection of *W. bancrofti* were conducted using PCR.

Results: A total of 242 mosquitoes were collected, with *Culex quinquefasciatus* (135, 55.8%) being the most dominant species, followed by *Anopheles gambiae* s.l. (79, 32.6%), *Mansonia uniformis* (26, 10.7%), and *Aedes aegypti* (2, 0.8%). Mosquitoes collected via PSC were more numerous than those from CDC traps, though the difference was not statistically significant ($p = 0.09$; $p > 0.05$). PCR analysis confirmed the presence of *W. bancrofti* sporozoites in *Anopheles coluzzii*, providing molecular evidence of active lymphatic filariasis transmission in the community.

Conclusions: These findings underscore the urgent need for targeted vector control and disease surveillance to mitigate the spread of filariasis in the region.

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PREVALENCE OF FLEAS AND TICKS INFESTING FREE-RANGE CHICKENS IN SELECTED RURAL COMMUNITIES OF SOUTH AFRICA.

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Background and Aims: Fleas and ticks are ectoparasites causing anaemia, skin dermatitis, and introduction of secondary infections in chickens. This compromise their health and productivity. Despite the negative impact, there is scarcity of information on ectoparasite of free-ranging chickens in South Africa. This study aimed to determine the identity and prevalence of flea and tick species infecting free-ranging chickens in KwaZulu-Natal (KZN) and Eastern Cape (EC) provinces of South Africa.

Methods: Seventy-nine chickens were collected from KZN and EC and screened for fleas and ticks.

Results: Twenty-four out of 79 chickens (30.4%) were infested with fleas (27.9%) and ticks (3.8%). *Echidnophaga gallinacea, Amblyomma hebraeum* and *Haemaphysalis* sp. were the only fleas and ticks species infesting chickens in both provinces respectively. In KZN, Shongweni had the highest prevalence of *E. gallinacea* infections (90%), followed by Maphumulo (50%), and Umzinto (10%). In EC, Tsolo showed the highest *E. gallinacea* infestation (40%), and Gcuwa recorded the lowest (10%). Low chicken numbers were infested with ticks in KZN, and none in the EC. This low infestation is attributed to that chickens are often secondary/accidental host for several tick species. Generally, females were highly infested by *E. gallinacea* than males however, males had the highest tick infestation than females. Overall, the highest fleas and ticks were recorded in KZN.

Conclusions: This study provided information on ectoparasite burden in free-range chickens, which contributes knowledge on the geographical distribution of these ectoparasites species in South Africa, and can be used to develop ectoparasites control and prevention strategies of free-ranging chickens in South Africa.

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OPTIMIZATION OF THE ARTIFICIAL FEEDING OF DERMACENTOR MARGINATUS TICKS BASED ON SILICON MEMBRANES

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Background and Aims: Artificial tick feeding systems are a valuable tool for studying tick physiology and pathogen transmission while reducing the need for vertebrate hosts, supporting the 3Rs in animal research. However, this technique requires optimisation to meet the specific needs of different tick species and life stages. *Dermacentor marginatus*, an expanding vector species in Europe, has proven challenging to maintain under *in vitro* conditions. The aim of the study was to feed *D. marginatus* ticks artifically with the purpose of investigating pathogen transmission dynamics.

Methods: In this study, we fed field-collected adult *D. marginatus* artificially using a feeding unit based on silicone membranes. The silicone membrane was attached to a glass cylinder and suspended in a six-well plate containing blood. Ticks were added to the units, and blood was changed every 12 hours. Throughout the feeding, blood samples were taken to monitor the transmission of tick-borne pathogens. After feeding, the ticks were stored at room temperature and screened for pathogens after oviposition, enabling assessments of vector capacity and the time required for transmission.

Results: We tested silicone membranes with increased thickness compared to previous trials. Additionally, we determined that, unlike *Ixodes ricinus*, *D. marginatus* requires mechanical stimuli to initiate attachment. The addition of horsehair to the feeding chamber supplied this mechanical cue and revealed this species-specific requirement.

Conclusions: Overall, this work shows that for successful *in vitro* feeding of *D. marginatus,* thicker membranes are required as well as mechanical stimuli, which led us to the first successful artificial feeding of *D. marginatus* in our lab.

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TICK DIVERSITY AND DISTRIBUTION OF PATHOGEN IN TICKS COLLECTED FROM WILD ANIMALS AND VEGETATION IN AFRICA

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Background and Aims: Ticks are hematophagous ectoparasites widely distributed in African ecosystems and parasitize a wide variety of wild animals. In addition to impact of climate change and migratory birds, which Influence the dissemination of ticks and their pathogens, the trade in wild animals also has an important role in the expansion of their geographical range. Thus, a better understanding of tick diversity is essential to anticipate epidemiological risks, strengthen surveillance, and guide prevention strategies.

Methods: PRISMA guideline was used for systematic search of literatures. We searched exhaustively, several electronic databases in English and French. The keywords used in online databases to select the articles. We included publications reporting on tick-borne pathogens from ticks collected in wild animals or the environment.

Results: Tick species infesting wild animals, as well as collected from the environment and their pathogens were reported in 17 countries in Africa between 2003 to 2023. Data on ticks resulting in a total of 40 different tick species from 35 different wild animal species. Among the ticks 34 infectious agents were noted including parasitic (Babesia, Theileria, Hepatozoon, Eimeria) bac-terial (Anaplasma, Bartonella, Borrelia, Candidatus Midichloria mitochondrii, Candidatus Allocrypto-plasma spp., Coxiella, Ehrlichia, Francisella, and Rickettsia) and a surprising high diversity of viral pathogens (Bunyamwera virus, Crimean Congo Haemorhagic Fever virus, Ndumu virus, Semliki Forest virus, Thogoto virus, West Nile virus)

Conclusions: This study reports relevant information on infectious pathogens in wildlife and highlights the need to understand diseases and their consequences on animal and human health. Such knowledge is essential for combating ticks and tick-borne diseases.

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TRANSMISSION DYNAMICS, EPIDEMIOLOGY, AND GLOBAL HEALTH / FIELD EPIDEMIOLOGY AND SURVEILLANCE IN HUMAN AND ANIMAL POPULATIONS

INTEGRATIVE USE OF CONVENTIONAL AND DNA METABARCODING METHODS REVEALS HIGH DIVERSITY, PREVALENCE AND INTENSITY OF GASTROINTESTINAL PARASITES IN PIGS FROM VICTORIA, AUSTRALIA

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Background and Aims: Understanding the diversity, prevalence and intensity of parasites in livestock is critical to animal health, production efficiency and sustainable farming. This cross-sectional study provides the first integrated parasitological and molecular survey of gastrointestinal parasites in pigs from 69 commercial and backyard farms across Victoria, Australia.

Methods: In total, 1,222 faecal samples were tested using the modified McMaster technique to determine faecal egg/oocyst counts. Samples test-positive for strongylid nematodes were characterised further using next-generation sequencing (NGS) targeting the second internal transcribed spacer (ITS-2) of ribosomal DNA.

Results: Based on faecal egg/oocyst count, 39.1% of farms tested positive for at least one parasite taxon. Coccidia were most prevalent (9.5%) in samples, followed by *Ascaris suum* (5.2%), *Trichuris suis* (4.5%) and strongylid nematodes (1.8%). Infection intensity was highest for coccidia, with notable burdens also for *Ascaris suum* and strongylid in individual samples. Eggs of *Metastrongylus* spp. were detected for the first time in Australian domestic pigs on an outdoor farm. Prevalence patterns varied substantially according to the production system and pig age group: coccidia dominated in mature breeders (17.8%) and piglets (15.4%) in backyard settings (33.7%), whereas *Ascaris* peaked in mature breeders (9.1%) and in semi-intensive systems (21.7%). Strongylid eggs were most frequently detected in gilts and mature breeders from backyard and extensive systems. Notably, NGS revealed a diverse community of strongylid nematodes, including *Oesophagostomum dentatum, O. quadrispinulatum* and *Trichostrongylus axei* and *Metastrongylus apri* and *M. salmi*.

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Conclusions: These findings uncover a considerable and underappreciated parasitic burden in pig populations across multiple production systems in Victoria.

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A PRELIMINARY STUDY ON THE EFFECTS OF FIELD-ACQUIRED HELMINTH INFECTIONS ON FECAL EGG COUNTS AND PACKED CELL VOLUME IN NIGERIAN DWARF GOATS WITH DIFFERENT COAT COLOUR TYPES

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Background and Aims: Gastrointestinal helminth infections are major constraints to small ruminant production, causing anemia, reduced growth, and economic losses. Nigerian Dwarf Goats (NDG) are considered relatively resilient, but field-based evidence is limited. This study assessed the effects of field-acquired helminth infections on fecal egg count (FEC) and packed cell volume (PCV) and explored associations with coat colour and sex in NDG under extensive management in southwestern Nigeria.

Methods: A cross-sectional study sampled 130 NDG from Ogun and Oyo States, representing Black, Brown, White, Black and White, Brown and Black, Black and White Dalmatian, and Brown and White coat types. Only goats not treated with anthelmintics within two months and free from other clinical diseases or hemoparasites were included. Fecal samples were analyzed by modified McMaster technique for FEC, and blood samples were assessed for PCV using the microhematocrit method. Associations between infection, FEC, PCV, sex, and coat colour were evaluated using chi-square and Student's t-tests ($p < 0.05$).

Results: Overall helminth prevalence was 80.8%. Females showed higher infection rates than males, though not significant. Mean PCV was significantly lower in infected goats (25.95 ± 5.24%) versus non-infected (28.64 ± 5.99%). Most goats (67.7%) had FEC <3,000 eggs per gram. Coat colour was not significantly associated with FEC or PCV.

Conclusions: This study demonstrates high helminth prevalence in Nigerian Dwarf Goats under extensive management, with infection associated with reduced packed cell volume. Despite infection, low fecal egg counts suggest resilience. Trends across coat colour types warrant further study to guide selective breeding and helminth control strategies.

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BEYOND BIOLOGY: EVALUATING CONTROL STRATEGIES AND SOCIO-ECONOMIC BURDEN OF CYSTIC ECHINOCOCCOSIS IN MOROCCO (A ONE HEALTH APPROACH)

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Background and Aims: Cystic echinococcosis (CE) remains a major public health and economic challenge in North Africa. Despite national control efforts, transmission persists. This study integrates clinical field trials of control strategies with a comprehensive nationwide socio-economic impact assessment to provide a holistic view of the disease burden in Morocco.

Methods: A dual approach was employed: Field Trial: A 4-year longitudinal study in the Middle Atlas evaluated three strategies: dog deworming (praziquantel), sheep vaccination (EG95), and a combined protocol. Outcomes were assessed via ultrasound and necropsy in 797 lambs and 595 owned dogs. Economic Analysis: Using Monte Carlo simulations, we estimated direct and indirect monetary losses, Disability-Adjusted Life Years (DALY), and zoonotic DALY (zDALY) for the 2011–2014 period across Morocco.

Results: The field trial demonstrated that EG95 vaccination is highly effective, reducing the risk of viable hydatid cysts by 97% in sheep ($p<0.001$). Conversely, 4-monthly dog treatment significantly reduced canine prevalence (35% to 12%) but failed to interrupt transmission to sheep, likely due to untreated wild and stray reservoirs.
Economically, CE imposes a staggering burden of 73 million USD annually, with significant regional variations. The health burden was estimated at 55 zDALY per 100,000 persons/year, primarily driven by unperceived losses in animal productivity and asymptomatic human cases.

Conclusions: EG95 vaccination is a game-changer for livestock protection in Morocco. To mitigate the severe public health and economic burden of CE, an intensified, region-specific 'One Health' strategy integrating sheep vaccination with wild reservoir surveillance is essential.

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XENOMONITORING OF PLASMODIUM SPECIES IN MALARIA VECTORS ACROSS AGRICULTURAL LANDSCAPES IN SOUTHERN GHANA

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Background and Aims: Although *Plasmodium falciparum* remains the predominant malaria parasite in Ghana, increasing reports of non-falciparum malaria species, particularly *Plasmodium malariae* and *Plasmodium ovale*, highlight the need for enhanced surveillance of malaria transmission dynamics. However, information on the occurrence of these parasites in mosquito vectors remains limited.

Methods: An entomological survey was conducted between 2019 and 2022 across agricultural communities located within different ecological settings in southern Ghana, including rice-growing and vegetable-growing areas in the Eastern and Central Regions. Mosquitoes were collected indoors . Molecular assays were used to identify the *Anopheles* species and *Plasmodium* parasites.

Results: A total of 1,362 *Anopheles* mosquitoes were collected, comprising the *Anopheles gambiae* complex (90.9%), *Anopheles funestus* complex (8.8%), and *Anopheles nili* group (0.3%). Within the *An. gambiae* complex, *Anopheles coluzzii* was the predominant species (70.0%), followed by *Anopheles gambiae sensu stricto* (16.3%). *An. coluzzii* predominated in irrigated rice-growing communities in both forest and coastal savannah zones, whereas *An. funestus* was more abundant in the upland farming community. The overall *Plasmodium* infection rate was 3.6%. Although *P. falciparum* was the dominant parasite detected, *P. malariae* and *P. ovale* were also identified in *An. coluzzii*, *An. gambiae*, and *An. funestus*.

Conclusions: The study demonstrates that the major malaria vectors in Ghana are involved not only in the transmission of *P. falciparum* but also non-falciparum malaria parasites. These findings suggest that current vector control interventions targeting primary malaria vectors may simultaneously reduce the transmission of *P. malariae* and *P. ovale*. Integrating xenomonitoring into routine surveillance systems could strengthen malaria elimination strategies in Ghana.

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ABATTOIR SURVEY OF CATTLE AND BUFFALO HAEMONCHOSIS IN GREECE AND ASSOCIATED RISK FACTORS

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Background and Aims: Although best known as a major parasite of sheep and goats, the blood-feeding abomasal nematode *Haemonchus contortus* can infect cattle and buffaloes under the mixed-grazing conditions prevalent in Greece. The aim of this study was to determine the prevalence of *H. contortus* infections in cattle and buffaloes in Greece through an abattoir survey, to evaluate potential host- and farm-related risk factors and to assess the likelihood of detecting homozygous benzimidazole (BZ)-resistant *H. contortus* in large ruminant populations in relation to these determinants.

Methods: A total of 213 abomasa were examined. A questionnaire provided additional animal- and farm-level information. *Haemonchus*-like helminths were molecularly identified at species level by amplifying a 321 bp fragment of the internal transcribed spacer 2 region of nuclear DNA. An allele specific multiplex PCR, targeting codon 200 of the β-tubulin gene, was applied to detect BZ-resistant alleles.

Results: The prevalence of *H. contortus* infection was 21.2% in cattle and 69.8% in buffaloes. In cattle, multivariable analysis revealed that mixed-species farming, productive orientation and slaughter age were significant predictors of increased *H. contortus* infection. Controversially, none of these factors were significantly associated with infection in buffaloes. Finally, multivariable modelling suggested that BZ resistance was more prevalent in intensively managed, older cattle, yet less common among older buffaloes and in herds where both species coexisted.

Conclusions: These findings indicate that *H. contortus* is an emerging and underrecognized threat to large ruminant health and productivity in Greece, particularly in grazing-dependent systems.

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DYNAMICS AND WITHIN-HOST COMPETITION OF THEILERIA LESTOQUARDI AND T. OVIS AMONG NAIVE SHEEP IN OMAN

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Background and Aims: Mixed species infection of livestock by *Theileria spp*. is common in nature. Experimental as well as epidemiological data suggest that interaction between mixed parasites elicits cross-immunity that can modulate pathogenicity and disease burden at the population level. T

Methods: he present study examined within host interaction and disease outcome of the two prevalent ovine *Theileria* spp (pathogenic [*T. lestoquardi*] and non pathogenic [*T. ovis*]) in Oman among a cohort of naive sheep, over a period of 11 months. The animals were raised in a tick-free farm and then transferred into a *Theileria* spp. endemic area. Species-specific qPCR was used to quantify *T. lestoquardi* and *T. ovis*.

Results: During the first two months, animals infected with *T. lestoquardi* alone exhibited high morbidity and mortality, after which disease levels declined and mixed infections with *T. ovis* became predominant. Overall, parasite densities were significantly higher in single infections than in mixed infections, indicating competitive interaction between the two species. The pathogenic *T. lestoquardi* showed a competitive advantage in co-infections, though densities of both parasites fluctuated over time without seasonal differences. A high level of genotype multiplicity was observed in *T. lestoquardi*, increasing with parasite density.

Conclusions: Our results illustrate a competitive interaction between ovine *Theileria* spp (*T. lestoquardi* and *T. ovis*) in co-infected animals that results in a substantial reduction in the risk of mortality due to presumed heterologous protection.

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POSTER ON BOARD SHIFT 01
TRANSMISSION DYNAMICS, EPIDEMIOLOGY, AND GLOBAL HEALTH / FIELD EPIDEMIOLOGY AND SURVEILLANCE IN HUMAN AND ANIMAL POPULATIONS

EPIDEMIOLOGY AND GENETIC DIVERSITY OF ALARIA ALATA MESOCERCARIAE IN SELECTED WILDLIFE HOSTS IN CENTRAL EUROPE (POLAND) DURING 2018–2025

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Background and Aims: The aim of this study was to investigate the epidemiology and genetic diversity of *Alaria alata* mesocercariae in selected wildlife hosts in Poland.

Methods: Muscle and adipose tissue samples were collected from 16 voivodeships. In total, 3250 wild boars sampled between 2018 and 2025 were examined, as well as 247 raccoon dogs and 106 badgers hunted in 2024–2025. In addition, 19 raccoons were examined in 2025. All samples were tested by *Alaria* migration technique. Species identification based on the amplification and analysis of fragments of the 18S rRNA gene and the mitochondrial COI gene.

Results: *A. alata* mesocercariae were detected in 351 of 3250 wild boars (10.8%), 81 of 247 raccoon dogs (32.8%), 22 of 106 badgers (20.8%), and 2 of 19 raccoons (10.5%). Based on morphological characteristics, all detected parasites were identified as *A. alata*. PCR amplification of the 18S rRNA (232 bp) and COI (450 bp) gene fragments yielded products of the expected sizes, and sequence analysis using BLAST, GenBank, and Geneious software confirmed species affiliation. Analysis of the COI gene revealed 1–7 single nucleotide polymorphisms, indicating considerable intraspecific genetic diversity.

Conclusions: These results confirm that wildlife act as important paratenic hosts of *A. alata* in Poland and may represent potential sources of human alariosis, particularly through the consumption of raw or undercooked game meat. The observed genetic variability highlights the need for continued molecular surveillance and may contribute to improving national and European regulations concerning the handling and inspection of game carcasses in which *Alaria* spp. are detected.

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TRANSMISSION DYNAMICS, EPIDEMIOLOGY, AND GLOBAL HEALTH / FIELD EPIDEMIOLOGY AND SURVEILLANCE IN HUMAN AND ANIMAL POPULATIONS

EFFICACY OF A FLURALANER–MOXIDECTIN–PYRANTEL CHEWABLE FORMULATION AGAINST CANINE INTESTINAL NEMATODES

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Background and Aims: Broad-spectrum antiparasitic products that are effective against endo and ectoparasites improve owner compliance to veterinary parasite control programs. This multicentre field study evaluated the safety and efficacy of a chewable combination product containing fluralaner, moxidectin, and pyrantel for the treatment of intestinal nematode infections in client-owned dogs in Europe.

Methods: Dogs (*n* = 258) from Albania, Bulgaria, Greece, and Italy were enrolled into this study. Animals with positive pretreatment fecal egg counts (FECs) were randomly allocated on Day 0 to receive an investigational product containing a combination of fluralaner (10–20 mg/kg), moxidectin (0.025–0.050 mg/kg), and pyrantel (5–10 mg/kg) (*n* = 172), or a control product containing afoxolaner (2.5–5.3 mg/kg) and milbemycin oxime (0.5–1.1 mg/kg) (*n* = 86). Fecal examinations were performed on Days 0 and 14, and efficacies against individual nematode species were assessed when FEC reduction was ≥ 90% in at least 10 initially FEC-positive dogs and when the reduction was statistically significant ($P \leq 0.05$).

Results: In the investigational group, geometric mean FEC reductions on Day 14 were significant ($P < 0.0001$) for hookworms, *Toxocara canis*, *Toxascaris leonina*, and *Trichuris vulpis*, with efficacy exceeding 99% for all evaluated species. The proportion of nematode-free dogs was significantly higher in the investigational group than in the control group ($P < 0.0001$). No treatment-related adverse events were observed.

Conclusions: The fluralaner–moxidectin–pyrantel combination was safe and highly effective for the treatment of major canine intestinal nematode infections under European field conditions, supporting its use in integrated parasite control strategies.

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STRENGTHENING FIELD EPIDEMIOLOGY THROUGH COMMUNITY-DRIVEN DIGITAL SURVEILLANCE: EVIDENCE FROM SOUTHERN NIGERIA

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Background and Aims: Routine surveillance for neglected tropical diseases (NTDs) in many endemic settings remains heavily facility-based, leading to under-reporting, delayed detection, and poor geographic resolution of transmission hotspots. Community-driven digital reporting may strengthen field epidemiology by improving case detection and real-time spatial mapping. This study evaluated the performance of a community-based, smartphone-enabled surveillance system for skin NTDs in Southern Nigeria.

Methods: A cross-sectional field epidemiology study was conducted across endemic communities in three southern Nigerian states. Trained community contact persons used a mobile reporting platform to capture geotagged images of suspected skin NTD cases, which were reviewed by trained health personnel. Surveillance outputs were compared with routine passive reporting from health facilities over the same period. Spatial cluster analysis was conducted to identify transmission hotspots, and system performance indicators including case detection yield, reporting timeliness, and geographic coverage were assessed.

Results: Over a 12-month period, 1,248 suspected cases were reported through the community-based digital system, compared to 312 cases identified through routine facility-based surveillance in the same locations. The digital platform increased case detection by 300% and expanded geographic reporting coverage from 41% to 87% of endemic wards. Median reporting time from case identification to database entry was reduced from 21 days (routine system) to 48 hours. Community-level reporting demonstrated high concordance (84%) with clinical validation.

Conclusions: Community-driven digital surveillance substantially improved case detection and hotspot identification compared to routine passive systems. Integrating participatory digital reporting into national NTD elimination programmes could strengthen field epidemiology capacity and enhance early detection of persistent transmission in endemic settings.

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IMPACT OF MISCLASSIFICATION ERROR OF SCREENING AND CLINICIAN DIAGNOSIS FOR EPILEPSY IN ESTIMATING THE ASSOCIATION BETWEEN SEROPOSITIVITY OF ANTIGENS TO T. SOLIUM CYSTS AND EPILEPSY IN COMMUNITY-BASED STUDIES.

Hélène Carabin[1], Ian Schiller[2], Athanase Millogo[3], Veronique Dermauw[4], Rasmané Ganaba[5], Zekiba Tarnagda[3], Pierre Dorny[4], Nandini Dendukuri[2]
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Background and Aims: The association between *Taenia solium* cystic serological antibodies or antigens and epilepsy is usually estimated assuming that the diagnostic made by a clinician is perfect. In community-based studies, epilepsy cases are identified through the clinician examination of people answering yes to at least one screening question on epilepsy. The aim of this study was to assess the impact that misclassifying epilepsy can have on the estimated association between cysticercosis and epilepsy.

Methods: Data from 3696 Burkinabés answering an epilepsy screening questionnaire and providing serum for the detection of antigens with an ELISA. The results of two models are presented: Model 1 assumed that the diagnostic of the clinician was perfect and ignored the results of the screening; Model 2 assumed that both the screening and clinician were imperfect and considered verification bias. Odds ratios (OR) of the association between the presence of serological antigens to cysticercosis and epilepsy are reported. Bayesian logistic models were used, with latent class and adjustment for verification bias included in Model 2

Results: Model 1 resulted in an OR of 1.95 (95% Bayesian Credible interval: 0.97-3.6) while Model 2 estimated an OR of 2.61 (95%BCI: 1.2-5.1).

Conclusions: The model adjusted for the imperfect diagnostic of epilepsy resulted in a stronger estimation of the association between cysticercosis and epilepsy, although the posterior credible interval was wider. These results could have important impacts on evaluating the role played by cysticercosis in epilepsy.

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ASSESSING CLIMATE-DRIVEN VULNERABILITY TO DENGUE FEVER IN INDONESIA USING MACHINE LEARNING MODELS

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Background and Aims: Indonesia experiences a persistent, high-burden dengue transmission that is increasingly shaped by climate change, rapid urban expansion, and hydrological variability. Although many studies have evaluated climatic determinants of dengue in Indonesia, the relative contributions of large-scale climate teleconnections, drought-related metrics, and landscape characteristics—and how these contributions vary geographically—remain insufficiently characterized.

Methods: We investigated spatiotemporal drivers of monthly dengue incidence across Indonesia using a comprehensive set of environmental and landscape covariates. We compiled monthly dengue incidence for 444 cities from 2010 to 2023. We then developed an extreme gradient boosting (XGBoost) model and applied SHapley Additive exPlanations (SHAP) to quantify the relative importance and direction of associations for candidate predictors during the study periods.

Results: The model showed robust predictive performance (AUC = 0.87) and revealed two epidemiologically distinct regimes: (1) predominantly climate-driven transmission in Kalimantan, Sulawesi, and Eastern Indonesia, and (2) predominantly landscape-driven transmission in Java and Sumatra. In climate-driven regions, precipitation, temperature, the Evaporative Stress Index, and ENSO-related variability were the dominant contributors to dengue dynamics. In contrast, built-up land cover emerged as the most influential factor in Java and Sumatra, underscoring the role of urban form and land-use intensity. Finally, we derived a Climate Vulnerability Index from climate-related SHAP contributions to identify locations that are most sensitive to climate variability and change.

Conclusions: This CVI can support public health agencies by helping prioritize surveillance and targeted vector control in climate-sensitive settings.

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IMPACT OF DIFFERENT INTEGRATED INTERVENTIONS ON RE-INFECTIONS BY SOIL-TRANSMITTED HELMINTHS, SCHISTOSOMES AND INTESTINAL PROTOZOA, SOUTH-CENTRAL CÔTE D'IVOIRE: A CLUSTER RANDOMIZED TRIAL STUDY.

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Background and Aims: Preventive chemotherapy is the main strategy to control helminthiasis, but rapid reinfection occurs in settings that lack clean water, sanitation and hygiene. This study aimed to assess the effect of preventive chemotherapy combined with either community-led total sanitation (CLTS) or community health education, or both, on infections with helminths and intestinal protozoa.

Methods: We conducted a 3-year repeated cross-sectional cluster-randomised trial within 56 communities in Côte d'Ivoire. Households that had at least one child aged 5-15 years were selected. After a baseline parasitological and KAPB questionnaire survey, localities were randomly assigned to one of the three intervention groups: (i) preventive chemotherapy plus CLTS; (ii) preventive chemotherapy plus health education; and (iii) all 3 interventions combined. Two follow-up surveys at 17 and 38 months post-intervention assessed the effects of the interventions on primary (soil-transmitted helminths) or secondary (schistosomes and intestinal protozoa) outcomes.

Results: The results revealed no significant differences in terms of hookworm prevalence in CLTS and control villages for both follow-up surveys: (i) follow-up 1 [59 (3.8%) of 1,552 vs 69 (4.3%) of 1,594; OR= 0.87, 95% CI 0.45 to 1.68, p = 0.69] and follow-up 2 [26 (2.1%) of 1,212 vs 24 (1.9%) of 1,278; OR 1.15, 95% CI 0.60 to 2.17, p = 0.68]. Subgroup analysis using localities that achieved at least 75% latrine coverage versus no-CLTS localities, showed on specific differences in reinfection.

Conclusions: During both follow-up surveys, no statistically significant synergistic effect of the integrative intervention packages compared to standard preventive chemotherapy could be observed, with any parasite's species.

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DIROFILARIA IMMITIS IN DOGS FROM ITALY AND GREECE: ENZOOTICITY AND OCCULT INFECTIONS

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Background and Aims: *Dirofilaria immitis* causes one of the most important parasitoses of dogs. The diagnosis may be challenging, because occult infections are common. This study has updated the occurrence of *D. immitis* in Italy and Greece with a focus on the frequency of occult infections (i.e., infections without circulating microfilariae).

Methods: In each country, 200 dogs were examined by the Knott's test for microfilariae and the SNAP™ Leish 4Dx™ Test (IDEXX Laboratories, Inc., USA) for circulating *D. immitis* antigen.

Results: Forty-two (21%) and 69 (34.5%) dogs from Italy and Greece, respectively, were positive for *D. immitis* upon at least one diagnostic test. Of those, 50 (17 and 33 dogs in Italy and Greece) had an occult infection.

Conclusions: This study demonstrates that *D. immitis* is stably present in Italy and Greece with high infection rates and confirmed that the detection of the antigen identifies higher number of infections compared to the microscopy. The high number of occult infections (~45% of infected animals) here recorded corroborate the indication of the American Heartworm Society and the European Society of Dirofilariosis and Angiostrongylosis, i.e., to use the antigen detection in combination with the Knott's test to reduce as much as possible the risk of false negative results, thus avoiding undiagnosed dogs in clinical practice and prevalence underestimations in epizootiological surveys.

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EMERGING ZOONOTIC CESTODES: ABUNDANCE AND GEOGRAPHICAL DISTRIBUTION OF ECHINOCOCCUS SPECIES IN WILD CANIDS OF NOVA SCOTIA, CANADA.

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Background and Aims: In Nova Scotia (NS), Canada, *Echinococcus canadensis* and *Echinococcus multilocularis,* are emerging in wildlife populations. Historically endemic to western and central Canada, they were presumed absent from NS due to wolf extirpation. These parasites are transmitted from wild canid definitive hosts to cervid or rodent intermediate hosts and pose public health and conservation concerns. Humans who interact with wildlife, or their habitats are at risk of zoonotic infection. In addition, *E. canadensis* infection is associated with predation in moose, further threatening the already endangered NS moose population. This ongoing project aims to screen wild canids for *Echinococcus* species, map positive infections, and identify potential hotspots. This will inform conservation and public health efforts.

Methods: Small intestines of coyotes and foxes were screened for *Echinococcus* species. Mucosal scraping and sieving were conducted to retain *Echinococcus* scolexes and proglottids. *Echinococcus* species were morphologically identified using stereomicroscopy. Specimens were preserved for future species confirmation by PCR and DNA sequencing.

Results: Intestinal screening of 113 coyotes and 62 foxes revealed 10 wild canids to be infected with an *Echinococcus* species. Preliminary morphological examination suggests 8 coyotes were positive for *E. canadensis*, and 1 coyote and 1 fox were positive for *E. multilocularis*.

Conclusions: The presence of *Echinococcus* species in NS suggests that coyotes and foxes can sustain the parasites' life cycles, in the absence of wolves. Humans who interact with wildlife and their habitats should take appropriate precautions. Further investigations are needed to better characterize the risk to endangered NS moose.

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MOLECULAR SURVEY OF TICK INFESTATION AND PIROPLASMOSIS IN DROMEDARY CAMELS FROM AL BATINAH GOVERNORATE, SULTANATE OF OMAN

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Background and Aims: Ticks and tick-borne diseases represent a significant constraint to livestock productivity in tropical and subtropical regions, however data regarding camel parasitism in Oman is still scarce. This study investigated the prevalence of tick infestation and piroplasm infection among dromedary camels in Al Batinah Governorate.

Methods: A cross-sectional survey was carried out in North and South Al Batinah, during which 425 camel blood samples and 872 adult ticks were collected and analyzed through conventional morphological identification and molecular generic primer targeting the hypervariable V4 region within the partial 18S rRNA of both *Theileria* spp. and *Babesia* spp.

Results: The polymerase chain reaction detected piroplasm infection in 24.7% (105/425) of samples, whereas stained blood smears detected only 5.6% (24/425), demonstrating the superior sensitivity of molecular screening. Tick infestation rate was recorded in 61.2% (260/425) of camels, with an average intensity of three ticks per animal (range 1–10). Piroplasm infection was significantly higher in tick-infested camels (30.4%, 79/105) compared with non-infested animals (15.8%, 26/105), highlighting the epidemiological role of ticks in transmission. Identified species included *Hyalomma dromedarii* (480 males and 260 females), *Hyalomma impletatum* (53 males and 44 females), and *Hyalomma anatolicum* (26 males and 9 females), with *H. dromedarii* being dominant.

Conclusions: To the best of our knowledge, this is the first comprehensive report on the prevalence of piroplasmosis and Ixodid ticks in dromedary camels in Oman. The findings recommend continued surveillance, species-level characterization of circulating piroplasms, and further evaluation of their clinical impact on camel health and productivity.

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ANALYZING FECAL SAMPLES FOR BACTERIAL AND PARASITIC INFECTIONS: INSIGHTS FROM UPPSALA COUNTY

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Background and Aims: In Uppsala County, we commenced a comprehensive analysis of all fecal samples submitted to our clinical diagnostic laboratory in 2025. Beyond characterizing the bacterial profiles of these samples, this initiative focused on the detection of underdiagnosed parasitic infections.

Methods: A total of 5,000 fecal samples were analyzed for bacterial content and tested for parasites, including *Giardia intestinalis*, *Cryptosporidium* spp., and *Entamoeba histolytica* by PCR. Comparative analyses were made with data from 2024 to assess changes in positivity rates for these parasites. We also performed subtyping of *Cryptosporidium* and *Giardia* to better understand their transmission dynamics.

Results: Preliminary results indicate a notable increase in the prevalence of *Cryptosporidium* spp., with positive samples rising from 50 in 2024 to 102 in 2025. Cases of *Giardia intestinalis* infection have also increased from 14 in 2024 to 55 in 2025, while *Entamoeba histolytica* infection showed no significant increase. When including results from the last five years, the number of *Cryptosporidium* spp. and *Giardia intestinalis* cases in 2025 still appear significantly higher. The data suggests that these parasites are frequently underdiagnosed. Furthermore, we observed a geographical distinction, where *Cryptosporidium* spp. cases predominantly occurred within Sweden, whereas *Giardia intestinalis* cases were primarily associated with international transmission.

Conclusions: Our findings highlight the importance of further investigating parasitic infections in fecal samples, emphasizing the need for enhanced diagnostic practices to address the rising incidence of these underdiagnosed organisms.

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CHAGAS-SPECIFIC DEATH AND COMPETING RISK OF MORTALITY IN CHILE: A NATIONWIDE RETROSPECTIVE COHORT STUDY, 2007-2021

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Background and Aims: Chagas disease (CD) in Chile shifted towards a post-vector-control, ageing-cohort scenario in which chronic sequelae and non-vectorial transmission increasingly shape outcomes. We quantified Chagas-specific mortality and its determinants using competing-risks methods in a national cohort.

Methods: We conducted a nationwide retrospective cohort study of confirmed CD cases notified in Chile (2007-2021), linked to national mortality and hospital discharge records using unique identifiers. The primary outcome was Chagas-specific death (underlying cause), with other-cause death as a competing event. We estimated cumulative incidence functions (CIFs), compared groups with Gray's test, and fitted Fine-Gray models for subdistribution hazard ratios (sHRs). Cause-specific Cox models were fitted as a complementary approach, including hospitalisation as time-fixed and time-varying severity markers.

Results: Among 17,508 individuals with CD, 261 (1.49%) died from CD and 1,021 (5.83%) from other causes. Ten-year CIF was higher in men (2.66%) and rose sharply with age (≥70 years, 8.00%). CIF peaked in chronic digestive disease (B57.3, 6.49%) and was elevated in chronic cardiac disease (B57.2, 2.93%) compared with Z22.8 (1.47%) (Gray $p<0.005$). In adjusted Fine-Gray models, age ≥70 years (sHR 8.24), male sex (sHR 1.59), B57.2 (sHR 2.03) and B57.3 (sHR 3.889 remained associated with higher Chagas-specific mortality. Cause-specific Cox models identified a strong dose-response with recurrent hospitalisations (≥3 admissions: HR 76.05). Proportional hazards assumptions were assessed using Schoenfeld residuals.

Conclusions: Although Chagas-specific death is uncommon in Chile, it is highly concentrated among identifiable high-risk groups. Risk-stratified chronic care and hospital-anchored follow-up should be prioritised to reduce preventable mortality.

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THE EPIDEMIOLOGY OF SNAKEBITE ENVENOMING IN HONDURAS

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Background and Aims: Snakebite envenoming (SBE) remains a significant global health burden that is poorly characterized in developing countries, such as Honduras. This study aimed to describe the epidemiology of snakebite envenoming in Honduras and comprehensively appraise the country's current efforts around SBE management.

Methods: The methodological approach for this study was two-pronged**.** A retrospective study based on a clinical chart review was conducted at a secondary regional hospital. Mapping the SBE landscape was premised on the World Health Organization's four strategic pillars to prevent and control. This was implemented through semi-structured interviews with stakeholders.

Results: A total of 96 medical charts were included in the study. Preliminary analyses show that there was no mortality, patients received adequate antivenom treatment, and most accidents were caused by Bothrops asper in rural locations. 23 key stakeholders were interviewed. Preliminary analyses show that there is a significant amount of effort being taken by a variety of stakeholders; however, there is fragmentation in such efforts. Main themes emerging from this analysis include the urgent need for citizen education, consistent availability of antivenom, and bridging the gaps among key stakeholders.

Conclusions: Honduras would benefit from a One Health approach to the problem of SBE. Under the stewardship of the national health system, bringing together medical doctors, biologists, herpetologists, agriculturalists, international stakeholders like the Pan American Health Organization, and other key stakeholders, the country will be in a better position to prevent and control SBE.

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UNVEILING THE HIDDEN BURDEN: EXPLORING THE PSYCHOSOCIAL IMPACT OF CUTANEOUS LEISHMANIASIS LESIONS AND SCARS IN SOUTHERN ETHIOPIA

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Background and Aims: Cutaneous leishmaniasis (CL) is a significant public health issue in Ethiopia, often causing lesions and scars on exposed body parts, which can lead to physical, social, and psychological challenges. This study seeks to evaluate the psychosocial impacts of CL by exploring the experiences of affected individuals, enhancing understanding of the condition and informing public health interventions.

Methods: Using a descriptive phenomenological design, the study examined the experiences of individuals with Cutaneous leishmaniasis (CL) lesions and scars. Participants were purposively selected, and data were gathered through open-ended interviews. The analysis utilised both inductive and deductive approaches to develop a coding framework with seven themes and subthemes, providing insights into the psychosocial impacts of CL. NVivo 12v facilitated the analysis process.

Results: The study revealed negative views and misconceptions about cutaneous leishmaniasis (CL) and its effects. Traditional herbal treatments often cause pus and foul odours, leading to embarrassment, pain, and anxiety, which contribute to discomfort and isolation. The stigma surrounding CL scars significantly impacted individuals' behaviour, resulting in school absenteeism and lowered self-esteem. These scars served as identifiers, affecting self-perception and overall happiness, particularly for females facing societal pressure and stigma regarding their marriage prospects and role performance.

Conclusions: The study highlights the need for improved education and awareness about CL to reduce misconceptions and negative attitudes towards affected individuals. Additionally, more effective treatment options and integrated preventive ways should be explored to minimize the physical and psychological impact of CL on affected individuals.

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SHIFT01-203

POSTER ON BOARD SHIFT 01
TRANSMISSION DYNAMICS, EPIDEMIOLOGY, AND GLOBAL HEALTH / FIELD EPIDEMIOLOGY AND SURVEILLANCE IN HUMAN AND ANIMAL POPULATIONS

SCREENING BLOOD FED SAND FLIES IDENTIFIES INFECTION RESERVOIRS TOWARDS INTERRUPTION OF LEISHMANIASIS TRANSMISSION

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Background and Aims: In many active foci of leishmaniasis, identity of reservoirs and their relative significance in maintenance and dissemination of disease remains uncertain. To enable the unmanipulated identification of leishmaniasis reservoirs, we developed a field-applicable molecular toolbox based on analysis of individual blood fed sand flies (IBFS).

Methods: Sand flies were infected with *Leishmania donovani* by feeding on a chicken membrane or on a clinically ill infected animal and fed subsequent blood meals (BMS^+) every 5-6 days after infection. DNA and RNA were co-extracted from membrane-preserved IBFS midguts. The DNA was used to identify low level *Leishmania* infections, using a sensitive kinetoplast probe-based qPCR, and the blood source. RNA was used to quantify live parasites by RT-qPCR of *ssrRNA*. A Bulk-RNAseq approach identified two target genes validated by qRT-PCR that distinguished first blood meal parasites from those present in BMS+. Machine learning predicted if our toolbox would identify reservoirs accurately.

Results: Compared to IBFS parasite counts, kDNA was highly sensitive but overestimated the midgut burden, while *ssrRNA*yielded similar numbers. The first infected blood meal was distinguished from BMS^+ by expression of either *sherp* or a novel hypothetical *Leishmania* gene (*HPB*), that had a significantly higher $\triangle$CT in BMS^+ than in BM1. Machine learning indicated that our toolbox has an accuracy of ~87% and ~82%, in membrane- or naturally-infected fed flies, respectively.

Conclusions: Screening IBFS with this adaptable combination of molecular tools enables reliable identification of probableleishmaniasis reservoirs towards a more effective targeted control strategy in leishmaniasis foci.

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TRANSMISSION DYNAMICS, EPIDEMIOLOGY, AND GLOBAL HEALTH / FIELD EPIDEMIOLOGY AND SURVEILLANCE IN HUMAN AND ANIMAL POPULATIONS

PREVALENCE AND RISK FACTORS FOR PIGEON TRICHOMONIASIS IN MYMENSINGH DISTRICT OF BANGLADESH

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Background and Aims: Trichomoniasis, also known as canker, is a common infectious disease of pigeons and squabs caused by Trichomonas gallinae. Adequate epidemiological knowledge is essential for designing effective control and prevention strategies for pigeon trichomoniasis.

Methods: A total of 90 pigeons and squabs were examined. Data on age, sex, and season were recorded. The presence of Trichomonas gallinae was detected through microscopic examination of oropharyngeal swabs. Post-mortem visceral examination was conducted to identify concurrent helminth infections. Bacterial co-infection in the oropharynx was assessed by culturing oral swabs on different media. Proportionate analyses were performed using Microsoft Excel.

Results: The overall prevalence of pigeon trichomoniasis was 46.67%. Infection rates were 48.64% in females and 45.28% in males. Age-wise prevalence was 29.41% in birds aged 0–3 months, 54.76% in >3–6 months, and 45.16% in >6–9 months. Seasonal prevalence was 50.00% in summer (March–June), 45.45% in the rainy season (July–October), and 52.94% in winter (November–February). Concurrent helminth infection was detected in 66.67% of T. gallinae-positive pigeons, while all samples were positive for one or more bacterial genera.

Conclusions: This study demonstrates a high prevalence of pigeon trichomoniasis in Bangladesh, with concurrent helminth and bacterial infections being common. These findings emphasize the need for integrated control measures, including improved hygiene, routine screening, and timely treatment to reduce disease burden in pigeon populations.

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TRANSMISSION DYNAMICS, EPIDEMIOLOGY, AND GLOBAL HEALTH / FIELD EPIDEMIOLOGY AND SURVEILLANCE IN HUMAN AND ANIMAL POPULATIONS

SEROPREVALENCE OF TOXOPLASMA GONDII AND NEOSPORA CANINUM IN INTENSIVELY MANAGED CATTLE IN UKRAINE

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Background and Aims: *Toxoplasma gondii* and *Neospora caninum* are closely related obligate intracellular protozoan parasites. In most intermediate hosts, including cattle, infections caused by these parasites are often subclinical. When clinical disease does occur, it can be characterised by neurological signs or reproductive failure. We aimed to estimate the seroprevalence of these protozoa in cattle in Ukraine.

Methods: We conducted a cross-sectional study to estimate the seroprevalence of *T. gondii* and *N. caninum* in intensively managed cattle in Ukraine prior to the full-scale war from December, 2021 to February, 2022. Sera from 345 cattle from 24 herds were tested using commercial ELISA.

Results: Altogether, 11.0% (95% CI 8.0-14.7) of the animals tested positive for antibodies to *T. gondii*, while 17.7% (95% CI 13.9-22.0) tested positive for antibodies to *N. caninum*. Six (1.7%, 95% CI 0.7-3.6) animals tested seropositive for both. At least one *T. gondii* seropositive animal was found in 14 of the herds, and within these herds the seroprevalence varied from 5.3% to 60.0%. At least one *N. caninum* seropositive animal was found in 14 of the herds, and within these the seroprevalence varied from 3.9% to 47.4%.

Conclusions: These data provide a baseline for future monitoring and planning of control strategies for protozoal reproductive losses in cattle in Ukraine.

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TRANSMISSION DYNAMICS, EPIDEMIOLOGY, AND GLOBAL HEALTH / FIELD EPIDEMIOLOGY AND SURVEILLANCE IN HUMAN AND ANIMAL POPULATIONS

MOLECULAR SURVEYS OF BORRELIA SPP. IN BATS AND SOFT TICK INFESTING BATS IN THAILAND

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Background and Aims: Bats are recognized reservoirs for a wide range of pathogens, including zoonotic agents, and host diverse ectoparasites such as ticks that may facilitate cross-species pathogen spillover. *Borrelia* spp. are spirochete bacteria responsible for vector-borne diseases and represent a major global public health concern. However, the diversity and distribution of *Borrelia* in Southeast Asia remain poorly characterized. This study aimed to investigate the prevalence and genetic diversity of *Borrelia* spp. in bats and their associated ticks in Thailand.

Methods: Bats were captured across ten provinces in Thailand between 2018 and 2023. Ticks were removed from bat hosts and identified morphologically using a stereomicroscope, with molecular confirmation. Screening for *Borrelia* was conducted using PCR amplification and sequencing targeting the flagellin B (*flaB*), glycerophosphodiester phosphodiesterase (*glpQ*), and 16S rRNA genes.

Results: A total of 1,031 bats were sampled. Morphological and molecular analyses confirmed 96 *Reticulinasus* ticks infesting *Craseonycteris thonglongyai*. Molecular screening of 48 pooled tick samples identified four *Borrelia*-positive pools originating from three individual bats. Additionally, PCR screening of 814 bats detected 13 *Borrelia*-positive individuals (1.6%; 95% CI: 0.9–2.7), including *Taphozous melanopogon*, *Hipposideros gentilis*, and *C. thonglongyai*. Multilocus sequence analysis of the 16S rRNA, *flaB*, and *glpQ* genes resolved three distinct genetic lineages: two clustering within the relapsing fever *Borrelia* group, suggesting potential zoonotic relevance, and a third highly divergent, bat-associated lineage indicative of cryptic speciation.

Conclusions: This study provides the first molecular evidence of *Borrelia* spp. in bats and bat-associated ticks in Thailand and underscores the need for continued surveillance to assess their pathogenicity.

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TRANSMISSION DYNAMICS, EPIDEMIOLOGY, AND GLOBAL HEALTH / FIELD EPIDEMIOLOGY AND SURVEILLANCE IN HUMAN AND ANIMAL POPULATIONS

EPIDEMIOLOGY OF SEA LICE IN IRISH SALMON AQUACULTURE: INSIGHTS FROM 30 YEARS OF SURVEILLANCE

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Background and Aims: Sea lice (*Lepeophtheirus salmonis*) are ectoparasites of Atlantic salmon that feed on blood, tissue, and mucus, causing dermal damage, impaired osmoregulation, secondary infections, and increased mortality risk. Sea lice represent a major constraint on salmon aquaculture in the Northern Hemisphere due to their negative effects on fish welfare and the substantial economic cost associated with treatment and management. To prevent sea lice outbreaks, salmon farms implement various management strategies, informed by monitoring programmes designed to assess infestation levels. The effectiveness of these strategies depends on a comprehensive understanding of sea lice epidemiology, including population structure, temporal dynamics, and assessment of the full range of risks associated with sea lice infections.

Methods: Using 30 years of Irish national sea lice surveillance data, combined with producer-level aquaculture records, we characterised sea lice population dynamics.

Results: Across infection metrics, abundance and prevalence showed a linear relationship up to prevalence levels of ~40%. Building on this relationship, we identified optimal sample sizes for monitoring programmes to ensure accurate estimation of lice levels while balancing resource constraints. Temporal analyses revealed substantial fluctuations alongside an overall declining trend in lice abundance, strongly influenced by environmental (temperature, dissolved oxygen) and geographical factors (bay morphology). Extending our work to examine the potential role for sea lice in pathogen transmission analysis of the sea lice gut microbiome detected several putative fish pathogens.

Conclusions: These epidemiological insights enhance our understanding of sea lice population dynamics and can directly inform adaptive management strategies, ultimately improving fish health and sustainability in salmon aquaculture.

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TRANSMISSION DYNAMICS, EPIDEMIOLOGY, AND GLOBAL HEALTH / FIELD EPIDEMIOLOGY AND SURVEILLANCE IN HUMAN AND ANIMAL POPULATIONS

UNEQUAL EXPOSURE OF DOGS TO LEISHMANIA DONOVANI; EVIDENCE FROM DOMESTIC AND STRAY DOGS IN SRI LANKA

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Background and Aims: Sri Lanka is endemic for cutaneous leishmaniasis caused by *Leishmania donovani,* and the reported number of human cases is rising. The role of dogs in this transmission cycle in Sri Lanka remains unclear, while they are known reservoir hosts for *L. donovani* in several endemic countries. This study aims to assess and compare the exposure in two dog populations, i.e., stray and domestic dogs, with distinct ecological characteristics and levels of human contact, as these differences can provide important insights into local transmission dynamics.

Methods: A cross-sectional serological survey was conducted in two leishmaniasis endemic districts, Matara and Hambantota. Blood samples were collected from 300 dogs (232 domestic, 68 stray). Sera were tested for anti-*L. donovani* antibodies using KMP11 antigen-based indirect ELISA. Seroprevalence was calculated for each group, and differences were assessed using the chi-square test ($p < 0.05$).

Results: Overall seropositivity to *L. donovani* was 62.0% (186/300). Stray dogs demonstrated a higher seroprevalence (72.1%, 49/68) compared to domestic dogs (58.7%, 137/232). The difference in seroprevalence between the two groups was statistically significant (Pearson chi-square = 4.336, df = 1, $p = 0.037$), indicating greater exposure among stray dogs in these endemic study areas.

Conclusions: The results provide a significantly higher seroprevalence of anti-*L. donovani* antibodies among stray dogs compared to domestic dogs in southern Sri Lanka, indicating differential exposure between canine populations. Further longitudinal and parasitological investigations, including molecular detection and vector–host interaction studies, are required to clarify the role of dogs in the transmission cycle of *L. donovani* in Sri Lanka.

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TRANSMISSION DYNAMICS, EPIDEMIOLOGY, AND GLOBAL HEALTH / FIELD EPIDEMIOLOGY AND SURVEILLANCE IN HUMAN AND ANIMAL POPULATIONS

PREVALENCE AND TAXONOMIC COMPOSITION OF TRICHINELLA SPP. IN TERRESTRIAL AND MARINE MAMMALS IN NORTHERN CANADA: HIGH CO-INFECTION RATES IN YUKON BEARS REVEALED BY ILLUMINA SEQUENCING

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Background and Aims: This study investigated the prevalence and taxonomic composition of *Trichinella* spp. in species of northern Canadian wildlife, which serve as primary sources of human trichinellosis.

Methods: Using artificial digestion, we analyzed muscle samples from Yukon grizzly bears (*n*=23) and black bears (*n*=102) collected in 2013-14 and 2024. We also isolated larvae from muscle samples of seven walruses and seven polar bears (Nunavut; 2019-25) that had tested positive by this method. Up to 100 pooled larvae per isolate (Range: 10-100) were genotyped by Illumina sequencing of an amplified fragment of the nuclear ribosomal internal transcribed spacer 1.

Results: *Trichinella* spp. prevalence was 69.6% (16/23) in grizzly bears and 18.6% (19/102) in black bears, while mean larvae per gram (LPG) of muscle was significantly higher ($p<0.05$, Mann-Whitney U) in black bears (221.2) than in grizzlies (34.4). Mean larval loads in walruses and polar bears measured 23.8 and 13.0 LPG. Genotyping was successful for 15 of 16 grizzly bear isolates (one failed to amplify); all 15 were mixed infections of *Trichinella nativa/Trichinella* T6. Eighteen of 19 isolates from black bears were *T. nativa*/T6 co-infections, while the remaining larval pool contained only *T. nativa*. Only *T. nativa* was detected in larval isolates from walruses and polar bears.

Conclusions: Our genotyping approach identified a higher frequency of *T. nativa*/T6 co-infections in terrestrial bears than previously reported, suggesting these taxa frequently co-occur in the Yukon sylvatic cycle. The presence of solely *T. nativa* in walruses and polar bears aligns with known Arctic transmission patterns.

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UNCOVERING DIAGNOSTIC ACCURACY IN THE ABSENCE OF A GOLD STANDARD: BAYESIAN ANALYSIS OF BATCH VARIATION AND THRESHOLDS IN SCHISTOSOMA POC-CCA TESTS

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Background and Aims: For many parasitic diseases, a perfect "gold standard" diagnostic is unavailable or infeasible, complicating study interpretations and the validation of new tests. Conventional comparisons with imperfect reference tests can lead to imperfect performance estimates. We present a Bayesian Latent Class Analysis (LCA) framework to address this challenge, using the evaluation of an updated *Schistosoma mansoni* rapid test (POC-CCA3) against its predecessor (POC-CCA) and microscopy (Kato-Katz), as a methodological case study.

Methods: We analysed data from 870 children in moderate and high *S. mansoni* endemicity settings in Uganda, tested over three days. We employed a Bayesian LCA, that utilises understanding of the dose-response relationship of the diagnostics, to estimate true disease prevalence and diagnostic performance metrics. The model specifically investigated sensitivity, specificity, inter- and intra-sample variation, batch-to-batch consistency, and the optimisation of diagnostic thresholds to align with World Health Organization Target Product Profiles for *Schistosoma* across different endemicity settings.

Results: The LCA successfully disentangled technical variation from diagnostic outputs. It quantified reduced batch-to-batch variation in POC-CCA3 compared to the original POC-CCA. By identifying an optimised G-score threshold of 4, the model estimated POC-CCA3 sensitivity/specificity at 86.3%/92.9% (moderate endemicity) and 88.1%/95.5% (high endemicity), against estimated true prevalences of 31% and 91% respectively.

Conclusions: Bayesian LCA provides a robust methodology for validating diagnostics when reference standards are imperfect. By explicitly modelling error sources, we demonstrated that while POC-CCA3 offers superior consistency, threshold adjustments are critical for accurate deployment. This framework is essential for reliable diagnostic evaluation and decision-making in diverse endemic settings.

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ASSESSING MOSQUITO-BORNE DISEASE RISK ACROSS AN URBAN-RURAL GRADIENT IN SCOTLAND.

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Background and Aims: Mosquito-borne diseases (MBDs), including West Nile virus (WNV) and Usutu virus (USUV), are an emerging threat in northern Europe, with transmission risk varying across landscapes. In Scotland, where mosquitoes remain understudied, it is important to understand how mosquito exposure and host activity may differ across urban and rural environments. This study aimed to assess the spatial and seasonal variation in MBD risk in West Scotland by integrating avian monitoring with entomological surveillance.

Methods: Sampling was conducted at four sites: one urban, one suburban and two forests. Adult mosquitoes were trapped between July and September 2023 and April and September 2024 using BG-Pro traps baited with CO_2 and a BG-lure. In 2024, Passive Acoustic Monitoring (PAM) was incorporated to quantify avian reservoir host activity and diversity across the vector season.

Results: A total of 363 mosquitoes representing 10 species were collected across both years. Urban and suburban sites had a higher abundance and species richness than forest sites. *Culex pipiens pipiens* dominated collections (78%) and emerged earlier in urban and suburban sites than at forest sites. *Culex pipiens molestus* was detected at both urban and suburban sites. Mosquito abundance and avian activity also differed significantly between sites across the season, with peak mosquito abundance in late July/early August in urban and suburban sites coinciding with higher avian activity than at forest sites.

Conclusions: Greater mosquito activity and higher MBD risk were detected in urban areas, highlighting the importance of targeted surveillance and public health planning in Scottish urban environments.

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TRANSMISSION DYNAMICS, EPIDEMIOLOGY, AND GLOBAL HEALTH / FIELD EPIDEMIOLOGY AND SURVEILLANCE IN HUMAN AND ANIMAL POPULATIONS

STUDY OF THE RELATIONSHIP BETWEEN MALARIA, GEOHELMINTHIASIS AND ANEMIA IN PREGNANT WOMEN IN THE KAOLACK HEALTH DISTRICT

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Background and Aims: Anemia is a public health problem in tropical regions. Children under 5 years of age and pregnant women are the most affected. Our study aims to investigate the determinants and main risk factors of anemia in pregnant women in a rural setting

Methods: A case-control study was conducted in Kaolack over a 3-month period in 2023. All recruited women underwent venipuncture, stool sampling, and thick blood smear testing for laboratory analysis. Data were entered into Excel and analyzed using STATA

Results: A total of 458 women were recruited. *Plasmodium falciparum* carriage was present in 7% of cases and 1.3% of controls. No significant association was found between the occurrence of anemia and parasite density. *Trichuris trichiura* was found in 0.9% of controls. The main risk factors for developing anemia in women were the presence of microcytosis (ORa=2.2, 95% CI (1.1–4.2; p=0.02)) and hypochromia (ORa=2.8, 95% CI (1.2–6.7; p=0.01)). Those who were overweight and/or obese had a lower risk of developing anemia (ORa: 0.6, 95% CI (0.3–0.9; p=0.03)).

Conclusions: Anemia remains common despite a decrease in malaria and soil-transmitted helminthiasis during pregnancy.

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PREVALENCE, INCIDENCE, CLINICAL MANIFESTATIONS AND FACTORS ASSOCIATED WITH PEDICULOSIS CAPITIS IN NURSERY SCHOOL CHILDREN OF A LOW-INCOME AREA FROM COLOMBIA

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Background and Aims: The hematophagous insect *Pediculus humanus capitis* (Anoplura: Pediculidae), colloquially known as head lice, causes pediculosis capitis and is the most common ectoparasite around the globe. Epidemiology of pediculosis capitis has not been described for each department (Colombian state). Aimes: the objective was to determine the prevalence, incidence, associated variables and the most common clinical manifestations in preschooler population.

Methods: Cross-sectional descriptive study based on a sample (356 children) aged 1 to 5 of a low-income area from Popayán, Colombia. Two observations were practiced: at the beginning and at the end of the year 2017. Hair, scalp, lymphatic nodules and frontotemporal, parietal, occipital, nuchal and retroauricular cutaneous regions were examined. Insects were mechanically removed by wetting the hair and using lice combs. Nits, nymphs and adult lice were gathered and stored for future studies.

Results: Prevalence and incidence of pediculosis capitis were 5.1 % and 20.2 %, respectively. The associated variables were mainly infestation antecedent, long hair, female sex and eliminating with shampoo (95 % CI: 15-20). Clinical variables: presence of adenopathies, hair scalp inflammation and nuchal adenopathies (25-35 %); nits and lice localized in occipital region, hair scalp itching and retroauricular itching
(20-25

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%).



Conclusions: Pediculosis capitis affects those nursery children studied. It is important to know the variables associated for prevention, control and eradication of head lice infestation.

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TOXOCARA CANIS: SEROPOSITIVITY IN PEDIATRIC PATIENTS AT A TERTIARY PEDIATRIC HOSPITAL OVER THE LAST 14 YEARS

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Background and Aims: In humans, T. canis larva cause ocular larva migrans (OLM), visceral larva migrans (VLM), and cerebral toxocariasis. In Mexico, the seroprevalence is 13.8% in children and 4.7% in adults; however, this zoonotic parasitosis is not subject to continuous epidemiological surveillance. This study analyzed the proportion of patients seropositive for secretions and excretions of third-stage T. canis larvae (L3TES) who were diagnosed with larva migrans over 14 years at the National Institute of Pediatrics.

Methods: Retrospective, transverse, and descriptive study. Thirty-one patients diagnosed with visceral or ocular larva migrans, eosinophilia, or toxoplasmosis were included over 14 years. An indirect ELISA was performed to detect anti-L3TES IgG, and a sandwich ELISA was performed on 12 serum samples to detect L_3TES. The proportions for each variable were analyzed.

Results: 17 girls and 14 boys aged 2 to 18 years. Fifteen children were diagnosed with VLM, 12 with OLM, three with eosinophilia, and one with toxoplasmosis. IgG was detected in nine children with VLM, in nine with OLM, three with eosinophilia, and one with toxoplasmosis. L3TES was detected in 4/5 of the children with OLM and in 4/7 of the children with VLM. Two children underwent enucleation; one of them tested positive for both IgG and L3TES.

Conclusions: Detection of IgG and L3TES supports the diagnosis of active larva migrans. This study may set a precedent for OLM screening in patients with suspected retinoblastoma.

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FIRST DETECTION OF LEISHMANIA INFANTUM IN DOGS AND WILD-CAUGHT CULICOIDES OXYSTOMA IN HAINAN, SOUTHERN CHINA

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Background and Aims: Leishmaniases are neglected parasitic diseases expanding globally. In China, *Leishmania infantum,* endemic in the west, has reemerged and spread to central China, raising concerns about further potential expansion. Given limited data from southern China, this study investigates the presence of *Leishmania* infection in dogs and potential vectors in Hainan, China's southernmost Province.

Methods: In 2024, blood, conjunctival swabs, and lymph node aspirates were collected from 52 dogs at two shelters in Haikou, Hainan, and four CDC light traps were placed. *Leishmania* DNA was amplified by kinetoplast minicircle DNA (kDNA) and internal transcribed spacer 1 (ITS1) PCR. A commercial rapid test (SNAP® Leish 4Dx®; IDEXX, USA) and immunofluorescence antibody test (IFAT) were used to detect antibodies against *L. infantum*. Insects were morphologically identified, and non-engorged females were tested for *Leishmania* by ITS1 PCR and sequencing. Phylogenetic relationships were inferred using Maximum likelihood and neighbour-joining trees constructed in MEGA X.

Results: Overall, 4 of 52 samples (7.7%) were serologically (n=3) or molecularly (n=1, conjunctival swab by kDNA PCR) positive for *Leishmania* species. Two were SNAP® positive, with one showing generalized lymphadenomegaly, and one was IFAT positive (1:160). No sand flies and 1573 *Culicoides* were captured. *Leishmania* DNA sequences (10 *L. infantum,* 1 *L. tarentolae*) were detected in 11/ 224 non-engorged female *Culicoides oxystoma* and clustered within their corresponding phylogenetic clades.

Conclusions: This study represents the first evidence of *L. infantum* infection in dogs and biting midges from Hainan Province. Ongoing surveillance of competent vectors and dogs in this region is essential.

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TRANSMISSION DYNAMICS, EPIDEMIOLOGY, AND GLOBAL HEALTH / FIELD EPIDEMIOLOGY AND SURVEILLANCE IN HUMAN AND ANIMAL POPULATIONS

MOLECULAR DETECTION AND EPIDEMIOLOGICAL ASSESSMENT OF CUTANEOUS LEISHMANIASIS IN ENDEMIC REGIONS OF PAKISTAN

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Background and Aims: Cutaneous leishmaniasis is a major neglected tropical disease and remains a persistent public health problem in Pakistan, particularly in endemic rural areas. This study aimed to determine the prevalence of cutaneous leishmaniasis using conventional microscopy and molecular techniques and to assess associated epidemiological factors.

Methods: Clinical samples were collected from patients presenting with suspected lesions and examined using microscopy, followed by polymerase chain reaction (PCR) for confirmation. Epidemiological variables such as age, gender, and geographic origin were analyzed.

Results: Microscopy identified *Leishmania* parasites in 38% of cases, while PCR confirmed infection in 56% of samples, demonstrating higher diagnostic sensitivity. A higher burden was observed among young individuals and residents of rural endemic areas. The continued transmission of cutaneous leishmaniasis highlights gaps in early diagnosis and surveillance. Incorporation of molecular diagnostic tools into routine public health programs can significantly enhance case detection and support effective control strategies.

Conclusions: This study underscores the need for strengthened surveillance, improved diagnostic capacity, and integrated public health interventions to reduce the burden of cutaneous leishmaniasis in Pakistan.

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BREAKING VERTICAL TRANSMISSION: A MOLECULAR STUDY ON THE PROTECTIVE EFFECTS OF BUPARVAQUONE FOR CATTLE AND THEIR NEWBORN CALVES

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Background and Aims: Tropical theileriosis, caused by *Theileria annulata*, affects 80% of cattle globally, threatening food security. While Buparvaquone is the standard treatment, its effectiveness is limited to early stages and challenged by rising drug resistance. Due to insufficient local data in Sudan, this study aims to evaluate the efficacy of Buparvaquone in naturally infected pregnant cows and its potential to protect their newborn calves.

Methods: A total of 100 cows were screened for *Theileria annulata* using PCR testing, and found that 75 tested positive. These positive cases were divided into a treated group of 55 cows and a non-treated group of 20. To evaluate the effectiveness and protective effects of Buparvaquone, we monitored the number of PCR-positive cases in both groups and their calves.

Results: The results showed that out of 55 cows treated with buparvaquone, 42 (76.4%) recovered, while 13 (23.6%) remained PCR-positive. In comparison, only 6 (30%) of the 20 cows that did not receive treatment recovered, and 14 (70%) remained PCR-positive ($\chi^2 = 13.684$, $p = 0.0002$). Regarding the calves, only 5 (9.1%) born to the treated cows tested positive, compared to 14 (70%) in the non-treated group ($\chi^2 = 28.766$, $p = 0.0001$). Notably, seven calves born to the non-treated group died within the first week of life.

Conclusions: Buparvaquone significantly decreased positive cases in cows and calves, enhancing recovery rates and calf survival. These results underscore the importance of treating pregnant cows for tropical theileriosis.

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THE ONE HEALTH PERSPECTIVE OF WOMEN FAMILY FARMERS IN BRAZIL, AND HOW CONSERVATION AGRICULTURE CONTRIBUTES TO CLIMATE RESILIENCE AND SOCIO-ECONOMIC BALANCE

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Background and Aims: Conservation agriculture (CoAg) is widely recognized by the UN as an effective method for increasing farmers' resilience to health and weather events. Although CoAg includes various ecological farming practices that protect the environment, some may disproportionately affect women family farmers (WFF) (i.e., Giardia spp., Strongyloides spp., etc.). WFF face barriers to adopting sustainable practices, largely due to limited technical training and persistent gender stereotypes. The objective of this study was to examine and co-produce a diagnostic on how the One Health concept can empower the livelihoods of WFF and promote health and food security in rural communities.

Methods: Through a network of researchers, 34 WFF in the Ribeira Valley, Brazil, were interviewed to identify practices that align with CoAg: livestock production, animal husbandry, and knowledge levels.

Results: Crop-livestock-forest perennial systems (banana, manioc, livestock) are used by 42% of WFF. Almost all WFF (92.3%) raised animals (chicken, fish, water buffalo). Milk from buffalo and meat from chicken and swine were the most prevalent food security factors. Although most WFF practices low-input, positive-ecosystem-service farming, detailed data on health protocols to control diarrhea, anemia, and parasites in humans and animals were not available. Water and soil practices and land use were not considered as environmental risk factors.

Conclusions: Technical training in CoAg is being scheduled despite financial constraints. This work provided evidence for the need for national and global public policies that align CoAg with the needs of socially and climate-vulnerable WFF in developing countries.

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EFFICACY OF SINGLE-DOSE BENZIMIDAZOLES AGAINST SOIL-TRANSMITTED HELMINTH INFECTIONS

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Background and Aims: Preventive chemotherapy with single-dose benzimidazoles remains the cornerstone of soil-transmitted helminth (STH) control. However, concerns are increasing regarding reduced drug efficacy and the potential emergence of resistance. We assessed the efficacy of albendazole and mebendazole across multiple endemic countries.

Methods: We conducted 11 prospective, open-label trials in 10 countries across Africa and Asia among schoolchildren aged 5-14 years. Participants received a single dose of albendazole (400 mg) or mebendazole (500 mg) through national deworming programs. Stool samples were collected at baseline and 14-21 days post-treatment. Egg reduction rates (ERRs) were calculated using duplicate Kato-Katz. Drug efficacy was classified using WHO point-estimate and formal hypothesis-testing frameworks.

Results: A total of 9,974 children were treated, with 2,987 included in efficacy analyses. Albendazole showed high efficacy against *Ascaris lumbricoides* (ERR range: 90.8% to 100%), although reduced efficacy was observed in three of five trials. Efficacy against hookworm was consistently reduced across all four trials (ERR: 44.1% to 69.5%). For *Trichuris trichiura*, efficacy was variable (ERR: 4.1% to 74.3%), with reduced efficacy observed in three of five trials. Formal hypothesis testing identified more trials with reduced efficacy compared to WHO point estimates. Molecular analyses indicated differential responses between hookworm species, with limited reduction observed for *Ancylostoma* spp.

Conclusions: Our findings demonstrate variable and often reduced efficacy of single-dose benzimidazoles, particularly against hookworm and *T. trichiura*, in settings with sustained preventive chemotherapy. These results underscore the need for routine efficacy monitoring, refinement of evaluation frameworks, and consideration of alternative treatment strategies to sustain progress toward STH control targets.

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EPIDEMIOLOGY OF SOIL-TRANSMITTED HELMINTH INFECTIONS IN URBAN SLUM AND TEA GARDEN COMMUNITIES IN NORTHEASTERN BANGLADESH

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Background and Aims: Soil-transmitted helminth (STH) infections remain prevalent in northeastern Bangladesh despite the national school-based deworming programme implemented since 2008. This study aimed to describe STH epidemiology and examine individual- and household-level factors associated with infection in slum and tea garden communities of Sylhet and Moulvibazar districts

Methods: A total of 772 stool samples from 16 communities were collected between May and October 2025. Structured questionnaires captured household- and individual-level risk factors. Stool samples were analysed using sodium-nitrate-flotation (SNF), Kato-Katz, and Baermann techniques. Descriptive statistics reported STH prevalence adjusted for community-level clustering. Multivariable regression to investigate factors associated with STH infection is underway.

Results: Using SNF, STH prevalence was 35.1% (271/772; 95%CI 26.2-45.2), with *Ascaris lumbricoides* predominating at 31.1% (240/772; 21.8-42.2), followed by hookworm (38/772; 4.9%, 2.6-9.1) and *Trichuris trichiura* (34/772; 4.4%, 2.0-9.5). All infections were of light to moderate intensity. STH prevalence ranged from 12.5% to 70.9% across communities, with similar levels in tea garden (57/162; 35.2%) and slum settings (214/610; 35.1%). Using Kato-Katz, overall STH prevalence was 28.4% (219/772; 20.1-38.4), with *A. lumbricoides* predominant at 25.0% (193/772; 16.5-36.1), followed by *T. trichiura* at 3.4% (26/772; 1.3-8.2) and hookworm (19/772; 2.5%, 1.4-4.4). SNF was more sensitive than Kato-Katz, with planned diagnostic comparison including concordance analysis. Baermann-based prevalence was 2.9% (22/772; 1.2-6.6) for hookworm and lower than expected for *Strongyloides* spp. (3/772; 0.4%, 0.1-1.6).

Conclusions: These findings highlight persistent transmission and community-level variation, supporting community-based control strategies targeted to high-risk areas, with further insights expected from ongoing risk factor analyses.

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FIRST REPORT OF GIARDIA DUODENALIS ASSEMBLAGE C IN NEW WORLD MONKEYS (CALLITHRIX SPP.) FROM RIO DE JANEIRO, BRAZIL

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Background and Aims: *Giardia duodenalis* is a globally distributed protozoan parasite causing gastrointestinal disease in humans and animals. This pathogen comprises various genetic assemblages (A–H) with diverse host ranges, including New World Monkeys (NWM) in the Brazilian Atlantic Forest. This study aimed to detect and genotype *G. duodenalis* in *Callithrix* spp. from Rio de Janeiro, Brazil, using molecular and histopathological methods.

Methods: Samples were collected from 22 *Callithrix* spp. at the Fiocruz Mata Atlântica Biological Station (EFMA). Small intestine tissues were formalin-fixed and paraffin-embedded (FFPE) for histopathological analysis, while stool samples were pooled into three groups and stored at -20°C for molecular processing. DNA was extracted from stool using the QIAamp PowerFecal Pro DNA Kit, and a nested PCR targeting the beta-giardin gene was performed for detection and assemblage differentiation. In addition, FFPE intestinal tissues were stained with hematoxylin and eosin (H&E).

Results: One stool pool (33.3%) tested positive for *G. duodenalis* Assemblage C. At H&E stained sections, trophozoites forms of *Giardia* spp. were visualized in the small intestines of one animal. To our knowledge, this is the first report of Assemblage C in NWM from Rio de Janeiro.

Conclusions: Although *G. duodenalis* Assemblage C is mostly found in dogs, humans, and environmental samples in Brazil, and is typically considered non-zoonotic. These findings highlight

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the need to further investigate its transmission cycles among humans, domestic animals, and wildlife through a One Health approach.

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MOLECULAR EPIDEMIOLOGY OF ZOONOTIC AND ANTHROPONOTIC CRYPTOSPORIDIUM SPECIES IN CHILDREN WITH DIARRHEA IN SOUTHWESTERN NIGERIA

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Background and Aims: *Cryptosporidium* is a globally distributed protozoan parasite of major public health importance, causing diarrheal disease in humans and animals and representing a key One Health concern due to its zoonotic transmission potential. However, molecular epidemiological data from Nigeria remain scarce. This study investigated the prevalence, species distribution, and genetic diversity of Cryptosporidium among children with diarrhea in Ogbomoso, southwestern Nigeria, to provide insights relevant to zoonotic transmission and parasite diversity

Methods: A total of 122 children presenting with diarrhea were enrolled from two health facilities in Ogbomoso between February 2023 and October 2024. DNA was extracted and screened using multiplex real-time quantitative PCR (RT-qPCR). Samples with cycle threshold values <30 were further analyzed by nested PCR and Sanger sequencing to identify species and subtypes, followed by phylogenetic analysis

Results: RT-qPCR detected *Cryptosporidium* spp. in 29 (23.8%) samples, while *Strongyloides* spp. was detected in one (0.8%) sample. Eight *Cryptosporidium*-positive samples were successfully genotyped. Molecular analysis identified *Cryptosporidium parvum* (62.5%), a species commonly associated with zoonotic transmission, and *Cryptosporidium hominis* (37.5%), which is primarily anthroponotic. Phylogenetic analysis revealed close clustering with isolates from diverse regions worldwide and limited amino acid variation among Nigerian *C. parvum* sequences, suggesting the circulation of conserved lineages with zoonotic relevance. No significant associations were observed between infection status and demographic or clinical variables ($p > 0.05$).

Conclusions: These findings highlight the co-circulation of zoonotic and anthroponotic *Cryptosporidium* species in Nigerian children, underscoring the importance of integrated One Health surveillance and improved water, sanitation, and hygiene interventions to reduce transmission

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TRANSMISSION DYNAMICS, EPIDEMIOLOGY, AND GLOBAL HEALTH / FIELD EPIDEMIOLOGY AND SURVEILLANCE IN HUMAN AND ANIMAL POPULATIONS

EPIDEMIOLOGY OF SOIL-TRANSMITTED HELMINTHIASIS INFECTION AMONG POPULATIONS IN AN ENDEMIC COMMUNITIES IN IBARAPA AND OKE-OGUN ZONES OF OYO STATE, NIGERIA.

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Background and Aims: This study assessed the epidemiology of STH infections among adult populations, examined soil and human behavioural factors contributing to transmission of STH in Ibarapa and Oke-Ogun zones of Oyo State.

Methods: A cross-sectional study was conducted between January and September 2025 where 1560 stool samples were collected from adult participants and 2480 soil samples collected. Structured questionnaire was administered to the participants to obtain socio-demographic risk factors, hygiene and sanitation practices and Mass Drug Administration (MDA) participation. Stool and soil samples were analysed using parasitological techniques and PCR amplification of parasites DNA. Descriptive statistics and inferential tests were done at $p<0.05$.

Results: A total of 20.5% of the participants were infected with at least one of the STH parasites. *Ascaris* had the highest parasite prevalence of 11.0%, followed by hookworm(6.9%) and *Strongyloides*(2.7%). 69.0% of soil samples were contaminated with STHs larva and eggs, with hookworm larva having the highest prevalence of 45.2%, followed by *Strongyloides* larva(40.1%), *Trichuris trichura* eggs(6.5%), and the least was *Ascaris* with prevalence of 5.3%. Water, Sanitation and Hygiene data revealed that 52.6% of participants had assess to portable drinking water, 43.8% lacked access to good toilet facilities, with reliance on open defaecation, while 42.5% reported walking barefoot during daily or occupational activities. About 68.5% of the participants claimed they practised proper hand washing with soap while the remaining 32.5% were inconsistent in the use of soap during hand-washing.

Conclusions: This study demonstrates that STHs infections remain prevalent among adults population in study areas due to widespread environmental contamination and inadequate sanitations.

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ACTIVE SURVEILLANCE OF SAND FLIES (DIPTERA: PSYCHODIDAE) IN ROMANIA

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Background and Aims: Phlebotomine sand flies (Diptera: Psychodidae) are the main vectors of *Leishmania infantum* in the Mediterranean basin. In Romania, three species are competent vectors; consequently, autochthonous canine leishmaniasis highlights the need for updated surveillance. Recent data have assessed the sand fly diversity, distribution and host-feeding preferences, as well as the prevalence of *L. infantum*, Toscana virus (TOSV) and Sandfly fever Sicilian virus (SFSV).

Methods: Between 2013 and 2025, sand flies were collected from 133 sites in multiple counties using CDC miniature light traps, mouth aspirators and sticky traps.

Results: Out of 1,868 collected specimens, 89.5% were *Phlebotomus neglectus* and 10.2% *Ph. perfiliewi*. Other minor species included *Ph. papatasi, Ph.balcanicus, Ph. sergenti* s. l., *Sergentomyia minuta* and *Ph. simonahalepae*. Seasonal dynamics showed a peak of activity for *Ph. neglectus* in July and *Ph. perfiliewi* in August. Sand flies were recorded for the first time in Călăraşi County in July 2025. Molecular screening for pathogens (*L. infantum*, TOSV and SFSV) yielded negative results for all specimens. Bloodmeal analysis (n = 74) indicated opportunistic feeding behavior on domestic and wild hosts: *Ph. neglectus* fed primarily on cattle (34%) and humans (22%), whereas *Ph. perfiliewi* showed a strong prference for horses (80%).

Conclusions: Current data indicates that six of the eight historically reported species remain present. While no pathogens were isolated, the risk of disease re-emergence persists due to the presence of competent vectors with opportunistic feeding habits in areas influenced by the Mediterranean climate. Therefore, long-term monitoring programs are crucial for understanding sand fly population dynamics and defining their vectorial role.

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INTESTINAL HELMINTH INFECTIONS IN HUMANS AND ANIMALS ALONG THE LAO-THAI BORDER, LAOS: A ONE HEALTH APPROACH

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Background and Aims: Intestinal parasitic infections remain a major global public health concern, particularly in regions where close interactions among humans, animals, and the environment facilitate transmission. The Lao-Thai border, centered around the Mekong River, represents a critical ecological and socio-economic region characterized by intense cross-border movement and shared ecosystems.

Methods: A cross-sectional study was conducted in seven villages located at established Lao-Thai border crossing points in Laos to assess the prevalence of intestinal helminths in humans and animal reservoirs. A total of 475 human participants and 75 animals (12 cats and 63 dogs) were enrolled. Stool samples were examined using the formalin-ethyl acetate concentration technique for ova and parasite detection.

Results: Six parasite species were identified in humans: *Opisthorchis viverrini*, hookworms, *Trichuris trichiura*, *Strongyloides stercoralis*, *Capillaria* spp., and *Taenia* spp. Hookworms showed the highest overall prevalence (36.6%), followed by *O. viverrini* (24.6%). Both parasites were most commonly detected in central Laos. Among animal reservoirs, eight parasite species were detected: *O. viverrini*, hookworms, *T. trichiura*, *S. stercoralis*, *Toxocara* spp., *Spirometra* spp., *Hymenolepis diminuta*, and *Taenia* spp. Hookworms were the most prevalent parasite in cats (66.7%) and dogs (73.0%). *O. viverrini* infection was detected only in cats (8.3%).

Conclusions: This study demonstrates ongoing transmission of intestinal helminths at the human-animal-environment interface along the Lao-Thai border. The findings highlight the importance of integrated surveillance and coordinated control strategies that address human populations, animal reservoirs, and shared environments within a One Health framework to reduce the burden of intestinal parasitic infections in this transboundary region.

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PREVALENCE, MORPHOLOGICAL AND MOLECULAR IDENTIFICATION OF PARAMPHISTOMIDS IN RUMINANTS FROM ÑUBLE, CHILE, AND THEIR ASSOCIATION WITH PRODUCTIVE TRAITS

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Background and Aims: Paramphistomosis is a neglected parasitic disease affecting ruminants. In Chile, information is limited to a single report in cattle. This study aimed to expand current knowledge by increasing sample size, including sheep, performing molecular identification, and evaluating the potential impact of infection on carcass traits (weight and fat cover).

Methods: The study was conducted at a slaughterhouse in the Ñuble Region, Chile. Trematodes were collected from the rumen/reticulum of 427 bovines and 112 sheep. Identification was performed morphologically (alum-carmine staining) and molecularly (ITS-2 sequencing). Pearson correlation was used to assess the relationship between infection status and carcass traits across cattle categories.

Results: Overall bovine prevalence was 22.0%, doubling previous national reports, while sheep remained negative (0%). Spatial analysis identified San Ignacio and San Nicolás as high-prevalence clusters (33.3%). Morphological and molecular analysis confirmed *Calicophoron microbothrioides* as the etiological agent. Regarding production, statistical analysis revealed no significant negative correlation between parasite presence and carcass weight or fat cover in the evaluated adult cattle categories ($p > 0.05$).

Conclusions: This study confirms *Calicophoron microbothrioides* presence in Ñuble with high prevalence in cattle, contrasting with the absence in sheep. Despite the high infection rate, the condition appears subclinical in finished cattle, showing no detrimental impact on carcass quality at slaughter. Further research should focus on younger categories where susceptibility might be higher.

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FIELD EPIDEMIOLOGY, PARASITE DYNAMICS AND HEALTH INDICATORS RELEVANT CONSERVATION OF TRANSBOUNDARY MOUNTAIN GORILLA (GORILLA BERINGEI BERINGEI) POPULATIONS, 2015–2025.

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Background and Aims: Les gorilles des montagnes menacés sont confinés à 416 km^2 de forêt transfrontalière dans le Grand Paysage du Virunga (RDC, Rwanda, Ouganda). En 2025, ~1 100 individus restent répartis dans 150+ groupes surveillés. Malgré une croissance de 10 à 15 % depuis 2000, les infections parasitaires persistent, nécessitant une surveillance soutenue de One Health.

Methods: De 2015 à 2025, une étude longitudinale a surveillé 48 groupes de gorilles. 1 250 échantillons fécales non invasifs ont été analysés à l'aide de la flottation, de la sédimentation et du diagnostic moléculaire par PCR. Les données ont été intégrées avec des variables démographiques, écologiques et anthropiques (densité de groupe, altitude, saisonnalité, sites de nidification, proximité entre humains et bétail). Les modèles linéaires et multivariés généralisés ont évalué les prédicteurs de l'infection.

Results: La prévalence des héminthiques a atteint 38-62 % (320-410 œufs/g), dominée par les strongylidés et les céstodes. La charge augmentait avec la densité des groupes (R^2=0,63 ; p<0,01) et variait selon l'altitude et la saison. La PCR détectait une plus grande diversité. Le chevauchement de l'habitat a entraîné des partages partagés, résistants aux antibiotiques *E. coli* (17 % gorilles ; 35 % humains ; 27 % bétail), en déclin avec une réduction du contact.

Conclusions: Les infections parasitaires principalement subcliniques sont des indicateurs sensibles des pressions écologiques et humaines. Une surveillance moléculaire et démographique intégrée, une collaboration transfrontalière renforcée et des diagnostics de terrain améliorés sont essentiels pour anticiper les risques émergents et garantir une conservation à long terme.

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POSTER ON BOARD SHIFT 01
TRANSMISSION DYNAMICS, EPIDEMIOLOGY, AND GLOBAL HEALTH / FIELD EPIDEMIOLOGY AND SURVEILLANCE IN HUMAN AND ANIMAL POPULATIONS

BAYESIAN ESTIMATION OF TRUE HERD-LEVEL PREVALENCE OF NEOSPORA CANINUM AND ASSOCIATED FACTORS: INSIGHTS FROM A SURVEILLANCE PROGRAM IN DAIRY HERDS IN ALBERTA, CANADA

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Background and Aims: Accurate estimates of infectious disease prevalence are essential for designing control programs that support dairy cattle health and productivity. *Neospora caninum* is a major cause of reproductive losses in Canadian dairy herds. This study estimated the true herd-level prevalence of *N. caninum* in Alberta dairy herds and explored factors associated with herd-level positivity.

Methods: Bulk tank milk samples were collected from all active dairy herds in Alberta, Canada, in April (n=459), August (n=457), and November 2025 (n=452). Samples were tested for *N. caninum* antibodies using a commercial indirect ELISA. Apparent prevalence was the proportion of positive herds at each time point. True herd-level prevalence was estimated with Bayesian latent class models to account for imperfect test performance. A voluntary questionnaire on management and biosecurity was distributed to all producers. Associations with *N. caninum* positivity are being evaluated using mixed-effect generalized linear models.

Results: Estimated true prevalence of herds with ≥15% within-herd seroprevalence was 23.3% (Bayesian credible interval [BCI]: 12.8–33.6), 42.4% (31.7–54.9), and 27.5% (17.2–38.3) in April, August, and November 2025. In total, 120 producers completed the questionnaire (response rate: 26.1%). Herds in the northern region and those using conventional milking systems were more frequently positive. Analysis of additional management factors is ongoing and will be presented at ICOPA 2026.

Conclusions: These findings provide updated estimates of the true prevalence of herds with ≥15% within-herd *N. caninum* seroprevalence in Alberta. Because herds above this threshold face increased risk of substantial reproductive losses, these data support development of evidence-based control strategies.

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TRANSMISSION DYNAMICS, EPIDEMIOLOGY, AND GLOBAL HEALTH / FIELD EPIDEMIOLOGY AND SURVEILLANCE IN HUMAN AND ANIMAL POPULATIONS

MAPPING TICK VECTORS IN GREEK RUMINANTS: SEASONAL PATTERNS AND GEOGRAPHIC DISTRIBUTION

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Background and Aims: Ticks and tick-borne pathogens pose a significant threat to livestock health, welfare, and productivity in the Mediterranean region, with potential implications for public health. This study aimed to assess tick presence and infestation intensity, and to describe their geographical and seasonal distribution across Greece, in order to identify areas at risk.

Methods: Seasonal tick collections were carried out on sheep, goat, and cattle farms in nine regions across the country. In each region and season, up to 20 farms were surveyed, with ticks collected from five animals per farm. Environmental sampling by flagging was also performed at grazing sites.

Results: From March 2024 to March 2025, 650 farms were visited; a total of 2,659 ticks were collected from 1,831 animals on 441 (68%) farms, and an additional 39 ticks from 21 (3%) flagging sites. Tick abundance peaked in spring (38.5%), followed by autumn (24.2%), winter (20.2%) and summer (17.1%). *Rhipicephalus* spp. was the dominant genus (56%), comprising *R. turanicus s.l.* (33%) and *R. bursa* (20%). *R. bursa* was prevalent across all regions during spring and summer. Other important species included *Hyalomma excavatum* (5%), *Ixodes ricinus* (3%), *I. gibbosus* (1%) and *H. marginatum* (1%), vectors of major zoonotic pathogens. *Ixodes* ticks were collected mainly in autumn and winter and predominantly from northern Greece, whereas *Hyalomma* spp. were detected sporadically across seasons and locations.

Conclusions: These findings improve our understanding of tick ecology in Greece and support targeted surveillance and prevention strategies to protect animal and human health.

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TRANSMISSION DYNAMICS, EPIDEMIOLOGY, AND GLOBAL HEALTH / FIELD EPIDEMIOLOGY AND SURVEILLANCE IN HUMAN AND ANIMAL POPULATIONS

ENVIRONMENTAL DNA SURVEILLANCE USING MULTIPLEX QPCR BRIDGES CRITICAL GAPS IN MONITORING AND TRANSMISSION HOTSPOT IDENTIFICATION OF SCHISTOSOMIASIS IN AN ENDEMIC PHILIPPINE VILLAGE

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Background and Aims: Schistosomiasis japonica remains a major public health concern in the Philippines, with 12.4 million people at risk from ongoing environmental transmission. Malacology-based surveillance inadequately captures focal *Oncomelania hupensis quadrasi* (*Ohq)* habitats, *Schistosoma japonicum* (*Sj)* transmission hotspots, and their spatiotemporal dynamics. To address this gap, we developed and applied environmental DNA (eDNA) multiplex real-time PCR (qPCR).

Methods: Multiplex qPCR assay targeting the COI gene of *Ohq* and *Sj* was applied to environmental samples from 30 sites in Ekiran, Leyte, Philippines. Water samples were collected quarterly from July 2023 to March 2024, while soil samples were collected during two cross-sectional surveys in December 2023 and March 2024. Results were compared with conventional malacological surveys, and associations with environmental parameters were assessed using nonparametric analyses.

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Results: Water eDNA system detected *Ohq* and *Sj* in 18/30 (60%) and 16/30 (53%) sites, respectively, whereas malacological surveys confirmed snail presence in five sites and *Sj*-infected snails in one site. Soil eDNA further identified microhabitats of both targets, including sites without observable snails, and consistently outperformed classical surveys. Detection frequencies increased during periods of high precipitation. Among water parameters, pH and temperature were significantly associated with *Sj* eDNA detection, while in soil, pH alone was significantly associated with combined *Ohq* and *Sj* eDNA detection.

Conclusions: Water- and soil-based eDNA detection bridges gaps in schistosomiasis surveillance by efficiently and sensitively identifying transmission hotspots. Simultaneous detection of *Ohq* and *Sj* captures fine-scale spatiotemporal dynamics, guiding interventions that support targeted transmission interruption when combined with human and animal surveillance in endemic Philippine communities.

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POSTER ON BOARD SHIFT 01
TRANSMISSION DYNAMICS, EPIDEMIOLOGY, AND GLOBAL HEALTH / FIELD EPIDEMIOLOGY AND SURVEILLANCE IN HUMAN AND ANIMAL POPULATIONS

TOXOPLASMA GONDII SEROPREVALENCE, SEROCONVERSION RATES AND GENETIC VARIABILITY IN HUMANS FROM URUGUAY

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Background and Aims: Toxoplasmosis, caused by *Toxoplasma gondii*, is a highly prevalent zoonotic infection. When acquired during pregnancy, it may be transmitted to the fetus, with outcomes influenced by gestational age and parasite genotype. Prenatal screening allows detection of seroconversion and timely intervention. In Uruguay, despite mandatory screening, national seroprevalence data are outdated and the genetic diversity of local strains remains poorly characterized. This study aimed to update seroepidemiological data and assess the genetic diversity of *T. gondii* in Uruguay.

Methods: Publicly available serological records from pregnant individuals attending Uruguay's largest public maternity hospital between 2019 and 2023 were analysed to estimate *T. gondii* seroprevalence and maternal seroconversion. Samples from confirmed cases were subjected to molecular detection, and parasite genotyping was performed using in silico PCR–RFLP based on established multilocus markers. Genetic profiles were compared with reference strains.

Results: Seroprevalence of *T. gondii* was 45.5%, a modest decline compared to 50% in 1998. The congenital transmission rate was estimated at 0.58%. Clinical evaluation of congenitally infected newborns identified chorioretinitis as the most frequent manifestation. Molecular analysis revealed

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substantial genetic diversity among circulating strains, including novel allele combinations not previously reported.

Conclusions: Despite the slight decline in seroprevalence, toxoplasmosis remains a relevant public health concern in Uruguay, with ongoing congenital transmission and clinically significant neonatal outcomes. The high genetic diversity observed highlights the dynamic population structure of *T. gondii* and underscores the need to strengthen prenatal surveillance and prevention of congenital toxoplasmosis in South America.

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TRANSMISSION DYNAMICS, EPIDEMIOLOGY, AND GLOBAL HEALTH / FIELD EPIDEMIOLOGY AND SURVEILLANCE IN HUMAN AND ANIMAL POPULATIONS

SUBTYPE DISTRIBUTION OF BLASTOCYSTIS SPP AND ITS POTENTIAL INTERACTION WITH THE INTESTINAL MICROBIOTA AND THE INTESTINAL EPITHELIUM

Alejandra Valentín-Decuadro[1,2,3], Denisse Bercovici[3,4], Florencia Peñalba[5], Carolina Olano[6], Nadia Riera[5,7], Romina Pagotto[4], Maria E Francia[1,3]
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Background and Aims: *Blastocystis spp.* is one of the most prevalent intestinal protists worldwide, yet its clinical relevance remains controversial, particularly in inflammatory bowel disease (IBD). Molecular studies have revealed extensive genetic diversity among *Blastocystis* subtypes (STs), which may contribute to differences in pathogenicity. This study aimed to determine the presence and subtype distribution of Blastocystis in IBD patients and non-IBD controls in Uruguay using a molecular approach. Additionally, microbiota composition in IBD patients with and without *Blastocystis* was analyzed, and interactions of different STs with intestinal organoids were explored.

Methods: Fourteen patients (n=14) with IBD and twenty-five (n=25) control individuals were included. Genomic DNA was extracted from stool samples, and *Blastocystis* detection was performed by end-point PCR targeting the SSU rRNA gene. Reference DNA from subtypes ST1, ST3, ST4, ST6 and ST8 was used as positive controls. Microbiota composition was analyzed by shotgun metagenomic sequencing of fecal DNA.

Results: *Blastocystis spp.* was detected in 38.5% of IBD patients and in none of the controls. Subtype analysis identified ST2 allele 09, ST3 allele 36, ST6 alleles 122 and 123, and ST8 allele 21, with ST6 being the most frequent subtype. ST6 isolates showed sustained *in vitro* growth, supporting their use in functional assays.

Conclusions: The exclusive detection of *Blastocystis* in IBD patients highlights the need to further investigate its potential pathogenicity. Ongoing work explores host–parasite–microbiota interactions

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using integrative approaches combining metagenomic microbiota profiling and *in vitro* human intestinal organoid co-culture models to assess epithelial responses and parasite behavior in different microbial contexts.

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TRANSMISSION DYNAMICS, EPIDEMIOLOGY, AND GLOBAL HEALTH / FIELD EPIDEMIOLOGY AND SURVEILLANCE IN HUMAN AND ANIMAL POPULATIONS

TAILORING TAENIA SOLIUM TARGET PRODUCT PROFILES: COST-EFFECTIVENESS OF DIAGNOSTIC TOOLS FOR PROGRAMME MONITORING AND EVALUATION

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Background and Aims: The WHO calls for intensified control of *Taenia solium* in hyperendemic areas, underscoring the need for robust monitoring and evaluation (M&E) systems to assess control programme performance towards the 2030 targets. Current survey recommendations suggest sampling pigs for detection of porcine cysticercosis using tongue palpation or meat inspection, or sampling humans for detection of taeniasis via stool microscopy (Kato-Katz) followed by molecular species confirmation. Existing target product profiles define minimal requirements for new *T. solium* diagnostics, however, are not tailored to the specific needs of M&E. This study assessed the cost-effectiveness of survey designs employing currently recommended and emerging diagnostic tools to correctly inform programme decision-making.

Methods: Zambian field datasets were used to inform a lot quality assurance framework. Minimal diagnostic performance requirements were explored across levels of spatial heterogeneity, along with survey design (number of villages and animals/individuals) and correct decision-making. Survey costs for emerging diagnostics (point-of-care and ELISA test formats) were compared with costs of the reference tongue palpation and Kato-Katz plus PCR.

Results: Diagnostic performance became less stringent with increasing sample sizes and greater tolerance for incorrect decision-making, with specificity as key determinant. Tongue palpation yielded survey costs more than three-fold lower than Kato-Katz plus PCR, suggesting cost-effectiveness of surveys focused on porcine cysticercosis. Point-of-care test formats showed particular usability for cost-effective survey designs.

Conclusions: Target product profiles should be updated to meet the needs for M&E of *T. solium* control programmes, emphasizing easy-to-use diagnostics with high specificity to support cost-effective decision-making.

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TRANSMISSION DYNAMICS, EPIDEMIOLOGY, AND GLOBAL HEALTH / FIELD EPIDEMIOLOGY AND SURVEILLANCE IN HUMAN AND ANIMAL POPULATIONS

OPERATIONAL DEPLOYMENT OF SMARTPHONE-BASED POC-CCA INTERPRETATION FOR SCHISTOSOMIASIS SURVEILLANCE: THROUGHPUT, STAFF WORKFLOW, AND DATA QUALITY ACROSS 148 SCHOOLS IN WESTERN KENYA

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Background and Aims: Smartphone-based diagnostic platforms promise to scale disease surveillance, but few publications report the operational realities—field workflow, staff productivity, and data quality—that determine real-world feasibility. We report operational metrics from a 13,165-test schistosomiasis POC-CCA deployment across Western Kenya using MEDSCAN, a smartphone-based interpretation platform.

Methods: Between 2025 and 2026, five field staff collected and analyzed POC-CCA tests at 148 schools across four Western Kenyan counties using the MEDSCAN smartphone app, which captures test images of the POC-CCA, automatically interprets the results, stores test images, and syncs to cloud server environments. The deployment combined cross-sectional and longitudinal cohorts.

Results: Five staff collected 13,165 samples and analyzed 12,591 POC-CCA tests (99.2% completion rate) across both cohorts. Median collection throughput was 1.3 samples/minute, with analysis rates of 1.1 tests/minute in active scanning windows. 96% of tests were analyzed within 8 hours of collection, with a median turnaround of 58 minutes. 52% of tests were collected and analyzed by different staff, demonstrating workflow flexibility not possible with microscopist-dependent methods. We additionally report per-test costs and travel times across the four-county deployment.

Conclusions: Smartphone-based POC-CCA interpretation supports population-scale schistosomiasis surveillance with high completion rates and flexible field workflow, offering a simpler and more scalable alternative to microscopy-based mapping. Offline-first architectures require explicit data harmonization, informing design choices for digital surveillance platforms in low-connectivity settings.

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EXPANDING INTO THE WILD HEART OF CANADA: DIROFILARIA IMMITIS INVASION INTO SASKATCHEWAN WILDLIFE

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Background and Aims: Canine heartworm (*Dirofilaria immitis*) is a mosquito-borne filarial parasite that causes cardiopulmonary disease in dogs and cats in some regions of Canada, but not Saskatchewan. Range expansion is possible due to climate change enabling development within local vectors and spread through movements of naturally infected domestic and wild canids. Therefore, we used a multi-pronged approach to determine if heartworm has newly established in Saskatchewan.

Methods: We examined blood and hearts of wild canids (coyotes, wolves, and foxes) (2024-26), screened trapped mosquitoes for filarial DNA (2024-26), and conducted a retrospective analysis of veterinary diagnostic testing of dogs and cats in Saskatchewan (2014-2025).

Results: Based on serum antigen testing, 12/71 (17%) wild canids were positive in 2024, and 15/40 (37.5%) were positive in 2025. Adult nematodes were detected in 1 of 40 hearts examined from 2025. From 2014-2025, 14 (0.4%) of 3497 dogs tested in SK were positive for antigen to *D. immitis*. The highest prevalence in dogs was seen in 2025 (1.4%), and this was also the first year that a positive cat was detected.

Conclusions: *Dirofilaria immitis* may be expanding into wildlife within Saskatchewan, with possible spillover into domestic dogs and cats. Surveillance in wild canids has advantages over that of dogs, who frequently receive prophylactic treatment and may acquire infections through travel. If newly established, this has implications for management and prevention in dogs and cats, and for public health, since the parasite can cause subcutaneous and pulmonary nodules in people.

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TRANSMISSION DYNAMICS, EPIDEMIOLOGY, AND GLOBAL HEALTH / FIELD EPIDEMIOLOGY AND SURVEILLANCE IN HUMAN AND ANIMAL POPULATIONS

UPDATE ON THE SPATIAL DISTRIBUTION OF FASCIOLOSIS IN SHEEP AND ALPACA HERDS IN THE HIGHLANDS OF THE CENTRAL AND SOUTHERN MACRO-REGIONS OF PERU

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Background and Aims: Fasciolosis, caused by *Fasciola hepatica*, is a major parasitosis affecting sheep and alpacas in the Peruvian highlands. Effective control programs require updated epidemiological information on the prevalence of infection. The objective of this study was to determine the prevalence of *F. hepatica* infection in sheep and alpacas across five central (5) and seven (7) southern Departments of the Peruvian highlands.

Methods: During November and December, 2024, pooled fecal samples were obtained from alpaca herds of 49 districts, from 2 central Departments (Lima and Pasco), and 7 southern departments (Apurimac, Arequipa, Ayacucho, Cusco, Huancavelica, Moquegua, and Tacna); and from sheep herds of 105 districts, from 5 central departments (Ancash, Huánuco, Junín, Lima, and Pasco) and 5 southern departments (Apurimac, Arequipa, Ayacucho, Cusco, and Huancavelica). The Flukefinder test was used to identify *F. hepatica* eggs and to determine the eggs per gram (epg).

Results: A total of 282 composite fecal samples from alpaca and 522 from sheep herds were evaluated. The general prevalence of fasciolosis was 13.8% (10-18.4%) and 29.48% (26.34-32.76%) for alpaca and sheep, respectively. The average epg in sheep was 5.8, ranging from 1 to 48. For sheep detected as positive, the average epg was 36.8, ranging from 0.5 to 1596. The maps of spatial distribution of fasciolosis (**Fig. 1** and **Fig. 2**) showed differences among the different departments, in both macro-
regions.

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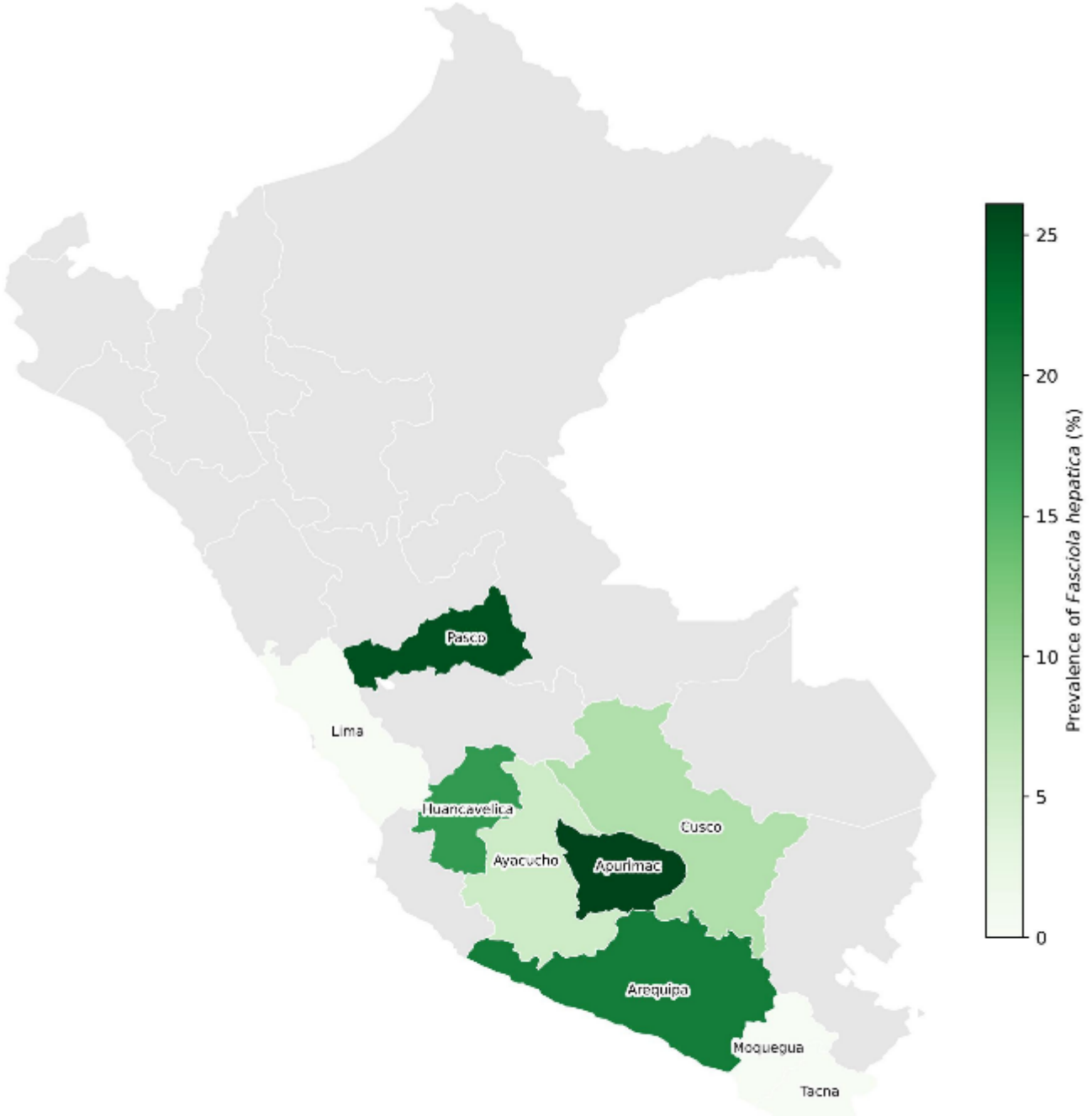


Fig 1. Prevalence of fasciolosis in alpaca herds in departments of the central (Lima and Pasco) and southern (Apurimac, Arequipa, Ayacucho, Cusco, Huancavelica, Moquegua, and Tacna), macro-regions of Peru. November-December, 2024

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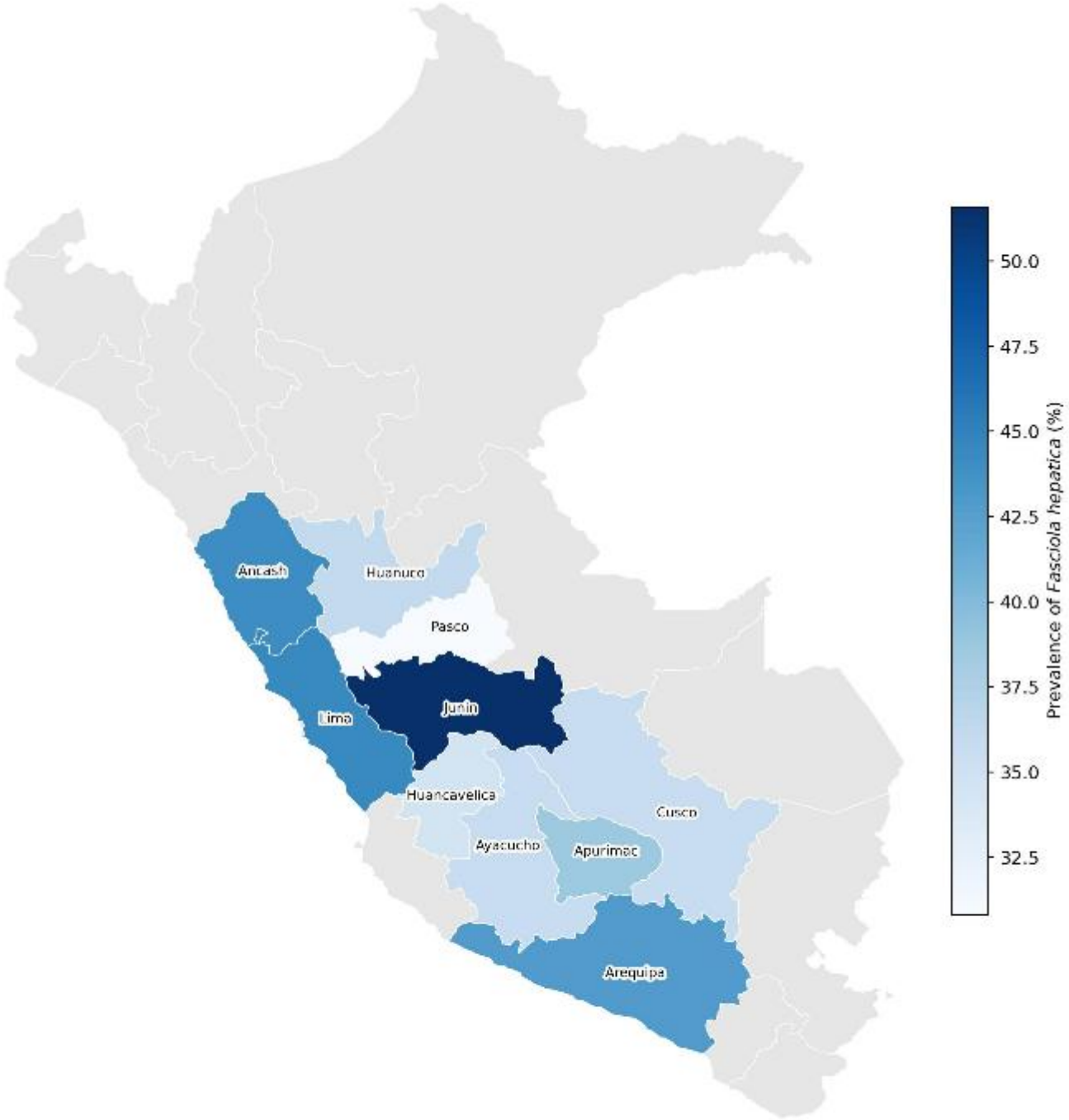


Fig 2. Prevalence of fasciolosis in sheep herds in departments of the central (Ancash, Huánuco, Junín, Lima, and Pasco) and southern (Apurimac, Arequipa, Ayacucho, Cusco, and Huancavelica), macro-regions of Peru. November-December, 2024

Conclusions: Fasciolosis remains a significant parasitosis affecting alpacas and sheep in the central and southern macro-regions of Peru, with a heterogeneous spatial distribution among departments. Sheep show more susceptibility than alpaca.

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TRANSMISSION DYNAMICS, EPIDEMIOLOGY, AND GLOBAL HEALTH / ONE HEALTH APPROACHES TO ZOONOTIC PARASITIC DISEASES

ONE HEALTH ASSESSMENT OF FASCIOLIASIS IN UGANDA: PREVALENCE, SPATIAL DISTRIBUTION, ECONOMIC BURDEN, AND RISK FACTORS AMONG FARM AND ABATTOIR WORKERS (2023–2024).

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Background and Aims: Fascioliasis is a zoonotic trematode infection caused by *Fasciola hepatic*a and *Fasciola gigantica,* and remains a challenge under the One Health umbrella; it conditions livestock productivity and a potential risk to people working directly with livestock, notably farm and abattoir workers. The current study assessed the prevalence, distribution, and economic effects associated with fascioliasis in domestic ruminants and its zoonotic potential to humans in six districts, Uganda.

Methods: A cross-sectional prevalence survey was conducted in 2,445 domestic ruminants (cattle, goats and sheep) at their farms and abattoirs. Faecal samples were collected and assessed using the Modified Mini-Flotac technique. Human stool samples (n=363) were collected and analyzed for fascioliasis and co-infection with other helminths among occupationally exposed workers. Structured questionnaire and focus group discussions were used to elicit behavioural and environmental risk factors.

Results: The prevalence of animal fascioliasis was highest in Jinja (26.8%) and lowest in Buliisa; animals that were female or aged 1- 2 years were also more susceptible. The ecology of residing near an open water body was also a significant predictive factor for fascioliasis in animals. A total of 741.4 kg of infected livers were condemned with an estimated economic loss of UGX 9.5 million (USD 2634). *F. hepatica* was detected in 1.45% of the human participants, all of whom were occupationally exposed. Other common helminths included Ascaris lumbricoides (14.6%) and *Schistosoma mansoni* (12.9%).

Conclusions: Fascioliasis occurs in Ugandan livestock and presents an occupational zoonotic risk. We must Integrate One Health interventions to sustainably control fascioliasis and improve livestock productivity.

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TRANSMISSION DYNAMICS, EPIDEMIOLOGY, AND GLOBAL HEALTH / ONE HEALTH APPROACHES TO ZOONOTIC PARASITIC DISEASES

SPATIAL VARIATION IN GIARDIA SPP. AND CRYPTOSPORIDIUM SPP. INFECTIONS IN AFRICAN BUFFALO (SYNCERUS CAFFER) IN KRUGER NATIONAL PARK

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Background and Aims: *Cryptosporidium* and *Giardia spp.* are zoonotic enteric protozoan parasites with a global distribution. Previous research has highlighted the potential for spillover at the wildlife-livestock interface between African buffalo (*Syncerus caffer*) and domestic cattle surrounding Kruger National Park (KNP), South Africa, driven by shared grazing areas and water sources. In KNP, *Cryptosporidium* has been detected in African Buffalo, but its spatial and temporal distribution within the park remain unexamined. Consequently, any existing geographic hotspots and their associated environmental drivers have not been identified. No published literature exists on *Giardia* infection in KNP African buffalo despite documentation in other hosts across South Africa. This lack of information on host demographic predispositions, environmental reservoirs, and potential geographic hotspots limits our understanding of the epidemiology and spillover potential of these parasites in this species.

Methods: This study uses fecal samples from approximately 600 African buffalo captured in KNP in 2023 (cross-sectional), along with recaptured individuals sampled every six months for over two years (longitudinal) which will be analyzed using digital PCR (dPCR).

Results: Using cross-sectional data, we characterized basic epidemiological patterns of infection, including associations with age, sex, and host body condition. We assessed spatial patterns of *Cryptosporidium* and *Giardia,* identifying areas within KNP with the highest spillover risk to livestock and human populations. Using longitudinal data, we extended this analysis to evaluate temporal changes in these potential spillover areas and identify associated environmental drivers.

Conclusions: Ultimately, this work will inform targeted surveillance and management strategies to mitigate zoonotic risk in and around KNP.

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TRANSMISSION DYNAMICS, EPIDEMIOLOGY, AND GLOBAL HEALTH / ONE HEALTH APPROACHES TO ZOONOTIC PARASITIC DISEASES

PREVALENCE OF TOXOPLASMA GONDII AND NEOPSORA CANINUM ANTIBODIES IN DOMESTIC DOGS FROM SÃO JOSÉ DO RIO PRETO, NORTHWEST SÃO PAULO, BRAZIL

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Background and Aims: *Toxoplasma gondii* is a zoonotic intracellular protozoan capable of causing disease in humans and animals, whereas *Neospora caninum* is an important pathogen of animals, particularly dogs and cattle. Dogs are the definitive host for *N. caninum* and may act as sentinels of environmental exposure to *T. gondii*. Investigating canine exposure contributes to understanding parasite circulation and supporting prevention strategies. This study aimed to determine the seroprevalence of anti-*T. gondii* and anti-*N. caninum* antibodies in domestic dogs from a municipality in northwest São Paulo State, Brazil.

Methods: Peripheral blood samples were collected from 595 dogs at a municipal Zoonosis Control Center. Detection of anti-*T. gondii* antibodies was performed using the modified agglutination test (MAT) with a cut-off of 1:25. Anti-*N. caninum* antibodies were detected by indirect fluorescent antibody test (IFAT) with a cut-off of 1:50. Epidemiological data, including sex, habitat, and area of residence, were recorded when available.

Results: Anti-*T. gondii* and anti-*N. caninum* antibodies were detected in 113/595 (19.0%) and 20/595 (3.4%) dogs, respectively. Antibody titers ranged from 1:25 to 1:1600 (MAT) and 1:50 to 1:200 (IFAT). Co-infection was observed in 10 (1.7%) animals. Most seropositive dogs for both parasites were female, domiciled, and from urban areas.

Conclusions: These findings indicate substantial exposure to *T. gondii* and lower circulation of *N. caninum* in the studied canine population. The results support integrated surveillance strategies aligned with the One Health framework to guide prevention and control measures. **Acknowlegements:** CAPES; FAPESP; INCT-CNPq; FAPERP.

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TRANSMISSION DYNAMICS, EPIDEMIOLOGY, AND GLOBAL HEALTH / ONE HEALTH APPROACHES TO ZOONOTIC PARASITIC DISEASES

EMERGENCE OF AUTOCHTHONOUS LEISHMANIA INFANTUM INFECTION IN DOGS FROM COSTA RICA CONFIRMED BY MULTIMODAL DIAGNOSTICS: A CASE SERIES

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Background and Aims: Canine visceral leishmaniasis (CVL) has been reported in humans and dogs from Costa Rica over the past four decades as sporadic reports. In this study, we analyzed eight cases of autochthonous infections in dogs from Costa Rica, none of which had a history of travel abroad.

Methods: Eight dogs with suspected CVL were analyzed using serological assays. Histopathological staining was applied in cases with spleen, dermal, and lymph node involvement to determine the presence of Leishmania amastigotes. Blood, lymph node aspirates or cutaneous lesion swabs were also analyzed for the presence of Leishmania spp. ITS1, hsp70, and kDNA fragments. Phylogenetic and haplotype network analyses were conducted for hsp70 and kDNA data.

Results: Dogs showed clinical manifestations that included persistent anemia, thrombocytopenia, splenomegaly, dermatitis, and onychogryphosis, whereas the other four dogs remained subclinical. Histopathological analysis revealed numerous intracellular amastigotes in lymph node aspirates, spleen sections, and ear skin biopsy. Some dogs were positive in the serological analysis, and the others to the Leishmania ITS PCR. Phylogenetic analysis revealed that sequences derived from our country clustered with those from the Old World, rather than with ones from Brazil, indicating a likely introduction from outside the Americas.

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Conclusions: Infection with L. infantum in Costa Rican dogs was confirmed by clinical and laboratory evidence and thus represents the first autochthonous cases of CVL in our country. This study highlights the urgent need for routine canine testing and increased awareness, emphasizing the importance of public health policies for controlling leishmaniasis in both animals and humans from a One Health perspective.

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COMMUNITY RISK OF ENVIRONMENTAL-BORNE CYSTIC ECHINOCOCCOSIS TRANSMISSION IN SOUTH AMERICA: RESULTS FROM THE MULTISTEP CROSS-SECTIONAL AND CASE-CONTROL PERITAS STUDY

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Background and Aims: Cystic echinococcosis (CE) is mainly described as a food/waterborne zoonosis. However, evidence about matrices contamination is scarce. We evaluated *Echinococcus granulosus* contamination of matrices in two areas of Chile and Peru.

Methods: In stage 1, areas with high active CE prevalence were identified through cross-sectional ultrasound surveys. Stage 2 was a case-control study encompassing matrices sampling in public places and households with and without CE cases in these areas, followed by (stage 3), matrices processing by sequential sieving and *E. granulosus* detection by PCR. Bayesian multilevel mixed-effects logistic regression analysis was used to identify factors associated with risk of contamination.

Results: In households, soil (19%-42%); dogs' fur (10%-30%); shoes' soles (5%-33%); and dogs' feces (0-50%) were highly contaminated. In public areas, ~30% of fecal and soil samples were contaminated. Overall, matrices from public areas were more contaminated than those from households. When examining households, there was no difference in risk of contamination according to presence of CE cases, while CE-free households had lower risk when considering households and public areas. There was no difference in risk of contamination according to matrix. Vegetables were PCR-negative.

Conclusions: Conclusion: Results suggest the need for a paradigm-shift towards considering CE an environmental-borne infection with a "community risk" to which people are exposed. This work was funded by EU-LAC Health (EULAC/FONIS T020067) through the PERITAS project (*Molecular epidemiological studies on pathways of transmission and long-lasting capacity building to prevent cystic echinococcosis infection*) (EULACH16/T02-0067).

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SURVEILLANCE OF MOSQUITO POPULATIONS IN POTENTIAL WILDLAND REFUGIA AREAS ACROSS EASTERN NORTH AMERICA

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Background and Aims: Most concern for severe outbreaks of mosquito-borne diseases centers on urban areas with dense human populations and mosquito species that flourish in synanthropic settings. Rural areas populated by humans are also areas of concern, often with emphasis on non-urban mosquito species. Surveillance and control programs are often deployed in both areas. However, wildland areas with little or no human habitation are generally ignored in surveillance, while control measures are not deployed especially in protected wetlands and other wild habitats. Such wildlands may serve as refugia for mosquitoes that can transmit zoonotic pathogens among wildlife, and thus pose risks for spillover and spread to nearby human populations under appropriate circumstances.

Methods: Thus, we surveyed mosquitoes in three large wildland areas that are among the least populated areas of the eastern United States, yet close to urban centers: The Everglades of south Florida; Okefenokee Swamp of south Georgia; Adirondacks of northern New York state. Adults were collected with CDC mini light traps baited with CO2 and octenol and human landing catches with handheld vials. Larvae were collected with standard dipping cups and valved basters. All were identified microscopically using various taxonomic keys.

Results: Competent vectors of concern included *Aedes aegypti* in Florida; *Aedes albopictus* in Florida and Georgia; *Aedes japonicus* in New York. We collected various *Anopheles* spp., *Culex* spp., and *Aedes* spp. in all three regions, with particular species varying with latitude and associated climate.

Conclusions: Thus, we propose that these potential refugium areas warrant increased attention in executing broad public health surveillance programs.

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MOLECULAR TYPING OF CRYPTOSPORIDIUM ISOLATES FROM HUMANS, ANIMALS, AND RAW VEGETABLES IN CALI, COLOMBIA

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Background and Aims: *Cryptosporidium* is an enteric protozoan that causes diarrheal disease in humans and a wide range of animal hosts. Chronic and severe disease occurs in young and immunosuppressed individuals. However, *C. parvum* and *C. hominis* have been frequently reported in food- and waterborne outbreaks worldwide, affecting the general population. In a previous study, we found a high prevalence of *Cryptosporidium* (24%) among asymptomatic carriers and their pets. AIM: To understand the virulence potential and transmission patterns of the infection, we have characterized *Cryptosporidium* isolates from humans, pets, raw vegetables, and cattle in the region.

Methods: Positive samples from diverse sources were subjected to DNA extraction. Humans and pets were positive only by PCR, whereas cattle and vegetables were positive by both microscopy and PCR. *Cryptosporidium* species identification and genotyping were performed using nested PCR targeting the *18S rRNA* and the *gp60* genes, respectively. Sanger sequences were analyzed with Cryptogenotyper1.5. Phylogenetic trees (maximum- likelihood) and haplotype analysis were performed.

Results: Sixty-one isolates (61/84) were identified: 60 *C. parvum* and 1 *C. bovis*. Marked differences in genetic diversity among hosts and environmental sources were seen (pets> cattle=vegetables> humans). Fifteen (25%) *C. parvum* isolates were genotyped, including 10 from calves (IIaA17G2R1), 3 from humans (2 IIaA17G2R1, 1 IIaA16G2R1), and 2 from raw vegetables (1 IIaA17G2R1, 1 IIaA12R1).

Conclusions: *C. parvum* is the predominant species detected across all sources, highlighting the high risk of transmission. This is the first detection of gp60 subtype IIaA17G2R1 in Colombia and a novel IIaA12 variant, expanding the known diversity of *C. parvum*.

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THE ONE HEATH APPROACH IN LATIN AMERICAN EDUCATION: THE CASE OF THE FIRST REGIONAL COURSE FROM AUGM ON ANIMAL HEALTH BASED ON THE ONE HEALTH APPROACH.

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Background and Aims: This work presents the activities of an international committee in Latina America connected at AUGM, responsible for the promotion of the course of animal health based on the concepts of the One Health. AUGM allowed the stage for the scientific meeting. Different regional universities were represented, in themes of research interest like environment, education, extension, or animal health. Based on frequent meeting of university delegates, actions in education, research and extension are performed, and some supported by national or colaborative LA calls.

Methods: Observatiry participation was the method used. The aim is to present an initiative with the lens of a delegate participant, member of the academic committee of Animal Health. The course was offered virtually, and was divided in two blocks of 30 hours each. The first block focuses on zoonotic and neglected diseases. The second block covers environment, biosecurity, and outbreak epidemiology. Each 6-hour session was divided into a 3-hour lecture, followed by collaborative group work. Classes were presented in Spanish or in Portuguese, depending on the origin of the teacher. It was used to discuss case studies depending on the topic of each class.

Results: It was observed a strong interest and curiosity from young adults registered (MS and PhD level) from LA in the One-Health approach. However, virtual classes are not sufficient to stimulate student participation and true knowledge comprehension.

Conclusions: The One Health approach has to be incorporated In the general curricula and resources must be allocated to train future professionals with experiences in animal, human and environmental health.

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RE-EMERGENCE OF HUMAN DIROFILARIA REPENS INFECTION IN ITALY: A ONE-YEAR MULTICENTRIC CASE SERIES

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Background and Aims: Dirofilariases are vector-borne parasitoses caused by nematodes of the genus *Dirofilaria*, primarily infecting wild and domestic animals but occasionally humans. *Dirofilaria repens* represents the leading cause of human dirofilariasis in the Old World and is traditionally endemic in Italy. This study aims to describe a multicentric case series of human D. repens infections diagnosed within a one-year period and to highlight their epidemiological relevance.

Methods: Nine human cases diagnosed between August 2024 and September 2025 were collected from different Italian regions. Patients' ages ranged from 15 to 80 years. Clinical specimens included whole worms or tissue sections preserved in ethanol, saline solution, or paraffin. Morphological identification was performed by direct microscopy and/or histological examination. Genomic DNA was extracted from all samples, and species-specific end-point PCR targeting the IpS region of *D. repens* was performed. Mitochondrial COI and 12S rDNA markers were also amplified to investigate genetic variability.

Results: Cases showed heterogeneous anatomical localizations, including subconjunctival, subcorneal, subcutaneous, facial, scalp, and epididymal sites. Microscopic examination revealed morphological features consistent with *D. repens* in all evaluable specimens. All samples tested

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positive for D. repens by PCR. Amplification of mitochondrial markers was successful for all cases. Sequence analysis and phylogenetic reconstruction are currently ongoing.

Conclusions: The occurrence of multiple confirmed cases within a short time frame, together with additional sporadic reports from central-southern Italy during the same period, supports the hypothesis of a genuine re-emergence of *D. repens* infection in Italy. Increased clinical awareness and integrated diagnostic approaches are essential for timely recognition of human dirofilariasis.

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FIRST EVIDENCE OF LOCAL CIRCULATION OF PLASMODIUM OVALE CURTISI AND RELIABILITY OF A MALARIA RAPID DIAGNOSTIC TEST AMONG SYMPTOMATIC OUTPATIENTS IN DOUALA, CAMEROON

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Background and Aims: The performances of a commonly used Plasmodium falciparum-detecting rapid diagnostic test (RDT) were determined in symptomatic individuals living in Cameroon. Discrepancies between RDT and light microscopy (LM) results were further investigated, with a focus on non-falciparum malaria (NFM) which are still largely understudied in sub-Saharan Africa (sSA) countries.

Methods: In the present study, a total of 355 individuals aged 1–65 years were enrolled in the study. Their signs/symptoms and sociodemographic characteristics were documented. The RDT reliability was evaluated using LM as gold standard method. Polymerase chain reaction (PCR) of Plasmodium 18S gene was performed for samples with discordant results between LM and RDT (i.e., RDT− /LM+, and RDT+/LM-).

Results: The PCR amplicons of NFM species were sequenced and BLASTed. The prevalence of malaria infection by LM was 95.7% (95% CI: 93.1–97.4%). The sensitivity and specificity of RDT for P. falciparum detection was 94.0% and 66.7%, respectively. By PCR assay, P. ovale curtisi (PoC) was found in 5 of the 30 discordant samples, and on sequence analysis these isolates were found to be phylogenetically closer to sequences reported from China-Myanmar border and Malaysia. This is the first report on molecular characterization of P. ovale subspecies in Cameroon.

Conclusions: The study also outlines the good diagnostic performances of the RDT for detection of P. falciparum. Though, the presence of PoC indicated the importance of having RDTs targeting the NFM species in malaria diagnosis and treatment, which is presently limited in the country.

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TRANSMISSION DYNAMICS, EPIDEMIOLOGY, AND GLOBAL HEALTH / ONE HEALTH APPROACHES TO ZOONOTIC PARASITIC DISEASES

MOLECULAR EPIDEMIOLOGY OF THE ZOONOTIC CRYPTOSPORIDIUM PARVUM IN DAIRY CALVES IN FINLAND

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Background and Aims: In Finland, cryptosporidiosis cases caused by *Cryptosporidium parvum* in calves have increased over fivefold since 2012. As part of the research project "Cryptosporidiosis – an emerging zoonosis from cattle", we conducted the epidemiological study to identify *C. parvum* subtypes.

Methods: A total of 101 calf faecal samples from 101 Finnish dairy herds were analyzed, all confirmed as *C. parvum* positive by real-time PCR. Between 2017 and 2020, 76 samples were obtained from farms with clinical cryptosporidiosis. An additional 25 faecal samples were obtained from dairy farms without a recent calf diarrhoea outbreak (control farms) in 2020. The gp60 gene was sequenced to determine *C. parvum* subtypes.

Results: Nine gp60 subtypes were found: seven on farms with clinical cryptosporidiosis and eight on control farms. The *C. parvum* subtype IIaA15G2R1 was detected in 68% of samples from farms with clinical cryptosporidiosis and 40% of control farm samples, while subtype IIaA13G2R1 was found in 17% and 20%, respectively. The poster will provide a more detailed analysis of the regional distribution of subtypes in Finland.

Conclusions: The gp60 subtypes identified on dairy farms with clinical cryptosporidiosis were, for the most part, the same as those detected on the control farms. The most common subtype was IIaA15G2R1, followed by IIaA13G2R1. Based on previous reports of human cryptosporidiosis in Finland, humans and dairy calves share the most common *C. parvum* gp60 subtypes, suggesting an occupational health risk of cryptosporidiosis for farm workers and veterinarians. Preliminary results of this study were also presented at the CSBSP10 conference.

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FIRST MOLECULAR DESCRIPTION OF CRYPTOSPORIDIUM RYANAE AND C. BOVIS IN CHILEAN CATTLE.

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Background and Aims: *Cryptosporidium* spp. is a protozoan parasite that causes diarrhea and gastrointestinal disorders in approximately 150 mammalian species, including humans. This parasite includes several zoonotic species that affect both cattle and humans, among which *Cryptosporidium parvum* is particularly relevant due to its pathogenicity and zoonotic potential, and because it is currently the only species characterized in Chilean cattle.

Methods: In this study, we detected the presence of *Cryptosporidium* spp. in cattle using fecal samples for DNA amplification and a specific nested PCR to amplify an 820-nucleotide region of the SSU-rRNA gene of *Cryptosporidium* spp. Positive samples were sequenced for molecular identification using BLAST.

Results: *Cryptosporidium* spp. was detected in 18/85 (21.18%) analyzed samples. Molecular characterization identified three species: *Cryptosporidium bovis* in 10/18 positive samples, *Cryptosporidium ryanae* in 6/18, and *Cryptosporidium parvum* in 1/18. The detection of C. *ryanae* and C. *bovis* represents the first report of these species in Chile using molecular methods. While the presence of C. *parvum* and C. *bovis* highlights the zoonotic relevance of *Cryptosporidium* spp. in cattle.

Conclusions: The detection of zoonotic *Cryptosporidium* species in cattle highlights the potential role of livestock as a source of environmental contamination and transmission to humans of this protozoan. The first report of *Cryptosporidium ryanae* in Chile reinforces the importance of conducting further studies to better understand the distribution, epidemiology, and zoonotic potential of *Cryptosporidium* species

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TRANSMISSION DYNAMICS, EPIDEMIOLOGY, AND GLOBAL HEALTH / ONE HEALTH APPROACHES TO ZOONOTIC PARASITIC DISEASES

A NATIONWIDE MULTICENTRIC SURVEY OF CANINE FECAL CONTAMINATION IN PUBLIC GREEN AREAS: SEASONAL AND ENVIRONMENTAL DRIVERS OF ZOONOTIC PARASITE EXPOSURE IN ITALY

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Background and Aims: Canine gastrointestinal parasites, including several zoonotic helminths and protozoa, contribute to environmental contamination of public spaces and represent an underrecognized One Health concern. This study aimed to quantify faecal contamination by canine parasites in urban green areas across Italy and to investigate seasonal, geographical and environmental factors potentially influencing transmission risk.

Methods: A 12-month prospective survey (January–December 2024) was conducted through a coordinated network of 11 veterinary academic units distributed nationwide. Each unit sampled five dog-frequented green areas once per month, collecting five fresh fecal samples per site. Overall, 3300 samples were analyzed using the Mini-FLOTAC copromicroscopic technique. Environmental and management variables were recorded, including temperature, humidity, sun exposure, ground cover (soil *vs* grass), and availability of faeces disposal facilities. Results were stratified by month and by macro-area (northern, central and southern Italy).

Results: In total, 262/3300 samples tested positive (7.9%). Helminths predominated, with zoonotic species representing the largest proportion; *Toxocara canis* was the most frequently detected parasite (76/262), followed by hookworms and helminths, plus protozoa and occasionally cestodes. Positive samples were detected throughout the year, with temporal fluctuations and higher contamination during cooler months.

Conclusions: This large-scale longitudinal study demonstrates that canine faecal contamination of public green areas is widespread and persistent throughout Italy. The consistent presence of zoonotic parasites highlights the need for continuous surveillance, responsible faeces removal and effective parasite control programs to reduce environmental exposure and protect both animal and public health.

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DEVELOPMENT OF AN EARLY WARNING SYSTEM FOR CANINE LEISHMANIASIS IN THE IBERIAN PENINSULA: A CASE STUDY FROM THE PLANET4HEALTH PROJECT

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Background and Aims: Leishmaniasis, caused by *Leishmania infantum* and transmitted by phlebotomine sand flies, remains endemic in the Mediterranean region, where dogs serve as primary hosts and reservoirs for human infection. In Iberia, canine seroprevalence varies widely, reflecting heterogeneous transmission dynamics. Climate change, environmental alterations, and globalization are expected to expand vector distribution and increase transmission potential. This study aims to co-develop, with veterinarians, dog owners, and other stakeholders, an Early Warning System (EWS) for canine leishmaniasis (CanL) to support early detection and prevention strategies.

Methods: The EWS integrates high-resolution hydrometeorological (temperature, humidity, precipitation, soil moisture), environmental (land variables), entomological predictors, and CanL data using statistical and machine learning models to identify conditions favourable for sand fly activity, seasonality, and parasite transmission. These datasets are combined to generate spatially explicit risk maps and short-term forecasts and projections, highlighting locations and time periods with elevated transmission risk.

Results: The first operational prototype integrates near real-time climatic and epidemiological data into dynamic risk products that enhance spatial and temporal understanding of CanL risk across the Iberian Peninsula, serving as a proof of concept for a scalable, data-driven EWS.

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Conclusions: Ongoing validation with stakeholders will assess predictive performance, refine functionality, and strengthen usability. Supported by a collaborative One Health approach, this work advances integrated disease monitoring and proactive prevention of leishmaniasis in animals and humans. Acknowledgements PLANET4HEALTH consortium is funded by the European Commission (101136652). The five Horizon Europe projects, GO GREEN NEXT, MOSAIC, PLANET4HEALTH, SPRINGS, and TULIP, form the Planetary Health Cluster.

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POSTER ON BOARD SHIFT 01
TRANSMISSION DYNAMICS, EPIDEMIOLOGY, AND GLOBAL HEALTH / ONE HEALTH APPROACHES TO ZOONOTIC PARASITIC DISEASES

HIGH PREVALENCE OF FASCIOLA SP. AND SEASONAL PARASITE DIVERSITY IN URBAN SIKA DEER REVEALED BY FECAL PCR AND METABARCODING

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Background and Aims: Fascioliasis is a zoonotic disease of increasing concern within the One Health framework. However, its prevalence in urban wildlife remains unclear. This study aimed to determine the prevalence of *Fasciola* sp. and characterize fecal parasite diversity in wild sika deer in Nara Park, Japan.

Methods: Fecal samples (n = 100) were collected at four time points between November 2023 and October 2024. *Fasciola* infection was assessed using PCR on unpurified stool samples. Microscopic examination was performed for comparison in a subset of samples (n = 50). To profile parasite diversity, 18S rDNA amplicon sequencing (metabarcoding) was conducted.

Results: *Fasciola* DNA was detected in 97% of samples using PCR on unpurified stool samples. In a subset of samples (n = 50), microscopy detected *Fasciola* eggs in 72%, whereas PCR detected 94%. Metabarcoding revealed diverse parasitic taxa, including trematodes and apicomplexans, with seasonal variation in community composition.

Conclusions: *Fasciola* sp. is highly prevalent in wild sika deer in Nara Park. These findings highlight the importance of urban wildlife as reservoirs of zoonotic parasites and support the need for continued surveillance in human–wildlife interactions.

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SUSTAINMENT STATUS OF COMMUNITY-BASED HEALTH EDUCATION DELIVERY TO CONTROL TAENIA SOLIUM INFECTION IN SELECTED DISTRICTS OF TANZANIA

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Background and Aims: Despite the proven effectiveness of health education intervention (HEI) in addressing *Taenia solium* infections, its sustained delivery remains a challenge. This study was conducted to determine the sustainment status of HEI delivery in selected districts of Tanzania.

Methods: A cross-sectional study was conducted from December 2024 to June 2025 in Kongwa, Mbulu, Mbinga, and Songwe districts, using the Sustainment Measurement System Scale (SMSS), which comprises a global sustainment outcome (GSO) and seven sustainment determinants (SDs). Composite scores (CS) of the GSO and SDs were used to determine the extent and determinants of HEI delivery sustainment, respectively. Internal consistency of SMSS subscales was assessed using composite reliability (CR). Based on the class interval the CS falls into, the GSO and SDs were considered: very low (1.00 - 1.80), low (1.81 - 2.61), moderate (2.62 - 3.42), high (3.43 - 4.23), or very high (4.24 - 5.04).

Results: In total, 485 respondents were interviewed. The CR was 0.742 for GSO, while ranging from 0.616 to 0.885 for SDs. The GSO for HEI delivery was low (CS = 2.58), with differences across districts, $F(3,142) = 22.998$, $p < 0.001$. Responsiveness to community needs and values were the two SDs with high CS (4.08 and 4.06, respectively), whereas financial support scored the least (1.19). Further, SDs were weakly to moderately correlated with GSO ($r = 0.17$ to 0.47).

Conclusions: Sustainment level of HEI delivery was low. A One Health approach should be adopted to improve resource efficiency and strengthen SDs to support long-term delivery of HEI.

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MOLECULAR EVIDENCE FOR HUMAN TRICHURIS TRICHIURA ACROSS MULTIPLE RODENT GENERA IN PAPUA NEW GUINEA

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Background and Aims: *Trichuris trichiura* is a globally important human whipworm with substantial public health impact, yet its circulation in wildlife reservoirs remains poorly understood. *In Papua New Guinea, ancient endemic rodents coexist with recently introduced species, creating opportunities for cross-species transmission at the human–wildlife interface.* Here, we investigated *Trichuris* diversity in rodents using an integrative morphological and genomic approach to assess host range and potential zoonotic involvement.

Methods: A total of 874 rodents and marsupials were examined by parasitological dissections in Papua New Guinea. *Trichuris* specimens were analysed using morphology and skim sequencing to resolve species-level diversity and host associations

Results: Integrative analyses revealed three distinct *Trichuris* lineages. Two were rodent-associated and detected in *Rattus* and *Hydromys*. The third lineage corresponded to the human parasite *Trichuris trichiura* and was detected across multiple phylogenetically distinct rodent genera, including *Pogonomys*, *Mallomys*, *Uromys* and *Hyomys*. Overall *Trichuris* prevalence was 1.8%, with no infections detected in marsupials. Morphological analyses were consistent with the genetic delineation of the three lineages.

Conclusions: Our findings show *Trichuris* diversity in Papua New Guinean rodents and provide molecular evidence for the circulation of human *T. trichiura* across multiple rodent genera. These results indicate that wild rodents may contribute to parasite maintenance in the environment and raise important questions about their potential role in shaping human transmission dynamics. This

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highlights a previously underappreciated wildlife component in human trichuriasis and the need for longitudinal surveillance to assess impacts on human infection risk.

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VIRAL AND PARASITIC PATHOGENS IN URBAN RATS ACROSS EUROPE: A SYSTEMATIC REVIEW FROM A ONE HEALTH PERSPECTIVE

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Background and Aims: Commensal rats (*Rattus norvegicus* and *Rattus rattus*) are common in European cities, where their close proximity to humans and ability to harbour zoonotic pathogens make them relevant indicators of urban environmental health. Despite increasing interest in their role within One Health frameworks, the diversity, distribution and epidemiological relevance of viral and parasitic infections in European rat populations remain insufficiently characterised.

Methods: This systematic review was conducted according to PRISMA 2020 guidelines. Inclusion and exclusion criteria were applied to identify studies focused on urban rats and associated viral and parasitic pathogens. Literature searches were performed using PubMed (MEDLINE), Embase, Web of Science Core Collection, Scopus and WILDbase to ensure broad coverage of relevant publications.

Results: Numerous studies reported zoonotic parasites, including *Calodium hepaticum* and *Hymenolepis nana*, as well as viruses such as Hepatitis E virus and Seoul virus in urban rat populations. Prevalence varied widely across Europe, with notable differences between countries and cities. Evidence was unevenly distributed geographically, highlighting substantial gaps in surveillance and limited data from many regions.

Conclusions: Across Europe, *R. norvegicus* and *R. rattus* harbour a range of viral and parasitic agents of known or suspected zoonotic relevance. Research efforts remain geographically biased and methodologically heterogeneous, often based on short-term, single-pathogen studies, limiting comparability and interpretation of absence data. Integrating routine rat surveillance into European One Health systems, alongside environmental, wastewater and clinical monitoring, could improve early detection of public health threats and support targeted interventions.

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INTEGRATED MAPPING OF TAENIA SOLIUM AND CO-ENDEMIC NTDS TO INFORM CONTROL AND SAFE MASS DRUG ADMINISTRATION IN AFRICA

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Background and Aims: Background and aims: *Taenia solium* taeniasis and cysticercosis remain neglected zoonotic infections in Africa, contributing significantly to epilepsy and posing rare but serious risks during praziquantel mass drug administration (MDA) for schistosomiasis. This study synthesizes multi-country evidence to map endemicity and inform integrated control strategies.

Methods: Cross-sectional and integrated surveys were conducted in Tanzania, Cameroon, and Namibia using World Health Organization (WHO) mapping tools. Data sources included desk reviews, field surveys, stool microscopy, molecular diagnostics, pig tongue inspection, and risk factor assessments. In Namibia, surveys were integrated with schistosomiasis, guinea worm tracing and scabies assessments.

Results: In Tanzania, *T. solium* transmission risk was reported in 36.4% of districts, with co-endemicity in humans and pigs linked to free-roaming pig systems and poor sanitation. In Cameroon, porcine cysticercosis prevalence reached 10.39% by tongue palpation, while human taeniasis prevalence was 0.58%, approaching intervention thresholds. In Namibia, *T. solium* was detected in four of five surveyed regions, with one region exceeding the 0.5% threshold for public health action. Integrated surveys highlighted widespread risk factors, including open defecation and limited awareness, and demonstrated operational feasibility of combined NTD surveillance.

Conclusions: *T. solium* remains under-recognized yet widely distributed across diverse African settings. Integrated mapping and surveillance are critical to guide targeted interventions, mitigate MDA-related adverse events, and strengthen One Health approaches. Embedding *T. solium* within NTD platforms enhances efficiency and supports safe and sustainable control strategies.

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MOLECULAR SURVEILLANCE IN URBAN PARKS ACROSS AUSTRALASIA REVEALS WIDESPREAD CANINE SOIL-TRANSMITTED HELMINTHS BUT NO EVIDENCE OF LUNGWORMS

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Background and Aims: Canine helminths include environmentally transmitted intestinal parasites and respiratory lungworms, yet their regional distributions in Australasia remain poorly defined. We performed large-scale molecular surveillance to assess environmental contamination with cSTHs and the presence of canine lungworms in urban parks.

Methods: In New Zealand, 739 canine faecal samples from 123 urban parks were screened for cSTHs using multiplex real-time PCR with sequencing confirmation. In Australia, 1581 canine faecal samples from 190 parks were collected and analysed using faecal floatation and multiplex real-time PCR. All 2,320 samples from both countries were additionally tested for *Angiostrongylus vasorum*, *Crenosoma vulpis*, and *Eucoleus* spp. using a validated multiplex qPCR assay.

Results: In New Zealand, 24.4% of parks were positive for at least one cSTH species, most commonly *Ancylostoma caninum* and *Uncinaria stenocephala*. Contamination was associated with higher dog density, closer proximity to city centres, and bioclimatic zone. In Australia, 44.2% of parks were contaminated with at least one species of cSTH. In contrast, lungworm DNA was not detected in any of the 2,320 samples.

Conclusions: Canine STHs represent some of the most prevalent dog parasites across Australasia, whereas canine lungworms appear absent or occur at extremely low prevalence. These findings provide an important regional baseline and support targeted surveillance for canine parasites. Controlling these parasites comprehensively will be essential to protecting companion animal welfare and ensuring the health and safety of shared urban spaces from a One Health perspective.

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WHAT LIES BENEATH: A ONE HEALTH INVESTIGATION OF INTESTINAL PROTOZOAN CONTAMINATION IN LAKE ECOSYSTEMS: THE CASE OF THE SEVEN LAKES OF SAN PABLO, LAGUNA, PHILIPPINES

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Background and Aims: Lake ecosystems sustain food sources, recreational activities, and community livelihoods, yet they remain vulnerable to contamination from enteric protozoans such as Cryptosporidium spp. and Giardia spp. These pathogens pose significant public health risks, particularly in areas where human, animal, and environmental interfaces overlap. This work has investigated the prevalence and potential sources of these enteric protozoans in lake waters and tributaries, and surrounding animal populations in the Seven Lakes of San Pablo.

Methods: Fecal samples were collected from companion animals, livestock, and poultry within a 200-meter radius of the lakes, concurrent with 10-L water samples from lake bodies and tributaries.

Results: Using immunofluorescence assay (IFA), initial findings revealed high prevalence rates of Cryptosporidium and Giardia across multiple hosts. For Cryptosporidium, infection rates reached 63.0% (dogs) and 71.0% (cats), 78.2% in poultry, and 50.0% in livestock. Giardia was similarly widespread, detected at 18.3%, 63.3% and 60.0%, respectively. Water samples, specifically in Sampaloc Lake, likewise showed contamination, with Cryptosporidium in over 70.0% of the sites and Giardia at 40.0%. Semi-nested PCR targeting the 18S rRNA of Cryptosporidium and ß-giardin gene of Giardia further confirmed the presence of these protozoans in fecal and environmental samples. Knowledge, Attitude, and Practices surveys among households highlighted gaps between awareness and hygiene behaviours among animal owners and lake communities, indicating that anthropogenic activity, and poor sanitation practices exacerbate contamination risks.

Conclusions: These findings emphasize the need for coordinated efforts on surveillance, source tracking, and community education within a One Health framework.

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UNRAVELLING THE ROLE OF LIVESTOCK AND RODENT IN SCHISTOSOMA INFECTIONS IN SOUTHERN BENIN: IS UROGENITAL SCHISTOSOMIASIS A ZOONOTIC DISEASE ?

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Background and Aims: Zoonotic diseases are challenging to control because untreated animal reservoirs sustain transmission. In southern Benin, rodents and livestock have been implicated in the circulation of *Schistosoma haematobium*, *S. bovis*, and their hybrids. This study aimed to assess the contribution of livestock and rodents to the local transmission of urogenital schistosomiasis in southern Benin, and to evaluate its zoonotic potential.

Methods: Urine samples from 117 humans and faeces from 44 cattle were collected in Késsounou and examined for *Schistosoma* infection. Ninety-six rodents were captured and examined for schistosome after perfusion and dissection. Molluscs vectors were collected monthly and tested for cercarial shedding. Miracidia and cercariae were preserved on FTA cards and used for RADseq library construction. Experimental infections of naïve *Mastomys natalensis* F1 and cattle are ongoing to assess interspecific variation in transmission competence.

Results: Overall, 23.9% of humans were positive, with higher prevalence in men ($p = 0.041$) and adults ($p < 0.00001$). Infection prevalence in cattle was 38.6%. No infections were detected in rodents (*M. natalensis*, *R. rattus*, *C. olivieri*). A total of 798 *Bulinus forskalii* and 57 *B. globosus* were collected. Mollusc abundance varied by month and season ($p < 0.001$), but infection prevalence remained low. Genomic analyses and experimental infections are ongoing.

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Conclusions: These preliminary results suggest a limited or highly seasonal role of rodents in transmission, likely due to rapid population turnover and short lifespan compared with humans and cattle. Integrating genomic and experimental data into mathematical models will help quantify reservoir contributions, zoonotic potential, and guide control strategies.

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TRANSMISSION DYNAMICS, EPIDEMIOLOGY, AND GLOBAL HEALTH / ONE HEALTH APPROACHES TO ZOONOTIC PARASITIC DISEASES

NINE YEARS OF MONITORING CRYPTOSPORIDIUM OCCURRENCE IN WILDLIFE IN BRANDENBURG GERMANY: CLIMATIC AND ANTHROPOGENIC FACTORS WITH ONE HEALTH IMPLICATIONS

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Background and Aims: Understanding *Cryptosporidium* dynamics in wildlife and their contribution to zoonotic transmission at wildlife–human–livestock interfaces requires long-term data, yet such data remain scarce. Based on monitoring data from Brandenburg, Germany, this study aims to (i) quantify long-term occurrence and temporal patterns of *Cryptosporidium* in wildlife; (ii) assess ecological and anthropogenic factors associated with parasite occurrence; and (iii) inform One Health risk assessment, including comparative spatial insights from peri-urban and urban subsets in Berlin.

Methods: Analyses are based on molecular detection results from approximately 1,600 fecal samples of free-ranging ungulates (roe deer, fallow deer, red deer, wild boar) collected over nine hunting seasons (2017–2026) in predominantly rural study areas in Brandenburg. Detection relied on nested PCR (18S rRNA, COWP) and sequencing. A stepwise exploratory statistical approach combined descriptive and bivariate analyses with multivariable logistic regression to evaluate host-related, climatic, and anthropogenic factors associated with parasite occurrence. Additional peri-urban (Berlin-Grunewald; wild boar and roe deer) and urban (Berlin; brown rat) subsets enabled spatial comparison.

Results: *Cryptosporidium* spp. were detected across host species and study periods, including taxa with zoonotic potential. Marked interannual variation was observed in the long-term dataset. Preliminary analyses indicate associations with selected climatic and host-related variables; multivariable analyses incorporating anthropogenic factors and spatial comparisons of the subsets are ongoing.

Conclusions: The findings underscore the dynamic and multifactorial ecology of *Cryptosporidium* in wildlife and emphasize the value of long-term wildlife surveillance that accounts for multiple influencing factors in integrated One Health risk assessment.

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TRANSFER OF ANISAKID ALLERGENS THROUGH FEED INTO FOOD: UNCOVERING PREVALENCE IN FISHMEAL AND AQUACULTURE PRODUCTS

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Background and Aims: Anisakid nematodes are a family of fishborne zoonotic parasites which have a global distribution in marine environments. Beyond the risk of anisakidosis due to infection with live larvae following consumption of raw or undercooked seafood, they also possess thermostable allergens which can elicit allergic reactions. Recent evidence suggests that these allergens can be transmitted through contaminated fishmeal feed into aquaculture fish and even livestock. This study investigates the prevalence of Anisakid proteins within the fishmeal supply chain and their subsequent transfer to aquaculture products.

Methods: To investigate allergen transfer, commercial fishmeal samples, as well as 384 seafood products were sampled from 8 different retail outlets over the course of 2025. This survey included 240 aquaculture products characterized by a high fishmeal inclusion rate in their diet. Protein extracts are made by homogenizing tissue in 5% SDS lysis buffer. A high-sensitivity proteomics method using parallel reaction monitoring (PRM) is currently being optimized to screen these products for the presence of Anisakid allergens.

Results: Preliminary proteomic analysis of commercial fishmeal samples has confirmed the presence of Anisakid-derived proteins such as Ani s 3, validating the risk of allergen entry into the feed chain. Ongoing PRM analysis of the 384 seafood samples will determine the extent of allergen carry-over into edible tissues.

Conclusions: These results will provide critical data for food safety risk assessments, as it could highlight a new route of allergen exposure. This will be relevant for the increasing amount of *Anisakis* sensitized individuals.

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CHIGGER MITE INDICATOR DESIGN AND SCRUB TYPHUS NOTIFICATIONS: EVIDENCE FROM NINE YEARS OF HABITAT BASED SURVEILLANCE IN YESAN, REPUBLIC OF KOREA

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Background and Aims: To evaluate whether species composition-based chigger mite indicators provide more stable associations with human scrub typhus notifications than total abundance during long-term surveillance.

Methods: Weekly chigger mite collections were conducted in four habitat types in Yesan, Republic of Korea, during weeks 36 to 51 from 2017 to 2025 and aggregated to monthly indicators. Four indicators were defined: total abundance, a literature-based key vector indicator comprising eight infection-associated taxa, an extended indicator adding the locally dominant taxon, and the dominant taxon alone. Associations with monthly scrub typhus notifications were assessed using negative binomial regression with month fixed effects and a linear year trend.

Results: A total of 3,672 chigger mites representing 13 species were collected. *Neotrombicula tamiyai* accounted for 51.9% of specimens. The key vector indicator showed a stronger association with notifications (incidence rate ratio [IRR] 1.34 per doubling) than total abundance (IRR 1.28). The dominant taxon alone was not associated (IRR 1.09).

Conclusions: Indicators incorporating species composition can improve interpretability of routine surveillance when pathogen detection is limited and may support more robust risk interpretation across years.

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TRANSMISSION DYNAMICS, EPIDEMIOLOGY, AND GLOBAL HEALTH / ONE HEALTH APPROACHES TO ZOONOTIC PARASITIC DISEASES

FACTORS RELATED TO KNOWLEDGE, MOTIVATION AND CONFIDENCE OF TEACHING PREVENTIVE EDUCATION ON OPISTHORCHIS VIVERRINI AMONG TEACHERS IN ENDEMIC AREA IN LAO PDR.

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Background and Aims: Opisthorchis viverrini (OV) is a foodborne trematode endemic in the Lao PDR. Infection is caused by the consumption of raw fish, a dietary habit often established in childhood. School-based preventive education is therefore a sustainable strategy for OV control, with teachers playing a critical role. However, opportunities for teacher training remain limited, and teachers' knowledge, motivation, and confidence regarding OV prevention have not been fully examined. This study aimed to identify factors influencing teachers' knowledge, motivation and confidence in providing OV preventive education.

Methods: A cross-sectional survey was conducted among 515 teachers attending in-service teacher upgrading programs at Savannakhet Teacher Training College in August 2025. A self-administered questionnaire assessed OV knowledge, perceptions, learning experiences, preventive behaviors, information sources, and attitudes toward OV preventive education. Ordinal logistic regression analysis was used to identify factors associated with teaching confidence. The dependent variable was measured on a 5-point Likert scale.

Results: The mean age was 35.8 ± 5.7 years, and 16.0% had received OV preventive training. Although 72.7% correctly understood the OV life cycle, only 35.4% strongly intended to teach OV prevention. Higher teaching confidence was significantly associated with prior OV training (OR = 0.29, 95% CI:

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0.18–0.46), belief in OV preventability (OR = 1.58, 95% CI: 1.36–1.85), recognition of difficulty in changing childhood raw fish–eating habits (OR = 1.23, 95% CI: 1.04–1.45), and higher OV knowledge (OR = 1.14, 95% CI: 1.07–1.20).

Conclusions: Strengthening teachers' knowledge, awareness of early-life prevention, and access to structured training is essential to enhance confidence in OV preventive education.

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TRANSMISSION DYNAMICS, EPIDEMIOLOGY, AND GLOBAL HEALTH / ONE HEALTH APPROACHES TO ZOONOTIC PARASITIC DISEASES

WHO ESTIMATES OF THE GLOBAL, REGIONAL, AND NATIONAL FOODBORNE BURDENS OF 14 INVASIVE PARASITIC DISEASES FROM 2000 TO 2021: AN UPDATED DATA SYNTHESIS

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Background and Aims: Over 10 years ago, WHO estimates of hazard-specific foodborne disease burdens showed that parasites exert considerable health burdens globally. We updated these estimates, focusing on 14 invasive parasitic diseases.

Methods: Incidences, deaths, and disability-adjusted life year (DALY) burdens were estimated for each parasitic disease from 2000 to 2021, using data from systematic reviews and the Global Burden of Diseases, Injuries, and Risk Factors Study. For some diseases, structured expert judgment was used to estimate proportions of foodborne infection. Data were pooled via hierarchical meta-regression models with uncertainty propagated through Monte Carlo simulations following disease-specific computational models defined by incidence rates and probability parameters.

Results: We estimated there were several hundred million illnesses caused by potentially foodborne invasive parasites, with approximately 64% million attributable to foodborne transmission. Considerable heterogeneity by parasite, in magnitude and uncertainty, was observed with these diseases. The highest contributions were from *Taenia solium* and *Clonorchis sinensis*, both also associated with most foodborne deaths. Burden was greatest in the region of the Americas,

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predominantly due to Chagas disease, followed by the African region, where neurocysticercosis-associated epilepsy caused most burden. Burdens decreased globally from 2000 to 2021, except in the Western Pacific region, where the burden, predominantly associated with clonorchiasis, is rising.

Conclusions: Foodborne parasitoses cause considerable suffering, with some populations and regions particularly at risk. These data provide a baseline by which effects of interventions can be assessed and emphasis directed to those parasites exerting the greatest burden.

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TRANSMISSION DYNAMICS, EPIDEMIOLOGY, AND GLOBAL HEALTH / ONE HEALTH APPROACHES TO ZOONOTIC PARASITIC DISEASES

CIRCULATION OF DIROFILARIA IMMITIS AND DIROFILARIA REPENS IN MOSQUITOES AND BLOOD MEAL HOST IDENTIFICATION: AN INTEGRATED APPROACH TO BETTER DEFINE DIROFILARIOSIS EPIDEMIOLOGY IN ENDEMIC AREAS

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Background and Aims: Dirofilariosis is a mosquito-borne disease expanding throughout Europe, including previously unaffected regions. Its transmission relies on competent mosquito vectors, favourable climatic conditions and larval development. Dogs are the definitive hosts, with cats and wild canids playing minor roles. Identifying blood-meal sources of infected mosquitoes may reveal possible additional reservoirs.

Methods: Mosquitoes were collected in 2025 by the Istituto Zooprofilattico (IZSLER) within the regional West Nile Virus (WNV) surveillance program in peri-urban and rural areas and by MF in urban areas near Parma. Specimens were trapped using CDC-CO_2 traps in August, identified morphologically, pooled and analyzed for *Dirofilaria immitis*, *D. repens* and WNV presence by Real-time PCR. For blood-meal source identification, ribosomal and mitochondrial gene fragments were amplified by PCR, Sanger sequenced and compared with GenBank entries.

Results: From IZSLER captures, 4/77 and 28/77 pools were positive for *D. immitis* and for *D. repens*, respectively. On Parma targeted captures, 11/80 pools were positive for *D. immitis* and 5/80 for *D. repens*. None was positive for WNV, while four were positive for Flavivirus. *Aedes caspius* was the species most frequently positive for the presence of both parasites, followed by *Ae. albopictus* especially in Ferrara and Parma provinces. Forty-eight individual mosquitoes were selected from traps positive for *D. immitis* or *D. repens*, based on clear evidence of recent blood meals observed microscopically. Blood-meal analysis showed *Homo sapiens* as the predominant host, followed by anthropophilic birds, domestic ruminants, and medium-sized mammals.

Conclusions: This approach offers a picture of the distribution of two parasites of public health concern.

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TRANSMISSION DYNAMICS, EPIDEMIOLOGY, AND GLOBAL HEALTH / ONE HEALTH APPROACHES TO ZOONOTIC PARASITIC DISEASES

A CASE REPORT OF RARE ORBITAL HYDATID CYST MASQUERADING AS UNILATERAL EXOPHTHALMOS

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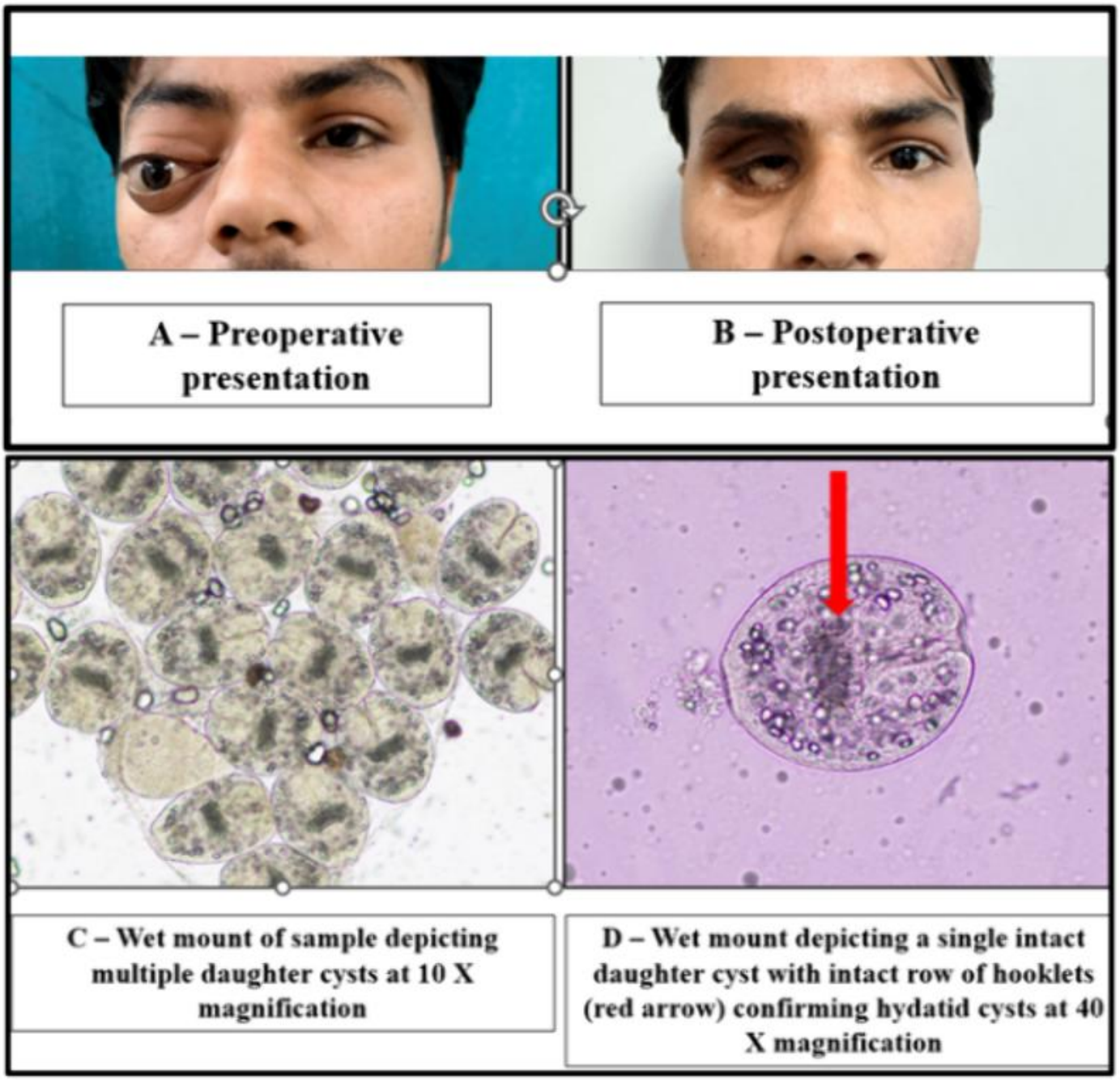
Background and Aims: Hydatid disease is a zoonotic infection caused by the larval stage of the tapeworm *Echinococcus granulosus*. It is endemic in the Indian subcontinent and prevalent in societies with high contact with livestock. Life cycle of *E. granulosus* involves dogs as the definitive hosts and sheep or cattle as intermediate hosts, with Humans being accidental hosts. The liver and lungs are the most commonly affected organs. Hydatid cysts of the central nervous system are rare, and orbital localisation is very uncommon.

Methods: An 18-year-old male presented with a painless protrusion of the right eye for 1.5 years. The onset was insidious, with gradual progression over the initial six months, followed by static phase for nearly one year. Visual acuity was 6/18 in the right eye, improving to 6/12 with pinhole. Right eye movements were restricted in the superior and medial directions. The right eye showed severe, non-pulsatile proptosis without bruit. MRI revealed a well-defined, medial extraconal orbital cystic lesion, T1 hypointense/T2 hyperintense, displacing the optic nerve and extraocular muscles, suggestive of an infective cyst.

Results: The lesion was completely excised via an eyelid approach with hypertonic saline irrigation. The excised cyst was sent for parasitological examination. Wet mount of the sample revealed multiple

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daughter cysts and numerous hooklets, confirming it to be a hydatid cyst.



Conclusions: Postoperatively, proptosis was reduced, vision was preserved, and ocular motility improved. But the patient is currently suffering from diplopia due to intraorbital space loss, which is planned to be managed in future in a staged reconstruction

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TRANSMISSION DYNAMICS, EPIDEMIOLOGY, AND GLOBAL HEALTH / ONE HEALTH APPROACHES TO ZOONOTIC PARASITIC DISEASES

RISK OF HUMAN EXPOSURE TO TICKS (ACARI: IXODIDA) AND TICK-BORNE PATHOGENS ALONG TOURIST TRAILS IN MAGURA NATIONAL PARK (SOUTH-EASTERN POLAND)

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Background and Aims: Ticks (Acari: Ixodida) are vectors and/or reservoirs of numerous pathogens of medical and epidemiological importance. In Europe, including Poland, the most common tick species is *Ixodes ricinus*, which is typically found in deciduous and mixed forests. Magura National Park (MNP) is a frequently visited tourist destination that provides favourable environmental conditions for the maintenance of tick populations. The aim of this study was to assess the potential risk of human exposure to ticks and tick-borne infections with selected pathogens along tourist trails within MNP.

Methods: Ticks were collected using the flagging method during the spring peak of their activity from four tourist trails in MNP. Specimens were identified to species and developmental stage. Genetic material (DNA/RNA) was isolated from individual ticks using a column method. For the detection of TBEV, *Borrelia burgdorferi* sensu lato, *Anaplasma phagocytophilum*, and *Babesia* spp., primers specific for the 5'UTR, 16S rRNA, and 18S rRNA were used, respectively.

Results: A total of 247 *I. ricinus* specimens and a single male *Dermacentor reticulatus* were collected. Molecular analyses for the presence of pathogens were conducted on 100 randomly selected ticks. *B. burgdorferi* s.l. was detected in 13 individuals, while *A. phagocytophilum* was identified in 5 ticks. *Babesia* spp. and TBEV were not detected in the examined material.

Conclusions: The results indicate a high potential risk of human exposure to ticks, a moderate potential risk of tick-borne infection with *B. burgdorferi* s.l., and a low risk of infection with *A. phagocytophilum* along the studied tourist trails in MNP.

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TRANSMISSION DYNAMICS, EPIDEMIOLOGY, AND GLOBAL HEALTH / WATER- AND FOOD-BORNE PARASITIC INFECTIONS

SPATIO-TEMPORAL ANALYSIS OF THE DISTRIBUTION OF WATERBORNE DISEASES IN CHILDREN UNDER 5 FROM 2018 TO 2022 IN KINSHASA, DR CONGO

Aymar Akilimali
Medical research circle, Department Of Research, Goma, Congo, Republic of

Background and Aims: The city of Kinshasa faces the problem of access to drinking water and sanitation; its municipalities and health zones are exposed to a proliferation of waterborne diseases, a problem for the public health of the population. This study aims to carry out a spatial and temporary analysis of the distribution of waterborne diseases

Methods: We carried out an environmental investigation followed by the collection of data that were processed by tools of the geographic and statistical information system using Pearson correlation to see the link between these diseases in space and time.

Results: The distribution of Malaria affects more intermediate zones, including Mbanza-Lemba with 9044 cases and an average of 1809 cases per year in the period studied; the same case is true for Typhoid Fever and Diarrhea which affect the flood zone including Gombele with 12420 cases with an average of 2484 cases per year of Typhoid Fever and 4931 cases for Diarrhea. The Salongo health area has the most recorded cases of Amoeba, including 2192, with an average of 438 per year. Malaria has a strong correlation with Diarrhea, which is 0.99, these two diseases have a strong to medium correlation with Amoeba. A negative correlation is observed with Typhoid Fever.

Conclusions: The distribution of waterborne diseases in space and time is linked to physical factors such as altitude and slope, creating flood zones likely to increase the spread of these diseases. The problem of access to drinking water and problem of sanitation are other factors facilitating the spread of these diseases

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TRANSMISSION DYNAMICS, EPIDEMIOLOGY, AND GLOBAL HEALTH / WATER- AND FOOD-BORNE PARASITIC INFECTIONS

COMMUNITY PREFERRED INTERVENTIONS TO MINIMIZING RISKS TO INFECTION WITH SCHISTOSOMIASIS IN MAYUGE DISTRICT.

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Background and Aims: Over 240 million people are infected with schistosomiasis. Infection with *Schistosoma mansoni* occurs when the infective stage of the parasite penetrates the skin of individuals. Governments and non-governmental organizations have mainly used praziquantel mass drug administration to control schistosomiasis. However, alternative interventions are required for communities to reach the World Health Organization 2030 goal of elimination as a public health problem. Understanding community preferences for existing and new interventions will help improve uptake and sustainable use. This study elicited communities' preferences for interventions aimed at minimizing exposure to schistosomiasis.

Methods: Stakeholder workshops, with school-age children, women, fisherfolks and opinion leaders, were held in three high-endemicity communities in Mayuge, Uganda. The life cycle and interventions, previously suggested by rapid ethnographic appraisals, were presented. Data were quantitatively and qualitatively analysed.

Results: Tap water providing multiple jerrycans per day, having a new community landing site and sensitization through village health teams were recommended for their popularity and affordability. Having a strong water committee, strict rules and regulations were major facilitators, alongside having many taps at each water-stand, affordable fees, coupled with village health team sensitization. Strong and large landing sites to accommodate many community members at a time, that are clean and fenced to protect from drowning and guided by a dedicated sensitization team were recommended.

Conclusions: Findings indicate preferences of communities who live around infected water bodies that are popular, affordable and sustainable for interventions to reduce human infection with schistosomiasis that implementers of control interventions should consider for endemic communities like these.

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TRANSMISSION DYNAMICS, EPIDEMIOLOGY, AND GLOBAL HEALTH / WATER- AND FOOD-BORNE PARASITIC INFECTIONS

PREVALENCE AND RISK RANKING OF PARASITES OF ONE HEALTH IMPORTANCE IN ZIMBABWE'S LIVESTOCK AND MEAT VALUE CHAIN

Jeffy Chivheya
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Background and Aims: Risk ranking of food safety hazards is crucial for authorities in low- and middle-income countries to prioritize scarce economic and human resources for addressing public health challenges. A study was conducted to identify, map the prevalence of, and rank the risks posed by zoonotic parasites in the livestock and meat value chain in Zimbabwe. This represents the first study of its kind conducted in Zimbabwe.

Methods: Twenty-one experts were recruited to participate in the study based on their expertise in veterinary sciences, medicine, agriculture, and policy. Records from the Veterinary Department were accessed, and four parasites were selected for prevalence assessment and risk ranking: *Taenia saginata*, *Taenia solium*, *Fasciola* spp., and *Echinococcus granulosus*. A multicriteria decision analysis approach recommended by the World Health Organization was employed to rank the parasites. The Delphi technique was used to establish risk ranking criteria and assign corresponding weights . Risk scores were calculated using the weighted sum model.

Results: The parasites were ranked in descending order of risk as follows: *Taenia saginata*, *Taenia solium*, *Echinococcus granulosus*, and *Fasciola* spp., with prevalences of 1.1%, 0.08%, 0.53%, and 0.64%, respectively. The rankings were influenced by public health impact, economic considerations, and ease of control.

Conclusions: This study provides public health authorities with evidence-based guidance for parasite prioritization. It also generates objective data to support the implementation of risk-based meat inspection systems.

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TRANSMISSION DYNAMICS, EPIDEMIOLOGY, AND GLOBAL HEALTH / WATER- AND FOOD-BORNE PARASITIC INFECTIONS

ASSESSMENT OF PHOTOELECTROCATALYSIS FOR THE INACTIVATION OF CRYPTOSPORIDIUM IN WATER

Seila Couso-Pérez[1,2,3], Sandra Martín-García[1,2,3], Stuart Mcmichael[3], Hipólito Gómez-Couso[1,2], Tony Byrne[3], Pilar Fernández-Ibáñez[3]
[1]Universidade de Santiago de Compostela, Department Of Microbiology And Parasitology, Santiago de Compostela, Spain, [2]Universidade de Santiago de Compostela, Aquatic One Health Research Center (arcus), Santiago de Compostela, Spain, [3]Ulster University, Nanotechnology And Integrated Bioengineering Centre (nibec), School Of Engineering, Belfast, United Kingdom

Background and Aims: The high resistance of *Cryptosporidium* oocysts makes this parasite resilient to most conventional water treatment processes. Consequently, the development of effective technologies for the inactivation of *Cryptosporidium* oocysts in water remains a critical challenge. Advanced oxidation processes have been recognised as highly effective for pathogen inactivation in aqueous media. Among them, photoelectrocatalysis (PEC) is an electrochemically assisted photocatalytic system that uses an n-type photoanode and a conductive counter-electrode under an externally applied bias. Given the limited knowledge regarding the ability of PEC to inactivate waterborne protozoa, this study evaluates its effectiveness against *Cryptosporidium parvum* oocysts.

Methods: A small-scale PEC reactor was used to assess the inactivation of *C. parvum* oocysts in different electrolytes at various concentrations. The system was irradiated with a 450 W Xe lamp under a +2.0 V applied bias for 3 h, using a WO_3 photoanode and carbon paper as the cathode. Oocyst viability was determined by quantifying *hsp70* mRNA through RT-qPCR.

Results: Initial tests yielded inactivation values of 0.68, 1.14 and 1.4 log reduction (LR) using 100 mM NaCl, 100 mM Na_2SO_4 and 0.5 mM peroxymonosulfate (PMS), respectively. Subsequent assays with increasing PMS concentrations showed that 1.0 mM PMS resulted in 2 LR, while >4 LR was achieved with >1.5 mM PMS.

Conclusions: The *Cryptosporidium* inactivation levels obtained using PMS meet WHO guideline requirements for protozoa (>4 LR), demonstrating the potential of PEC as a promising water disinfection technology. However, further optimisation and research are required to enhance its applicability for water treatment.

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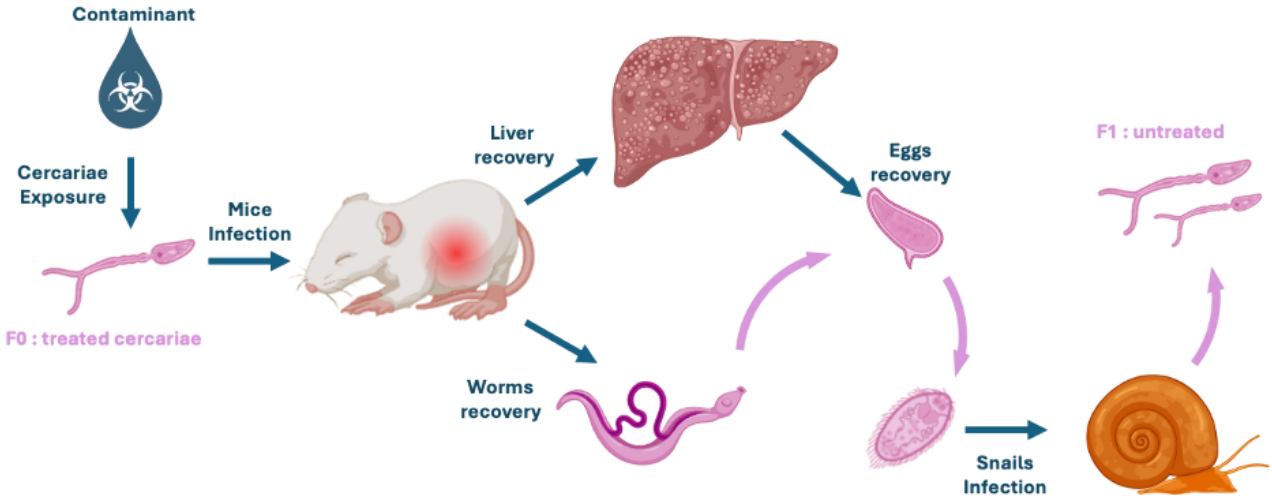
POSTER ON BOARD SHIFT 01
TRANSMISSION DYNAMICS, EPIDEMIOLOGY, AND GLOBAL HEALTH / WATER- AND FOOD-BORNE PARASITIC INFECTIONS

EARLY AQUATIC EXPOSURE TO WATER CONTAMINANTS MODULATES SCHISTOSOMA MANSONI VIRULENCE AND TRANSMISSION

Bertrand De L'Isle, Christoph Grunau, Ronaldo De Carvalho Augusto
Université de Perpignan Via Domitia, Perpigan, France

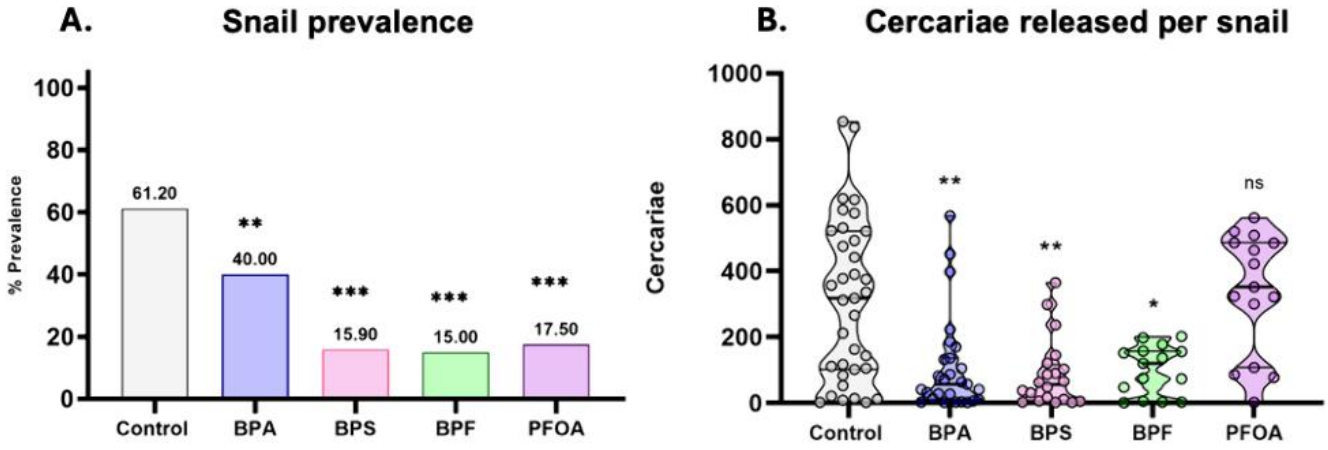
Background and Aims: Schistosomiasis is a major water-borne parasitic disease in which transmission and pathology are tightly linked to parasite reproduction and egg-induced host damage. Although environmental changes are known to affect hosts and vectors, the direct influence of aquatic chemical pollution on parasite virulence remains largely unexplored. Here, we tested whether short-term exposure of *Schistosoma mansoni* cercariae to environmentally relevant water contaminants could durably alter parasite virulence and transmission.

Methods: Cercariae were exposed for two hours to environmentally relevant concentrations of bisphenol A (BPA), bisphenol S (BPS), bisphenol F (BPF), or perfluorooctanoic acid (PFOA), and subsequently used to infect mice. Adult worm burden, reproductive output, egg quality, and hepatic pathology were assessed. Transmission potential was evaluated by infecting *Biomphalaria glabrata* snails with miracidia derived from exposed parasites.



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Results: Early cercarial exposure induced pronounced and contaminant-specific effects on parasite virulence. Adult worms displayed structural alterations of the male tegument and a strong reduction in egg production by mature females, accompanied by a sharp decrease in egg hatching rate, indicating impaired egg quality. These reproductive changes were associated with modified host pathology, with BPA and BPF increasing hepatic granuloma area, while PFOA reduced granuloma size. Importantly, eggs produced by exposed parasites showed a reduced ability to sustain transmission, with lower snail infection prevalence and decreased cercarial output in the next generation, despite no direct exposure of the intermediate
host.



Early aquatic exposure of cercariae reduces parasite transmission to the intermediate host.
A. Infection prevalence of *Biomphalaria glabrata* snails following exposure to miracidia derived from contaminant-exposed parasites. **B.** Number of cercariae released per infected snail in the subsequent generation. Statistical significance is indicated relative to control conditions.

Conclusions: These results reveal that environmentally relevant water contaminants act as biologically active drivers of parasite virulence, with potential consequences for disease severity and transmission in endemic settings.

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TRANSMISSION DYNAMICS, EPIDEMIOLOGY, AND GLOBAL HEALTH / WATER- AND FOOD-BORNE PARASITIC INFECTIONS

INVESTIGATION OF INTESTINAL PARASITE CONTAMINATION IN GREEN VEGETABLES SOLD AT NEIGHBORHOOD MARKETS, GREENHOUSES, AND SUPERMARKET SHELVES IN ESKIŞEHIR USING MICROSCOPIC AND MOLECULAR METHODS

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[1]Eskişehir Osmangazi Üniversitesi, Microbiology, Eskişehir, Turkey, [2]Eskişehir osmangazi üniversitesi, Microbiology, Eskişehir, Turkey

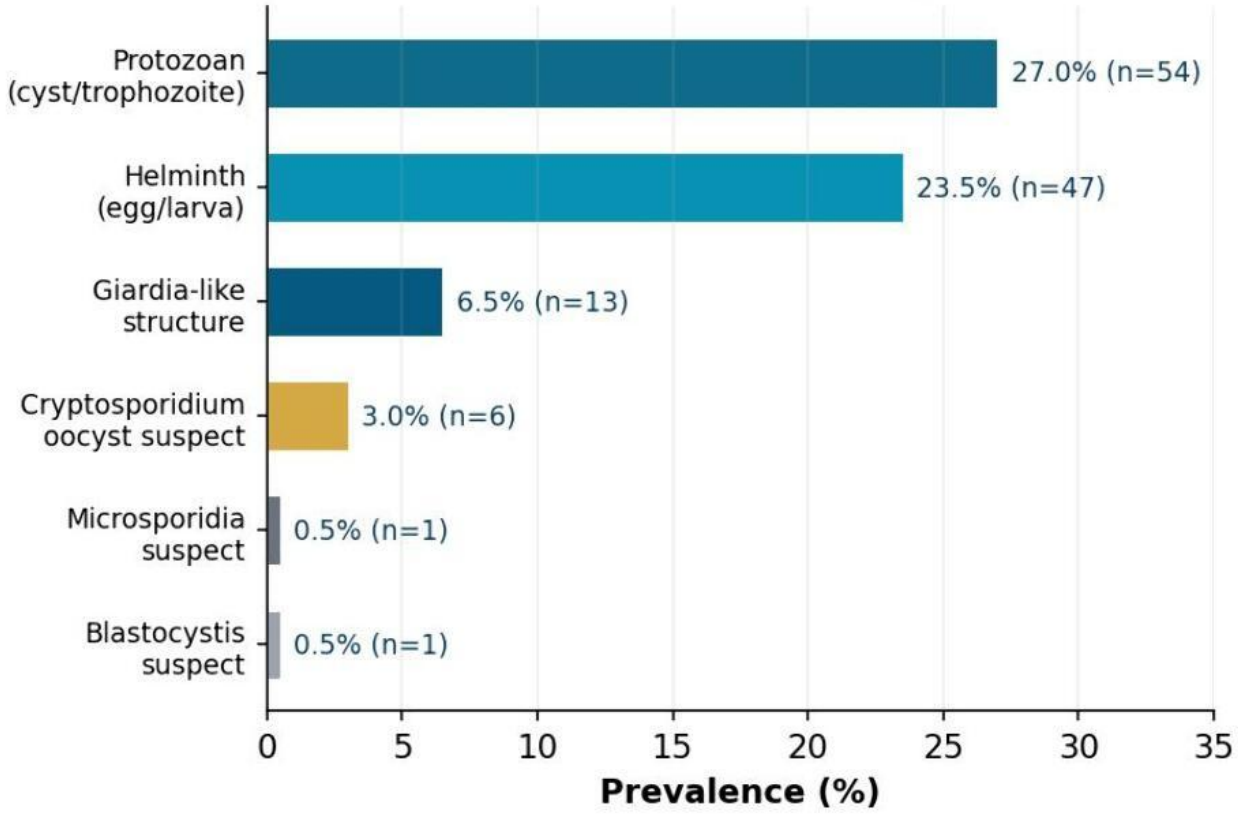
Background and Aims: Leafy green vegetables consumed raw pose a risk for foodborne parasitic infections. Most studies in Turkey rely solely on microscopy, which lacks species-level discrimination. This study aimed to investigate the presence of six intestinal parasites in vegetables from three sales points in Eskişehir using both microscopy and qPCR.

Methods: Two hundred vegetable samples (21 types) were collected from farmers' markets (n=134), greenhouses (n=51), and supermarkets (n=15) between November 2025 and February 2026. Sediments were examined by Lugol staining and modified Kinyoun acid-fast staining. DNA was extracted using the DNeasy Plant Pro Kit (Qiagen). Six parasites were screened by qPCR (Rotor-Gene Q): *Cryptosporidium* spp., *Cyclospora cayetanensis* and *Entamoeba histolytica* using a multiplex kit; *Toxoplasma gondii*, *Echinococcus granulosus* and *Toxocara canis* using singleplex kits.

Results: Microscopy detected parasitic structures in 89/200 samples (44.5%), while qPCR identified parasite DNA in 34/200 (17.0%). The most frequently detected parasites were *T. gondii* (7.0%), *T. canis* (5.0%), *E. granulosus* (2.5%), and *Cryptosporidium* spp. (2.5%). *C. cayetanensis* was not detected. Greenhouse samples showed the highest any-method positivity (62.7% vs. 26.7% supermarket; p=0.013). Parsley had the highest qPCR positivity (44.4%; p=0.023).McNemar's test confirmed that microscopy and qPCR yielded complementary results (p<0.001; κ=0.32), with 59 samples positive only by microscopy and 4 samples positive only by qPCR, supporting the use of both methods in combination for comprehensive surveillance.

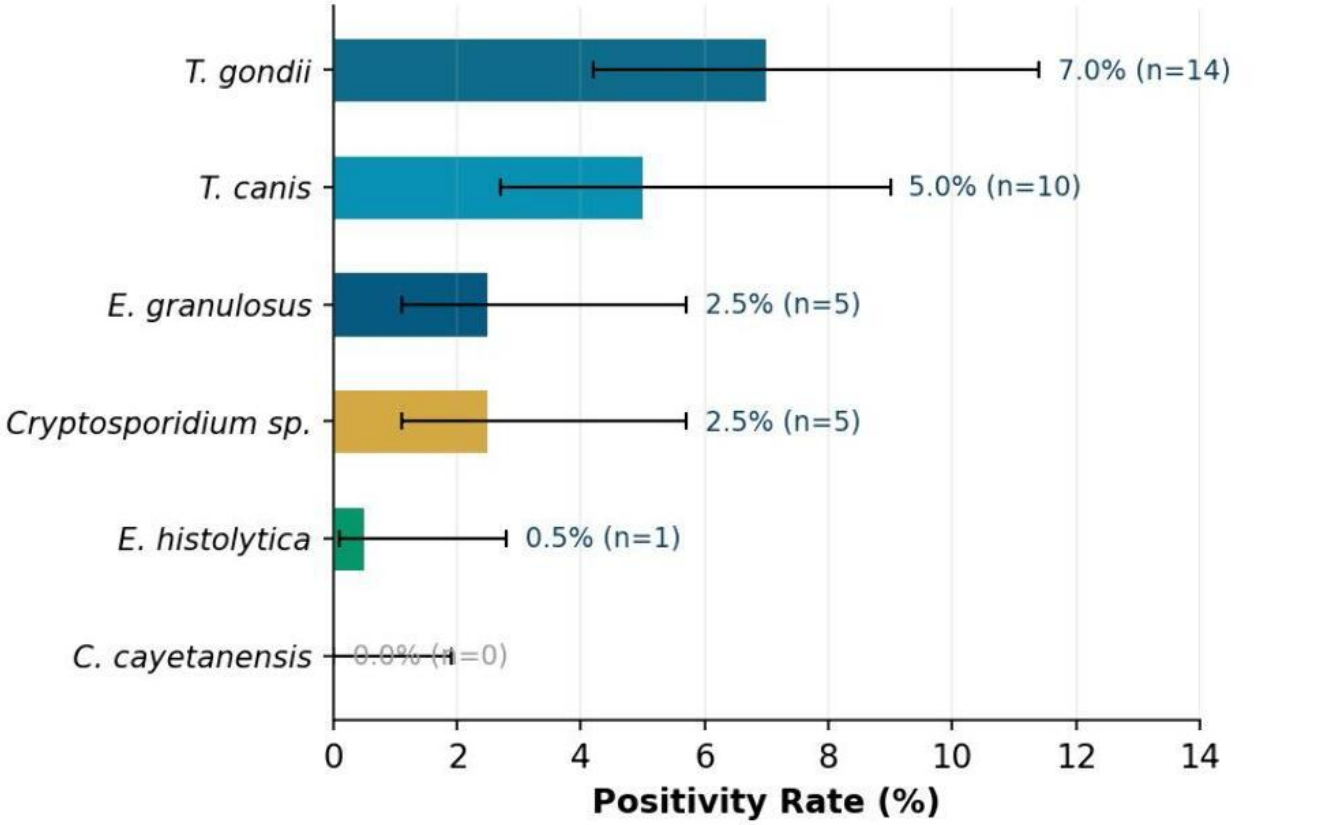
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Table 1. Parasitic Structures Detected by Microscopic Examination (n = 200 vegetable samples)



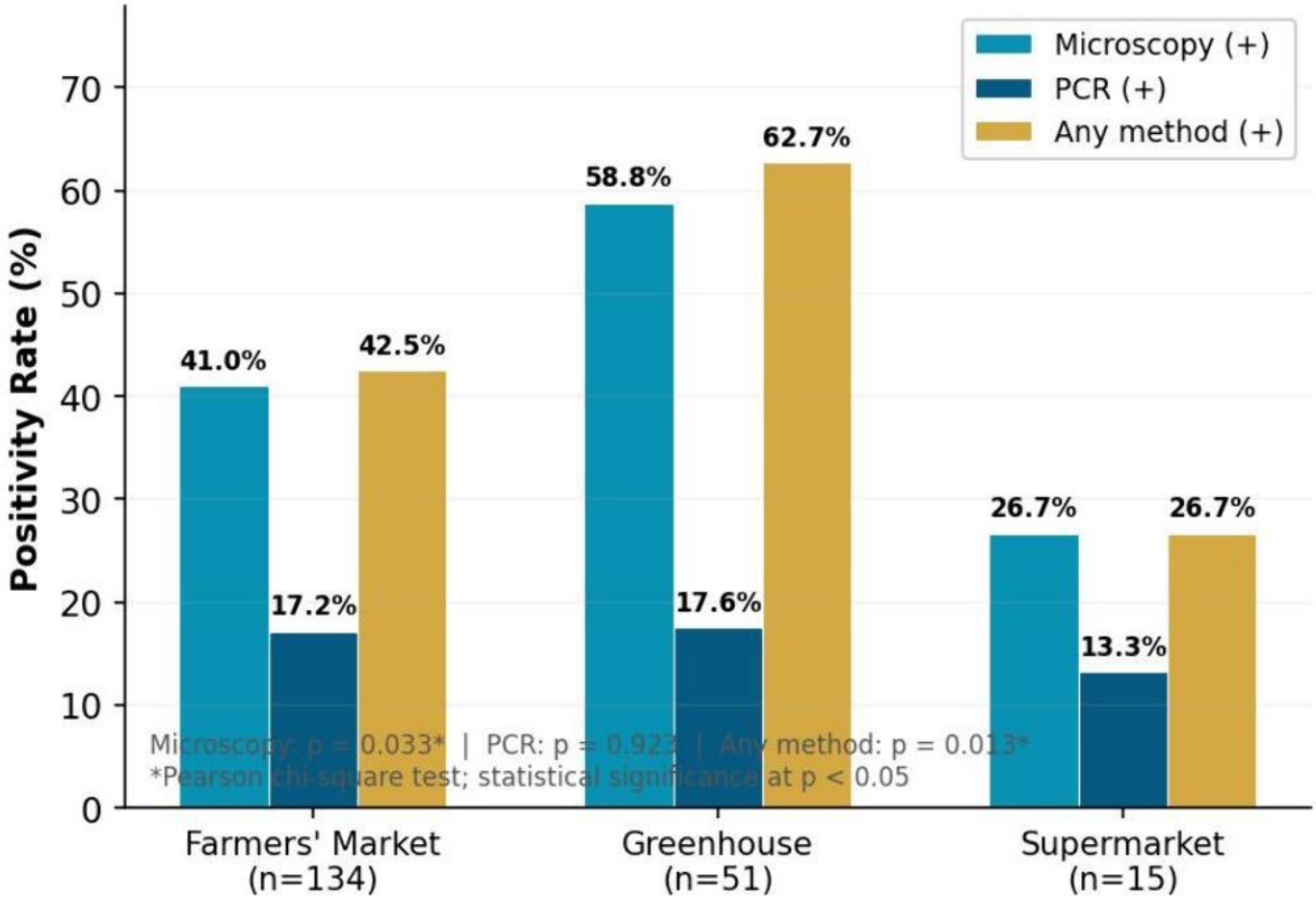
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Table 2. Distribution of Parasites Detected by qPCR (n = 200; Total PCR+ = 34/200, 17.0%; 95% CI with Wilson method)

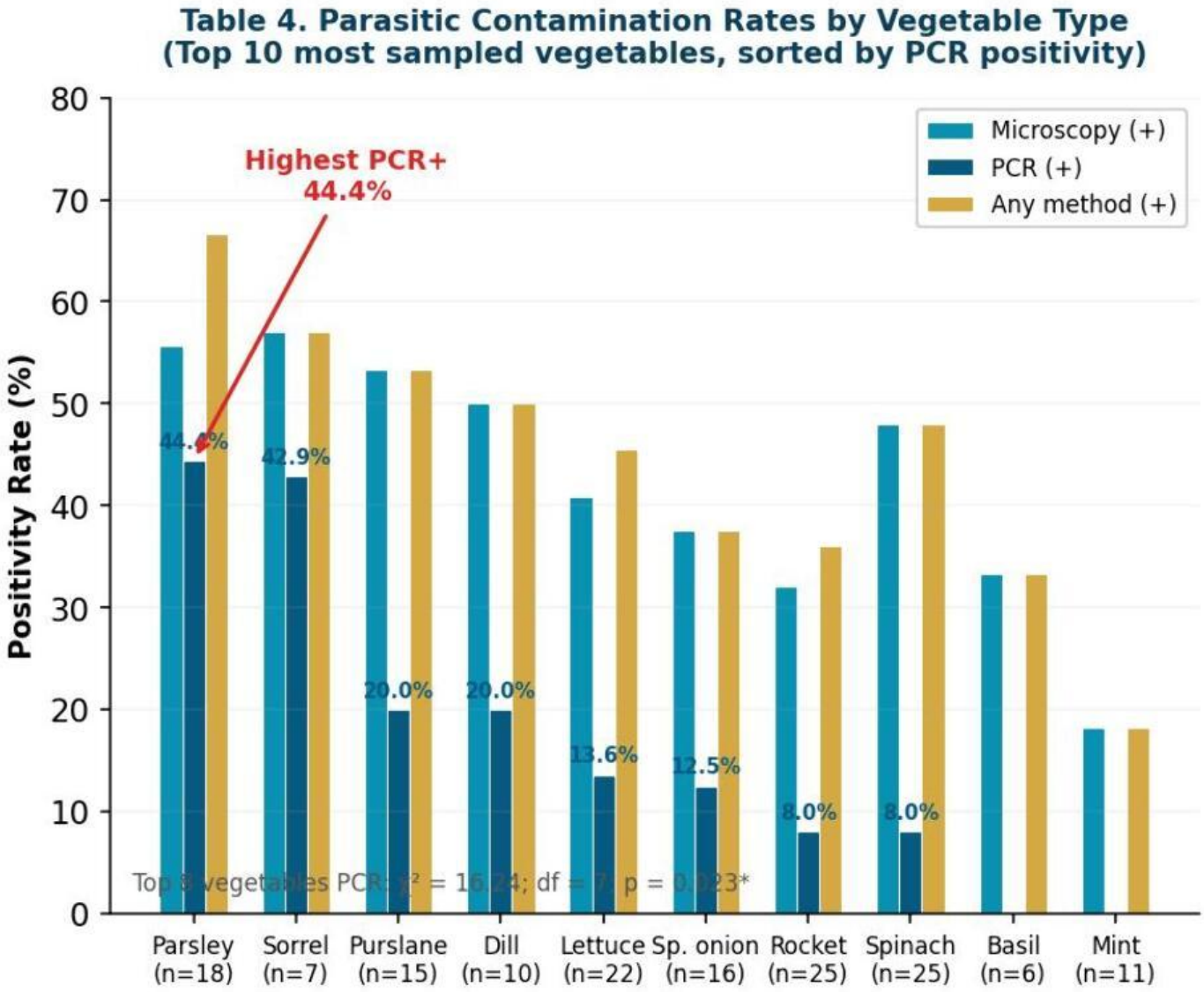


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Table 3. Parasitic Contamination Rates by Sales Point



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Conclusions: Nearly half of the vegetables were contaminated. Combining microscopy and qPCR is recommended for comprehensive surveillance. Parsley and greenhouse-sourced vegetables warrant particular attention for public health interventions.

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SHIFT01-275

POSTER ON BOARD SHIFT 01
TRANSMISSION DYNAMICS, EPIDEMIOLOGY, AND GLOBAL HEALTH / WATER- AND FOOD-BORNE PARASITIC INFECTIONS

MONITORING THE PRESENCE AND TRENDS IN THE PREVALENCE OF BLASTOCYSTIS SPP. IN CHILDREN AGED 0-18 YEARS

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Background and Aims: Bu çalışmanın amacı, son on yılda 0-18 yaş arası çocuklarda görülme sıklığı artan ve patojenitesiyle ilgili süregelen tartışmalara konu olan *Blastocystis spp.'nin* yaygınlığını, yaş, cinsiyet ve mevsimsel değişkenlikle ilişkisini geriye dönük olarak değerlendirmektir.

Methods: ESOGÜ Hastanesi Parazitoloji Laboratuvarı'na başvuran 0-18 yaş arası çocuk hastalardan alınan dışkı örnekleri, Blastocystis spp. varlığı açısından retrospektif olarak incelenmiştir. Bulguların istatistiksel analizleri, IBM SPSS Statistics for Windows, sürüm 20 kullanılarak yapılmıştır. Nitel verilerin karşılaştırılması için ki-kare testleri kullanılmış ve p değeri <0,05 istatistiksel olarak anlamlı kabul edilmiştir.

Results: 0-18 yaş grubundaki toplam 10.921 örnek analiz edildi. Blastocystis spp. varlığı 578 örnekte (%5,29) tespit edildi. Tespit edilen vakaların 298'i (%52,7) erkek, 280'i (%47,3) kız çocuğuydu. Yaygınlığın 0-3 yaş grubunda daha yüksek olduğu görülüyor. Veriler yıllara göre analizlere göre, COVİD-19 salgını döneminde önemli bir artış kaydedildi. Mevsimsel analizler, Blastocystis spp. varlığı ile hem sonbahar hem de sonbahar ayları, ayrıca yaş ve cinsiyet arasında anlamlı bir ilişki ortaya çıkıyor. Bununla birlikte, mevsim ve yaş arasında anlamlı bir ilişki yoktu. Çalışmanın tüm verileri tablolarda kullanıma sunuldu.

Conclusions: Bölgemizde gastroenteritli çocuklarda en sık tespit edilen parazit etkenin *Blastocystis spp* . belirlenmişti. En yüksek pozitif vaka sayıları çocuk acil servislerinden gönderilen örneklerde gözlemlenmiştir. Parazitin varlığı erkek çocuklarda daha sık görülür. Kanadadaki sürgün parazitlerine ilişkin verilerin çeşitliliği göstermekte olup, özellikle çocuklar arasında ulusal düzeyde sürveyans seçeneklerinin olduğunu vurgulamaktadır.

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TRANSMISSION DYNAMICS, EPIDEMIOLOGY, AND GLOBAL HEALTH / WATER- AND FOOD-BORNE PARASITIC INFECTIONS

HIGH FREQUENCY OF DNA DETECTION OF TOXOPLASMA GONDII AND ZOONOTIC SARCOCYSTIS SPP. IN READY-TO-EAT MEAT PRODUCTS PURCHASED IN SWITZERLAND

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Background and Aims: *Toxoplasma gondii* and *Sarcocystis* spp. infect a wide range of animal species, and humans. They can be transmitted to hosts through food sources, such as infected meat. Resulting infections may pose serious health risks, especially for immunocompromised individuals and developing foetuses. This study aimed to assess the presence of these parasites in ready-to-eat (RTE) meat products sourced from Swiss supermarkets, retail stores and local butcher shops.

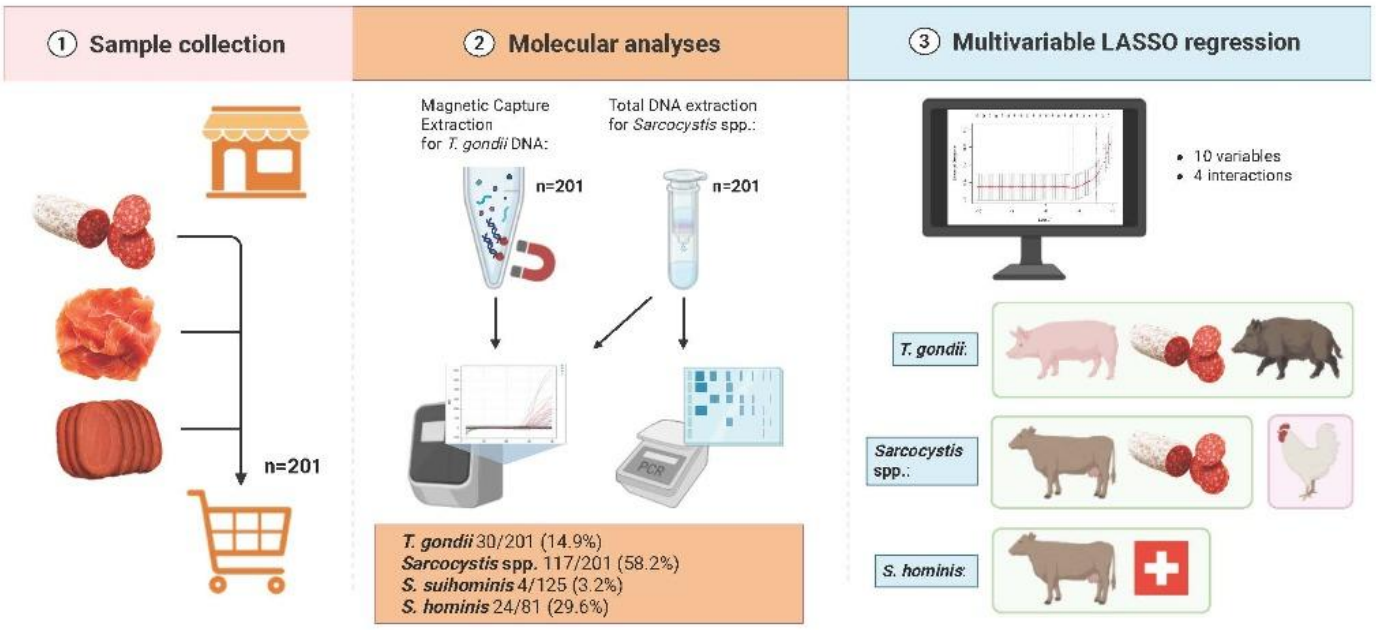
Methods: A total of 201 RTE meat products consisting of pork, beef, poultry, game, and equine meat, as well as mixes thereof, were tested. Hundred-gram samples were homogenized, followed by a sequence-specific magnetic capture and real-time PCR for *T. gondii* DNA, and a crude DNA extraction and real-time PCR - Sanger sequencing for *Sarcocystis* spp. Variables associated with the presence of these parasites were identified by multivariable LASSO regression.

Results: *Toxoplasma gondii* DNA was detected in 14.9 % (30/201) of the samples, while *Sarcocystis* spp. DNA was present in 58.2 % (117/201). Zoonotic *S. suihominis* DNA was found in 3.2 % (4/125) of the samples containing pork, and *S. hominis* DNA in 29.6 % (24/81) of the samples containing beef. The presence of *T. gondii* DNA was associated with the variables pork, salami-like products, and wild boar. *Sarcocystis* spp. DNA was positively associated with beef and salami-like products, and negatively with chicken and Swiss pork. *Sarcocystis hominis* was positively associated with Swiss beef.

Conclusions: These findings reveal a notable rate of RTE meat products positive for DNA of zoonotic parasites, suggesting a potential public health

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risk.



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TRANSMISSION DYNAMICS, EPIDEMIOLOGY, AND GLOBAL HEALTH / WATER- AND FOOD-BORNE PARASITIC INFECTIONS

ENHANCING CRYPTOSPORIDIUM INACTIVATION IN SOLAR WATER DISINFECTION USING A PEROXYMONOSULFATE-BASED GRANULAR FORMULATION

Sandra Martín-García[1,2], Seila Couso-Pérez[1,2], Victoria Díaz-Tomé[3,4], Franscisco Otero-Espinar[3,4], Hipólito Gómez-Couso[1,2]
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Background and Aims: Access to safe drinking water remains a major challenge in many parts of the world. Household water treatment approaches offer a practical, low-cost and sustainable option to improve microbiological safety of drinking water. Solar water disinfection (SODIS) consists of exposing contaminated water in polyethylene terephthalate (PET) bottles to direct sunlight for 6 hours. While SODIS has proven highly effective against bacteria, its efficacy is more limited for viruses and protozoa. *Cryptosporidium* is one of the leading causes of diarrhoeal diseases in low and middle-income countries and, due to its resistance to conventional disinfection processes, it is used by the World Health Organization (WHO) as the reference pathogen for the protozoan group in the evaluation of household water treatment technologies. The present study aimed to develop and evaluate peroxymonosulfate-based granular formulation to improve the inactivation of *Cryptosporidium*.

Methods: Granules were formulated with peroxymonosulfate and iron as the main components. Water samples containing $2x10^6$ *Cryptosporidium parvum* oocysts/mL were exposed to simulated solar radiation (40 W/m^2, 280-400 nm) at 40°C for 6h under a PET plastic cover. Oocyst viability was evaluated after 1, 2, 4 and 6h of exposure by quantifying 70 kDa heat shock protein (HSP70) mRNA using RT-qPCR.

Results: The formulation reduced oocyst viability in >2 log reduction (LR), whereas the formulation without iron achieved >4 LR, exceeding the WHO criteria for high protection against waterborne protozoan diseases.

Conclusions: This study demonstrates that incorporating a peroxymonosulfate-based formulation can be a promising strategy to enhance SODIS procedures and improve biological quality of drinking water, particularly in resource-poor settings.

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TRANSMISSION DYNAMICS, EPIDEMIOLOGY, AND GLOBAL HEALTH / WATER- AND FOOD-BORNE PARASITIC INFECTIONS

URBAN HOTSPOTS AND SOCIOECONOMIC DETERMINANTS FOR SPECIFIC ETIOLOGICAL AGENTS OF PEDIATRIC DIARRHOEA: A GEOSPATIAL ANALYSIS IN THE COLOMBIAN COFFEE-GROWING REGION

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Background and Aims: Pediatric acute diarrheal disease (ADD) is evolving in response to climatic, migratory, and social determinants. To date, no studies have been published that integrate social, bacteriological, and temporal trends for specific parasitic and bacterial etiologies.

Methods: A prospective cohort of 249 children (0–15 years) with ADD was followed from 2022 to 2025 at Hospital Universitario San Juan de Dios. Clinical, demographic, and laboratory data—including stool parasitology (modified Ziehl–Neelsen stain) and bacterial identification (BD Phoenix™ M50)—were collected. Multivariate logistic regression and Principal Component Analysis (PCA) assessed risk factors and epidemiological patterns. Geospatial and temporal analyses correlated pathogen distribution with environmental and socioeconomic variables.

Results: Etiological agents were identified in 81% of cases, with *Cryptosporidium* predominating (58% monoinfection) and a high rate of multi-infection (33.3%).
Whereas *Blastocystis* and *Cyclospora* point toward a stronger correlation with the subsidized regime, their separation from the daycare vector suggests that their transmission is more likely linked to domestic water-handling practices (e.g., unboiled water for juices) rather than congregate social settings. Seasonal peaks of *Cryptosporidium* coincided with rainfall, implicating water safety. *Cryptosporidium* confirms its role as a distinct, explosive pathogen that often occurs in monoinfections particularly during rainfall peaks. Bacterial and pathogenic *Entamoeba* vectors point in a similar direction particularly in children attending daycares where person-to-person spread is common

Conclusions: Pediatric ADD in this region is shaped by protozoal and bacterial infections, socioeconomic inequities, and diagnostic gaps. Targeted public health interventions addressing water safety, nutritional support, and daycare infection control, alongside rapid diagnostics, are critical to reduce morbidity.

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TRANSMISSION DYNAMICS, EPIDEMIOLOGY, AND GLOBAL HEALTH / WATER- AND FOOD-BORNE PARASITIC INFECTIONS

CRYPTOSPORIDIOSIS TRANSMISSION IN ONTARIO, CANADA AND CHANGING MOLECULAR PROFILES DUE TO COVID TRAVEL RESTRICTIONS

Rebecca Guy[1,2], Scott Lambie[1], Laura Martin[1], Justin Carpani[1], Antoine Corbeil[3], Mohammad Bhuiyan[4], Fiifi Agyabeng-Dadzie[5], Travis Glenn[6], Jessica Kissinger[7]
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Background and Aims: Cryptosporidiosis, caused by the gastrointestinal protozoan parasite *Cryptosporidium,* is a nationally reportable disease in Canada with over 400 cases reported per year in the province of Ontario. The aim of this study was to identify potential animal sources of *Cryptosporidium* to better understand transmission dynamics.

Methods: Molecular analysis of the 18S gene and the subtyping marker glycoprotein 60 (gp60) in stool specimens identified *Cryptosporidium* species/genotypes and subtypes. DNA hybridization capture data analysis is in progress to enable greater discrimination between isolates.

Results: Our previous surveillance of Ontario specimens from 2008-2017 revealed *C. parvum* and *C. hominis* in 71% and 19% of genotyped cases, respectively. In the present study of 261 cases from 2018-2024, 80% of cases were *C. parvum*. *C. mortiferum* (Chipmunk Genotype II) was the second most prevalent species at 6% and *C. hominis* was observed in only 5% of cryptosporidiosis cases. Five new species/genotypes were also observed. Travel restrictions in Canada from 2021-22 due to COVID resulted in no *C. hominis* detected during that time and emergence of new wildlife genotypes. GP60 genotyping revealed new subtypes; however, *C. parvum* subtype IIaA15G2R1 remained the most common, observed in 63% of cases from 2008-2024. Analysis of bait capture genotyping data is in progress with the aim to provide greater discrimination of these isolates.

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Conclusions: Zoonotic transmission of *Cryptosporidium* is the most common transmission in Ontario and it appears that *C. hominis* infection is mainly acquired through travel and then spread within communities.

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TRANSMISSION DYNAMICS, EPIDEMIOLOGY, AND GLOBAL HEALTH / WATER- AND FOOD-BORNE PARASITIC INFECTIONS

DIENTAMOEBA FRAGILIS CYSTS AND PRE-CYSTS IN HISTORIC SLIDE COLLECTIONS AND A REVIEW OF CYST FORMATION AMONG THE PARABASALIA

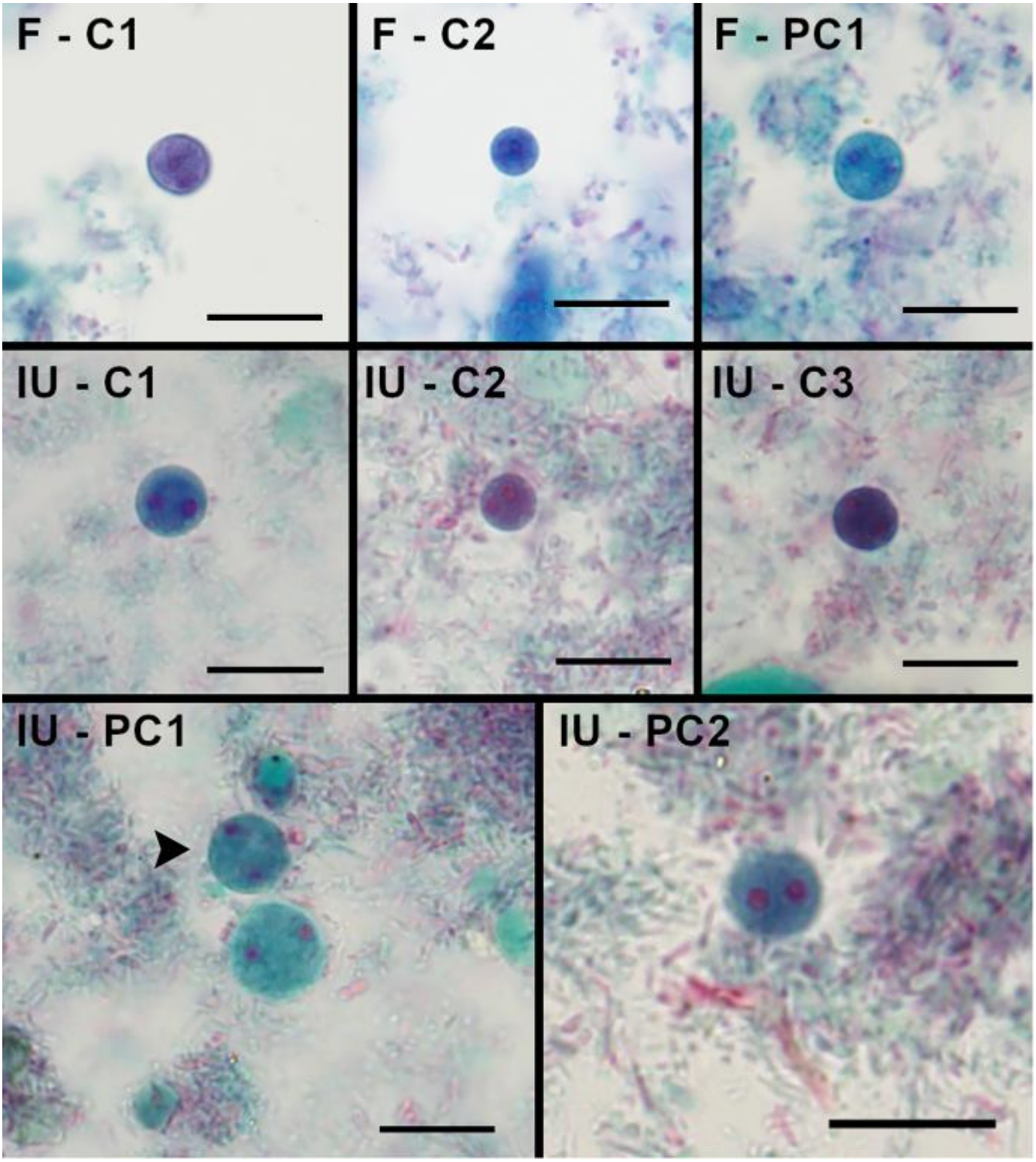
Luke Hall[1,2], Joel Barratt[3], John Ellis[1], Damien Stark[2]
[1]University of Technology Sydney, Life Sciences, Ultimo, Australia, [2]SydPath, St Vincent's Hospital Sydney, Microbiology, Darlinghurst, Australia, [3]Emory University School of Medicine, Pathology And Laboratory Medicine, Atlanta, United States of America

Background and Aims: Transmission is a basic aspect of gastrointestinal parasites' biology; however, for *Dientamoeba fragilis,* it remains poorly understood. Early descriptions of *D. fragilis* report the absence of a cyst stage and are often cited in debates supporting the lack of *D. fragilis* cysts. Since Dobell's original description, cysts of *D. fragilis* have been reported, though their existence is not universally accepted. One of the main challenges to the wider acceptance of a cyst stage in the life cycle of *D. fragilis* is its rarity in human specimens. In this study, we aimed to review historical faecal smears containing *D. fragilis* with the current understanding of this species, identifying the life cycle stages present.

Methods: Here, Dobell's, Wenyon's and Hoare's collection of historical faecal smears stored at the Natural History Museum (London), dating back to the 1890s and the early 1900s, were examined for forms consistent with modern descriptions of *D. fragilis* cysts. We also examined decades-old faecal smears archived at the United States Centers for Disease Control and Prevention. Finally, we compared *D. fragilis* cysts from these historic slide collections to *D. fragilis* cysts observed in experimentally infected rodents and to examples of binucleate cysts from other trichomonads.

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Results: We detected multiple forms compatible with the description of cysts in the historical slides examined.



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Conclusions: Considering published literature on *D. fragilis* cysts and the broader picture of cyst formation across Parabasalids, we recommended that future investigations on *D. fragilis* transmission consider the role of a true cyst despite its rarity in human faecal specimens.

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TRANSMISSION DYNAMICS, EPIDEMIOLOGY, AND GLOBAL HEALTH / WATER- AND FOOD-BORNE PARASITIC INFECTIONS

DEMONSTRATING THE IMPACT OF MODELLING CHOICES ON DISABILITY-ADJUSTED LIFE YEARS ESTIMATION FOR NEUROCYSTICERCOSIS: FROM EPILEPSY ALONE TO MULTI-SEQUELAE BURDEN

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Background and Aims: Disability-adjusted life years (DALYs) have been used to quantify the health burden of neurocysticercosis (NCC). Current global burden estimates include only DALYs attributable to NCC-associated epilepsy, even though NCC patients can present with other manifestations, such as severe headaches. This study aimed to demonstrate the impact of adding headaches to the overall NCC-associated DALYs-estimates.

Methods: We first estimated NCC-associated DALYs for India, Brazil and Tanzania considering epilepsy as the only sequela (Base Model). Headache data were then included using three modeling approaches: (Model 1) calculating headache cases only among people with NCC-associated epilepsy, (Model 2) assuming that a proportion of all headache cases in a population are attributable to NCC, and (Model 3) calculating the total number of NCC cases from sero-survey data and assuming that a proportion have epilepsy or headaches.

Results: In Model 1, the mean DALY-estimates increased by 13% to 69% depending on the country; however, the 95% uncertainty intervals overlapped with those from the Base Model. In contrast, Model 2 yielded substantially higher estimates (ranging from 90% to 940%), accompanied by wider uncertainty intervals. The results from Model 3 were highly sensitive to the assumed sensitivity (Se) and specificity (Sp) of the serological tests used to detect NCC. While some values of Se and Sp produced DALY estimates similar to or lower than those from the Base Model, other values resulted in estimates even higher than those from Model 2.

Conclusions: Overall, NCC-associated headaches likely contribute substantially to disease burden, but better epidemiological data are needed to improve DALY-estimates.

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HISTOPATHOLOGY AND ANTIBODY RESPONSES DESCRIBE THE SEASONAL PATTERN OF DICROCOELIOSIS IN SMALL RUMINANTS IN THE HIMALAYAN RANGES OF PAKISTAN

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Background and Aims: The aims of the present study were to describe the histopathology and to investigate the transmission patterns of Dicrocoelium amongst ten sheep and goat farms in Khyber Pakhtunkhwa and Gilgit Baltistan, Pakistan.

Methods: The liver histology and indirect enzyme-linked immunosorbent assay (ELISA) analyses followed standard procedures.

Results: The liver histopathology showed intensive tissue destruction and biliary hyperplasia associated with presence of adult flukes, severe inflammatory cell infiltration, congestion of blood vessels, damaged hepatocytes, and sinusoids in the infected areas. The time of onset of infection was investigated by ELISA detection of antibodies in sheep (n = 164) and goats (n = 152). Colostral transfer of *Dicrocoelium* antibodies from seropositive mothers was detected in sheep and goats up to 16 weeks of age. In both sheep and goats, the estimated time of infection differed between farms and years. Infection was seen in both sheep flocks and goat herds, with high variation between flocks and herds, and the highest infection rate in lambs. *Dicrocoelium* infection was most prevalent in sheep and goats in September (n = 84) and August (n = 63) respectively. This study concluded *Dicrocoelium* causes severe inflammation and necrosis of liver tissues in sheep and goats. Colostral transfer of antibodies can be detected up to about ten weeks of age. Higher infection rates are observed during August and September in sheep than in goats, putatively due to effects of different grazing and browsing behaviors on the ingestion of ants.

Conclusions: Results will inform disease control strategies for optimal sheep-goat productivity

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TRANSMISSION DYNAMICS, EPIDEMIOLOGY, AND GLOBAL HEALTH / WATER- AND FOOD-BORNE PARASITIC INFECTIONS

FASCIOLIASIS, A NEGLECTED TROPICAL DISEASE IN JAPAN

Mio Kokubo-Tanaka[1], Fukumi Nakamura-Uchiyama[2], Kei Yamamoto[3], Mio Sanada[3], Haruhiko Maruyama[1]
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Background and Aims: Fascioliasis is a food-borne trematodiasis categorized in NTD. Humans acquire *Fasciola* infection by ingesting metacercaria-contaminated aquatic plants. In Japan, fascioliasis is a rare infectious disease, with only sporadic cases reported. The objective of this study is to elucidate the clinical and epidemiological features of fascioliasis in Japan.

Methods: We analyzed fascioliasis cases consulted to the Research Group on Chemotherapy of Tropical Diseases (RG-CTD) between 2001 and 2024 in Japan. Clinical data were provided from the attending physicians on the antibody test application forms. Diagnosis was based on *Fasciola* worms/eggs detection in feces or bile, and/or positivity of serum anti-*Fasciola* antigen IgG by ELISA. Diagnosed patients were recommended to participate in a clinical trial conducted by RG-CTD, within which they received triclabendazole treatment.

Results: 99 patients were diagnosed during the period, of which 71% were Japanese. Among foreign patients, Vietnamese constituted the majority. Most cases were serologically diagnosed, with worms/eggs detected only in seven individuals. Chief complaints were nonspecific, such as abdominal pain and fever. Marked peripheral blood eosinophilia was observed, and nearly half exhibited severe eosinophilia (> 5,000/μL). 91% had liver image abnormalities including abscesses and nodules. The source of infection was often unidentified, yet 28 patients had consumed vegetables such as watercress. In some cases, rice straw exposure was suspected as an infection source. In all patients who completed post-treatment follow-up, triclabendazole was effective.

Conclusions: Fascioliasis transmission is continuously occurring in Japan, affecting both Japanese and foreign patients. Clinical characteristics were typical, while epidemiological feature reflected regional variations and local lifestyle habits.

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TRANSMISSION DYNAMICS, EPIDEMIOLOGY, AND GLOBAL HEALTH / WATER- AND FOOD-BORNE PARASITIC INFECTIONS

PREVALENCE OF SOIL-TRANSMITTED HELMINTHS FROM SELECTED PUBLIC ELEMENTARY SCHOOL GROUNDS IN LOS BAÑOS, LAGUNA

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[1]University of Santo Tomas, Senior High School, Manila, Philippines, [2]University of Santo Tomas, Faculty Of Pharmacy, Manila, Philippines

Background and Aims: Soil-transmitted helminths (STHs) remain a significant public health concern in the Philippines, particularly in public elementary schools where children's outdoor activities increase infection risks. This study compared STH contamination (*Ascaris lumbricoides*, *Trichuris trichiura*, *Necator americanus*, *Ancylostoma duodenale*, and *Strongyloides stercoralis*) in two schools in Los Baños, Laguna.

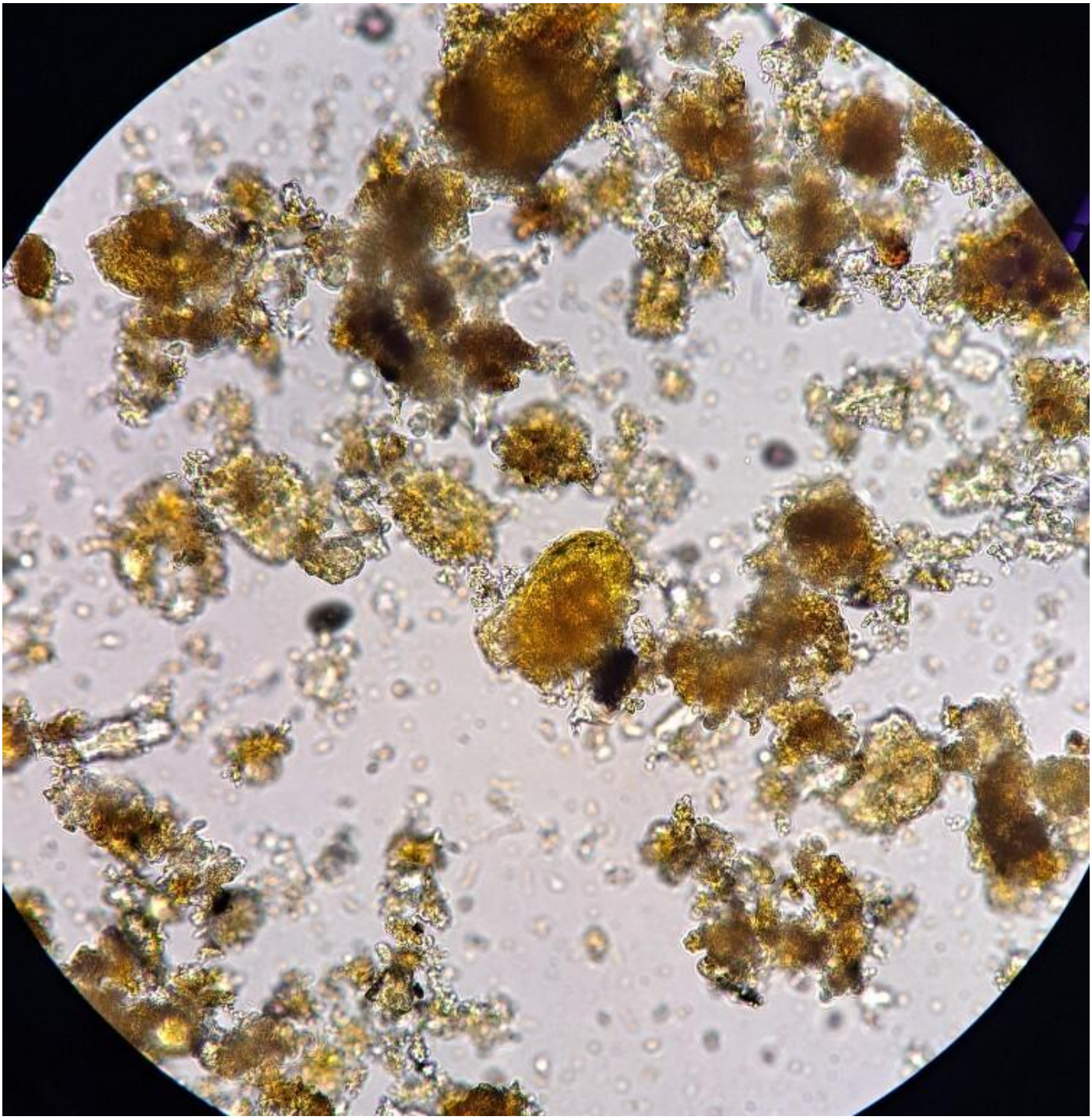
Methods: Thirty soil samples (n=15 per school) were collected using simple random sampling and quadrat sampling. On-site pH and temperature were recorded. At the University of Santo Tomas, samples were weighed, homogenized with distilled water, and filtered using a kitchen filter and 105-micron sieve before undergoing sucrose flotation. To maximize detection, both supernatant and settled sediment layers were microscopically examined. A spiked composite control sample validated the procedure. Fisher's Exact Test and descriptive statistics were used for analysis.

Results: On-site monitoring showed a mean soil pH of 6.7 and temperature of 29.8°C. Flotation supernatant yielded a 0% detection rate. However, sediment examination revealed a 6.67% overall prevalence; School A had two samples (13.33%), one containing an STH egg and another with three larvae, while School B showed 0% detection ($p = 0.483$). *Blastocystis*-like cysts were identified in both school
sediments.

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Conclusions: The study confirms the presence of STH contamination within the soil of at least one public school ground in Los Baños. Parasite recovery exclusively in the sediment indicates strong soil adherence, highlighting the necessity of including sediment analysis in environmental surveys. Crucially, the water-borne *Blastocystis*-like cysts serve as biological indicators of fecal contamination, necessitating an urgent review of water, sanitation, and hygiene (WASH) protocols.

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TRANSMISSION DYNAMICS, EPIDEMIOLOGY, AND GLOBAL HEALTH / WATER- AND FOOD-BORNE PARASITIC INFECTIONS

CAN PHOTOSENSITIZERS IMPROVE CRYPTOSPORIDIUM INACTIVATION IN SOLAR WATER DISINFECTION?

Sandra Martín-García[1,2], Marina Díaz-Domínguez[1,2], Seila Couso-Pérez[1,2], Hipólito Gómez-Couso[1,2]
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Background and Aims: Safe drinking water remains a global public health challenge, particularly in regions relying on household-level treatment systems. *Cryptosporidium* is a waterborne protozoan pathogen with a high resistance to water disinfection processes. This parasite is a leading cause of diarrhoeal diseases and responsible for 500000 child deaths annually in low and middle-income countries. Therefore, it has been selected by the WHO as a reference organism for evaluating household water treatments. Solar water disinfection (SODIS) is a simple, low-cost method that uses sunlight to inactivate microorganisms. Although it is highly effective against bacteria, some viruses and protozoa may persist. The addition of photosensitizers can generate reactive oxygen species under solar radiation, thereby enhancing disinfection. Moreover, photobleaching make them potentially suitable as indicators of the disinfection process. This work aims to evaluate the enhancement of SODIS by the addition of different photosensitizers against *Cryptosporidium*.

Methods: Samples containing different concentrations of erythrosine, riboflavin and methylene blue were spiked with *Cryptosporidium parvum* oocysts and exposed to simulated solar radiation at 40°C for 6h under a polyethylene terephthalate (PET) cover. Oocyst viability was evaluated by quantifying hsp70 mRNA using RT-qPCR. Photobleaching was monitored by spectrophotometry.

Results: After 6h, oocyst viability decreased ≥2.5 log with erythrosine (≥100 µM), >4 log with riboflavin (50-100 µM) and >2 log with methylene blue (≥0.5 µM). Photobleaching was observed for all dyes, with complete colour loss for riboflavin and methylene blue.

Conclusions: These photosensitizers significantly enhanced SODIS performance against *C. parvum*, meeting the WHO criteria for protection against waterborne protozoan diseases.

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TRANSMISSION DYNAMICS, EPIDEMIOLOGY, AND GLOBAL HEALTH / WATER- AND FOOD-BORNE PARASITIC INFECTIONS

RECENT TRENDS IN FOOD-BORNE PARASITIC INFECTIONS IN JAPAN

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Background and Aims: In Japan, national surveillance system for parasitic diseases is only targeting malaria, amoebiasis, cryptosporidiosis, giardiasis, and echinococcosis. In order to comprehend the total picture of food-borne parasitic diseases in Japan, we summarized and analyzed patient demographics and clinical manifestation in the last 20 years, from 2006 to 2025, consulted to our laboratory for antibody tests for parasitic diseases.

Methods: Clinical information of individual patient was provided by the attending physicians on a form of consultation sheet when the physicians consulted us about antibody tests for parasitic diseases. Information included age, sex, address (city or county), home country, chief complaint, brief summary of the present history, clinical diagnosis, dietary history, overseas traveling history, medical imaging findings and laboratory data including white blood cell (WBC) count (/μL), peripheral blood eosinophil count (%), blood chemistry, and serum total IgE (IU/mL).

Results: Among analyzed, the most frequent parasitic disease was larva migrans syndrome, caused by *Toxocara* and *Ascaris* nematodes. The next common infection was paragonimiasis, counting over 600 patients, with recent increase from patients from Indochina peninsula. Annual number of fasciolosis, gnathostomiasis, and sparganosis patients were basically less than ten, but a kind of ‘spike’ was observed in fasciolosis and gnathostomiasis. Immigrants from Indochina peninsula accounted for the recent increase of fasciolosis patients.

Conclusions: It would be impossible to eliminate food-borne parasitic infections from Japan. Medical professionals have to be aware of the risk of these diseases, especially patients are from Indochina peninsula.

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TRANSMISSION DYNAMICS, EPIDEMIOLOGY, AND GLOBAL HEALTH / WATER- AND FOOD-BORNE PARASITIC INFECTIONS

INCIDENTAL DIAGNOSIS OF HEPATIC PENTASTOMIASIS DURING RESECTION OF A NEUROENDOCRINE TUMOR: A CASE REPORT HIGHLIGHTING DIAGNOSTIC CHALLENGES

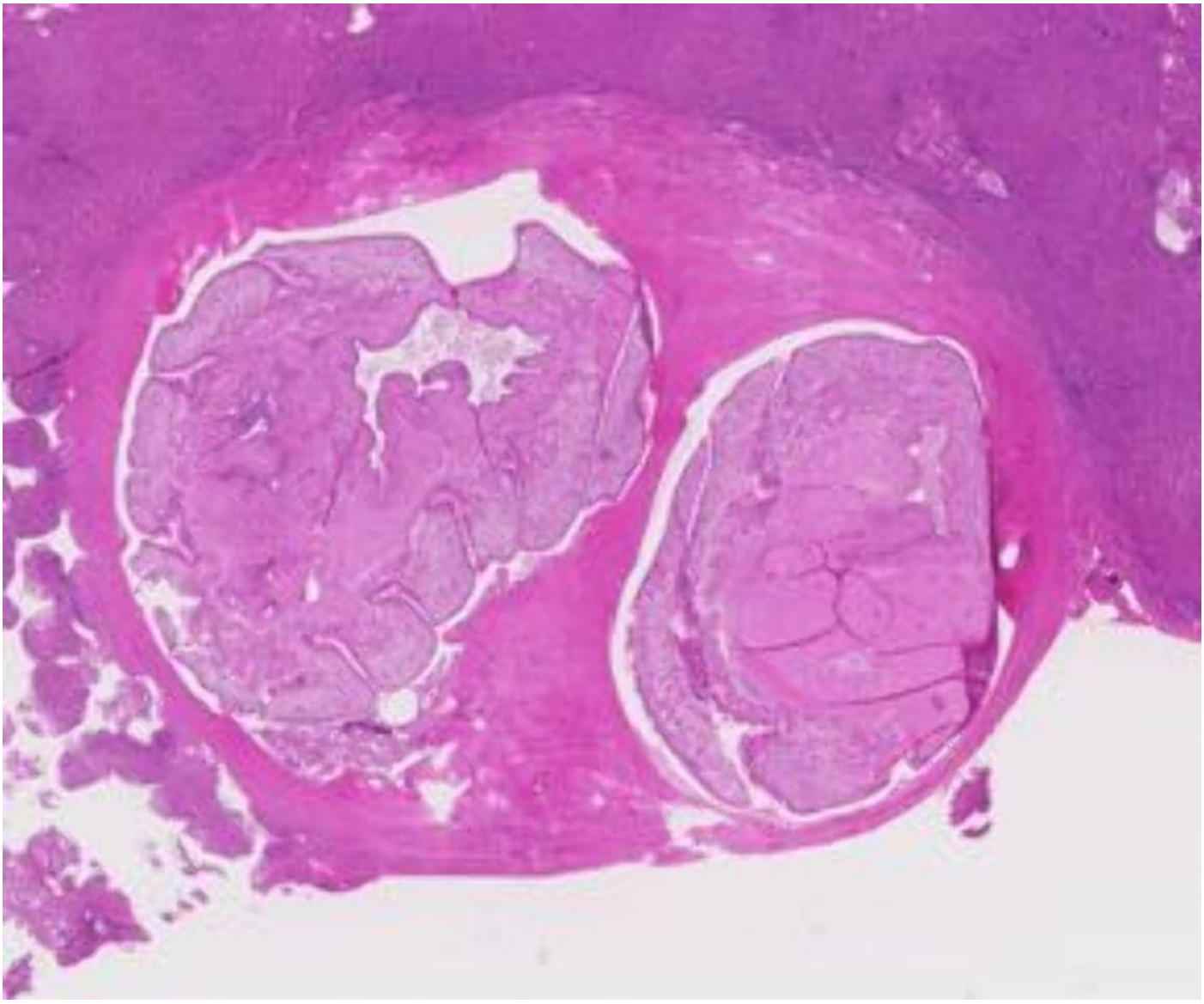
Arpita Chakravarti[1], Jean-Charles Mourot[1], Richard Bradbury[2], David Héneault[3]
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Background and Aims: Pentastomiasis is a rare zoonotic parasitic infection caused by snake lung worms, predominantly affecting individuals from endemic regions in Africa and Asia. Transmission to humans occurs through handling or consumption of undercooked meat from infected snakes or by oral ingestion of environmental ova. We report an unusual case of hepatic pentastomiasis discovered during oncological surgery.

Methods: We report an unusual case of hepatic pentastomiasis discovered during oncological surgery.

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Results:



A 60-year-old immigrant from Cameroon was diagnosed with a duodenal neuroendocrine tumor. During surgical resection of a persistent mass, multiple whitish hepatic lesions of uncertain etiology were seen. Due to suspicion of metastatis, partial hepatectomy was performed. Initial histopathological examination suggested hepatic fascioliasis. However, negative serology for *Fasciola* species prompted further expert pathology consultation. Revised histopathological analysis revealed an arthropod with a smooth thick, eosinophilic, acellular cuticle, lacking spines and with prominent annular rings.Frontal acidophilic glands, two oral hooks, and other morphological features consistent with a pentastomid nymph were observed. Based on the patient's geographical origin, *Armillifer armillatus* was considered the most likely etiological agent. Molecular sequencing is currently pending. The patient reported no symptoms attributable to parasitic infection and no visual disturbances. He disclosed frequent consumption of snake meat as part of cultural practices. No specific antiparasitic therapy was initiated, and the patient is being followed up clinically.

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Conclusions: This case illustrates the diagnostic difficulty of pentastomiasis in non-endemic settings, where hepatic lesions may mimic other parasitic infections or metastatic disease. Increased awareness is essential to avoid misdiagnosis and to guide appropriate patient management

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TRANSMISSION DYNAMICS, EPIDEMIOLOGY, AND GLOBAL HEALTH / WATER- AND FOOD-BORNE PARASITIC INFECTIONS

ASCARIASIS AND ITS RELATIONSHIP WITH SELECTED PSYCHO-NEUROLOGICAL SYMPTOMS AMONG CHILDREN AND ADULTS IN EUROPE.

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Background and Aims: Ascariasis is typically regarded as a public health problem of tropical regions, and existing research has focused predominantly on pediatric populations. Consequently, evidence linking Ascaris lumbricoides infection with psycho-neurological or psychomotor disturbances, particularly in European children and adults, remains limited. This study aimed to test the hypothesis that ascariasis in both children and adults is associated with selected psycho-neurological symptoms, including motor and vocal tics, compulsive habits, and inattention with hyperactivity.

Methods: A multicenter observational study was conducted between 2010 and 2025 involving 2,000 participants aged 1–82 years from Poland, Spain, Sweden, Germany, the United Kingdom, Ireland, Latvia, Hungary, Bulgaria, Romania, and Ukraine. Participants of both sexes were divided into a case group (ascariasis-positive) and a control group. Stool examinations performed by certified external laboratories were used to detect A. lumbricoides eggs. Psycho-neurological symptoms were assessed using a validated questionnaire. Associations were analyzed in the total cohort and stratified by age (children vs. adults) and sex using the Mann–Whitney U test.

Results: Individuals infected with A. lumbricoides exhibited a significantly higher prevalence of all evaluated symptoms compared with controls. Ascariasis was associated with increased rates of simple motor tics, simple vocal tics, compulsive habits, and inattention with hyperactivity. Symptom intensity was greater among women and children.

Conclusions: Ascariasis is significantly associated with a broad spectrum of psycho-neurological symptoms in both children and adults in Europe, with higher severity observed in women and pediatric populations. These findings highlight the need to consider neurobehavioral manifestations in the clinical assessment of ascariasis.

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TRANSMISSION DYNAMICS, EPIDEMIOLOGY, AND GLOBAL HEALTH / WATER- AND FOOD-BORNE PARASITIC INFECTIONS

CERCARIAL DERMATITIS: ENVIRONMENTAL DNA–BASED DETECTION AT RECREATIONAL FRESHWATER SITES

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Background and Aims: Cercarial dermatitis (CD; swimmer's itch), caused predominantly by avian schistosomes of the genus *Trichobilharzia*, is an emerging public health problem in freshwater recreational environments. Reliable detection of these parasites in natural bathing sites remains challenging, due to the reliance on labour-intensive snail collection and cercarial shedding. This study aimed to validate our newly developed environmental DNA (eDNA)–based *Trichobilharzia* detection toolkit (qPCR and isothermal amplification methods: LAMP and RAA) in freshwater environments.

Methods: Eight freshwater localities (representing different habitats) with a documented history of CD were sampled three times during the transmission season. Water was filtered using three filter types: Sterivex (0.45 µm) and Sylphium (0.45 µm and 5 µm). eDNA extracts were analysed using qPCR, LAMP, and RAA to detect *Trichobilharzia* spp. At all sites, freshwater snails were also collected and examined for emergent *Trichobilharzia* cercariae as a reference method for contextual comparison.

Results: We detected *Trichobilharzia* eDNA at all study localities at least once during the sampling period. Detection success varied among filtration and analytical workflows, with the combination of Sylphium 5 µm filters and qPCR providing the most consistent results across field samples.

Conclusions: This study demonstrates that eDNA–based molecular approaches enable sensitive and reliable detection of *Trichobilharzia* spp. at freshwater sites affected by CD. Validation of both qPCR and isothermal amplification methods supports their use in routine surveillance for parasite presence, improving the assessment of CD risk at natural bathing sites.

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TRANSMISSION DYNAMICS, EPIDEMIOLOGY, AND GLOBAL HEALTH / WATER- AND FOOD-BORNE PARASITIC INFECTIONS

DETECTION OF CRYPTOSPORIDIUM SPP. IN ENVIRONMENTAL SAMPLES FROM COASTAL BIRDS IN THE VALPARAÍSO REGION, CHILE DETECTION OF CRYPTOSPORIDIUM SPP. IN ENVIRONMENTAL SAMPLES FROM COASTAL BIRDS IN THE VALPARAÍSO REGION, CHILE

Laura Casoni-Miranda, Anaís France-Calfueque, Sofía Jara-Méndez, Nikita Enciso, Juliette Brante-Bernier, Fernando Fredes, Claudio Abarca, Patricio Retamal, Galia Ramírez-Toloza
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Background and Aims: Cryptosporidium spp. are gastrointestinal protozoa with zoonotic potential and a wide range of hosts, frequently transmitted through contaminated water. The epidemiologic role of shorebirds as potential reservoirs in Chilean coastal environments is limited. The aim of this study was to determine the positivity rate of Cryptosporidium spp. in coastal birds in the Valparaíso Region, Chile. This study offers an opportunity to understand the relationship between Cryptosporidium spp., shorebirds, and the potential role of shorebirds in the transmission of the agent.

Methods: A total of 290 environmental samples from eight localities across five communes in the Valparaiso region of Chile were analyzed using the modified Ziehl-Neelsen technique. Cryptosporidium spp. oocysts were identified microscopically by size, color, form, and the presence of sporozoites.

Results: The overall positivity rate was 11.7%. Algarrobo had the highest positivity rate, followed by Zapallar, Valparaiso, and San Antonio. Prevalence in penguins was significantly higher (26.3%) than in other bird species (5%).

Conclusions: This study reports the occurrence of Cryptosporidium spp. in wild shorebirds in the Valparaíso Region, Chile. Positivity rates were higher than the worldwide average.

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TRANSMISSION DYNAMICS, EPIDEMIOLOGY, AND GLOBAL HEALTH / WATER- AND FOOD-BORNE PARASITIC INFECTIONS

ADVANCING ELIMINATION-ORIENTED ENVIRONMENTAL MONITORING OF SCHISTOSOMA MEKONGI USING ENVIRONMENTAL DNA SURVEILLANCE SYSTEM

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Background and Aims: *Schistosoma mekongi* (Sm) remains endemic along the Mekong River in Cambodia despite long-term mass drug administration. As transmission declines, human infection surveys alone may not fully capture ongoing environmental risk. This study evaluated environmental DNA (eDNA) as a complementary molecular surveillance for detecting Sm transmission in riverine ecosystems.

Methods: Water samples were collected from six villages in Kratie Province, Cambodia in March 2025 and analyzed using a multi-marker qPCR assay targeting Sm eDNA. Historical village-level human prevalence data (2020–2023, Kato–Katz) were compared with eDNA detection patterns.

Results: Environmental DNA was detected in multiple villages, with spatial patterns partially aligning with human prevalence. Villages with higher prevalence, such as Koh Chbar (16%), showed higher eDNA detection (42%), whereas villages with lower prevalence, including Achen (1.4%), had lower detection (10%). This suggests environmentally suitable conditions for ongoing transmission that may be reflected in stool-based surveys. Logistic regression identified water temperature as a significant predictor of Sm eDNA detection (OR=0.424, 95%CI:0.208-0.865, $p<0.05$), indicating lower detection at higher temperatures. Proximity to agricultural land showed a positive but non-significant association ($p=0.11$). Model performance was robust ($p<0.05$, pseudo-$R2=0.3$, AUC=0.785), indicating discriminatory ability in identifying environmental suitability.

Conclusions: Environmental DNA surveillance revealed spatial heterogeneity and identified potential environmentally suitable sites even where recent human prevalence was low. These findings indicate

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that environmental monitoring can complement human infection surveys by capturing ecological conditions linked to parasite persistence. Integrating eDNA surveillance into elimination-phase programs may improve identification of residual transmission foci and support more targeted environmental and public health interventions.

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TRANSMISSION DYNAMICS, EPIDEMIOLOGY, AND GLOBAL HEALTH / WATER- AND FOOD-BORNE PARASITIC INFECTIONS

PULSED POWER TREATMENT IS A PROMISING ALTERNATIVE TO FREEZING FOR PREVENTING ANISAKIASIS: EVIDENCE FROM IN VIVO AND IN VITRO TESTS

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Background and Aims: Anisakiasis is a parasitic zoonosis caused by ingestion of raw seafood contaminated with anisakid larvae. Current preventive measures rely mainly on freezing, which compromises fish quality. Pulsed power (PP) technology has recently emerged as a promising alternative; however, conventional evaluation of larval viability based on mechanical stimulation may inaccurately classify temporarily inactivated larvae as dead. This study aimed to verify the inactivation efficacy of PP using in vitro and in vivo tests.

Methods: Anisakid larvae were subjected to PP treatment consisting of 10 pulses of 13 kV and 10 μs pulse width at 0.5 Hz. Larval invasiveness was assessed using an agar penetration assay with four replicates of 10 larvae each. Infectivity was evaluated by intragastric administration of 50 PP-treated or untreated larvae into rabbits (n = 4 per group). Differences between groups were analyzed using the Mann–Whitney U test, and relative risk was calculated.

Results: In the in vitro assay, none of the 40 PP-treated larvae invaded agar, whereas 35/40 untreated larvae did ($p = 0.0202$). In the in vivo model, no PP-treated larvae penetrated the gastric wall; only three non-viable larvae were recovered from the gastric lumen of a single rabbit. Conversely, untreated larvae exhibited extensive gastric wall penetration (mean: 21/50 larvae). This difference was statistically significant ($p = 0.0211$), corresponding to a relative risk of 0.006 (95 % CI: 0.0004–0.0947).

Conclusions: PP treatment completely abolished the invasiveness and infectivity of anisakid larvae in the agar penetration assay and the experimental infection, respectively. These findings validate PP treatment as an effective non-freezing strategy to prevent anisakiasis.

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TRANSMISSION DYNAMICS, EPIDEMIOLOGY, AND GLOBAL HEALTH / WATER- AND FOOD-BORNE PARASITIC INFECTIONS

PARASITOLOGICAL, PHYSIOCHEMICAL, MICROBIOLOGICAL, MINERAL AND HEAVY METAL CONCENTRATION OF FRESH FRUITS AND VEGETABLES BOUGHT FROM LUSADA LOCAL MARKET

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Background and Aims: Vegetables are referred to as fresh edible portions of roots, stems, leaves or fruits of herbaceous plant. Many people from different origin consume vegetables raw or lightly cooked. They are highly nutritious, sources of vitamin, minerals, fibres etc and these are part of our daily diet. In recent time, there have been more production of vegetables and fruits as well as supply in local markets however, the main problem is associated with the quality of supplied vegetables and fruits due to heavy metals, microbiological contamination. The aim of this study is to analyse the parasitological, Microbiological count, heavy metal, and physiochemical investigation of selected fruits and vegetables sold in various markets in Ado Odo Ota Local Government

Methods: Ten different fresh fruits and vegetable samples were gotten, cleaned, oscillated, and centrifuged for the collection of eggs, larva, and oval using modified method of Adejayan and Olajumoke (2014). Microbiological plate count using Nutrient agar and Eosine Methylene Blue agar, microscopic examination for larva, cyst, oval, and eggs, while physiochemical properties were also analysed.

Results: Percentage availability of parasites on collected samples ranges from 20% to 80% while the total coliform count and plate count ranged from $2.1\text{-}71*10^2$ and $4\text{-}56*10^2$ respectively. Furthermore, the isolated parasites includes Ascaris lubricoides, Moniezia spp, Teania spp, Planaria, Unfertilized egg of ascaris; varying concentration of chloride, nitrate, sulphate and phosphate but with no traces of heavy metals.

Conclusions: vegetables and fruits are vehicles for the transmission of disease and other chemicals if not properly washed.

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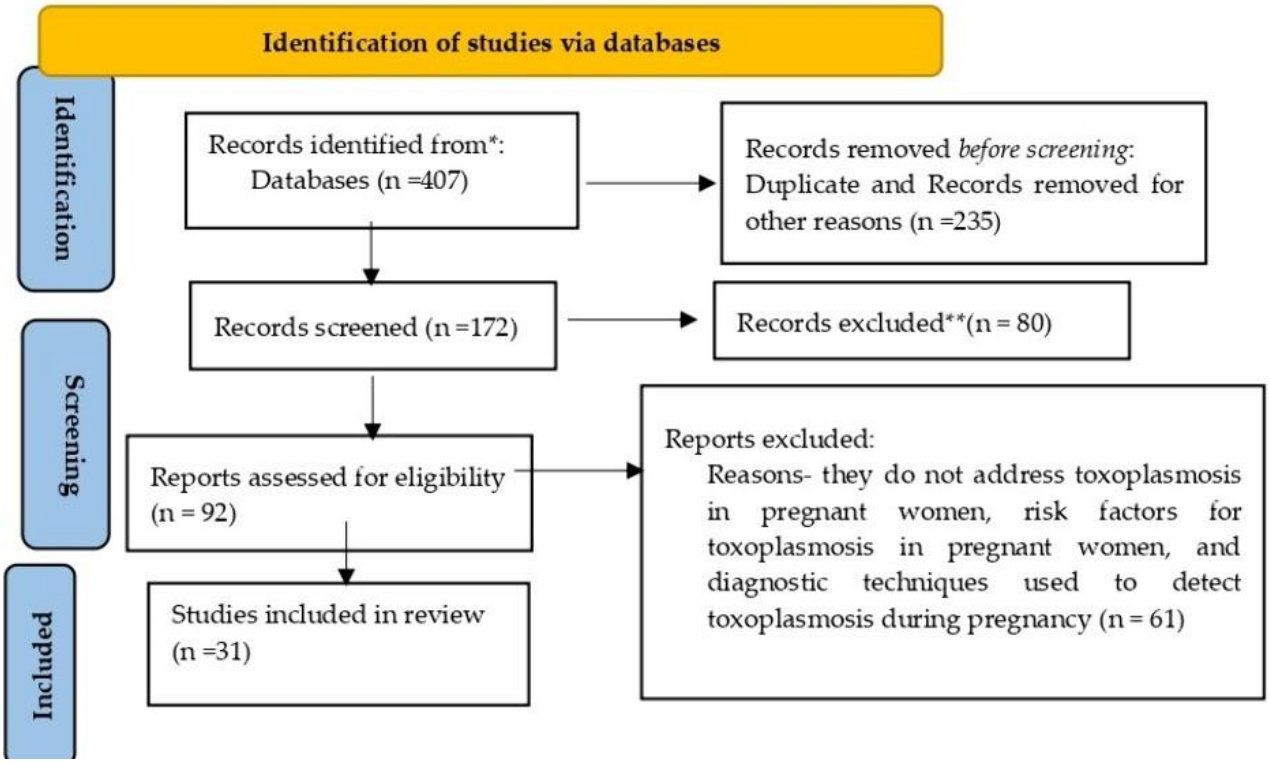
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TRANSMISSION DYNAMICS, EPIDEMIOLOGY, AND GLOBAL HEALTH / WATER- AND FOOD-BORNE PARASITIC INFECTIONS

TRANSMISSION DYNAMICS, EARLY WARNING SIGNS AND DIAGNOSIS OF TOXOPLASMA GONDII INFECTION IN PREGNANCY: A PRISMA-BASED SYSTEMATIC REVIEW

Cherotich Tangus
University of Nairobi, Kenya, Veterinary Pathology, Microbiology And Parasitology, Nairobi, Kenya

Background and Aims: *Toxoplasma gondii* is a globally distributed water- and food-borne zoonotic parasite causing congenital toxoplasmosis with severe foetal consequences. Maternal infections are frequently asymptomatic or present with mild, non-specific signs, limiting early detection in low-resource settings. This systematic review synthesised evidence on early warning signs, transmission-related risk factors, and diagnostic approaches in pregnancy.

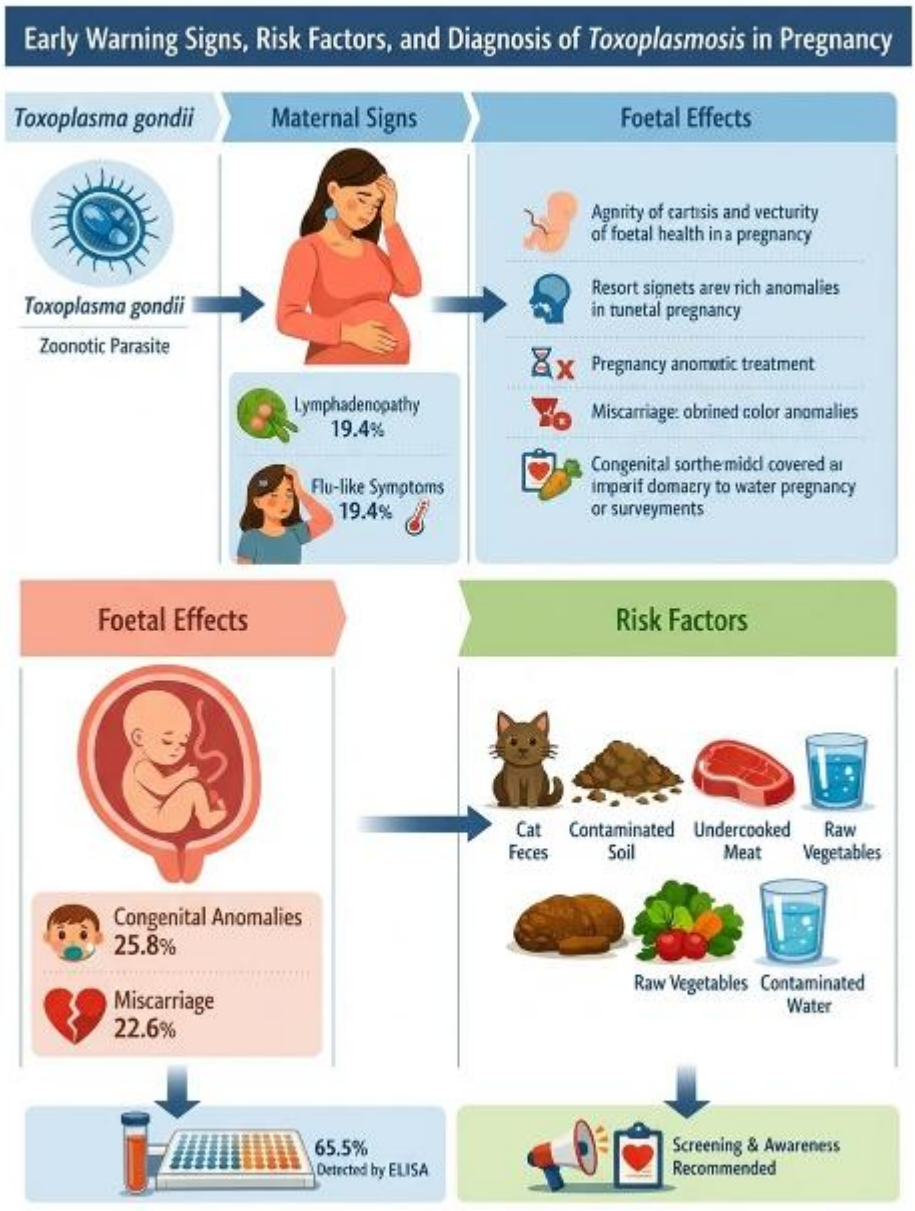
Methods:



The review followed PRISMA 2020 guidelines. Literature searches were conducted in PubMed, Scopus, ResearchGate, and Google Scholar for studies published between 2000 and 2025. Records were managed using Zotero; duplicates were merged. Non-English articles and studies lacking

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reference integrity were excluded. After screening 407 records, 31 studies met the inclusion criteria for analysis.



Results: Major maternal manifestations (lymphadenopathy (19.4%) and flu-like symptoms (19.4%)). Foetal outcomes (congenital anomalies (25.8%) and miscarriage (22.6%)). Key transmission pathways

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(contact with cat faeces and contaminated soil, consumption of undercooked meat, raw vegetables, and contaminated water). Predominant diagnostic method: ELISA (65.5%).

Conclusions: Congenital toxoplasmosis remains a significant global health challenge driven by food- and water-borne exposure and limited screening coverage. Strengthened African regional collaboration, integrated One Health surveillance, and risk-based antenatal screening are essential to reduce maternal–foetal morbidity in high-risk regions.

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TRANSMISSION DYNAMICS, EPIDEMIOLOGY, AND GLOBAL HEALTH / WATER- AND FOOD-BORNE PARASITIC INFECTIONS

IDENTIFYING ENVIRONMENTAL FACTORS INFLUENCING THE CARCINOGENIC FOODBORNE TREMATODE OPISTHORCHIS VIVERRINI AND INTERMEDIATE HOSTS IN NORTHEAST THAILAND.

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Background and Aims: Opisthorchiasis caused by the trematode *Opisthorchis viverrini* remains endemic to Southeast Asia, including Northeast Thailand, posing increased cholangiocarcinoma risk. Control remains challenging due to the parasite's complex lifecycle, with current monitoring methods lacking early detection. Advances in environmental DNA detection, including the application of droplet digital PCR (ddPCR), offer a new, sensitive approach for monitoring parasite presence in environmental matrices. This study aimed to apply a new molecular assay for the environmental detection of *O. viverrini* and intermediate hosts in the Northeast of Thailand.

Methods: We developed and validated a multiplex ddPCR workflow before implementation on field samples collected in December 2024. Active and passive water and soil samples were collected from a total of 19 sites within Chaiyaphum province. Biotic factors and abiotic influences on parasite transmission and intermediate host habitat choice were concurrently measured and analysed.

Results: Laboratory validation indicated *O. viverrini* eDNA was readily detectable in water during active cercarial shedding from the snail-intermediate-host (t = 4.391). Field analysis detected the snail-intermediate-host *Bithynia siamensis goniomphalos* within 16/19 sites, a secondary fish-intermediate-host *Barbonymus altus* was detected in 6/19, while 2/19 indicated *O. viverrini* positivity. Biotic factors, such as microbial source tracking indicated faecal contamination as a detectable risk correlated with *O. viverrini* positive sites.

Conclusions: Our findings present a novel eDNA ddPCR assay capable of detecting *O. viverrini* and intermediate hosts concurrently with environmental risk factors. Our results indicate a promising new monitoring approach, and development of a new risk model for *Opisthorchis viverrini*.

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TRANSMISSION DYNAMICS, EPIDEMIOLOGY, AND GLOBAL HEALTH / WATER- AND FOOD-BORNE PARASITIC INFECTIONS

ASSESSMENT OF THE CONTAMINATION BY ZOONOTIC PARASITES OF IMPORTED BERRIES IN FRANCE

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Background and Aims: Fresh fruits and vegetables can be a vehicle for parasites such as protozoan or Taeniidae, with estimated contamination rates between 1.9-9.3% and 1-19%, respectively. France imports annually several tons of berries coming from endemic regions, such as North-Africa or Eastern Europe. The aim of this study was to investigate the contamination rates of some imported berries in France for significant helminths (i.e. *Echinococcus* spp., *Taenia* spp. and *Toxocara* spp.) and protozoans (i.e. *T. gondii, Cryptosporidium* spp. and *Giardia* spp.).

Methods: In total, 74 imported berry batches, including 43 for fresh berries (i.e. blueberry and strawberry) and 19 for dates, were purchased at supermarkets. In addition, 12 frozen berry batches were only analysed for helminths detection. Several samples (each weighed 100g or 200g) per batch were analysed for the detection of helminths and protozoans.

Results: No contamination by *E. granulosus* sensu lato, *E. multilocularis* or *Giardia* spp. was observed. *Cryptosporidium* spp. and *T. gondii* were respectively detected in 9.6% (5/52) and 3.8% (2/52) of the samples. Three helminth species have been identified: *Taenia pisiformis*, *T. hydatigena* and *Hydatigera taeniaeformis* for a global rate of 5.4% (4/74).

Conclusions: This study highlights the presence of parasitic contamination on imported berries in France as it may be the case in many others European countries. Moreover, the identified *Taenia* species have parasitic cycles similar to *Echinococcus*, making contamination by these species a possibility. However, the viability of the parasites couldn't be evaluated, preventing to go further in risk assessment.

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TRANSMISSION DYNAMICS, EPIDEMIOLOGY, AND GLOBAL HEALTH / HIGH-BURDEN DISEASES: MALARIA, TRYPANOSOMIASIS, SCHISTOSOMIASIS, AMOEBIASIS, LEISHMANIASIS

AN OVERLOOKED PUBLIC HEALTH THREAT: FEMALE GENITAL SCHISTOSOMIASIS IN KOGI STATE, NORTH-CENTRAL NIGERIA

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Background and Aims: Female Genital Schistosomiasis remain a poorly recognized public health problem in Kogi State and Nigeria at large. This study assessed socio-demographic status, knowledge of schistosomiasis and FGS, and prevalence of FGS among reproductive females aged 15–49 years from 13 communities in of Kogi State, Nigeria. This cross-sectional study was conducted between the month of June 2024 and May 2025. The aim of the study was to determine the prevalence of female Genital Schistosomiasis in Kogi state, Nigeria

Methods: Structured questionnaires and clinical examination (colposcopy) were used in this study while 686 females participated in the questionnaire, only 222 among them consented to the clinical examination.

Results: This study shows that majority of participants were under 40 years and predominantly married (68.8%). Educational attainment was low, with (22.7%) having no formal education. Most women engaged in informal occupations such as business (43.7%) and farming (26.7%) and (89.6%) were low-income earners. Behavioural risk factors revealed high-risk exposure. Knowledge of schistosomiasis (14.3%) and FGS (4.1%) was low, only (11.2%) knew FGS could be prevented or treated. Clinical examination revealed high FGS prevalence (80.2%), most common lesions observed were abnormal blood vessels (51.8%), homogenous sandy patches (24.8%) and grainy sandy patches (23.4%). Statistical analysis shows no significant association with age as ($p = 0.297$).

Conclusions: This study reveals a high burden of FGS among participants and low awareness of the disease was observed.The findings show the urgent need for integrated control strategies, including community-based health education and inclusion of women of reproductive age in schistosomiasis control programs.

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TRANSMISSION DYNAMICS, EPIDEMIOLOGY, AND GLOBAL HEALTH / HIGH-BURDEN DISEASES: MALARIA, TRYPANOSOMIASIS, SCHISTOSOMIASIS, AMOEBIASIS, LEISHMANIASIS

PREVALENCE AND ASSOCIATED FACTORS OF CO-INFECTION OF URINARY SCHISTOSOMIASIS AND MALARIA AMONG CHILDREN AGED 3 TO 14 YEARS IN KLOUEKANME, SOUTHERN BENIN

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Background and Aims: Urinary schistosomiasis–malaria co-infection represents a significant health risk for children in sub-Saharan Africa, yet remains understudied in endemic settings such as Benin. This study assesses the prevalence and determinants of co-infection among children aged 3–14 years in rural Klouékanmè, southern Benin.

Methods: A cross-sectional study was conducted in August 2024 among 508 children aged 3–14 years in the Lanta and Ahogbeya villages in the Klouékanmè district, southern Benin. Capillary blood and urine samples were collected for microscopy-based diagnosis of malaria and schistosomiasis, and the prevalence of co-infection and associated factors were analysed using logistic regression

Results: The prevalence of urinary schistosomiasis and malaria co-infection was 15.8% (77/487; 95% CI: 13.0–19.0), with malaria was 61.3% (299/488) and schistosomiasis 24.3% (122/502). Significant associations were found with residence in Lanta compared to Ahogbeya (OR = 3.37; 95% CI: 1.7–6.30), frequent exposure to swamps (OR = 10.53; 95% CI: 3.22–64.88), presence of hematuria (OR = 77.62; 95% CI: 36.81–185.54), use of an unprotected water source (OR = 1.95; 95% CI: 1.12–3.56), and being over five years old compared to under five (OR = 3.22; 95% CI: 1.53–7.91).

Conclusions: Despite existing control strategies, malaria–schistosomiasis co-infection remains highly prevalent, exceeding rates reported in Senegal (1.1% in 2023), Nigeria (3.3% in 2016), and Gabon (9% in 2017). Its association with multiple risk factors highlights the need for integrated, cross-strategy approaches within global health frameworks to support elimination efforts.

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TRANSMISSION DYNAMICS, EPIDEMIOLOGY, AND GLOBAL HEALTH / HIGH-BURDEN DISEASES: MALARIA, TRYPANOSOMIASIS, SCHISTOSOMIASIS, AMOEBIASIS, LEISHMANIASIS

TEMPORAL ANALYSIS AND DISTRIBUTION OF LEISHMANIASIS BASED ON BIOMES IN BRAZIL

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Background and Aims: Leishmaniasis has been reported in all macro regions of Brazil, requiring epidemiological surveillance to contain the spread of these diseases. Analyses based on biomes provide a less fragmented view than usual studies and help understand epidemiological characteristics from different perspectives. Herein, we describe the temporal trends and spatial distribution of American cutaneous leishmaniasis (ACL) and visceral leishmaniasis (VL) in Brazilian biomes from 2007 to 2020.

Methods: This ecological survey used data from the Notifiable Diseases Information System. The Prais–Winsten method was used for temporal analyses. The incidences of ACL and VL were calculated based on sex and age groups, relative risk considering these two variables, and mortality, lethality, cure, and treatment abandonment rates per biome.

Results: The Amazon, Atlantic Forest, Cerrado, and Caatinga biomes showed a decreasing temporal trend, varying from −1% to −3% for ACL and VL, respectively. In other biomes, a stationary temporal trend was observed for both diseases. VL presented a similar transmission profile in all biomes, affecting children aged 0–4 years regardless of gender. In contrast, ACL mainly affected the elderly, except in the Amazon and Pampa biomes, where working-age males were most commonly affected. In all biomes, being a male was a risk factor for acquiring these diseases, abandoning treatment, and dying, whereas being a female was a protective factor against lethality and for cure.

Conclusions: The findings indicate a slight reduction in the incidence of ACL and VL in most of these biomes, and stability in the incidence in the others.

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UNDERSTANDING INVASION MECHANISMS IN ZOONOTIC PLASMODIUM KNOWLESI

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Background and Aims: Malaria remains a major global health burden, with over 282 million cases reported in 2024 according to the World Health Organization. While human malaria is predominantly caused by *Plasmodium falciparum* and *P. vivax*, zoonotic species such as *P. knowlesi* – a simian malaria parasite, has emerged as a significant zoonotic threat across Southeast Asia. Its ability to invade both human and macaque erythrocytes suggest adaptive mechanisms that facilitates cross-species transmission. Here, we investigate the invasion mechanisms of *P. knowlesi* strain A1-H1, which is adapted for culture in both human and macaque erythrocytes, using antibody-blocking invasion assays and erythrocyte digestion assays.

Methods: Briefly, human and macaque erythrocytes were treated with various enzymes to selectively remove surface sialic acid residues. Parallel antibody blocking invasion assays of known invasion ligands and putative host cell receptors were also carried out to assess invasion efficiency and sialylation-dependent interactions. Invasion rates were then quantified using flow cytometry and microscopy.

Results: Our preliminary data show a key role for erythrocyte sialylation in parasite entry and show that Pk A1-H.1 engages distinct, host-dependent receptor interactions during species-specific invasion.

Conclusions: Understanding these mechanisms may provide insights into how simian malaria parasites are able to infect human hosts.

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THE SCHISTOSOME AND SNAIL RESOURCE (SSR) – ENABLING ACCESS TO SCHISTOSOMIASIS MATERIAL AND CURRENT RESEARCH

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Background and Aims: Schistosomiasis is a parasitic disease transmitted via freshwater snails. Maintaining *Schistosoma* lifecycles and snail hosts requires specialised facilities, expertise and cost, resulting in very few resources worldwide. The Schistosome Snail Resource (SSR) is a Wellcome Trust–funded open access biomedical resource that supplies live and preserved schistosomes and snail material for applied and clinical research.

Methods: We currently maintain life-cycles of standard *S. mansoni*, *S. haematobium* and their snail hosts as well as maintaining less common African snail species. SSR also coordinates and contributes to interdisciplinary projects across diagnostics, ecology, behaviour, digitisation and education, positioning the resource centrally within global schistosomiasis research.

Results: We present some SSR-based projects include schistosome-snail compatibility, machine learning approach to study behaviour of infected snails, digitisation and educational tools for intermediate snail host identification.

Conclusions: By maintaining unique parasite life-cycles, providing specialist facilities and fostering collaborative networks, SSR enhances access to biomaterial and enables novel research directions in schistosomiasis. Our laboratory programmes and external partnerships aim to accelerate translational research and training. We hope that our laboratory-based projects as well as our external partnerships have helped to pave the way for new avenues in schistosomiasis research.

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EVALUATION OF THE MALARIA CONTROL STRATEGY FOR PREGNANT WOMEN IN BENIN

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Background and Aims: Gestational malaria remains a major public health issue in Africa, notably Benin. The WHO recommends a preventive strategy based on the use of long-lasting insecticidal nets (LLINs) throughout pregnancy and intermittent preventive treatment (IPTp) with sulfadoxine-pyrimethamine (SP) from the second trimester onward. To assess the implementation of this strategy among pregnant women in Benin by estimating key indicators and identifying associated factors.

Methods: A cross-sectional observational study was conducted from May to October 2023. A total of 1,829 women at their first antenatal care visit (ANC1) and 1,734 parturients were included using stratified sampling in public and private maternity facilities across three regions (North, Center, South), in both rural and urban settings. Data were collected via digitalized questionnaires and analyzed using mixed multivariate logistic regression models.

Results: 72.0% of women received an LLIN at ANC1, but only 40.7% actually used it. Women in the South were much less likely to receive one compared to those in the Center (OR=0.04; 95% CI [0.01–0.11]), as were urban women compared to rural women (OR=0.12; 95% CI [0.05–0.28]). Use was more frequent in public facilities (OR=13.2; 95% CI [1.52–114]) and among sensitized women (OR=1.99; 95% CI [1.22–3.25]). Regarding IPTp, only 48.3% received 3 or more doses. Coverage was higher among urban women, those in public maternity facilities, and those who attended 4 or more ANC visits (OR=69.1; 95% CI [44.4–108]).

Conclusions: Preventive coverage remains insufficient. Better integration of the private sector and strengthened antenatal care are needed to achieve the universal coverage recommended by the WHO.

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QUANTIFYING THE EFFECTS OF PRAZIQUANTEL TREATMENT ON SCHISTOSOMA INFECTION DYNAMICS USING IMPERFECT DIAGNOSTICS AND SIBSHIP ANALYSIS.

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Background and Aims: Schistosomiasis is a debilitating parasitic disease that infects 230 million people. While mass drug administration (MDA) has decreased disease burden, there is growing evidence of persistent treatment failure. Investigating drivers of treatment failure is difficult without measures of 'true' infection status and understanding of infection dynamics. Due to the inaccessible anatomical locations of worms, indirect diagnostics are used as infection status and intensity proxies. However, differences in specificity and sensitivity cause discrepancies between observed egg (Kato-Katz) and antigen-based (POC-CCA) diagnostic levels. These are enhanced post-treatment, affecting drug monitoring and evaluation efforts and our understanding of infection dynamics.

Methods: We combine longitudinal Kato-Katz and POC-CCA, previously collected from high- and low-endemicity settings in Uganda, with stool quantitative PCR and miracidia hatching success to parameterise newly-developed HMMs predicting infection status and differentiating between infected individuals shedding or not shedding parasites. We also perform microsatellite genotyping on hatched miracidia, generating multilocus genotypes for sibship analysis, to predict worm burdens (infection intensity) and differentiate whether post-MDA infections are caused by new infections or worms surviving MDA.

Results: Preliminary findings from a high-endemicity setting indicates MDA temporarily decreases prevalence and shedding, however, re-infection rates are high six months post-MDA. We now aim to measure prevalence and shedding rates pre/post-MDA in a low-endemicity setting and apply sibship analysis to measure infection intensities and observe infection dynamics across endemicity and time.

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Conclusions: This longitudinal study will lay the groundwork for exploring drivers of diagnostic discrepancies and treatment failure, informing epidemiological models and understandings of how MDA impacts *Schistosoma* infection dynamics.

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PERMISSIVE INTERSPECIFIC INTERACTIONS BETWEEN SYMPATRIC LEISHMANIA (VIANNIA) BRAZILIENSIS AND LEISHMANIA (VIANNIA) NAIFFI MAINTAIN PARASITE FITNESS IN VITRO

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Oswaldo Cruz Foundation, Oswaldo Cruz Institute, Rio de Janeiro, Brazil

Background and Aims: The maintenance of microbial communities relies on interactions among their components, yet such interactions remain poorly explored in *Leishmania*. In endemic regions of Brazil, *Leishmania (Viannia) braziliensis* and *Leishmania (Viannia) naiffi* circulate sympatrically, raising the question of whether interspecific interactions impact parasite fitness.

Methods: We evaluated the growth dynamics of *L. braziliensis* and *L. naiffi* in monocultures and co-cultures using Schneider's medium 20% FBS. Parasite densities were monitored daily for 10 days and quantified by species-specific qPCR targeting the G6PD gene with TaqMan probes. Growth kinetics were analyzed using $\log_{10}$-transformed values. Metacyclic promastigotes were quantified during stationary phase using *Bauhinia purpurea* lectin, and their species origin in co-cultures was determined by qPCR. Clump formation was assessed using fluorescently transfected parasites cultured with or without human serum.

Results: Both species maintained sustained growth in co-culture despite being initiated with half the inoculum used in monocultures. Parasites quantification revealed comparable overall population dynamics between mono- and co-cultures, with reduced amplitude of daily fluctuations in co-cultures and no evidence of competitive exclusion. *L. braziliensis* consistently reached lower parasite densities than *L. naiffi* but displayed higher proportions of metacyclic forms under both culture conditions. Clump formation occurred independently of human serum concentration, and in co-cultures, aggregates were consistently interspecific.

Conclusions: The interaction between *L. braziliensis* and *L. naiffi* is not deleterious to either species, supporting a permissive, non-antagonistic interaction that enables stable coexistence. Such sustained physical association may also create opportunities for genetic exchange between these sympatric species, potentially contributing to their evolutionary dynamics

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GEOSPATIAL INTELLIGENCE AND NEXT GENERATION SURVEILLANCE: A WEB-BASED SURVEILLANCE FRAMEWORK FOR MALARIA MAPPING AND HAZARD ANALYSIS IN IMO STATE, NIGERIA

Sylvia Egbom[1], Martin Iwuji[1], Victor Okorondu[1], Ikechukwu Chigozie[2], Alexander Opara[1], Chris Nwoko[1], Gloria Chukwudebe[1], Joseph Ikechukwu Nwachukwu[3], Sidney Nzeako[4], Florence Nduka[4]
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Background and Aims: Malaria remains a major public health challenge in Nigeria, which bears the highest global malaria burden. Effective control requires surveillance systems capable of capturing spatial and temporal transmission patterns. However, malaria surveillance in many Nigerian states is constrained by limited geospatial and predictive tools. This study presents a web-based, GIS-driven surveillance framework for malaria mapping and hazard analysis in Imo State, Nigeria.

Methods:

To ensure the AHP model for **Malaria Risk Assessment** is mathematically sound, the **Priority Weights** (normalized importance) were calculated and the **Consistency Ratio (CR)** for the values assigned.
CI=CONSISTENCY INDEX; λmax = the Principal Eigenvalue of pairwise comparison matrix
A **CR < 0.10 (10%)** indicates that the pairwise comparisons are consistent and the model is reliable.

1. Hazard Factors (Environmental Suitability)

Priority order: Temperature > Precipitation > Drainage > Elevation > Slope.

Factor	Priority Weight
Temperature	42.40%
Precipitation	27.20%
Drainage Network	17.50%
DEM (Elevation)	7.80%
Slope (Degrees)	5.10%

- **Consistency Check:** $\lambda_{max} = 5.10, CI = 0.025, CR = 0.022$ **(2.2%)**
- **Status:** ✓ Consistent (Pass)

2. Vulnerability Factors (Human Exposure & Access)

Priority order: Pop. Density > Proximity to PHCs > Proximity to Roads.

Factor	Priority Weight
Population Density	68.10%
Proximity to PHCs	20.10%
Proximity to Roads	11.80%

- **Consistency Check:** $\lambda_{max} = 3.02, CI = 0.012, CR = 0.021$ **(2.1%)**
- **Status:** ✓ Consistent (Pass)

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3. Elements at Risk (Land Cover Exposure)

Priority order: Built-up > Crops > Water Bodies > Trees > Bare Ground

Factor	Priority Weight
Built-up Areas	43.60%
Range Land & Crops	27.20%
Water Bodies	16.00%
Trees	8.30%
Bare Ground	4.90%

- **Consistency Check:** $\lambda_{max} = 5.05, CI = 0.013, CR = 0.012$ **(1.2%)**
- **Status:** ✓ Consistent (Pass)

Conclusion for GIS Model

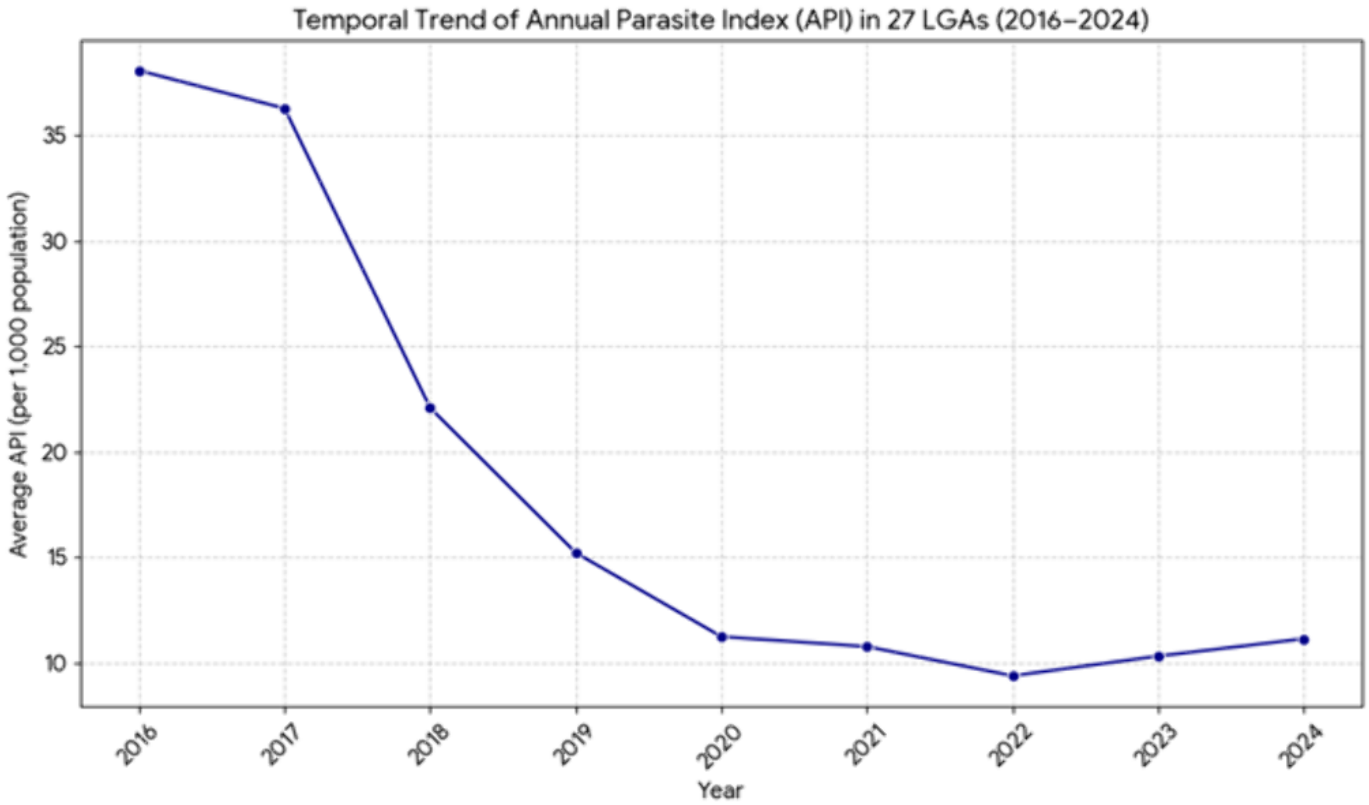
The **Priority Weights** were used to perform a **Weighted Overly Analysis** in GIS. For example, the final Malaria Hazard map formula:

Hazard = (Temp * 0.424) + (Precip * 0.272) + (Drainage * 0.175) + (DEM * 0.078) + (Slope * 0.051)
Vulnerability = (Pop. Density * 0.681) + (Proximity to PHCs * 0.201) + (Proximity to Roads * 0.118)
Risk = (Built Up Areas * 0.436) + (Range land & Crops * 0.272) + (Water Bodies * 0.16) + (Trees * 0.0830) + (Bare Ground * 0.049))

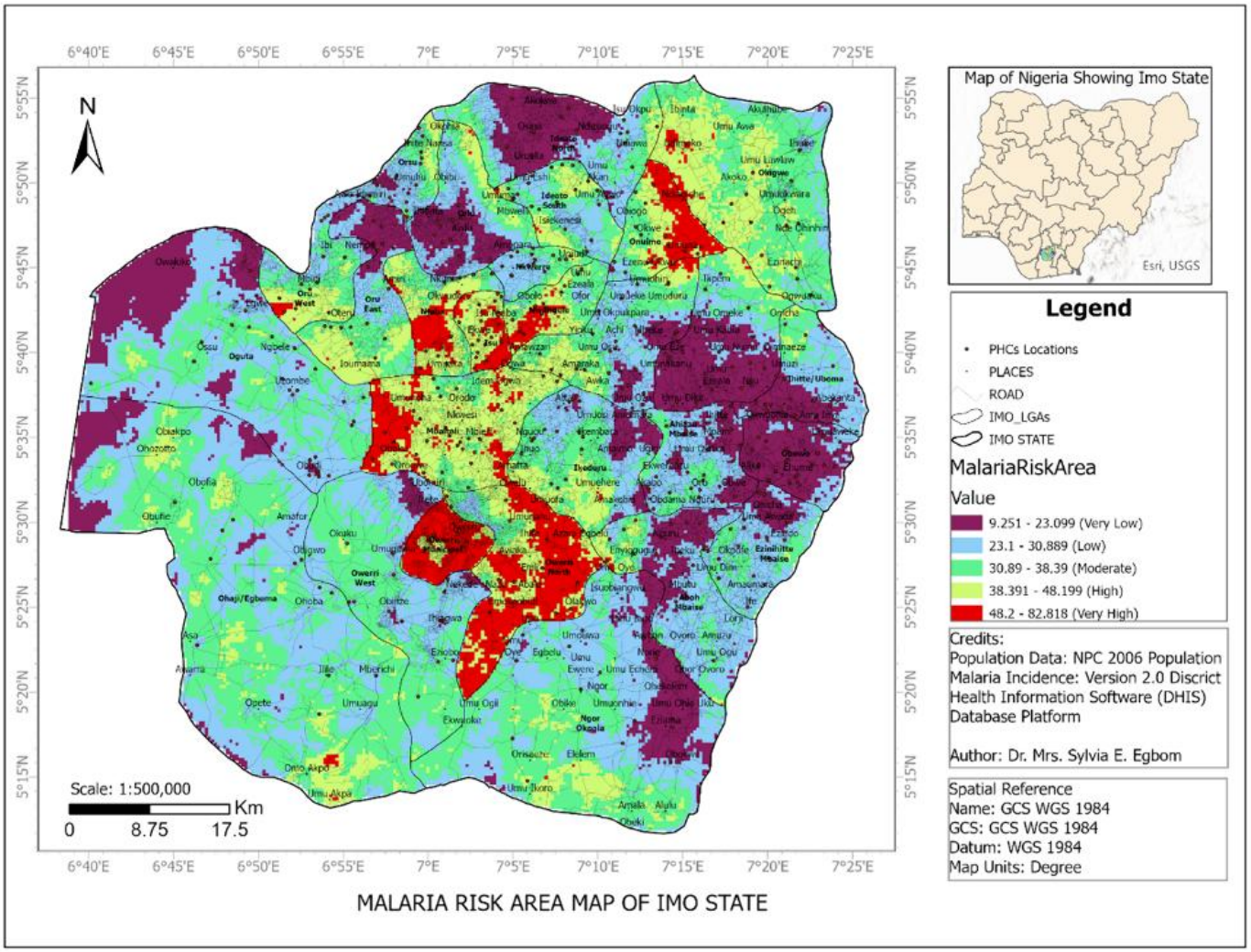
("Reclass_TEMP_new" * 0.424) + ("Reclass_Preci_new" * 0.272) + ("Reclass_Drainage_new" * 0.175) + ("Reclass_DEM_new" * 0.078) + ("Reclass_Slope_new" * 0.051)
MALARIA RISK AREA = HAZARD * VULNERABILITY * ELEMENT AT RISK

Malaria Annual Parasite Incidence data from 2016–2024 were aggregated at the Local Government Area (LGA) level and analyzed alongside environmental and vulnerability-related spatial factors. Spatiotemporal mapping was conducted to assess geographic and temporal trends in transmission. A Multi-Criteria Analysis–Geographic Information System (MCA-GIS) approach, incorporating the Analytical Hierarchy Process (CR < 0.10), was applied to generate a composite malaria risk map.

Results:



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The analysis revealed significant spatial heterogeneity in malaria incidence ($p < 0.05$), with Owerri-Municipal, Orlu, Orsu, and Obowu LGAs identified as persistent hotspots. Average API declined markedly from approximately 20 per 1,000 in 2016 to about 5 per 1,000 in 2020, followed by a slight increase between 2021 and 2024. The MCA-GIS model classified 21.0% of the state as high to very high malaria risk. Strong spatial agreement between observed incidence patterns and modelled risk zones supports the robustness of the framework.

Conclusions: The primary output is an interactive, open-access malaria risk web map hosted on ArcGIS Online. The framework is scalable and demonstrates the value of geospatial intelligence and web-based GIS technologies for strengthening malaria surveillance and guiding targeted interventions in Nigeria and similar endemic settings.

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CLIMATE DRIVEN DYNAMICS OF CUTANEOUS LEISHMANIASIS IN A MOROCCAN REGION

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Background and Aims: Cutaneous leishmaniasis (CL) remains a major public health concern in Morocco, where environmental and climatic changes increasingly influence transmission. While historically confined to established endemic foci, recent trends indicate expansion into regions such as Casablanca–Settat. We aimed to characterize the spatiotemporal dynamics of CL and assess their relationship with environmental and climatic factors.

Methods: We analyzed CL surveillance data from the Moroccan Ministry of Health and Social Protection, integrating demographic, environmental, and climate variables, including temperature, precipitation, and vegetation indices. Spatiotemporal patterns, seasonal trends, and demographic distributions were explored using Geographic Information Systems (GIS) and statistical approaches. We also compared multiple machine learning models to predict CL incidence in the El Brouj focus, assessing their performance using standard metrics.

Results: CL incidence increased steadily until 2019, followed by a sharper rise. All cases were attributed to *Leishmania tropica*. Higher incidence was observed in females and children aged 5–14 years. A clear seasonal pattern was identified, with peaks in spring. Spatial analysis highlighted El Brouj as the main and persistent hotspot. CL incidence was significantly associated with climatic factors, particularly lower temperatures and precipitation. Among the tested machine learning models, linear regression showed the best predictive performance.

Conclusions: By combining spatiotemporal analysis and machine learning, this study improves understanding of CL dynamics in an emerging Moroccan region. We believe that climate-informed models could help anticipate risk and support more proactive, targeted, and data-driven public health interventions.

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SPATIAL, AGE-RELATED, AND SEASONAL DYNAMICS OF UROGENITAL SCHISTOSOMIASIS AMONG RIPARIAN INDIGENES IN THE ASE-NIGER RIVER BASIN, DELTA STATE, NIGERIA.

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Background and Aims: Urinary schistosomiasis remains a major public health challenge in endemic regions of Sub-Saharan Africa. This study assessed the spatial distribution, age-specific prevalence, seasonal dynamics, and gender specific prevalence and infection intensity in eleven communities along the Ase River catchment, Delta State, Nigeria.

Methods: This study employed a longitudinal study design for parasitological investigations using swinnex urine filtration techniques. Prevalence data were mapped using Quantum GIS software, capable of visualization, analysis, and interpretation of geospatial data like prevalence and intensity.

Results: A total of 7,219 participants were screened across six age groups (<10, 11–20, 21–30, 31–40, 41–50, >51 years), of which 3,472(48.10%) were found infected. Spatial analysis revealed significant clustering of infections (Moran's I = 0.46, Z = 3.11, $p < 0.01$), with Ashaka, Igbuku, Lagos-Iyede, and Iyede-Ame identified as hot spots, and Awah and Ivrogbo as cold spots. Age-specific prevalence peaked in children (<10 years; 57.7%) and adolescents (11–20 years; 59.2%) with ensuing decline as the age increased. Parasite load was highest in <10 years (5,125 eggs/10 ml, mean 5.02 eggs/10 ml) and 11–20 years (4,472 eggs/10 ml, mean 4.14 eggs/10 ml), with heavy infection intensity observed across all age groups. Seasonal variation in prevalence was minimal, except in Awah and Ibedeni. Prevalence strongly correlated with parasite load ($r = 0.963$, $p = 0.002$, $R^2 = 0.927$).

Conclusions: These findings demonstrate heterogeneous transmission patterns influenced by hydrological and ecological gradients along the river. Targeted interventions focusing on identified hotspots and high-risk age groups are recommended.

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THE BURDEN OF CHAGAS DISEASE IN CHILE, 2007-2021: TRENDS IN MORTALITY, DISABILITY, AND DALYS.

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Background and Aims: After the interruption of domiciliary vector transmission in Chile (1999), Chagas disease (CD) is increasingly expressed as a chronic condition with long-term cardiac and digestive sequelae. We quantified the national burden of chronic CD in Chile (2007-2021) and described temporal, age- and sex-specific patterns under alternative structural assumptions.

Methods: Nationwide retrospective burden of disease study linking confirmed chronic CD notifications with mortality and hospital discharge microdata from the Department of Health Statistics and Information (DEIS) using the national identifier (RUT). Annual Years of Life Lost (YLL), Years Lived with Disability (YLD), and Disability-Adjusted Life Years (DALYs) were estimated under alternative attribution scopes, life-table frameworks, and clinical care-mix profiles (α scenarios), with sensitivity analyses for disability weights and discounting.

Results: From 2007 to 2021, Chile accumulated 7,565.6 DALYs attributable to chronic CD (INE), dominated by YLD 6,627.4 (82.6%) and YLL 938.3 (17.4%). The fatal share declined from 55.4% (2007) to 5.9% (2021). Annual DALYs increased from 19.75 (2007) to 1,051.96 (2021), driven by the progressive accumulation and ageing of the chronically infected population and the rise in non-fatal burden (YLD 8.8 to 989.9). YLL concentrated in ages 55–74 (57.5%) with marked male predominance (78.15%). Disability was essentially cardiac: heart failure 50.8% (3,366.9 YLD) and arrhythmias 29.2% (1,937.1 YLD); digestive complications contributed 20.0% (1,323.3 YLD).

Conclusions: These findings support policy beyond transmission control towards integrated chronic Chagas care, focusing on cardiomyopathy management, rehabilitation, and disability prevention. Surveillance and prevention efforts should be strengthened for non-vector routes.

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MISIDENTIFICATION, UNDER ESTIMATION AND BURDEN OF PLASMODIUM MALARIAE AND PLASMODIUM OVALE SPECIES IN LOW-TRANSMISSION SETTING TARGETED FOR ELIMINATION IN ETHIOPIA

Hallelujah Getachew Gebreyohannes[1], Chloe Wang[2], Kassahun Habtamu[3], Assalif Demissew[4], Teshome Degefa[1], Solomon K. Birhanie[5], James Kazura[6], Christopher King[6], Guiyun Yan[2], Delenasaw Yewhalaw[1]
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Background and Aims: Malaria caused by *Plasmodium malariae* and *P. ovale sensu lato* (*s.l*) infection, has been largely overlooked in Ethiopia due to their milder clinical symptoms and lower prevalence as compared to *P. falciparum* and *P. vivax*. This study aimed to determine the prevalence of *P. malariae* and *P. ovale* infections misdiagnosed as *P. vivax* in selected health facilities in southwestern Ethiopia.

Methods: A cross-sectional study was conducted on study participants who were positive for *P. vivax* by microscopy or rapid diagnostic test (RDTs) and attending health facilities. A total of 244 *P. vivax* positive samples were re-evaluated using quantitative polymerase chain reaction (qPCR).

Results: All 244 cases were re-confirmed *Plasmodium* positive by qPCR however, none of the tested samples were reported as *P. malariae* or *P. ovale* by microscopy or RDTs. Of these 244 PCR-confirmed cases, 214 (87.7%, 95% CI: 82.91-91.55) were positive for *P. vivax*, 9 (3.7%, 95% CI: 1.70-6.88) for *P. falciparum*, 8 (3.3%, 95% CI: 1.42-6.36) for *P. malariae*, 7 (2.8%, 95% CI: 1.16-5.82) had mixed infections (*P. falciparum* and *P. vivax*), and 6 (2.5%, 95% CI: 0.91-5.27) were positive for *P. ovale*.

Conclusions: The findings underscore the need for a strategic shift to integrate *P. malariae* and *P. ovale s.l* into Ethiopia's malaria control and elimination agenda. Strengthening diagnostic capacity, including training of miscroscopists and deployment of highly accurate diagnostic tools capable of detecting all *Plasmodium* species at lower levels of the primary health care system in resource-

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limited settings, is essential to strengthen malaria control and accelerate progress toward elimination.

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TRANSMISSION DYNAMICS, EPIDEMIOLOGY, AND GLOBAL HEALTH / HIGH-BURDEN DISEASES: MALARIA, TRYPANOSOMIASIS, SCHISTOSOMIASIS, AMOEBIASIS, LEISHMANIASIS

IMPACT OF HOST MICRONUTRIENT STATUS AND PRE-TREATMENT FOOD TYPE ON PRAZIQUANTEL-RELATED ADVERSE EVENTS IN SCHOOL-AGED CHILDREN IN CÔTE D'IVOIRE: A RANDOMISED-CONTROLLED TRIAL

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Background and Aims: Mass drug administration (MDA) using praziquantel is the cornerstone of schistosomiasis control. However, fear of adverse events contributes to treatment refusal and suboptimal MDA coverage. The primary aims of this study are to examine the impact of the micronutrient status and the co-administration of different food types on the occurrence and severity of praziquantel's adverse events.

Methods: This study is embedded within a larger randomised controlled trial in Côte d'Ivoire of 840 school-aged, *Schistosoma*-infected children. Children's micronutrient status for vitamin A, vitamin D, and iron pre-treatment will be assessed, before random assignment into three different food arms, receiving either a standardised high-carbohydrate, or high-fat meal, or instructed to eat at home following standard MDA procedures. Following praziquantel treatment (40 mg/kg), the occurrence and severity of observed and reported adverse events recorded after swallowing praziquantel will be compared across micronutrient statuses and between food groups.

Results: We will present findings on the associations between micronutrient deficiency and the occurrence and severity of drug-related adverse events and what type of food co-administered with praziquantel can best mitigate adverse events.

Conclusions: Our findings will help inform how best to mitigate praziquantel-associated adverse events by identifying micronutrient deficiencies that could potentially benefit from supplementing existing feeding programmes in endemic areas and optimising the type of food co-administered with praziquantel. This could help improve MDA uptake and performance, contributing towards the WHO goal of eliminating schistosomiasis as a public health problem by 2030.

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PERFORMANCE OF PARTICIPANTS IN THE MALARIA MICROSCOPIC PROFICIENCY TESTING PROGRAM IN VIETNAM

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Background and Aims: Malaria is a serious infectious disease requiring exactly diagnosis and effective treatment. Because microscopic examination of thick and thin blood films remains the gold standard for laboratory diagnosis, the performance of technician plays a crucial role in quality of the diagnosis.

Methods: A retrospective research was used to describe the quality of participants in the malaria microscopic proficiency testing program in Vietnam.

Results: The result of 3 recent years shown that participants were predominantly public units (71.9%), Grade I hospitals (50%), provincial and equivalent levels (62.5%), and located in the Southeast region (68.8%). Over three years, approximately 95% of units achieved a total score over 50%, with only 5% scoring less than 50%. Analyzing performance by component, the proportion of units achieving ≥50% for identification steadily increased (from 96.4% in 2022 to 97.8% in 2024), reaching 100% for individual parasite analysis in both 2023 and 2024. However, the density counting estimation component consistently had a lower proportion of units achieving ≥50%, remaining around 88% throughout the three years.

Conclusions: Within this research, the main characteristics are: belonging to units of the public type, ranked I, to the provincial level and equivalent, in the Southeast region. The quality of participation is increasingly improved when evaluating the overall and individual components, but the component always accounts for a lower proportion than the density counting.

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GOVERNMENT HEALTH CARE WORKER TRAINING NEEDS FOR SCHISTOSOMIASIS MORBIDITY MANAGEMENT

Phyllis Isaiah[1], Betty Nabatte[2], Lauren Wilburn[1], John Oryema[3], Noah Ukumu[3], Morris Okumu[4], Juma Nabhonge[5], Hilda Kyarisiima[2], Prudence Beinamaryo[2], Victor Anguajibi[6], Christopher Opio[7], Narcis Kabatereine[2], Goylette Chami[1]
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Background and Aims: Schistosomiasis causes substantial chronic morbidity in sub-Saharan Africa, yet case definitions, clinical management guidance, and health worker training for schistosomiasis-related morbidity remain limited.

Methods: We conducted a qualitative needs assessment on schistosomiasis morbidity management through one-day workshops in Pakwach, Buliisa, and Mayuge Districts, Uganda, in October 2024. A total of 105 government health workers participated including clinicians, nurses, laboratory technicians, sonographers, and district health managers from different levels of care. Workshops comprised six structured sessions: presentations on schistosomiasis burden in Uganda and the SchistoTrack cohort, an expert-led clinical case report, interactive discussion of patient case studies, mapping of patient care pathways, anonymous feedback, and demonstrations of schistosomiasis diagnosis. Discussions were documented in English and analysed thematically.

Results: Health workers showed major gaps in understanding schistosomiasis case definitions, particularly in differentiating current infection from chronic morbidity and in grading disease severity. Patient pathways were fragmented and inconsistent, with weak triage systems, unclear referral and feedback mechanisms, and limited follow-up between facility levels. Health facilities lacked essential resources, including routine access to praziquantel outside mass drug administration, diagnostic reagents, functional ultrasound equipment, trained sonographers, and standardized training or reference materials. These gaps contributed to inconsistent clinical decision-making and under-recognition of severe schistosomiasis-related morbidity.

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Conclusions: Integrating schistosomiasis morbidity management into routine health services through standardized case definitions, clearer patient pathways, and targeted practical training is essential to reduce preventable morbidity. The engagement framework and case studies used here may support needs-based assessments in other endemic settings to inform context-appropriate clinical guidance and training programmes.

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LYMPHATIC FILARIASIS, MORBIDITIES AND SOCIO-ECONOMIC IMPACT AMONG POPULATION OF ENDEMIC COMMUNITIES

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Background and Aims: Lymphatic Filariasis (LF), an extremely disfiguring and painful disease, commonly referred to as Elephantiasis, is a Neglected Tropical Disease (NTD) as described by World Health Organization (WHO), known for causing serious societal stigmatization and disablement globally. *Wuchereria bancrofti,* the causative parasite responsible for above 90% cases, is transmitted by blood-sucking *Culex, Aedes and Anopheles* mosquitoes in rural and semi-urban areas. This study investigated LF prevalence, associated morbidities and socio-economic impact

Methods: Nocturnal intravenous blood collection between 11pm and 1am due parasite's circadian clock, for parasitological examination of thick blood films, including children. Structured questionnaire provided demographic information and specifics to evaluate LF impact on quality of life, financial burden, access to education, etc. Participants were also physically examined for disease manifestation on limbs, breast and scrotum and documented for signs of Lymphoedema, limb elephantiasis and Hydrocoele

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Results:

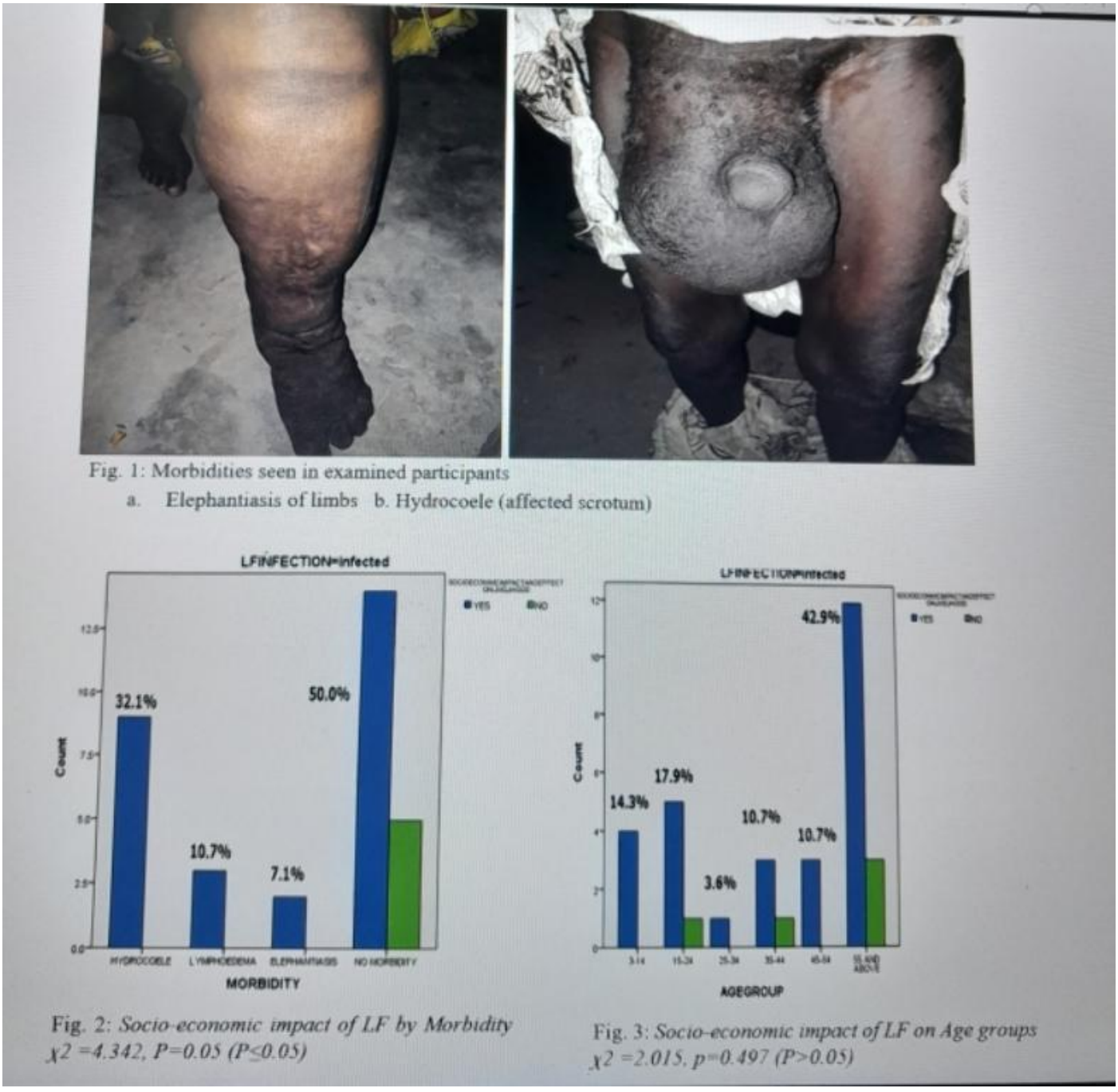


Fig. 1: Morbidities seen in examined participants
a. Elephantiasis of limbs b. Hydrocoele (affected scrotum)

Fig. 2: *Socio-economic impact of LF by Morbidity*
$\chi 2 = 4.342$, $P = 0.05$ ($P \leq 0.05$)

Fig. 3: *Socio-economic impact of LF on Age groups*
$\chi 2 = 2.015$, $p = 0.497$ ($P > 0.05$)

Overall prevalence of infection was 22.1%, and the burden of morbidities and disabilities due to *W. bancrofti* was 42.4%; with hydrocoele constituting about 66.7%, while Lymphoedema and limb elephantiasis mildly contributed to other clinical manifestations among women and children. Approximate socio-economic impact was 84.8% (P=0.01; P<0.05), as hydrocoele significantly impacted the population (P=0.05 $P \leq 0.05$), including children of ages 3-14years. Infection was predominant across all age groups, although higher among male subjects and elderly above ages 55 years

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Conclusions: Prompt diagnosis and immediate disease management is advocated to reduce transmission and associated morbidities arising due to neglect overtime. The social, economic and public health impact of LF is enormous, preventing school-aged children access to education, deprive victims of livelihood, relationships and comfort.

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TWO-YEAR PERSISTENCE WITHOUT CLINICAL PROGRESSION OF ASYMPTOMATIC SUBMICROSCOPIC MALARIA IN INDIGENOUS COMMUNITIES IN MALAYSIA

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Background and Aims: Asymptomatic submicroscopic malaria (SMM) represents a potentially hidden reservoir of *Plasmodium* infection that may challenge malaria elimination efforts, particularly in low-transmission settings where routine microscopy and symptom-based surveillance can miss infections. This study aimed to determine the clearance, persistence, recurrence, and clinical progression of untreated SMM among indigenous communities in Malaysia over a 24-month follow-up period.

Methods: A prospective longitudinal cohort enrolled 58 asymptomatic, polymerase chain reaction (PCR)-positive/microscopy-negative participants from Kota Marudu and Keningau, Sabah, and RPS Kemar, Peninsular Malaysia. Finger-prick dried blood spots and blood smears were collected during eight scheduled follow-up visits over 24 months. Samples were assessed using nested PCR targeting 18S rRNA and microscopy; participants were screened for symptoms. Outcomes were clearance, PCR persistence/recurrence, and progression to microscopy-positive or symptomatic malaria.

Results: At baseline, infections comprised *Plasmodium malariae* (24/58, 41.4%), *P. vivax* (16/58, 27.6%), *P. knowlesi* (15/58, 25.9%), one each of *P. falciparum* and *P. cynomolgi*, and one mixed *P. vivax/P. knowlesi* infection. Overall, 218/464 planned follow-up visits were completed (47.0%). Among 54 participants with at least one follow-up sample, 43 (79.6%) had no subsequent PCR-detectable infection, while 11 (20.4%) showed at least one PCR-positive follow-up. Three participants (5.6%), all initially infected with *P. malariae*, had recurrent PCR positivity across multiple visits. No microscopy-positive infection or symptomatic malaria was observed during follow-up; incomplete retention limits inference on late outcomes.

Conclusions: Most SMM infections cleared below PCR detection, but a minority, particularly *P. malariae* infections, remained intermittently detectable without clinical progression. Molecular active surveillance incorporating non-falciparum and zoonotic *Plasmodium* species may identify hidden reservoirs missed by symptom-based and microscopy-based surveillance in Malaysia's malaria elimination setting.

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MIXED SCHISTOSOME INFECTIONS CAUSING GENITAL SCHISTOSOMIASIS IN MEN AND WOMEN ACCESSING SEXUAL REPRODUCTIVE HEALTHCARE SERVICES AT A RURAL MPONDASI HEALTH CENTRE IN AN ENDEMIC AREA IN MANGOCHI DISTRICT, SOUTHERN MALAWI.

Sekeleghe Kayuni
Malawi Liverpool Wellcome Programme, Blantyre, Malawi

Background and Aims: Genital schistosomiasis remains an ignored chronic consequence of urogenital schistosomiasis, affecting male and female genitalia. Since discovery, there is still under-diagnosis, misdiagnosis and mismanagement, including zoonotic schistosomiasis. A cross-sectional study was conducted to assess prevalence of female and male genital schistosomiasis (FGS and MGS) among those accessing existing sexual reproductive healthcare (SRH) services at Mpondasi Health Centre, Mangochi district.

Methods: Urine, cervicovaginal fluid and swabs, as well as semen were collected and analysed by urine filtration and microscopy, cervicovaginal lavage (CVL) and semen microscopy to visualise the *Schistosoma* eggs and sexually transmitted infections (STIs). Real-time PCR and portable colposcopy were also conducted.

Results: Sixty-four women and 60 men were recruited, median age: 31.0 years and 32.5 years respectively. Seven women (11.3%) had *Schistosoma* eggs in urine and six in stool (10.2%). Three had FGS detected by CVL microscopy (5.2%), 30 on colposcopy (49.2%) and 11 on PCR (20.8%). Eleven women (18.0%) had STIs and four (6.6%) had FGS-STI co-infection. Twenty men (36.8%) had *Schistosoma* eggs in urine and eleven (21.6%) in stool. Nineteen men (35.2%) had MGS detected by semen microscopy (range: 24-1368 eggs) and 33 (61.1%) by PCR (n=54) of which 30 had *S. haematobium*, three *S. mansoni*, 12 *S. mattheei*. while 3 had mixed infection of *S. haematobium*, *S. mansoni* and *S. mattheei*.

Conclusions: Genital schistosomiasis caused by human and zoonotic schistosomes shows to be prevalent among those accessing SRH services in schistosomiasis-endemic areas, together with STIs. Adequate knowledge and diagnostic capacity building among healthcare personnel are essential to curb misdiagnosis and mismanagement.

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MICRO-EPIDEMIOLOGY OF ANAEMIA IN UROGENITAL SCHISTOSOMIASIS-ENDEMIC COMMUNITIES IN SOUTHERN MALAWI: A GEOSTATISTICAL ANALYSIS IN A CHANGING TRANSMISSION LANDSCAPE

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Background and Aims: Climate-driven shifts in *Schistosoma haematobium* transmission are reshaping schistosomiasis burden across sub-Saharan Africa. As part of the Hybridisation in Urogenital Schistosomiasis (HUGS) investigation, examining health consequences of hybrid schistosome species, we aimed to characterise the micro-epidemiology of anaemia, quantify the association between infection intensity and haemoglobin levels, and identify high-risk spatial clusters at fine geographical scales in two endemic communities in southern Malawi.

Methods: Urine samples from 1,149 participants (Samama, Mangochi District) and 977 (Mthawira, Nsanje District) were examined by microscopy to quantify *Schistosoma haematobium* infection intensity: low (0–9 eggs/10 mL), moderate (10–49 eggs/10 mL), and heavy (≥50 eggs/10 mL). Haemoglobin was measured by HemoCue photometer. Linear geostatistical models incorporating individual-level and spatial covariates estimated anaemia prevalence at fine geographical scales.

Results: Moderate anaemia (Hb 80–109 g/L) was most prevalent in children aged 6–12 years (45.3% Samama; 39.5% Mthawira); severe anaemia disproportionately affected adults ≥19 years. Heavy infection intensity was significantly associated with greater moderate-to-severe anaemia risk. Spatial modelling revealed divergent transmission ecologies: longer-range correlation in Nsanje (ϕ = 66.7 km) indicated landscape-driven, persistent risk, while localised heterogeneity in Mangochi (ϕ = 25.5 km) suggested microhabitat-level transmission foci. Predicted anaemia prevalence among female children aged 6–12 years with moderate infection ranged 30–65% (Mangochi) and 22–34% (Nsanje).

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Conclusions: Infection intensity is a strong determinant of anaemia severity, with spatial heterogeneity suggesting that environmental and climate-related factors are differentially shaping transmission. Geostatistical mapping effectively identifies high-risk clusters, supporting targeted preventive chemotherapy, iron supplementation, and locally-adapted water and sanitation interventions.

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A NEW APPROACH TO SUSTAINABLE CONTROL OF SCHISTOSOMIASIS WITH SPIRULINA FEED.

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Background and Aims: Schistosomiasis control currently relies primarily on mass drug administration with praziquantel. However, persistent reinfection and associated costs highlight the need for complementary strategies targeting infective larvae and their intermediate host snails. Although niclosamide is widely used as a molluscicide, its environmental toxicity and expense limit its applicability. We incidentally observed that a feed derived from the cyanobacterium *Arthrospira (Spirulina) platensis* markedly reduced the release of infective larvae from *Schistosoma mansoni*–infected snails.

Methods: We evaluated the effects of a spirulina-based feed on *S. mansoni*–infected snails under laboratory conditions. Cercarial release was quantified to assess the impact of spirulina feeding. The safety of spirulina was examined in both snails and Japanese medaka (*Oryzias latipes*). In addition, the direct effects of spirulina-derived components on cercarial viability were investigated.

Results: Feeding infected snails with spirulina reduced cercarial release by up to 88%, with the most pronounced effect observed during early infection stages. Spirulina exhibited no detectable toxicity to either snails or fish, indicating a high safety margin. Direct exposure experiments demonstrated increased cercarial mortality. Chemical analyses identified linoleic acid, a constituent present in both the spirulina-based and base feeds, as the primary factor responsible for this effect, and its direct antiparasitic activity against cercariae was confirmed.

Conclusions: These findings demonstrate that spirulina-based feeding can suppress larval development within infected snails and reduce the viability of released larvae without causing environmental toxicity. This feed-based approach represents a sustainable and nontoxic alternative to conventional molluscicidal strategies and may contribute to more effective and environmentally responsible control of schistosomiasis.

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HIGHER BLOOD CONCENTRATIONS OF R-TRANS-4- HYDROXY PRAZIQUANTEL, THE MAIN METABOLITE OF PRAZIQUANTEL, ARE ASSOCIATED WITH LOWER SCHISTOSOMA MANSONI EGG COUNTS POST-TREATMENT

Rachel Francoeur[1], Moses Arinaitwe[1], John Tetteh[2], Andrina Nankasi[3], Lydia Trippler[1], Alon Atuhaire[3], Alison Thompson[2], David Watson[2], Diana Ajambo[3], Moses Adriko[4], Aidah Wamboko[3], Fred Besigye[3], Candia Rowel[3], Christina Faust[1], Lauren Carruthers[1], Poppy Lamberton[1]
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Background and Aims: Schistosomiasis is a major parasitic disease caused by blood flukes acquired from contaminated water sources. The World Health Organization recommends mass drug administration (MDA) with praziquantel to reduce prevalence and control morbidity. However, despite widespread MDA implementation highly endemic areas persist and reductions in egg counts following treatment are variable. We hypothesize that differences in drug absorption and metabolism may be associated with differences in down stream drug efficacy.

Methods: This study investigated whether blood concentrations of praziquantel and its main metabolite were associated with faecal egg count reductions in school-aged children following treatment in an Ugandan community highly endemic for *Schistosoma mansoni*. One hundred and ninety-seven children aged 6 to 14 years were fed and administered a single oral dose of praziquantel (40 mg/kg). Dried blood spots were collected before treatment and at multiple time points within 24 hours post-treatment, with each child providing up to four samples. Drug and metabolite concentrations were measured using offline extraction followed by LC-MS/MS analysis.

Results: Generalized linear mixed models were fitted to assess associations between blood concentrations of praziquantel and analytes over the 24-hours post treatment and faecal egg counts measured at three and nine weeks post-treatment. Higher concentrations of the main metabolite, R-*trans*-4-hydroxypraziquantel, were significantly associated with lower absolute faecal egg counts at follow-up.

Conclusions: These findings suggest that host pharmacokinetic variability may influence praziquantel treatment outcomes and highlight the importance of optimising drug exposure in schistosomiasis control programmes.

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HIGH BURDEN OF MALARIA, SCHISTOSOMIASIS, AND ANEMIA AMONG CHILDREN IN KIFWA VILLAGE, DEMOCRATIC REPUBLIC OF CONGO.

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Background and Aims: Malaria and schistosomiasis remain highly endemic in the Democratic Republic of the Congo (DRC). Although integrated control is recommended in co-endemic settings, evidence on malaria–schistosomiasis coinfections and their contribution to anemia remains limited. Currently, schistosomiasis mass drug administration (MDA) targets only school-aged children (SAC), while preschool-aged children (PSAC) are excluded.

Methods: We analyzed data from children aged 1–15 years enrolled in a community-based cross-sectional study conducted in Kifwa, western DRC. Malaria was diagnosed using rapid diagnostic tests; schistosomiasis and soil-transmitted helminths (STH) using Kato–Katz thick smears and urine filtration; and anemia by hemoglobin measurement with HemoCue. Polyparasitism was defined as ≥2 concurrent parasitic infections. Associations between anemia and parasitic infections were assessed using logistic regression.

Results: Among 453 children (247 boys, 206 girls; median age 7 years, IQR 4–11), prevalence was 83.2% for malaria, 78.8% for schistosomiasis, 20.5% for STH, 81.0% for anemia, and 69.1% for polyparasitism. Malaria prevalence was similar in PSAC and SAC (83.2% vs. 83.2%). Schistosomiasis prevalence was 63.9% in PSAC and 87.0% in SAC; STH 8.1% and 27.4%, respectively. Anemia prevalence was 84.5% in PSAC and 79.1% in SAC. Polyparasitism affected 54.0% of PSAC and 77.4% of SAC. Schistosomiasis and STH infection intensity increased with age. Anemia was not significantly associated with parasitic infections.

Conclusions: Both PSAC and SAC in Kifwa bear an exceptionally high burden of malaria and helminth infections. These findings support integrated malaria–schistosomiasis control strategies and consideration of extending schistosomiasis MDA to preschool-aged children.

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CO-OCCURRENCE OF ALCOHOL USE AND SCHISTOSOMA MANSONI INFECTION IN RURAL UGANDA

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Background and Aims: Despite being known independently as important aetiological agents of liver disease, little is known about the prevalence, patterns, and risk factors associated with co-occurring alcohol use and *Schistosoma mansoni* infection in rural endemic settings.

Methods: A cross-sectional analysis was completed of 3198 individuals aged 10-90 years from 52 rural villages in Uganda. Alcohol use was assessed using the World Health Organisation STEPwise Approach to Surveillance survey, and *S. mansoni* infection was diagnosed using Kato-Katz microscopy. Logistic regressions with biomedical, demographic, socioeconomic, and spatial variables were run.

Results: The overall prevalence of *S. mansoni* infection was approximately 44% (1405/3198) and current alcohol use was reported by over 12% (392/3198) of participants. Among males aged ≥20 years, the prevalence of co-occurring current alcohol use and *S. mansoni* infection was 18.7% (148/791). Females were less likely to have *S. mansoni* infection (odds ratio [OR] 0.83, 95% CI 0.69 – 0.99) or report current alcohol use (OR 0.44, 95% CI 0.32 – 0.62). Smoking (OR 9.43, 95% CI 6.66 – 13.37) and participating in fishing activity (OR 3.50, 95% CI 2.57 – 4.76) were associated with current alcohol use. Age (OR 0.95, 95% CI 0.94 – 0.97), fishing activity (OR 1.82, 95% CI 1.16 – 2.84), and smoking (OR 5.25, 95% CI 3.49 – 7.90) were associated with co-occurring current alcohol use and *S. mansoni* infection.

Conclusions: Co-occurrence of alcohol use and schistosome infection is common. Future research should investigate multi-sectoral interventions that simultaneously address the risk factors associated with both alcohol use and *S. mansoni* infection.

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LEISHMANIASIS IN PAKISTAN: FROM ETIOPATHOGENESIS TO EMERGING THERAPEUTIC STRATEGIES

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Background and Aims: Leishmaniasis is a neglected tropical disease caused by *Leishmania* parasites and transmitted by infected sandflies. Pakistan is a recognized endemic country, with cutaneous leishmaniasis being the most prevalent form in all provinces. Visceral leishmaniasis, although less common, remains a serious concern due to its high mortality if untreated. The disease is associated with significant morbidity, social stigma, and economic burden. The aim of this review is to provide a comprehensive overview of the etiopathogenesis of leishmaniasis with a focus on disease patterns in Pakistan, while also evaluating current treatment options and emerging therapeutic approaches.

Methods: Literature published after 2015 was searched in PubMed, Google Scholar, and Scopus using keywords related to leishmaniasis, Pakistan, etiopathogenesis, and novel therapies. Relevant peer-reviewed studies were analyzed.

Results: The findings showed leishmaniasis cases in Pakistan, with *Leishmania tropica* & *Leishmania major* being the predominant species. Transmission is influenced by factors such as poverty, population displacement, poor housing conditions, and limited vector control. Current treatment regimens, including pentavalent antimonials and amphotericin B, remain effective but are associated with toxicity, high costs, and emerging drug resistance. Recent advances in novel drug delivery systems, particularly nanoparticle-based formulations, have shown improved therapeutic efficacy and reduced adverse effects in experimental and early clinical studies.

Conclusions: Leishmaniasis remains a major public health problem in Pakistan. Drug resistance and treatment toxicity demand improved therapies. Strengthened surveillance, vector control, community awareness & continued research are essential to reduce disease burden.

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SPATIAL HOTSPOTS OF MALARIA–SCHISTOSOMIASIS CO-ENDEMICITY IN MISUNGWI DISTRICT, NORTHWESTERN TANZANIA: IMPLICATIONS FOR INTEGRATED CONTROL

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Background and Aims: Malaria and schistosomiasis remain major parasitic diseases in sub-Saharan Africa, with overlapping transmission in communities near freshwater ecosystems. Despite shared ecological drivers, control programmes are often implemented independently, limiting opportunities for integrated intervention. This study aimed to identify spatial hotspots of malaria–schistosomiasis co-endemicity in Misungwi District, northwestern Tanzania, using integrated ecological, climatic, and environmental data combined with ecological niche modelling.

Methods: Malaria and schistosomiasis remain major parasitic diseases in sub-Saharan Africa, with overlapping transmission in communities near freshwater ecosystems. Despite shared ecological drivers, control programmes are often implemented independently, limiting integrated intervention. This study aimed to identify spatial hotspots of malaria–schistosomiasis co-endemicity in Misungwi District, northwestern Tanzania, by integrating ecological, climatic, environmental, and parasitological data from ongoing and planned surveys among school-aged children, combined with ecological niche modelling.

Results: A total of 4,196 freshwater snails were collected, dominated by *Bulinus* spp. (3,614), followed by *Biomphalaria* spp. (340) and *Lymnaea* spp. (242). Schistosome cercariae were detected in 21 *Bulinus* snails across six villages, indicating active transmission, while *Fasciola* cercariae were identified in five *Lymnaea* snails. Mosquito larvae were present in 88% of snail-positive aquatic habitats, demonstrating strong ecological overlap between malaria and schistosomiasis transmission sites. Molecular analysis confirmed *Anopheles funestus* and members of the *Anopheles gambiae* complex.

Conclusions: This study demonstrates fine-scale spatial overlap between malaria and schistosomiasis transmission systems. Integrating ecological, climatic, and entomological data with ecological niche modelling provides a predictive framework for identifying co-endemic hotspots and supports integrated, multi-disease risk mapping for targeted control in climate-sensitive setting.

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POPULATION GENETIC DYNAMICS OF PLASMODIUM FALCIPARUM WITH TRANSMISSION DECLINE, RESURGENCE AND EMERGENCE OF PARTIAL ARTEMISININ RESISTANCE IN PAPUA NEW GUINEA

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Background and Aims: Malaria genomic surveillance is transforming how we track, target, and eliminate transmission. In Papua New Guinea, where four *Plasmodium* species co-circulate, intensified control measures after 2006 initially drove infections down. Yet since 2014, prevalence has rebounded and early signs of artemisinin resistance have emerged.

Methods: To understand how *Plasmodium falciparum* populations responded to these shifting pressures, we analysed parasite transmission dynamics using Nanopore sequencing of SNP barcodes from 624 isolates collected between 2005-2020 across Madang and East Sepik Provinces.

Results: Our results reveal striking temporal and spatial contrasts. Madang showed fluctuations in genetic diversity and clustering, while East Sepik remained stable. Over time, population structure between the provinces increased, reflecting fragmentation during the control scale-up. As transmission resurged, parasite relatedness increased, with several clonal lineages expanding and recombining, providing evidence of population recovery from a genetic bottleneck. We detected a high-prevalence (10%) of the *kelch13*-C580Y mutation in Madang, signalling emerging partial-artemisinin resistance. In contrast, *mdr1*/*pm2* copy numbers remained stable, suggesting limited partner-drug selection. All C580Y mutants clustered with a lineage of local wild-type parasites, consistent with a recent incursion and local establishment of the lineage through recombination with local parasites. A single C580Y mutant detected in East Sepik (2019) belonged to the same lineage, indicating early spread but no local establishment.

Conclusions: These findings show that while control efforts initially fragmented parasite populations, the subsequent rebound enabled expansion of residual lineages and the spread of resistance-

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associated variants. Sustained pressure and continuous molecular surveillance are essential to protect gains and detect emerging threats early.

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ASSESSING THE PREVALENCE OF SCHISTOSOMA HAEMATOBIUM INFECTIONS AMONG SCHOOL-GOING CHILDREN AGED 5-14 YEARS OLD IN NELSON MANDELA BAY: A COMPREHENSIVE DIAGNOSTIC APPROACH

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Background and Aims: Urogenital schistosomiasis caused by *Schistosoma haematobium* is frequently underestimated in low-transmission areas because traditional diagnostics, such as urine filtration, have low sensitivity. Haematuria can act as an initial marker of infection but lacks specificity. More sensitive methods like DNA amplification are required to detect infections when eggs are absent. This study determined the prevalence of *S. haematobium* among school-going children in Nelson Mandela Bay (NMB) using a comprehensive diagnostic approach.

Methods: A cross-sectional study included 759 children aged 5–14 years from 15 primary schools. Each child provided one urine sample. Dipstick urinalysis was used to detect haematuria, and urine filtration was performed to identify *S. haematobium* eggs. Samples showing 2+ or 3+ haematuria were purposively selected for further confirmation using conventional Polymerase Chain Reaction (cPCR). The cPCR targeted the *S. haematobium* Dra1 repeat sequence to detect cell-free DNA (cfDNA).

Results: Of the 759 participants, 58% were male and 42% female. Haematuria was detected in 33.6% of samples. Urine filtration identified only one egg-positive case, reflecting a prevalence of 0.1%. To improve detection, 121 haematuria-positive samples were analysed using cPCR, with the egg-positive sample included as a control. cPCR detected *S. haematobium* cfDNA in 31.4% of these samples, revealing infections missed by traditional methods.

Conclusions: The large discrepancy between urine filtration (0.1%) and cPCR positivity (31.4%) demonstrates substantial underestimation of schistosomiasis when relying solely on conventional diagnostics. Many infections were likely low-intensity. These findings highlight ongoing transmission in

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NMB and emphasize the need for sensitive molecular diagnostics to guide effective public health interventions.

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PREVALENCE OF SCHISTOSOMA MANSONI,SOIL-TRANSMITTED HELMINTHS AND INTESTINAL PROTOZOA IN ORPHANS AND STREET CHILDREN IN RURAL MWANZA NORTHERN TANZANIA

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Background and Aims: The study aimed to gather data on parasitic infections in orphans and street kids in rural Mwanza, Tanzania, to inform targeted interventions. These groups are often excluded from preventive programs due to limited data.

Methods:
A cross-sectional study was conducted involving 144 orphans and 112 street children in Mwanza City, northern Tanzania. Participants were screened for Schistosoma mansoni, Schistosoma haematobium, soil-transmitted helminths, and intestinal protozoa using POC-CCA tests, urine filtration, and the Kato-Katz technique. Data on nutrition, water contact, and hygiene practices were collected through questionnaires, and ultrasonography was used to assess organ damage.

Results:
The prevalence of S. mansoni was high in both groups but significantly higher among street children. POC-CCA testing showed infection rates of 65.9% in orphans and 94.5% in street children, while Kato-Katz detected infections in 19.2% of orphans and 77.1% of street children. Most street children reported frequent use of Lake Victoria for washing, unlike orphans. Intestinal protozoa infections, including Giardia intestinalis and Entamoeba histolytica/dispar, were found at similar levels in both groups. Ultrasonography did not reveal severe liver damage.

Conclusions: Conclusion:
Street children showed a substantially higher burden of S. mansoni infection compared to orphans, likely due to poor sanitation and increased contact with contaminated lake water. Infection rates of intestinal protozoa were comparable between the groups. Overall, the findings highlight poor living conditions and emphasize the need for targeted public health interventions for both orphans and street children.

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MAPPING OF MALARIA PARASITAEMIA AND TREATMENT SEEKING BEHAVIOR OF SELECTED RESIDENTS OF ADAMAWA AND RIVERS STATES, NIGERIA.

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Background and Aims: This study was carried out to map prevalence of malaria in selected residents of Adamawa and Rivers States, Nigeria.

Methods: Total of 2400 blood samples was examined; 1200 samples from each State. Thick (stained with Field stain A and B) and thin (stained with Giemsa) blood films were examined by oil immersion microscopy. *Plasmodium* species molecular identification carried out in Adamawa State. Questionnaires were issued to obtain relevant information.

Results: *Plasmodium falciparum* was the only species detected. Out of the 1200 blood samples examined, 469(39.1%) were infected with total parasite density(PD) 1633048/µl in Adamawa State whereas in Rivers State out of 1200 samples examined 530(44.2%) were infected with PD 2107518/µl. By location, in Adamawa State, Yola North LGA recorded highest infection 68.8% with statewide infection 4.6% and PD 208896/µl whereas least infection was recorded in Ganye LGA 22.5% with statewide infection 1.5% and PD 95456/µl. Difference in percentage infected was not significant as $P>0.05$ whereas difference in PD showed $P<0.05$, in Rivers State; the highest infection was 61% with statewide infection of 5.1% and PD 248616/µl in Abua/Odual LGA; the least infection was recorded in Ikwerre LGA 24% with statewide infection 2.0% PD 137696/µl $P<0.05$. By zones, Adamawa North had the highest prevalence with statewide infection 15.2% PD 523648/µl whereas in Rivers State the highest statewide prevalence was 17.9% PD 802100/µl. In both States the use of herbs for prevention and cure of malaria infection was common.

Conclusions: There is need to step up data driven interventions to realize zero malaria communities.

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ASSESSMENT OF FACTORS INFLUENCING PATIENT REFUSAL OF TRACHOMATOUS TRICHIASIS (TT) SURGERY IN GAMBELLA , AFAR, SOMALI, AND BENSANGUL GUMUZ REGIONS OF ETHIOPIA

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Background and Aims: Trachomatous trichiasis (TT) remains a major public health problem in Ethiopia despite free surgery availability. Surgery refusal is driven by socio-demographic, knowledge, psychosocial, logistical, and cultural barriers, especially among rural and vulnerable groups. This study examines key drivers of TT surgery refusal in Afar, Gambella, Somali, and Benishangul-Gumuz to inform targeted interventions.

Methods: A cross-sectional study was conducted among 180 trachomatous trichiasis patients in Afar, Somali, Benishangul-Gumuz, and Gambella regions of Ethiopia between May and June 2025. Using convenience sampling, patients who were diagnosed and offered TT surgery but refused were interviewed during ongoing surgery programs.

Results: revealed that 71.1% of respondents were female, 68% illiterate, and 90.5% rural dwellers, with a mean age of 52. While 72% understood TT and 78% knew surgery was a treatment option, key refusal drivers included fear of pain (56%), lack of family support (28%), and doubts about efficacy (25%). Age significantly correlated with refusal reasons, with older patients citing lack of support and younger patients expressing uncertainty. Despite high satisfaction with healthcare communication (93%), psychosocial and logistical barriers persisted. Recommendations include targeted education for rural, illiterate women, community-based advocacy, and financial/logistical support. These findings highlight the need for interventions addressing both knowledge gaps and psychosocial factors to improve surgery uptake.

Conclusions: TT surgery refusal was mainly driven by fear of pain, lack of social support, and doubts about effectiveness. Although most patients had basic knowledge, gaps in awareness and psychosocial factors—especially among women, rural residents, and older patients—continued to limit acceptance.

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ASSOCIATIONS OF COMPLETE BLOOD COUNT DIFFERENTIALS WITH SCHISTOSOMAL FIBROSIS: A SYSTEMATIC REVIEW AND META-ANALYSIS

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Background and Aims: A severe complication of intestinal schistosomiasis is periportal fibrosis (PPF). Routine indicators are needed to monitor PPF in areas with limited resources, however little is known about the clinical utility of complete blood count (CBC) with differential analysis (w/diff) for suspected cases of PPF. We aim to assess the association between CBC w/diff and schistosomiasis.

Methods: In this systematic review and meta-analysis, we searched MEDLINE, Embase, Global Health, Global Index Medicus and Web of Science Core Collection – Science Citation Index Expanded on March 3, 2026.The protocol was prospectively registered (PROSPERO CRD420251244233, 2025). The main outcome was author-defined fibrosis related to Schistosoma infection or in a population endemic with schistosomiasis and the key exposure being CBC parameters. The pooled standardized mean differences (SMD) for white blood cell count (WBC), platelet count (PLT), red blood cell count (RBC) and hemoglobin (Hb) were calculated using a random effects model. The modified National Institute of Health risk of bias (ROB) tool was used for assessing study quality.

Results: The electronic search retrieved 2460 records, 1787 titles and abstracts were screened and 16 studies were included in the meta-analysis. When the findings were pooled, a decrease in PLT is associated with more severe liver fibrosis (SMD 2.09 95% CI 1.00-3.18; $p<0.0001$). No effects were found for WBC, RBC and Hb. Upon ROB assessment, most studies 81% (13/16) were rated as mid-quality.

Conclusions: Further work is needed to assess the association between other CBC w/diff parameters and schistosomal fibrosis.

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COMPLETE BLOOD COUNT ANALYSIS IDENTIFIES NEUTROPHILS AS PREDICTORS OF SCHISTOSOMIASIS SEVERITY

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Background and Aims: Schistosomiasis affects approximately 240 million people worldwide and can cause periportal fibrosis (PPF), a severe complication characterised by inflammatory scar tissue surrounding the portal vein of the liver. Despite its clinical importance, there are no early diagnostic indicators or effective strategies to manage PPF, and its immunopathogenesis remains poorly understood. In this study, we used complete blood count with differential (CBC w/diff) as an immune profiling tool to investigate the pathogenesis of schistosomiasis.

Methods: To investigate the role of host genotype on schistosomiasis pathogenesis, BALB/c and C57BL/6 mice were infected with *S. mansoni* cercariae and monitored using weekly CBC w/diff measurements. To assess the role of neutrophils in disease progression, infected BALB/c mice were treated at 8 weeks post-infection with praziquantel, dexamethasone, or tofacitinib citrate. Liver pathology was evaluated at 12 weeks post-infection using histology, flow cytometry, and confocal microscopy.

Results: Genotype and infection status significantly influenced CBC w/diff parameters. Elevated baseline neutrophil counts prior to infection predicted mortality in infected BALB/c mice. Anti-inflammatory treatment reduced liver and spleen weights and decreased neutrophil accumulation within liver tissue and granulomas.

Conclusions: These findings support an association between elevated neutrophils and schistosomiasis disease severity. This murine model provides a framework for my future epidemiological studies assessing CBC w/diff as a biomarker of disease progression and for identifying therapeutic interventions to improve clinical outcomes in schistosomiasis.

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CANINE LEISHMANIOSIS IN ENDEMIC AND SILENT MUNICIPALITIES OF SÃO PAULO STATE, BRAZIL: EPIDEMIOLOGICAL AND DIAGNOSTIC INSIGHTS

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Background and Aims: Canine leishmaniosis (CanL) caused by *Leishmania (Leishmania) infantum* is a public health concern in Brazil, with heterogeneous transmission dynamics across regions. This study aimed to assess the occurrence of *L. (L.) infantum* infection in dogs from municipalities classified as endemic or silent for visceral leishmaniasis (VL) and to evaluate the performance of multiple diagnostic methods.

Methods: One hundred dogs from five municipalities in São Paulo State underwent clinical examination and testing by parasitological (cytology, histology, culture), serological (DPP, ELISA, IFAT), and molecular (PCR targeting ITS1 and kDNA) methods. Biological samples included lymph node, bone marrow, skin, blood, and conjunctival and oral swabs. Parasite isolates were identified by ITS1-RFLP.

Results: Evidence of *Leishmania* infection was found in 46% of dogs, all of which exhibited clinical signs compatible with CanL. *L. (L.) infantum* was identified in 27 isolates recovered from lymph node, bone marrow, and blood. Infected dogs were detected in endemic areas and in a municipality (Pirassununga) previously classified as silent. Serological tests showed high sensitivity but limited specificity, with ELISA and IFAT outperforming DPP in sensitivity. Molecular diagnosis using kDNA PCR demonstrated the highest sensitivity, especially in lymph node, bone marrow, skin, and conjunctival swab samples, whereas blood and oral swabs showed lower performance.

Conclusions: Our findings confirm the active circulation of *L. (L.) infantum* in both endemic and silent municipalities and highlight the importance of integrating molecular diagnostics for accurate detection across diverse epidemiological contexts.

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THE BURDEN OF VISUALLY DIAGNOSED FEMALE GENITAL SCHISTOSOMIASIS AMONG WOMEN WITH INFERTILITY IN THE VOLTA REGION OF GHANA

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Background and Aims: Female genital schistosomiasis (FGS) is the outcome of the deposition of Schistosoma haematobium egg in the genital tract of women in schistosomiasis endemic areas, which can result in increased risk of infertility. This study aimed to evaluate the burden of visual FGS among women with infertility in the Volta region of Ghana.

Methods: This comparative cross-sectional study involved infertile women and nursing mothers in the Volta Region. Sociodemographic and clinical data were collected via questionnaires, urine samples were examined for *S. haematobium* eggs, and colposcopic genital tract assessments were performed. Data analysis in SPSS included descriptive statistics, Chi-square tests for group differences and associations, and logistic regression to identify independent risk factors for FGS.

Results: Of the 265 sampled women 132 (49.8%) were infertile and 133 (50.2%) were nursing mothers (fertile women). More women had visual FGS (155, 58.5%) and most with FGS were fertile [96, 76.1%; infertile, 59(45.3%); p<0.001], with infertile women having lower odds of FGS in this study (AOR, 0.29 [95% CI 0.17–0.50]; p<0.001); adjusted for childhood and current contact with rivers, availability of toilets facility, practice of open def- ecation and age. More women with FGS had childhood contact with rivers (68.4%, p=0.007) with lower odds of FGS seen in women without childhood contact with rivers and streams (AOR, 0.52 [95% CI 0.31–0.88]; p=0.015).

Conclusions: In this study, infertile women unexpectedly had lower odds of FGS suggesting the need for more rigor- ous research on this topic to elucidate the true contribution of FGS on infertility.

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EVALUATION OF NOVEL ANTIBODY BIOMARKERS FOR DETECTION OF SCHISTOSOMA HAEMATOBIUM INFECTION

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Background and Aims: We previously identified two antibody biomarkers for detecting *Schistosoma haematobium* infection using an ELISA platform: rSh_SAP-1 for IgG1 (68% sensitivity, 98% specificity) and rSh_quadruplet for IgG4 (93% sensitivity, 97% specificity). rSh_quadruplet performance remained robust in field evaluations but the performance of rSh_SAP-1 was unsatisfactory. To improve IgG1-based detection, we investigated additional candidate antigens from previous studies. Tetraspanin (TSP), a protein that was identified in an *S. mansoni* antibody biomarker study and cross-reacted with *S. haematobium*-positive sera was selected and two of its homologs were identified within the *S. haematobium* proteome. In parallel, two multi-epitope proteins (MEP) were designed based on previously identified peptide candidates.

Methods: These four proteins were expressed in a Chinese Hamster Ovary mammalian expression system and evaluated using ELISA and multiplex bead assay (MBA).

Results: Both homologs (rSm_TSP and rSh_TSP) antigens outperformed rSh_SAP-1 on both platforms with sensitivities of 85–86% and specificities of 96.2–96.7%. The newly designed multi-epitope proteins (rSh_MEP-1 and rSh_MEP-2) showed slightly higher specificities (97–98%) but approximately 10% lower sensitivities compared to rSh_quadruplet in the MBA platform.

Conclusions: These novel antigens enhance IgG1-based detection of *S. haematobium* infection and may improve serological assay diagnosis of urogenital schistosomiasis in multiplex platforms.

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UNLOCKING THE BLUEPRINT TO ELIMINATING NEGLECTED TROPICAL DISEASES (NTDS): LESSONS FOR SCHISTOSOMIASIS

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Background and Aims: Background: By January 2026, 58 countries had achieved the milestone of eliminating at least one neglected tropical disease (NTD), with some countries including Togo, eliminating multiple. Learning from these successes is pivotal for accelerating global elimination efforts of NTDs including schistosomiasis. This study aimed to systematically analyse successful NTD elimination programmes, identify key enabling factors, and develop a strategic blueprint to inform schistosomiasis elimination efforts.

Methods: We conducted a comprehensive review of published and grey literature on the 50 countries that eliminated at least one NTD in the past two decades and by March 2024. Data on elimination durations, organizers, strategies, partnerships, programmatic integration, and historical control efforts were extracted, analysed, and synthesised. A blueprint for strengthening elimination efforts was developed. We further determined what lessons can be learnt for schistosomiasis elimination.

Results: Successful elimination efforts involved strong country ownership, sustained commitment, and stakeholder partnerships. Key challenges in historical elimination attempts included limited resources, de-prioritisation and socio-political instability. The blueprint emphasises the need for cross-cutting interventions, increased funding, mainstreaming within health systems, and multisectoral approaches like One Health, particularly for NTDs with complex life cycles like schistosomiasis.

Conclusions: Conclusion: Notably, none of the 50 countries studied had eliminated schistosomiasis. Contributing factors include its complex life cycle, lack of integration into health systems, limited resources, and high re-infection rates. Our recommendations for elimination emphasise a holistic approach, country ownership, health system strengthening, cross-cutting strategies like WASH and One Health.

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ECLIPSE: AN INTERVENTION PROGRAMME TO IMPROVE PATIENT JOURNEY AND REDUCE STIGMA FOR PEOPLE WITH CUTANEOUS LEISHMANIASIS

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Background and Aims: Cutaneous leishmaniasis (CL) affects highly marginalized and underserved communities across the globe. We present findings from the five-year interdisciplinary ECLIPSE programme which aimed to understand the barriers to accessing healthcare and to improve the CL patient journey and reduce stigma in the most underserved communities in Brazil, Ethiopia and Sri Lanka.

Methods: We used qualitative, quantitative and creative approaches to investigate the impacts of CL on individuals and communities, levels of disease awareness and the barriers and facilitators for accessing diagnosis and treatment. Through dedicated Community Advisory Groups and Communities of Practice we ensured that our approach, research tools and interventions were appropriate for each context.

Results: Findings from over 200 interviews and 2,500 surveys across three countries showed that understanding of the causes and transmission of CL was low and that misinformation contributed to fear and stigmatization of affected individuals and, in some cases, of whole communities. Barriers to accessing healthcare included distance of travel to clinics, lack of childcare, fear of drug side-effects, inability to work, and low disease awareness in communities and healthcare professionals. The burden and impacts of CL were particularly high in Tigray, Ethiopia. We addressed identified challenges through co-production and implementation of bespoke community-facing interventions, including awareness campaigns, traditional theatre, podcasts, and training courses for healthcare professionals.

Conclusions: We have engaged with policymakers across multiple sectors and influenced policy change around decentralisation and increasing capacity and access for CL diagnosis and treatment. Work with the Tigrayan Regional Health Bureau has resulted in the establishment of new treatment centres for CL in Tigray, Ethiopia.

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FIRST REPORT OF ENTAMOEBA BANGLADESHI IN IRAN: APPLICATION OF TETRAPLEX REAL-TIME PCR FOR DIFFERENTIAL DIAGNOSIS OF ENTAMOEBA SPECIES

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Background and Aims: Accurate differentiation of *Entamoeba histolytica* from its morphologically identical species is critical for clinical management. While *E. dispar* and *E. moshkovskii* are well-documented, data on the emerging pathogen *E. bangladeshi* are lacking in Iran. This study aimed to evaluate a tetraplex real-time PCR assay for detecting the *Entamoeba* complex species.

Methods: A total of 552 stool samples were analyzed, 146 from symptomatic patients referred to the hospital in Tehran and 406 from working children in Tehran, mostly migrated from Afghanistan. An 18S rRNA-based tetraplex real-time PCR was performed to detect *E. histolytica*, *E. dispar*, *E. moshkovskii*, and *E. bangladeshi*.

Results: Real-time PCR identified *Entamoeba* species in 26 (4.7%; 95% CI: 3.2–6.8) samples, comprising 10 (1.8%; 95% CI: 1.0–3.3), 16 (2.9%; 95% CI: 1.8–4.7), 2 (0.4%; 95% CI: 0.1–1.3), and 6 (1.1%; 95% CI: 0.5–2.4) cases of *E. histolytica*, *E. dispar*, *E. moshkovskii*, and *E. bangladeshi*, respectively. Single and double *Entamoeba* infections were observed in 18 and 8 cases. Among the 10 *E. histolytica*-infected cases, 8 (80.0%; 95% CI: 49.0–94.3%) were co-infected with *E. dispar* (n=7) or *E. moshkovskii* (n=1). Most notably, this study identified *E. bangladeshi* in 6 (1.1%) samples, marking the first report of this species in Iran.

Conclusions: Our findings confirm the presence of *E. bangladeshi* in Iran for the first time, highlighting an overlooked diversity of *Entamoeba* species in the region. The developed multiplex real-time PCR is a robust tool for epidemiological surveillance and is essential for accurate diagnosis, especially in identifying emerging pathogens that are undetectable by conventional methods.

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ACCEPTABILITY AND FEASIBILITY OF RADIOFREQUENCY THERMOTHERAPY FOR CUTANEOUS LEISHMANIASIS IN A ROUTINE CLINIC IN SRI LANKA

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Background and Aims: Cutaneous leishmaniasis (CL) is an established public health problem in Sri Lanka, with suboptimal responses to intralesional sodium stibogluconate. Radiofrequency-induced heat therapy (RFHT) using the ThermoMed device is a safe and effective alternative. This study assessed the acceptability and feasibility of RFHT for uncomplicated CL as perceived by stakeholders in routine clinic settings.

Methods: CL patients treated at the District General Hospital, Hambantota, who received a single RFHT session (50 °C for 30 seconds per application, covering the entire lesion surface) were followed for up to 180 days. Acceptability and feasibility were explored using focus group discussions (FGDs) conducted approximately 105 days post-treatment. FGDs included 19 patients (9 males and 10 females) and 17 clinical staff from the dermatology clinic.

Results: RFHT was acceptable to patients and healthcare workers due to minimal discomfort, rapid administration, and early visible lesion improvement. Staff reported ease of use and good integration into routine clinic workflows. Operational challenges included prolonged clinic waiting times, transportation barriers affecting follow-up attendance, and competing family responsibilities. Patients reported high satisfaction, improved confidence, and perceived improvements in quality of life.

Conclusions: RFHT is acceptable and feasible for treating uncomplicated CL in routine Sri Lankan clinic settings. Addressing logistical barriers and pre-planning operations to minimize delays may facilitate RFHT scale-up within national treatment programmes. **Acknowledgements**
Byron Arana and Marina Boni are thankfully acknowledged for providing training, facilitating the funds and the ThermoMed devices through the Drugs for Neglected Diseases initiative (DNDi) and Thermo Surgery Technologies Inc. respectively.

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MULTI-COUNTRY ASSESSMENT OF PREVALENCE FOR FEMALE GENITAL SCHISTOSOMIASIS (MAP-FGS)

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Background and Aims: Female genital schistosomiasis (FGS) is a neglected presentation of Schistosoma haematobium that results in a variety of chronic gynecological symptoms in women and girls living in endemic areas. To date, there are no standardized methods to screen for and diagnose FGS, and FGS prevalence in sub-Saharan Africa (SSA) has not been estimated. The Multi-Country Assessment of Prevalence for Female Genital Schistosomiasis (MAP-FGS) is the first large-scale study to estimate the burden of FGS across SSA, filling in these crucial data gaps to guide regional interventions for at-risk women and girls.

Methods: Nearly 10,000 women and girls aged 10-60 were enrolled across six countries: Tanzania, Mali, Madagascar, Senegal, Ghana, and Nigeria. Women were examined in the field via colposcopy, and all women and girls were given general and gynecological-specific questionnaires. Study data includes FGS prevalence, spatial modeling, reported HIV, HPV and STI results, laboratory-based serology, and health-seeking behavior.

Results: In Tanzania, Ghana, and Nigeria, the estimated FGS prevalences in 2024 were 808,737, 2.1 million, and 10.4 million, respectively. These three countries account for approximately 24% of the total previously estimated number of FGS cases in SSA (56 million).

Conclusions: Prior to this study, the burden of FGS in SSA has not been estimated, and this study represents a first-of-its-kind prevalence estimation in women and girls. This study represents the

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most accurate prediction model for FGS, and the current estimates show a significant burden of FGS in SSA.

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CORD BLOOD MALARIA PARASITEMIA IN NEONATES FROM MALARIA AND HIV CO-INFECTED PREGNANT MOTHERS IN ABUJA NIGERIA

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Background and Aims: This was a hospital based study aimed at assessing the possible effects of Malaria and HIV co-infections on the babies from pregnant women co-infected with these diseases who are on routine retrovirals from the hospitals as well as on routine anti-malaria or malaria preventive therapy. The study assessed malaria parasitemia in the peripheral, and placental blood, of the pregnant women pre and post delivery, malaria parasitemia in cord blood post-delivery as well as the birth weights of the neonates of these women on delivery.

Methods: Informed and duly signed consents were obtained from the relevant agencies as well as from the study participants. Peripheral, placental and cord blood samples were collected pre - delivery, and post delivery respectively by the nursing midwives in the hospitals where this study was carried out. Thick and thin smears stained with 3% buffered Giemsa were prepared from the collected blood samples and examined under x1000 oil immersion microscope objective lens. Birth weights of the babies were measured with a standard weighing scale and categorized as normal baby birth weights (>2.5kg) and low birth weights (<2.5kg) respectively.

Results: A total of 237 pregnant women (116: 48.94% HIV+) and (121: 51.05% HIV- control). From the HIV+ group, 84 (72.41%) had peripheral malaria parasitemia; 76(65.52%) had placental malaria parasitemia and 73 (62.93%) had both peripheral and placental malaria, while 60(51.72%) had cord blood parasitemia. Low birth weights babies were not observed in the study group.

Conclusions: Cord blood malaria parasitemia often ignored deserves to be given priority attention.

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"BEYOND CANCER: TIRAPAZAMINE AS A POTENTIAL ANTIAMEBIC AGENT"

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Background and Aims: In Mexico and in other tropical countries worldwide, amoebiasis caused by *Entamoeba histolytica* remains a disease with high prevalence and mortality, characterized by its persistence and difficulty to eradicate. Although the parasite generally affects the gastrointestinal tract, amoebic liver abscess (ALA) represents the lethal form of the disease. The ALA model is reproduced in the Syrian golden hamster (*Mesocricetus auratus*), that allows monitoring of disease progression and the evaluation of new therapeutic alternatives with fewer adverse effects than current drugs. Tirapazamine (TPZ) is an anticancer drug that has been shown to have additional applications like antimicrobial agent. Our preliminary studies demonstrated that TPZ exerts an *in vitro* antiamoebic effect; therefore, the aim of the present study was to evaluate the effect of TPZ on the progression of ALA in hamsters.

Methods: ALA was induced in hamsters for 72 hours, then animals received nine doses of TPZ (25 mg/kg) administered intraperitoneally every 48 hours. On day 21, the hamsters were sacrificed, and abscess progression, as well as morphology and histopathology, were evaluated.

Results: Animals treated with TPZ showed smaller ALA, with morphological and histopathological features that did not correspond to the expected stage of disease progression. Additionally, the percentage of ALA Was 10% in TPZ-treated group, being comparable to the 6% exhibited by the metronidazole-treated group, while it was 70% in the untreated group.

Conclusions: These results demonstrate that TPZ has a significant effect on the progression of ALA in hamsters.

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BEYOND THE TRAP: INTEGRATING ENVIRONMENTAL DNA, CLASSICAL ENTOMOLOGY AND XENOMONITORING TO TRACE MALARIA VECTORS AND TRANSMISSION

Cecilia Wambui[1,2], Benjamin André[1,3], Léon Minga[4], Papy Ansobi[4], Jean-Paul Banzolele[5], Dalton Bakadila[6], Charly Mabaya[6], Patrick Mitashi[4], Michiel Jorissen[7], Mostafa Bentahir[7], Josué Zanga[4], Joule Madinga[6], Hugo Gante[1,2], Tine Huyse[1,2]
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Background and Aims: Malaria transmission remains exceptionally high in the DRC, driven by weak and fragmented surveillance systems that limit timely responses. Conventional entomological methods may miss cryptic or low-abundance vector species, particularly within *Anopheles* species complexes. This study integrates environmental DNA (eDNA), larval dipping, and adult mosquito surveillance to characterise malaria vector communities, *Plasmodium falciparum* infection, and vector competence at village scale in the Kimpese Health Zone.

Methods: In October 2025, 51 aquatic habitats across eight villages were sampled for eDNA using Sylphium-based filtration alongside larval dipping. eDNA samples were purified using the Sylphium Extraction Kit, and the internal transcribed spacer 2 (ITS2) region was amplified to detect the *Anopheles gambiae* complex and *Anopheles funestus* group. Samples positive at the complex or group level were screened using species-specific LAMP assays targeting principal malaria vectors. In parallel, adult mosquitoes were collected using CDC light traps and identified morphologically and molecularly. Adult *Anopheles* were dissected into abdomen and head-thorax fractions to distinguish midgut- and sporozoite-stage *P. falciparum* infections using LAMP-based xenomonitoring.

Results: Larval dipping yielded 95 mosquito larvae. Ongoing analyses are expected to reveal discordance between eDNA detection and larval dipping, with larval dipping being less sensitive and more prone to false negatives, particularly at sites harbouring low-abundance or cryptic species. Analysis of 445 adult mosquitoes from sentinel households is expected to reveal village-level variation in sporozoite prevalence and species-specific differences in parasite developmental success.

Conclusions: Integrating eDNA with classical entomology and molecular xenomonitoring strengthens fine-scale malaria vector surveillance and supports targeted control strategies in high-burden settings.

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EVALUATION OF LEGACY ANTIGENS OF ONCHOCERCA VOLVULUS IN MULTIPLEX BEAD ASSAY PLATFORM

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Background and Aims: We previously published the performance of an rOV16 ELISA using mammalian expressed protein for detecting *Onchocerca volvulus* infection with 89% sensitivity and 98.5% specificity. While sensitivity met the target product profile (TPP) requirements for stopping mass drug administration (MDA) decisions, specificity remained below the required threshold (99.8%). We sought to identify additional markers that, alone or in combination with rOV16 antigen, could meet the TPP targets.

Methods: Three legacy *O. volvulus* antigens — OV_FABP, OV_33.3, and OV_MSA — were expressed in a Chinese Hamster Ovary system and evaluated in a multiplex bead assay.

Results: For IgG1 detection, rOV_FABP performed best (92% sensitivity, 91% specificity) but cross-reacted with *Wuchereria bancrofti* (9%), *Loa loa* (66%), and *Mansonella perstans* (9%). For IgG4 detection, rOV_FABP again led (96% sensitivity, 96% specificity), followed by rOV_33.3 (93% sensitivity, 97% specificity) and rOV16 (89% sensitivity, 99.4% specificity). Notably, rOV16 showed minimal cross-reactivity, with no IgG4 recognition by *L. loa* or *M. perstans* sera and only 0.8% reactivity with *W. bancrofti*-infected people. In contrast, 48% of *L. loa* sera cross-reacted with rOV_FABP for IgG4. To evaluate the performance for IgG4 detection, antigen combinations were assessed using logistic regression and ROC analysis. The optimal pairing of rOV16 and rOV_FABP achieved 91% sensitivity and 99.88% specificity (AUC = 0.988), meeting the TPP specificity threshold.

Conclusions: rOV16 remains the most specific antigen for *O. volvulus* serology, while rOV_FABP serves as a complementary marker that, in combination, may enhance serologic testing in programmatic settings.

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EMERGING PARASITIC DISEASE BURDEN AND COMMUNITY HEALTH CHALLENGES IN GHANA: STRATEGIES FOR CONTROL IN DEVELOPING COUNTRIES

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Background and Aims: Parasitic diseases remain a major public health concern in many developing countries, particularly in sub-Saharan Africa. In Ghana, emerging and re-emerging parasitic infections continue to place significant pressure on healthcare systems and vulnerable communities. This study aimed to assess the growing burden of parasitic diseases in Ghana, identify associated community health challenges, and explore sustainable control strategies suitable for developing countries.

Methods: A descriptive review and community-based assessment approach were used to evaluate the prevalence and impact of parasitic diseases in selected communities in Ghana. Data were obtained from healthcare records, public health reports, environmental observations, and community health outreach activities. Key factors examined included sanitation, access to clean water, health education, and healthcare accessibility.

Results: The findings revealed increasing cases of malaria, intestinal helminths, schistosomiasis, and other parasitic infections, particularly among children and low-income populations. Poor sanitation, inadequate waste disposal, unsafe water sources, limited health education, and climate-related environmental changes were identified as major contributing factors. The burden of these infections has resulted in increased morbidity, reduced productivity, school absenteeism, and economic hardship within affected communities.

Conclusions: Emerging parasitic diseases continue to threaten community health and socio-economic development in Ghana. Strengthening preventive healthcare systems, improving sanitation infrastructure, expanding public health education, promoting early diagnosis and treatment, and enhancing community participation are essential strategies for effective control. Collaborative efforts between governments, healthcare institutions, and international partners are critical in reducing the parasitic disease burden in developing countries.

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TRANSMISSION DYNAMICS, EPIDEMIOLOGY, AND GLOBAL HEALTH / PARASITIC INFECTION OF GLOBAL HEALTH AND BIOMEDICAL IMPORTANCE

A SYSTEMATIC REVIEW OF THE EFFECTS OF GIARDIA AND CRYPTOSPORIDIUM ON CHILDHOOD GROWTH FALTERING

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Background and Aims: *Giardia lamblia* and *Cryptosporidium spp.* have been associated with childhood growth stunting; however, studies have found variable effects. We conducted a systematic review examining the effect of *Giardia* and *Cryptosporidium* on longitudinal growth in children under five, considering detection method, geography, and other study variables.

Methods: Studies required *Giardia* or *Cryptosporidium* detection, children under five, a comparison group, and growth measurements at ≥2 timepoints. Seven databases were searched through April 2025: PubMed, CINAHL via EBSCO, Cochrane Database, EMBASE.com, Global Health via EBSCO, Scopus, and LILACS. Two independent reviewers screened abstracts using Covidence, reviewed full-text, and extracted data. Data for growth associations (including height-for-age, weight-for-age, and weight-for-height z-scores) were synthesized. Of 10,872 articles identified, 810 underwent full-text review and 32 met extraction criteria. Studies were categorized by evidence quality: high-confidence (multivariate analysis with significant association in ≥1 subgroup) or low-confidence (univariate analysis, nominally significant, or borderline results) for negative or null growth associations.

Results: For *Giardia*, 13/21 (62%) showed high-confidence and 2/21 (10%) low-confidence negative growth associations. For *Cryptosporidium*, 10/19 (53%) showed high-confidence and 6/19 (32%) low-confidence negative growth associations. Studies represented South Asia (17/32, 53%), sub-Saharan Africa (15/32, 47%), and Latin America (9/32, 28%); 6/32 (19%) studies spanned multiple regions.

Conclusions: A minority of published studies on *Giardia* and *Cryptosporidium* childhood growth impact met pre-defined quality criteria. However, among these studies, most found negative growth associations for both pathogens, the majority with high confidence. Further analysis will examine

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potential clustering by measured baseline characteristics, detection method, symptomatic/asymptomatic infection, geography, and analysis strength.

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GEOGRAPHIC, DEMOGRAPHIC AND ECONOMIC BURDEN OF HUMAN CYSTIC ECHINOCOCCOSIS IN ITALY BASED ON HOSPITAL DISCHARGE RECORDS (2015–2022)

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Background and Aims: Cystic echinococcosis (CE) is a neglected zoonotic disease that remains a public-health concern worldwide. However, poor surveillance and incomplete reporting still hinder the calculation of the CE burden. This study evaluated national hospital discharge records (HDRs) from 2015 to 2022 to update CE epidemiological situation and economic impact of human CE in Italy.

Methods: We reviewed 4,295 HDRs with an echinococcosis diagnosis during 2015-2022. Data extracted included age, sex, residence, nationality, primary versus secondary diagnosis, treatment type, length of stay and discharge outcome. The average annual hospital prevalence was calculated per 100,000 inhabitants and direct hospital costs were derived from records of CE as the primary diagnosis. The 2015-2022 trend was assessed and compared to an extended data set (2001-2022).

Results: From 2015 to 2022, 2,663 cases were identified nationally. The national average annual hospital prevalence was 0.56 cases/100,000 inhabitants. Patients were predominantly adults (>90%) and divided into the following age categories: 25–44 (20.4%), 45–64 (30.2%) and ≥65 years (41.1%); pediatric cases were rare (<3%). National direct hospital costs amounted to €10,726,413. A downward trend in hospitalised CE cases was observed from 2015 (326 cases) to 2022 (165 cases), with a notable decline in 2020 linked to the COVID-19 pandemic. Analysis of the longer time period (2001–2022) revealed 1250 fewer cases.

Conclusions: These findings confirm CE's heterogeneous distribution, with persistent hyperendemic foci in traditional pastoral areas driving disproportionate healthcare costs. Sustained One Health surveillance, targeted regional prevention and robust national reporting are critical to further reduce CE burden in Italy.

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THE RISE OF THE EUROPEAN UNION REFERENCE LABORATORIES: EURL-PARASITES AND EURL-PUBLIC HEALTH ON HELMINTHS AND PROTOZOA

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EU Reference Laboratory for Parasites (EURL-P) - EU Reference Laboratory for Public Health on Helminths and Protozoa (EURL-PH-HP). ISTITUTO SUPERIORE DI SANITA', Rome, Italy

Background and Aims: As regards the "European Union Reference Laboratories (EURLs) for food, feed and animal health" (Regulation 2017/625 on "Official controls"), the European Commission (EC) nominated the "*EURL for Parasite"* (EURL-P) in the field of food safety, which was established at Istituto Superiore di Sanità (ISS; Rome, Italy) in 2006 (Regulation 776/2006), with a focus on *Echinococcus, Trichinella, Anisakis* and other parasites. Recently, the EC nominated the new "*EURL for Public Health on Helminths and Protozoa"* (EURL-PH-HP), established at ISS in 2025, in the field of public health (Regulation 2022/2371 on "Serious cross-border threats to health"), with a focus on *Echinococcus, Toxoplasma, Trichinella* and *Plasmodium*.

Methods: The EURLs have the following main objectives: 1) Provision of reference diagnostics and characterisation services. 2) Provision of reference material. 3) Provision of scientific advice and technical support. 4) Provision of PT schemes. 5) Organisation of trainings. 6) Communication and dissemination. 7) Organization of annual meetings.

Results: During 2006-2024, the EURL-P tested 23,514 samples of various foodborne parasites, of which 16,889 were molecular/serological tests targeting *Echinococcus* spp., *Toxoplasma gondii* and *Trichinella* spp. During 2013-2024, the EURL-P delivered 1,585 PT panels (3 samples each, for a total of 4,755 samples) on various foodborne parasites. Of these, 1,176 panels were molecular/serological PT schemes targeting *Echinococcus* spp., *Toxoplasma gondii* and *Trichinella* spp. The new EURL-PH-HP was operational on 1st January 2026 for a period of seven years.

Conclusions: The main purpose of these EURLs will be to contribute to the development of harmonized, high-quality tests for parasites in One Health perspective.

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European Union Reference Laboratory for Parasites (EURL-P; food safety)

EURL
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POSTER ON BOARD SHIFT 01
TRANSMISSION DYNAMICS, EPIDEMIOLOGY, AND GLOBAL HEALTH / PARASITIC INFECTION OF GLOBAL HEALTH AND BIOMEDICAL IMPORTANCE

DIRECTED ACYCLIC GRAPHS (DAGS) FOR CAUSAL INFERENCE OF THE IMPACT OF MATERNAL AND CHILD HELMINTH INFECTIONS ON CHILD GROWTH

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Background and Aims: As of 2025, an estimated 150.2 million children under five years globally were stunted – falling <-2 standard deviations below the World Health Organization (WHO) Child Growth Standards median. Helminth infections have frequently been proposed to be associated with childhood stunting. However, empirical evidence remains inconclusive and conflicting, with some studies demonstrating significant associations while others report negligible or null effects. This study aimed to construct directed acyclic graphs (DAGs) to clarify the causal relationships between helminth infection and birth weight/childhood stunting, incorporating maternal and child helminth infections as exposures and birth weight and childhood stunting as outcomes.

Methods: We utilised Evidence Synthesis for Constructing DAGs (ESC-DAG) methodology, as a transparent and systematic approach. Three temporal DAGs were constructed: maternal infection, infant infection during exclusive breastfeeding, and child infection post-weaning. DAGs were built using diagrammeR package, acyclicity checked using DAGitty package, and minimally sufficient adjustment sets (MSAS) identified (representing confounding variables), all in R (version 3.1).

Results: DAG models presented, and MSAS determined: core adjustments for all DAGs included geographical location, healthcare access, and Water, Sanitation and Hygiene (WASH) factors. The maternal infection model additionally incorporated maternal age, socioeconomic status (SES), and maternal education. For child models, both age groups included child sex, age, birth weight, and parental education. The infant model additionally incorporated breastfeeding status, whilst the post-weaning model included SES alongside other shared variables.

Conclusions: These MSAS provide researchers with standardised confounder adjustment frameworks, helping resolve inconsistencies in prior literature stemming from incomplete confounder control and advancing methodologically rigorous causal inference.

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DO MATERNAL AND/OR CHILD INTESTINAL PARASITIC PROTOZOAL INFECTION INFLUENCE BIRTH OUTCOMES AND LINEAR GROWTH FALTERING?

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Background and Aims: Gastrointestinal protozoan infections remain highly prevalent among children, particularly across LMICs, but their impact on maternal-child outcomes- including birth outcomes and childhood stunting (height-for-age z-score [HAZ] more than 2 standard deviations below the WHO median)- remains largely undetermined. This review evaluated the potential association of gastrointestinal parasitic protozoa infection of mother and/or child with birth outcomes and stunting.

Methods: Following PRISMA guidelines, six databases were searched using a PECO-based strategy. Meta-analyses stratified by parasite species were conducted for cross-sectional studies, with binary outcomes used odds ratios (ORs)/adjusted odds ratios (AORs) for stunting, while continuous outcomes used mean differences (MD) in HAZ. Longitudinal studies were narratively synthesised using the Synthesis Without Meta-analysis (SWiM) framework. Symptomatic versus asymptomatic infections were examined where data permitted.

Results: 99 studies (1976–2023) were included. Meta-analyses demonstrated significant associations between child infections and stunting: *Cryptosporidium* spp. (AOR=2.38, 95%CI: 1.55–3.64) and *Giardia* spp. (AOR=1.70, 95%CI: 1.12–2.58), with *Giardia* spp. also reducing HAZ (MD: –0.42, 95%CI: –0.53 to –0.30). Notably, asymptomatic infections were significantly associated with reduced growth across multiple species, not only symptomatic disease. SWiM of longitudinal studies supported these associations. Evidence on maternal protozoan infections and birth outcomes was limited and heterogeneous.

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Conclusions: Gastrointestinal parasitic protozoa are significantly associated with childhood stunting via symptomatic (e.g. diarrhoea causing nutrient loss) and asymptomatic (e.g. via environmental enteric dysfunction) infections. That asymptomatic infections are also associated, suggests the true burden on child growth is underestimated. Further research is required to clarify the impact of maternal infections on birth and subsequent childhood stunting outcomes.

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PREVALENCE AND RISK FACTORS OF CRYPTOSPORIDIUM SPP. AND GIARDIA INTESTINALIS INFECTIONS IN THE CITY OF OUAGADOUGOU, BURKINA FASO

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Background and Aims: *Cryptosporidium* spp. and *Giardia intestinalis* are intestinal parasites that cause gastroenteritis worldwide. These parasites infect animals and humans in rural and urban areas alike. This study aims to assess the prevalence and risk factors of these parasite infections in the city of Ouagadougou.

Methods: The study was conducted from August 2022 to July 2023 in three healthcare facilities in the city of Ouagadougou. Demographic data were collected from participants using a questionnaire. Stool samples were examined using parasitological techniques, including direct examination, the Ritchie concentration technique, the Ziehl-Neelsen staining technique, and by real-time PCR.

Results: A total of 341 participants were enrolled in this study, including 167 males (48%) and 174 females (52%). The sex ratio was 0.96. The overall parasite prevalence was 46.33%. The prevalences of *Cryptosporidium* spp *and Giardia intestinalis* were 22.58% and 7.62% respectively. Real-time PCR revealed 37 positive cases out of 56, including 18 for *Cryptosporidium*, 14 for *Giardia*, and 5 cases of co-infection with Giardia and *Cryptosporidium*. Rumbling, abdominal bloating, loose stools, and diarrhea are associated with cryptosporidiosis. In contrast, gender and drinking water source are associated with Giardiasis.

Conclusions: This study showed high prevalences of *Cryptosporidium spp* and *Giardia* spp among patients affected by digestive troubles in the city of Ouagadougou. The insufficiency of individual and collective hygiene measures is strongly associated with the occurrence of these parasites. The success of these parasitic disease control required a holistic engagement.

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TWELVE-YEAR PARASITOLOGICAL RESPONSE AND STABLE CARDIAC STATUS AFTER BENZNIDAZOLE FOR CHAGAS DISEASE DETECTED VIA BLOOD DONOR SCREENING IN A NON-ENDEMIC SETTING: A CASE REPORT

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Background and Aims: In non-endemic settings where reinfection is unlikely, Chagas disease is increasingly detected through blood donor screening. We report long-term parasitological and cardiac outcomes after antitrypanosomal therapy in a patient with mild cardiac involvement.

Methods: A seropositive blood donor was evaluated for Trypanosoma cruzi infection and cardiac involvement. Benznidazole was administered at 400 mg/day for 60 days (started January 2014) and completed. Parasitological monitoring included serial blood culture and quantitative PCR. Cardiac follow-up included electrocardiography and echocardiography.

Results: At diagnosis, the patient had second-degree atrioventricular block and mildly reduced left ventricular ejection fraction (LVEF 49.5%). Competing etiologies were assessed: exercise stress testing showed no inducible ischemia, and cardiac sarcoidosis was considered unlikely based on normal serum ACE and the absence of hilar lymphadenopathy or lung lesions on chest–abdomen CT. Blood culture and PCR became negative 6 months after treatment and remained negative for 12 years (January 2014 to January 2026; qPCR, n=12; culture, n=12). Cardiac status remained stable without progression of conduction disease, heart failure hospitalization, or device implantation. LVEF remained stable throughout follow-up (46–55%; final 48.7% in January 2026).

Conclusions: This case demonstrates the feasibility of long-term parasitological monitoring and supports the potential value of antitrypanosomal therapy to prevent progression of mild cardiac involvement in non-endemic settings where reinfection is unlikely.

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SYSTEMATIC REVIEW OF GASTROINTESTINAL INFECTION RISKS FROM SMALLHOLDER FARMING: POTENTIAL NEGATIVE HEALTH IMPACTS FROM INTENSIFYING AGRICULTURE FOR NUTRITION

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Background and Aims: Much research has highlighted the role of agriculture in nutrition of rural households in developing countries, and the role of agricultural interventions to improve household food security and diet; however, there is limited evidence of improved nutritional status. In these contexts, undernutrition commonly co-occurs with environmentally transmitted gastrointestinal parasites. The purpose of this paper is to review existing literature to highlight agricultural risk factors for infections.

Methods: Search terms encompassing smallholder farming and parasite infections were entered into Web of Science, PubMed, and SCOPUS, from start of records until May 2020. Inclusion criteria were: peer-reviewed; English language; primary research; agricultural practices including smallholder or subsistence production of crops and livestock, home gardening, irrigation, consumption of home-grown foods; studies of human gastrointestinal infections; located in LMICs. Exclusion criteria were: unpublished abstracts or briefs; reviews; meta-analyses; papers describing only food systems, food safety, or aquaculture. Citations were managed using Mendeley Reference Manager.

Results: Initially, 17,930 articles were returned, with 623 excluded as duplicates, leaving 17,307. Article titles and abstracts were screened first to remove articles that did not mention human health, leaving 3732 records. They were screened a second time, according to the full inclusion and exclusion criteria, leaving 445. Articles were then downloaded and read for a final decision on inclusion.

Conclusions: Reviewed publications were in two categories: those comparing infection risk between agriculture-related and non-related occupations, and those analyzing infection risks associated with farming activities/behaviours, including contact with water, wastewater, domestic animals, human and animal feces used as fertilizers, and hygiene practices.

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PREVALENCE OF INTESTINAL PARASITIC INFECTIONS FROM 2016 TO 2020 AT THE FANN UNIVERSITY HOSPITAL CENTER

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Background and Aims: Intestinal parasitic infections (IPIs) are considered a serious and widespread public health problem worldwide, mainly in urban and rural environments of tropical and subtropical countries. The objective of this study was to determine the prevalence of digestive parasitic infections diagnosed at a university hospital in Dakar, Senegal. Between 2016 and 2020

Methods: direct examination and Ritchie techniques were performed on 3,873 parasitological stool samples from patients with suggestive symptoms at the parasitology-mycology laboratory of the Fann Hospital in Dakar.

Results: A total of 1,056 samples tested positive for parasites, representing a prevalence of 27.27%. Protozoa were predominant in 94.52% of samples, while helminthiasis accounted for 5.48%. The main species encountered were *Blastocystis hominis* (38.83%), followed by *E. coli* (36.07%), *E. nana* (7.76%), *Giardia intestinalis* (7.39%), *Ascaris lumbricoides* (3.13%), *Trichocephale* (0.76%), *Teania* (0.76%) and *Ankylostoma* (0.28%).Males were more represented (52.36%) than females (47.63%), giving a sex ratio of 1.04. Children aged 10 to 15 were the most affected, with a prevalence of 27.27%.

Conclusions: These results confirm that intestinal parasitic infections are still endemic in Dakar, hence the need to strengthen deworming and health education strategies, as well as to improve the environment

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STUDY OF THE PREVALENCE OF TOXOPLASMA GONDII INFECTION IN PSYCHIATRIC POPULATIONS AND ASSOCIATIONS WITH SYMPTOMS

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Background and Aims: *Toxoplasma gondii* (Tg) infection has been related to several psychiatric disorders, with a particular emphasis on schizophrenia. However, no study has compared Tg infection across major psychiatric diagnostic categories. We aimed to compare Tg prevalence among psychiatric diagnostic categories and assess Tg infection associations with symptoms (overall and by sex).

Methods: Tg infection was measured in 1901 psychiatric patients from the *Signature* Biobank. Diagnostic groups included *substance use* (n=196)*, psychotic disorder* (n=622), *mood disorder* (n=470), *anxiety disorder* (n=228), *personality disorder* (n=236) disorders and community controls (n=149). Tg infection status was determined by IgG ELISA, and symptoms were measured with validated questionnaires.

Results: show that the Tg infection prevalence differ between diagnostic ($X^2(6) = 29.55$; $p < 0.001$). Tg infection is higher in mood (OR= 2.2, IC95% [0.74 ; 6.57], p=0.03) and anxiety disorders (OR=2.4, IC95% [0.66 ; 9.09], p=0.01) when compared to controls. Tg infection was associated with lower depression scores (Est.= -1.704, p=0.04), higher psychosis scores (Est.= 0.27, p=0.02) and lower suicidality scores (Est.= -1.42, p=0.01) only in mood disorder patients.

Conclusions: Our study reveals that when we compare psychiatric diagnostics within the same study, Tg prevalence is higher in mood and anxiety disorders, a result that stands in contrast with previous studies reported higher Tg prevalence in schizophrenic disorder. Moreover, Tg infection in mood disorder is associated with an intriguing clinical picture of low depression and suicidality, but high psychosis score.

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MOLECULAR IDENTIFICATION OF HELMINTH PARASITES OF THE HETERAKIDAE AND ASCARIDIDAE FAMILIES OF FREE-RANGING CHICKENS FROM SELECTED RURAL COMMUNITIES OF KWAZULU-NATAL PROVINCE OF SOUTH AFRICA

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Background and Aims: Free-range chickens are predisposed to diverse parasitic infections during scavenging. Accurate identification of these parasites using morphological characters has been a challenge. Therefore, this study aimed to identify nematodes from the Heterakidae and Ascarididae family infecting free-ranging chickens from KwaZulu-Natal province of South Africa using a combination of morphological and molecular techniques.

Methods: Forty-two free-ranging adult indigenous chickens were purchased from randomly selected households in Shong- weni (n=12), Umzinto (n=10), Gingindlovu (n=10) and Ozwathini (n=10) rural villages and examined for nem- atodes of the Heterakidae and Ascarididae family. Col- lected specimen were identified morphologically and confirmed using mitochondrial and nuclear ribosomal markers.

Results: showed that *Ascaridia galli* occurred at all sampling locations with an overall prevalence of 58.3%, being most prevalent in Shongweni, followed by Gingindlovu, Ozwathini and Umzinto. *Heterakis gallinarum* was recorded in three locations, with high prevalence in Gingindlovu, Ozwathini and Shongweni, while *Heterakis beramporia* occurred at low prevalence and was absent in Umzinto. Mixed infections of *A. galli* and *H. gallinarum* were common, whereas co-infections involving *H. beramporia* were limited to Gingindlovu. Molecular analyses confirmed species identification, with *A. galli* closely related to South African GenBank isolates and forming five novel haplotypes. Phylogenetic and haplotype network analyses supported clear species clustering, and ITS analysis confirmed *H. beramporia*, representing its first molecular record in South Africa documented.

Conclusions: The prevalence of gastrointestinal nematodes of fam- ily Ascarididae and Heterakidae in free-range chickens is high in 3 of the 4 sampled rural communities of Kwa- Zulu-Natal province of South Africa.

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MANAGEMENT OF MORBIDITY AND DISABILITY RELATED TO LYMPHATIC FILARIASIS IN AKWA IBOM STATE, NIGERIA

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Background and Aims: Lymphatic filariasis (LF) is a leading cause of chronic disability in endemic countries, with hydrocele and lymphedema imposing substantial physical, social, and economic burdens. Disease Management, Disability, and Inclusion (DMDI) is central to the global LF elimination strategy, yet evidence on sub-national variation in morbidity burden and service coverage remains limited. This study assessed LF-related morbidity patterns and DMDI coverage in Akwa Ibom State, Nigeria.

Methods: Secondary analysis of Akwa Ibom State LF DMDI program data collected between 2022 and 2025 was conducted across 31 Local Government Areas (LGAs). Data included reported cases of hydrocele, lymphedema, and elephantiasis, as well as access to DMDI services. Descriptive statistics were used to summarise morbidity burden, service coverage, and inter-LGA variation.

Results: A total of 1,632 LF morbidity cases were recorded, comprising 1,582 (96.9%) hydrocele, 35 (2.1%) lymphedema, and 15 (0.9%) elephantiasis cases. Among hydrocele patients, 1,134 (71.7%) received surgical care, leaving a backlog of 448 cases. Coverage was higher for lymphedema and elephantiasis, with 42 patients (84.0%) treated. LF morbidity per LGA ranged from 1–127 cases (mean = 49.5; SD = 33.1; CV = 76.7%), indicating marked geographical heterogeneity, with the highest burdens in Uyo, Nsit Atai, and Ibiono Ibom LGAs.

Conclusions: LF morbidity and DMDI coverage vary substantially across LGAs in Akwa Ibom State, highlighting inequities in access to care Targeted scale-up of hydrocele surgery, strengthened referral systems, and sustained integration of DMDI into primary healthcare are essential to reducing LF-related disability and achieving elimination goals.

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MOLECULAR IDENTIFICATION AND GENOTYPING OF FREE-LIVING AMOEBAE IN SOIL AND SAFFRON SAMPLES COLLECTED FROM MAJOR SAFFRON-GROWING AREAS IN IRAN

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Background and Aims: Free-living amoebae (FLA) are ubiquitous protozoa that may act as opportunistic pathogens and reservoirs for various microorganisms. Despite the widespread cultivation and manual harvesting of saffron, no study has investigated the presence of FLA in saffron-related environments in Iran. This study aimed to identify and characterize FLA in soil and saffron samples collected from major saffron-producing regions of Iran.

Methods: A total of 126 samples, including 66 soil and 60 saffron flower samples, were collected from Gonabad, Ferdows, Qaen, and Torbat-e Jam. Samples were cultured on non-nutrient agar enriched with heat-killed *Escherichia coli*. Molecular identification was performed using PCR targeting the 18S rRNA and ITS regions. Representative isolates were sequenced and analyzed phylogenetically. Thermotolerance and osmotolerance assays were also conducted on selected *Acanthamoeba* isolates.

Results: FLA were detected in 81 samples (64.3%). *Acanthamoeba* was the predominant genus (50%), followed by *Vermamoeba* (32.5%) and Vahlkampfiidae (8.7%). Sequence analysis of 31 *Acanthamoeba* isolates identified all as genotype T4, closely related to clinical and environmental reference strains. All tested isolates grew at 37 °C, whereas none tolerated 40 °C or 1.0 M mannitol.

Conclusions: This study provides the first molecular evidence of FLA, particularly *Acanthamoeba* T4, in saffron-associated environments in Iran. The detection of genotype T4, closely related to known clinical strains, suggests that local agricultural soils may contain amoebae with pathogenic potential. The presence of potentially pathogenic genotypes highlights the importance of monitoring agricultural environments and adopting protective measures during saffron harvesting.

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BURDEN AND DEMOGRAPHIC DETERMINANTS OF INTESTINAL PARASITIC INFECTIONS AMONG SELECTED INDIGENOUS COMMUNITIES IN MALAYSIA

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Background and Aims: Intestinal parasitic infections remain an important public health problem among indigenous communities or known as Orang Asli (OA) in Malaysia. Updated epidemiological data from remote settlements such as Pos Kemar, Perak, Malaysia is limited. This study aimed to determine the prevalence and demographic correlates of intestinal parasitic infections among OA communities in Pos Kemar, Perak, Malaysia.

Methods: A cross-sectional study was conducted among 141 OA residents from Kampung Katong and Kampung Lerlar. Sociodemographic information was collected using questionnaires. Stool samples were examined using Formalin-Ethyl Acetate Concentration Technique (FECT), Wheatley's trichrome staining, modified acid-fast staining and Gram chromotrope staining. Data were analysed using descriptive statistics and chi-square tests.

Results: The overall prevalence of intestinal parasitic infections was 52.5%. Monoparasitism accounted for 9.9% while polyparasitism accounted for 42.6%. *Trichuris trichiura* was the most common helminth detected (5.7%), followed by *Ascaris lumbricoides* (1.4%) and hookworm (0.7%). Protozoan parasites detected included *Entamoeba histolytica/dispar* and *Entamoeba coli*. The highest percentage of intestinal parasitism was observed among children aged 6–10 years (33.8%), likely due to increased outdoor activity and environmental exposure.

Conclusions: Intestinal parasitic infections remain highly prevalent among OA communities in Pos Kemar, Perak, Malaysia. Mass antihelminthic treatment targeting preschool- and school-aged children should be sustained to reduce both infection and reinfection with soil-transmitted helminths (STH). Continuous collection of baseline data on STH infections is essential to support public health authorities in developing effective intervention strategies, particularly among indigenous communities in Malaysia.

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INTEGRATED ASSESSMENT OF TRICHOMONAS VAGINALIS, OTHER STI ETIOLOGICAL AGENTS, CO-INFECTION, AND SEXUAL BEHAVIOUR AMONG STI AND OTHER CLINICAL POPULATIONS IN EASTERN INDIA

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Background and Aims: Sexually transmitted infections (STIs) remain a major public health concern worldwide. Recent years showed an increase in certain STIs among the groups like antenatal women and MSMs. Conventional approaches often fail to identify fastidious organisms and co-existing infections, underscoring the need for sensitive molecular methods. Data on STI agents with any co-infection patterns from Eastern India are limited.To determine the etiological agents of *T. vaginalis* versus other STIs, assess their co-infections, and evaluate associated sexual behavioural characteristics among patients attending the STI clinic along with other departments.

Methods: In this cross-sectional study any patient presenting with genital discharge, ulcers or warts were enrolled with consent. Clinical samples were subjected to microscopy and molecular testing. Multiplex real-time PCR was performed for detection of *N. gonorrhoeae*, *T. pallidum* subspecies *pallidum*, *C. trachomatis*, *M. genitalium*, *T. vaginalis,* Herpes simplex virus-1 and -2, and Human papillomavirus-16 and -18. Serological testing for HIV, hepatitis B & C viruses with syphilis was carried out. Sexual behaviour and risk factors were documented. Data were analysed.

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Results:

Table 1: Table showing the type of samples and positivity

Clinical presentation or sample type	Count (N)	Percentage (%)
HVS (High vaginal swab)	16	36.4%
Genital / Penile warts (including Molluscum contagiosum)	10	22.7%
Urine samples	6	13.6%
Urethral discharge (± urine)	3	6.8%
Ulcer-related samples (ulcer swab, penile/oral/vulval ulcer)	3	6.8%
Urethral swab	2	4.5%
Rectal swab	1	2.3%
Perianal abscess swab	1	2.3%
Other / mixed swab	2	4.5%
Total	**44**	**100%**

Table 2: Samples positive for 1 or more STI pathogens

Pathogens	Positive samples (N)	Percentage (%)
HPV-18	4	20%
HSV-2	4	20%
TV(Trichomonas vaginalis)	3	15%
CT (Chlamydia trachomatis)	3	15%
MG (Mycoplasma genitalium)	3	15%
NG (Neisseria gonorrhoeae)	2	10%
TP (Treponema pallidum)	2	10%

The study showed high T. vaginalis and viral STI pathogens among symptomatic clinic attendees with seropositivity. Co-infections were observed, highlighting the limitation of syndromic management alone.

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Conclusions: This study demonstrates the utility of multiplex real-time PCR in accurately identifying STI etiological agents and co-infections in a single clinical sample. Integration of molecular diagnostics with behavioural assessment provides a comprehensive approach to STI management. The findings emphasize the need for routine laboratory-based diagnosis to improve patient outcomes and guide public health interventions in resource-limited settings.

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POSTER ON BOARD SHIFT 01
TRANSMISSION DYNAMICS, EPIDEMIOLOGY, AND GLOBAL HEALTH / PARASITIC INFECTION OF GLOBAL HEALTH AND BIOMEDICAL IMPORTANCE

THE SILENT BURDEN UNHEARD: PROFOUND IGNORANCE OF FEMALE GENITAL SCHISTOSOMIASIS AMIDST SUPERFICIAL AWARENESS OF UROGENITAL SCHISTOSOMIASIS IN NORTHERN NIGERIA

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Background and Aims: In North-western Nigeria, urogenital schistosomiasis (UGS) remains a persistent public health threat, particularly for adolescent girls and women. This study assessed community awareness of UGS and its gendered complication, female genital schistosomiasis (FGS), among women and girls in Jigawa State.

Methods: This study enrolled 648 females aged 15years and above in Jigawa. A pre-tested questionnaire assessed knowledge of UGS and FGS. Data analysis involved proportions, chi-square (Fisher's exact test), and logistic regression. Knowledge scores ranged from 0 to 4, with 1 point for correct answers. Scores < 2 were poor, 2-3 average, and 4 good.

Results: The findings showed 73% of participants heard of UGS, but deep knowledge was scarce. Only 11.9% correctly identified water contact as the cause, and just 8.5% demonstrated good overall understanding. Information sources were largely informal, relying on town criers (31.1%) and family networks (29.6%). A critical disparity emerged regarding FGS, a severe reproductive health consequence. Here, awareness collapsed: 69.4% had never heard of FGS. Of the few who had, 58.1% cited personal experience, indicating knowledge is often acquired only through advanced suffering. Fundamental misconceptions persisted: only 0.3% knew the cause, 8.3% cited modern prevention; consequently, 91.8% had poor knowledge of FGS.

Conclusions: The findings reveal a dangerous chasm between general UGS recognition and specific FGS understanding. This knowledge gap, compounded by reliance on informal information sources, directly undermines control efforts. Thus, urgent gender-sensitive education campaigns are needed to transform awareness and combat this neglected health burden.

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POSTER ON BOARD SHIFT 01
TRANSMISSION DYNAMICS, EPIDEMIOLOGY, AND GLOBAL HEALTH / PARASITIC INFECTION OF GLOBAL HEALTH AND BIOMEDICAL IMPORTANCE

BIOACTIVE SUBSTANCES IN SPENT COFFEE GROUNDS FROM FILTERED SPECIALTY COFFEES MAY AFFECT ENDOPARASITES IN LAMBS

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Background and Aims: Spent coffee grounds (SCGs) are a by-product of coffee brewing. We quantified the phenolic compounds in Ethiopian specialty coffee (ECOF), SCGs from ECOF (SCG-ECOF), and mixed SCGs (SCG-M), which were prepared by filtration. We investigated the anthelmintic activity of ECOF and the effectiveness of SCG-M as a feed supplement in lambs infected with *Haemonchus contortus*.

Methods: The phenolic compounds of SCGs were analysed quantitatively by UHRMS; the concentrations were calculated as chlorogenic acid (CGA) equivalents. An *in vitro* larval development test was performed in 96-well microtiter plates. Larvicidal activity was expressed as the mean lethal dose (LD50 or LD99). Sixteen lambs (outdoor groups) and fourteen lambs (indoor groups) were infected with *H. contortus* larvae and divided into two control and two experimental groups. The experimental groups were fed a concentrate diet with SCGs. The number of eggs per gram (EPG) of feces was quantified from days 18 to 84 *post*-infection.

Results: Quantitative analysis of the ECOF, SCG-ECOF, and SCG-M samples revealed the presence of high levels of mainly CGAs and their isomers. The larvicidal effect of the aqueous ECOF extracts was evident after 48 hours of incubation. The EPG in lambs was highest on day 31. Except for the IN-SCG group, egg shedding was significantly lower in the other groups from day 59 onwards.

Conclusions: SCG are sources of bioactive compounds with anthelmintic activity, which may affect endoparasites in lambs. The study was supported by the MESRS of the Slovak Republic and the SAS (VEGA 1/0038/25).

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POSTER ON BOARD SHIFT 01
TRANSMISSION DYNAMICS, EPIDEMIOLOGY, AND GLOBAL HEALTH / URBANIZATION, MIGRATION, AND PARASITE SPREAD

PATCHY SPREAD OF A CO-INTRODUCED STRONGYLOIDES NEMATODE IN A CITY: THE COMBINED ROLES OF GREENSPACE COVERAGE AND HOST TRAITS IN AN INVASIVE SQUIRREL POPULATION

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Background and Aims: Biological invasions reshape parasite dynamics in urban environments, but the drivers of establishment and spatial spread of co-introduced parasites remain poorly understood. We investigated the non-native nematode *Strongyloides callosciureus*, introduced with invasive Pallas's squirrels in Hamamatsu City, Japan, to evaluate host- and environment-related correlates of infection.

Methods: We examined 174 invasive Pallas's squirrels (80 males, 94 females) collected from 14 sites between June 2019 and January 2021. Specimens were stored frozen and later thawed in the laboratory. Organs were dissected and examined under a stereomicroscope. The respiratory tract and gastrointestinal tract were inspected for nematodes using forceps and dissection scissors. We compared spatial variation in infection across an urban–rural gradient within the city.

Results: Overall prevalence was 31.0% (54/174). Infection formed spatial hotspots rather than a gradient from the long-established urban core to recently colonized edges. Negative binomial models indicated that host sex, host density, and forest cover were key predictors. Abundance was higher in males than in females, increased at lower host densities, and peaked at intermediate forest cover (~45%).

Conclusions: Greenspace configuration and host factors jointly generate patchy within-city spread. Low-density host populations, often deprioritised in control programmes, may sustain relatively high infection risk. These findings show how urban landscapes interact with host biology to shape the spread of co-introduced parasites, informing invasive species management and urban One Health planning.

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SHIFT01-362

POSTER ON BOARD SHIFT 01
TRANSMISSION DYNAMICS, EPIDEMIOLOGY, AND GLOBAL HEALTH / URBANIZATION, MIGRATION, AND PARASITE SPREAD

ASIAN VARIANTS OF ECHINOCOCCUS MULTILOCULARIS IN EASTERN EUROPE: WHERE DO THEY COME FROM?

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Background and Aims: Ingestion of *Echinococcus multilocularis* eggs can lead to the development of an infiltrative larva that causes a life-threatening disease of the liver. Reports demonstrate differences in the virulence of *E. multilocularis* depending on its geographical origin. We tested the hypothesis that the presence of Asian mitochondrial haplotypes in Eastern Europe is a result of their introduction with raccoon dogs from Northeast Asia.

Methods: We sequenced the cytochrome c oxidase subunit 1 gene of 252 tapeworms from 59 red foxes hunted in northern and northeastern Poland, calculated several measures of genetic variation, generated a histogram of nucleotide site differences between pairs of individuals, constructed a median-joining haplotype network, and created a RelTime timetree to estimate the divergence time of the European and Asian haplogroups in Poland. To quantify genetic differentiation, we calculated the fixation index (F_{ST}).

Results: We identified six haplotypes, including two Asian ones. The mismatch distribution was bimodal. F_{ST} between the European and Asian haplogroups was 0.97; the observed genetic differentiation was greater than expected by chance under panmixia, as assessed by permutation tests ($\alpha = 0.05$, all $P < 0.001$), and the divergence time was estimated to be 26,050 years ago.

Conclusions: Our findings suggest secondary contact between two previously isolated metapopulations with distinct genetic lineages. The clustering pattern and historical records appear to support the hypothesis that Asian variants were introduced into Eastern Europe as a consequence of the importation of raccoon dogs from the Soviet Far East in the 20th century, although alternative explanations remain plausible.

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TRANSMISSION DYNAMICS, EPIDEMIOLOGY, AND GLOBAL HEALTH / URBANIZATION, MIGRATION, AND PARASITE SPREAD

PREVALENCE OF INTESTINAL PARASITIC INFECTIONS IN REFUGEES MIGRANTS IN A BORDER CITY OF MEXICO

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Background and Aims: Approximately half the population lacks adequate health coverage, a situation more critical among migrants who often receive no medical care in host countries. Around 3.5% of the global population migrates intentionally, and recent increases in migration—driven by social and economic factors—have heightened the risk of infectious diseases in areas with previously low prevalence. Studies link migration to hepatitis B, Chagas disease, malaria, tuberculosis, and rising intestinal parasitic infections. To determine the prevalence and distribution of intestinal parasitic infections among refugee migrants in a shelter in Reynosa, Tamaulipas, Mexico, and to analyze associations with demographic characteristics and country of origin.

Methods: A total of 67 stool samples were analyzed using the concentration method, and structured interviews were conducted to collect sociodemographic data, countries traversed during migration, and potential risk exposures.

Results: 67% tested positive for at least one parasite, with *Blastocystis sp.* being the most prevalent, followed by *Giardia intestinalis* and *Entamoeba histolytica*. These infections were more common in women than in men. Of the participants, 38% were from Guatemala and 26% from Honduras, with smaller percentages from Nicaragua, El Salvador, and Haiti. No significant differences ($p < 0.05$) were found based on length of stay in Mexico.

Conclusions: The results suggest that the migrant population arriving in northern Mexico has a high prevalence of intestinal parasitic infections

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TRANSMISSION DYNAMICS, EPIDEMIOLOGY, AND GLOBAL HEALTH / URBANIZATION, MIGRATION, AND PARASITE SPREAD

THE ONGOING SPREAD OF THE ORIENTAL EYE WORM IN DOGS AND CATS IN FRANCE

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Background and Aims: *Thelazia callipaeda*, the "oriental eye worm", is a parasite of the conjunctival pouches of different mammalian hosts, including dogs (figure 1), cats, wildlife and sometimes humans. This nematode, frequently reported in East and Southeast Asia, is now recognized as an emerging pathogen in many European countries, including France. A former investigation demonstrated endemic regions in southwest of France. We recently conducted a questionnaire-based study to assess the current distribution and investigate a possible spread of the parasite. Figure 1: A case of canine thelaziosis © F. Beugnet



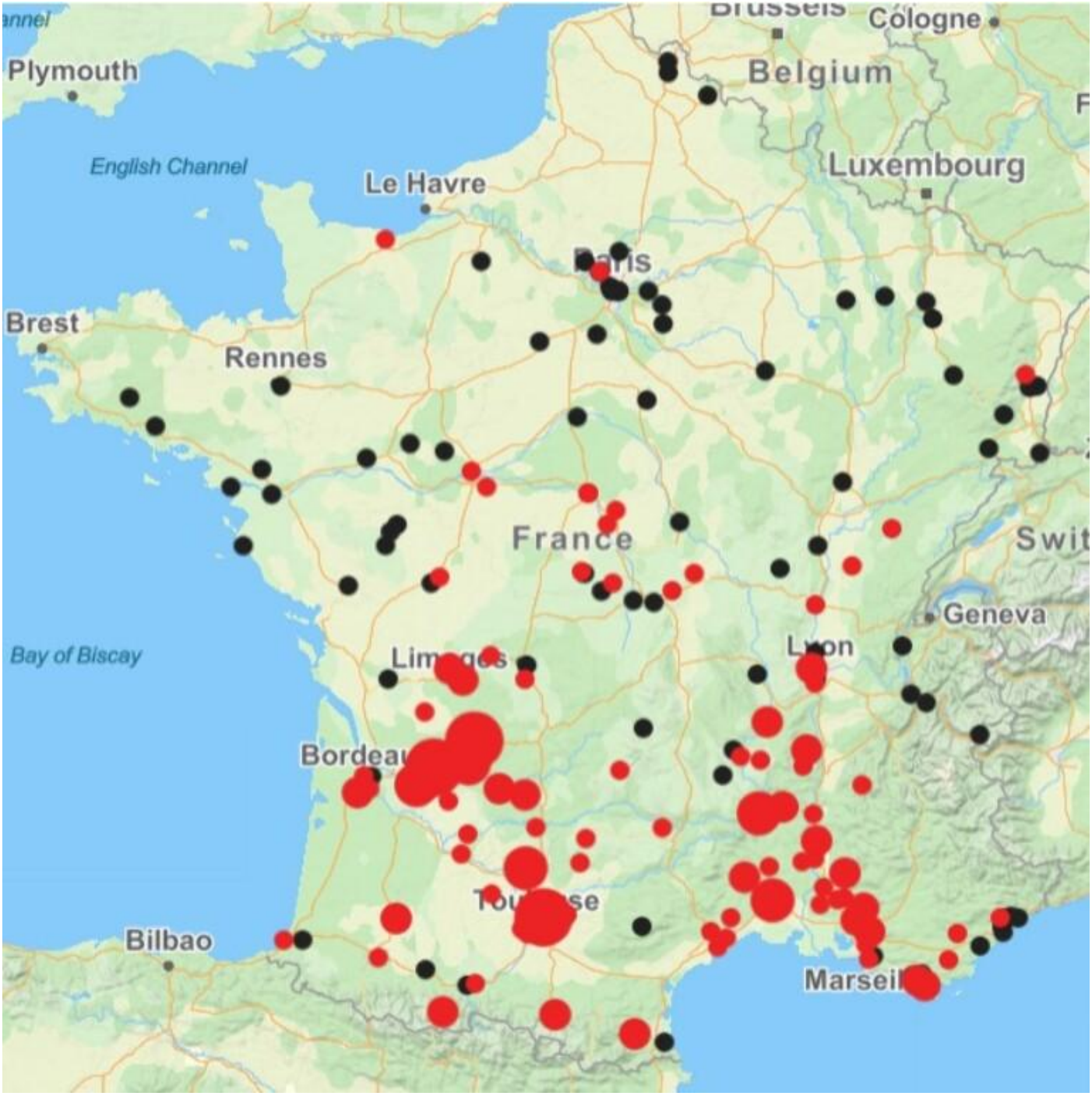
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Methods: In March 2026, an electronic questionnaire was sent to French veterinary practitioners to collect data on canine and feline thelaziosis in 2020-2025.

Results: Half of the veterinarians (109 out of 194 responses) observed clinical cases. Three veterinarians saw more than 50 cases, 8 between 20 and 50, 27 between 5 and 20 and 71 between 1 and 5 cases in 2020-2025. One third of veterinarians reported an increase in cases. Most cases were from Nouvelle-Aquitaine, Occitanie and Provence-Alpes-Côte d'Azur (figure 2). Feline thelaziosis was reported by 20% of the veterinarians and the presence of canine cases was significantly associated with the presence feline thelaziosis ($p<0.05$). Figure 2: Geographical distribution canine and feline thelaziosis. Red dots: cases (from 1 to more than 50) (Size of the dot is proportional to the number of cases); Black dots: no reported cases. ©

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Oniris



Conclusions: These results indicate a clear expansion of thelaziosis to the north and east of France. Due to zoonotic concerns, surveillance data is needed.

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TRANSMISSION DYNAMICS, EPIDEMIOLOGY, AND GLOBAL HEALTH / URBANIZATION, MIGRATION, AND PARASITE SPREAD

DETECTION OF POTENTIALLY ZOONOTIC GASTROINTESTINAL PARASITES IN CANINE FECES FROM URBAN PUBLIC PARKS IN SOUTHERN SANTIAGO, CHILE

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Background and Aims: Zoonotic gastrointestinal parasites in dogs are a public health concern in urban settings, particularly in public spaces with frequent human–animal interaction. This study aimed to assess the prevalence, diversity, and spatial distribution of potentially zoonotic gastrointestinal parasites in canine feces collected from urban parks in southern Santiago, Chile.

Methods: A total of 204 canine fecal samples were collected from urban public parks across 17 communes. Samples were analyzed by flotation coproparasitological techniques and Ziehl–Neelsen staining. Spatial analysis was conducted using SaTScan®. A multivariate logistic regression was used to explore associations between environmental variables and parasite positivity.

Results: Parasite positivity was 13.23%, with 10.78% attributable to zoonotic species. Protozoa predominated over helminths, with Giardia spp. (7.84%) and Cryptosporidium spp. (2.45%) as the most frequently detected zoonotic agents. Helminths were detected at low frequency, including Toxascaris leonina (0.49%). Spatial analysis showed a random distribution of positive samples, with no statistically significant clusters. Logistic regression identified a positive statistical association between parasite positivity and temperature and the ultraviolet radiation index, two correlated variables. These associations are non-causal and may reflect indirect behavioral factors, such as increased use of public spaces and higher animal activity under warmer conditions.

Conclusions: The findings indicate a sustained reduction in zoonotic helminths in urban public spaces compared with previous studies, alongside the persistence of protozoan parasites of public health relevance. Strengthening epidemiological surveillance, promoting responsible pet ownership, and providing public health education are essential to reducing zoonotic parasite transmission in urban settings.

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TRANSMISSION DYNAMICS, EPIDEMIOLOGY, AND GLOBAL HEALTH / URBANIZATION, MIGRATION, AND PARASITE SPREAD

NATIONWIDE ASSESSMENT OF INSECURITY AS A BARRIER TO PREVENTIVE CHEMOTHERAPY-NEGLECTED TROPICAL DISEASES ELIMINATION IN NIGERIA

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Background and Aims: Nigeria's Federal Ministry of Health is committed to eliminating Neglected Tropical Diseases (NTDs) by 2030 through large-scale preventive chemotherapy supported by donated medicines. These interventions target onchocerciasis (ivermectin), schistosomiasis (praziquantel), soil-transmitted helminths (albendazole), lymphatic filariasis (ivermectin plus albendazole), and trachoma (Zithromax alongside water, sanitation, and hygiene interventions). Despite notable progress, growing insecurity across the country poses a major threat to achieving elimination targets. This study assessed the impact of insecurity on the elimination of preventive chemotherapy NTDs (PC-NTDs) in Nigeria.

Methods: In April 2025, the Parasitology and Public Health Society of Nigeria coordinated a nationwide operational research study covering all 36 States and the Federal Capital Territory. Using a standardized questionnaire, the study examined how conflict and insecurity affect PC-NTD elimination efforts. Data were collected by state-level research teams, collated by zonal coordinators, and pooled for national analysis.

Results: Findings showed that approximately 209,105,075 Nigerians are at risk of one or more of the five PC-NTDs. Baseline surveys have been conducted in all States, and mass administration of medicines is ongoing; however, only 11 States have completed impact assessments for all PC-NTDs. Key security challenges identified included Boko Haram insurgency, herder–farmer conflicts, banditry, secessionist agitation, activities of unknown gunmen, illegal mining–related violence, communal clashes, and kidnapping. Insecurity has also led to the establishment of 75 internally displaced persons camps housing 3,066,718 persons.

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Conclusions: This study highlights the profound impact of insecurity on PC-NTD elimination in Nigeria and provides evidence-based lessons and recommendations to strengthen programme implementation in fragile and conflict-affected settings.

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TRANSMISSION DYNAMICS, EPIDEMIOLOGY, AND GLOBAL HEALTH / URBANIZATION, MIGRATION, AND PARASITE SPREAD

BAYLISASCARIS PROCYONIS IN EUROPE: THE SPREAD OF AN INTRODUCED ZOONOTIC PARASITE

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Background and Aims: The raccoon roundworm (*Baylisascaris procyonis*) was introduced by its final host, the North American raccoon (*Procyon lotor*), which has been spreading continuously in Europe since the early 20th century. *B. procyonis* is pathogenic to humans and can cause severe disease. The aim of this study was to give an overview of the current distribution in Europe and to identify possible research needs.

Methods: 146 raccoons from central and western Germany were examined via necropsy for *B. procyonis*. Additionally, an extensive literature search on *B. procyonis* in Europe was conducted.

Results: Of the 146 raccoons examined in this study, 66.4% were infected with *B. procyonis*. The literature review revealed the presence of *B. procyonis* in wild raccoons in nine European countries, with locally high prevalences. In three additional countries, infections were detected in raccoons or other animal species in captivity. Genetic studies confirm several dispersal points and an expansion of the range of the parasite. The distribution area is probably underestimated due to insufficient or absent data collection. Five animal species have been identified as paratenic/accidental hosts in Europe, with all infections documented in zoos/animal husbandry. There is a lack of data on paratenic hosts in the wild in Europe. There are three documented cases of human Baylisascariasis in Europe.

Conclusions: The results of the present study highlight the need for further research into *B. procyonis* in Europe, particularly in relation to ongoing urbanization and growing raccoon populations, with a strong possibility of further spread across Europe.

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TRANSMISSION DYNAMICS, EPIDEMIOLOGY, AND GLOBAL HEALTH / URBANIZATION, MIGRATION, AND PARASITE SPREAD

THE ORIGIN AND SPREAD OF THE NORTHEN HOUSE MOSQUITO CULEX PIPIENS IN ALBERTA, CANADA

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Background and Aims: The Northern house mosquito, *Culex pipiens,* is an invasive species in Canada capable of transmitting many pathogens of human and veterinary concern in North America, including West Nile virus. Within Canada, *Culex pipiens*'s distribution has been largely limited to eastern Ontario, Quebec, the Maritimes, and British Columbia, but global climate change is expected to result in significant changes to its distribution. Notably, we have already detected *Culex pipiens* in Alberta, well ahead of the predicted timeline, highlighting its rapid spread into new areas and its status as a considerable invasive vector threat. Despite its public health importance and clear evidence of potential as a significant disease vector in most of its range, relatively little is known about the public health risk *Culex pipiens* may pose in areas it has recently invaded in Canada.

Methods: We carried out whole genome sequencing on *Culex pipiens* from Calgary, Edmonton and Red Deer, and supplemented the dataset with genomes from Genbank and collaborators. We employed genotype likelihood methods to call and analyse variants to improve the inference accuracy of low-coverage whole genome sequencing.

Results: *Culex pipiens* populations across Alberta likely originated from one introduction event and surprisingly exhibits a population structure that is genetically similar to eastern US populations.

Conclusions: These results are important to estimate disease transmission risks, infer invasion pathways and effectively identify target sites and dispersal routes for effective *Culex pipiens* vector control.

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POSTER ON BOARD SHIFT 01
RESISTANCE AND DEVELOPMENT OF DRUGS AND VACCINES / DRUG DISCOVERY AND DEVELOPMENT PIPELINES - PART I

PROTEIN CRYSTALLIZATION AND HIGH-RESOLUTION STRUCTURE DETERMINATION FACILITATED BY TELSAM FUSION CRYSTALLIZATION

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Background and Aims: Malaria remains a critical global health challenge, worsened by rapid emergence of resistance to existing therapies. Developing new antimalarials with novel mechanisms requires detailed structural characterization of parasite proteins, as high-resolution structures guide rational drug design and reveal resistance-associated mutations. Many Plasmodium proteins are difficult to study due to low solubility, poor expression, intrinsic disorder, and conformational flexibility, particularly novel or parasite-specific targets lacking close homologues. Crystallization chaperones can overcome these barriers by stabilizing proteins and promoting ordered crystal lattice formation. TELSAM (Translocation ETS Leukemia Sterile Alpha Motif) is a genetically encodable, self-assembling helical polymer that facilitates protein crystallization. When fused to target proteins, TELSAM provides a rigid scaffold that promotes crystallization. This study evaluates TELSAM-assisted crystallography for solving structures of challenging Plasmodium proteins, assesses fusion construct effectiveness in promoting crystallization, and demonstrates its potential for structure-guided antimalarial drug discovery.

Methods: Target genes were cloned and expressed in *E*. Proteins were purified and fused to TELSAM using short-linker designs. Crystallization trials were performed under varied conditions, followed by X-ray diffraction at SSRL. High-resolution structures were determined.

Results: TELSAM-assisted crystallography markedly improved crystallization efficiency and structure determination. Non-crystallizing constructs formed high-quality crystals when fused to TELSAM. High-resolution structures of multiple client proteins confirmed preservation of native folds and functional architectures within the TELSAM lattice. The method was most effective for small to moderately sized, well-folded proteins, while highly disordered proteins were less successful.

Conclusions: Our findings demonstrate that TELSAM fusion reliably promotes crystallization without compromising protein integrity, enabling rapid access to previously intractable targets.

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RESISTANCE AND DEVELOPMENT OF DRUGS AND VACCINES / DRUG DISCOVERY AND DEVELOPMENT PIPELINES - PART I

DISCOVERY OF NOVEL DUAL ENZYME INHIBITORS FROM ANOPYXIS KLAINEANA AGAINST PLASMODIUM FALCIPARUM

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Background and Aims: Malaria remains a significant global health concern, with increasing resistance limiting the effectiveness of current antimalarial drugs. Exploring ethnopharmacological plants presents a valuable opportunity for developing new, safe, and effective therapies. This study investigates Anopyxis klaineana, a medicinal plant from Ghana traditionally used to treat febrile and parasitic diseases, for its potential antiplasmodial activity.

Methods: Methanolic extracts and solvent fractions of the A. klaineana stem bark were evaluated against Plasmodium falciparum 3D7 (chloroquine-sensitive) and DD2 (chloroquine-resistant) strains using the SYBR Green I fluorescence assay. Cytotoxicity was assessed on HepG2 and PNT2 cell lines via the MTT assay. Liquid chromatography with electrospray ionization tandem mass spectrometry (LC-ESI-QTOF-MS) was used to identify metabolites, which were then subjected to molecular docking against validated P. falciparum targets plasmepsin X (PfPX) and purine nucleoside phosphorylase (PfPNP).

Results: The methanolic extract and its fractions demonstrated potent antiplasmodial activity, with IC_{50} values ranging from 2.0 to 12 µg/mL against both strains. LC-MS profiling identified 24 metabolites, including cinncassiol A, lippioside II, dubamine, and mitoxantrone. Docking analyses revealed lippioside II, dubamine, and mitoxantrone as dual inhibitors of PfPX and PfPNP, exhibiting strong binding affinities (≤ –8.8 kcal/mol). The cytotoxicity evaluation indicated a high selectivity index, suggesting good safety potential.

Conclusions: This study provides the first integrated in vitro and in silico evidence of Anopyxis klaineana as a promising source of novel antiplasmodial compounds. The identification of dual inhibitors of essential parasite enzymes highlights its potential as a foundation for the development of new antimalarial drugs.

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RESISTANCE AND DEVELOPMENT OF DRUGS AND VACCINES / DRUG DISCOVERY AND DEVELOPMENT PIPELINES - PART I

CHEMICAL-GENETIC SCREENING IDENTIFIES ANTIMALARIAL LEADS TARGETING CPA-SENSITIVE INTRACELLULAR CALCIUM STORES IN PLASMODIUM FALCIPARUM

Waheed Ahmed[1], Mateus Pecenin[1], Rute Honorio[1], Andrew Thomas[2], Francisco Gamo[3], Célia Garcia[1]
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Background and Aims: Calcium signaling plays a central role in Plasmodium falciparum homeostasis and stage progression, yet the organization and druggability of intracellular calcium stores remain poorly understood. This study aimed to identify antimalarial leads that perturb parasite calcium homeostasis and to functionally characterize their effects on intracellular calcium signaling pathways.

Methods: A chemical-genetic phenotypic screen of 13,211 compounds from the GSK Tres Cantos Antimalarial Set (TCAMS) was performed using PfSR25-deficient P. falciparum parasites. Active compounds were prioritized for dose–response profiling, cytotoxicity assessment, and evaluation of gametocytocidal activity. Cytosolic calcium dynamics were monitored in live parasites expressing the genetically encoded calcium sensor PfGCaMP3 following compound treatment, with cyclopiazonic acid (CPA) and ionomycin used to probe intracellular calcium store responsiveness.

Results: Screening identified multiple inhibitory compounds, including a subset with nanomolar potency and favorable selectivity. Several compounds retained activity against mature stage IV/V gametocytes, indicating multistage efficacy. Live-cell calcium imaging revealed that selected compounds induced rapid cytosolic calcium elevations with kinetics comparable to CPA-mediated release. Importantly, these responses were markedly reduced following prior CPA treatment, indicating targeting of CPA-sensitive intracellular calcium stores. Distinct compound-specific calcium signatures further suggest selective perturbation of parasite calcium homeostasis.

Conclusions: These findings identify CPA-sensitive intracellular calcium stores, likely associated with SERCA-mediated regulation, as a tractable vulnerability in P. falciparum. This work highlights calcium homeostasis as a promising target for antimalarial intervention and provides a framework for linking phenotypic screening to mechanistic insights in parasite cell biology.

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RESISTANCE AND DEVELOPMENT OF DRUGS AND VACCINES / DRUG DISCOVERY AND DEVELOPMENT PIPELINES - PART I

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MARKHAMIA LUTEA (BENTH.) K. SCHUM. EX ENGL., A PLANT WITH HIDDEN INHIBITORY COMPOUNDS IN LEAF AND STEMBARK EXTRACTS AGAINST TNF-ALPHA PRO-INFLAMMATORY CYTOKINES

Clement Ajayi[1,2,3], Casim Tolo[4], Pauline Byakika-Kibwika[5]
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Background and Aims: Inflammation is one of the major challenges encountered in tumor or cancer growth, during which pro-inflammatory cytokines, including Tumor Necrosis Factor-α (TNF-α) is triggered. Interestingly, *Markhamia lutea* possesses several compounds that can inhibit cell proliferation, induce apoptosis, and exhibit cytotoxic activity against various cancer cell lines. However, some compounds in this plant remained unexplored for their anti-inflammatory potential.

Methods: *M. lutea* stembark and leaf compounds were extracted into water and ethyl acetate. The water extract was further partitioned into hexane before the extracts were subjected to GC-MS. The anti-inflammatory effect of the identified compounds from *M. lutea* was evaluated on TNF-α. In this study, a pharmacophore model was generated based on the target protein-co-crystallized ligand interaction before applying it to the identified compounds. Their activity was thereafter ranked using molecular docking, Molecular Mechanics/Generalized Born Surface Area (MM/GBSA), and SWISS pharmacokinetics modeling.

Results: Four compounds in the class of ether (compound A), phenol (compound B), aminoquinolines (compound C), and ester (compound D) exhibited high binding on TNF-α with the scores of -7.11, -6.62, -6.51, and -6.20 kcal/mol, respectively, while that of the co-crystallized ligand was -7.21 kcal/mol. The MM/GBSA scores for compounds A, B, C, D, and co-crystallised ligand were -53.24, -44.53, -48.52, -51.91, and -32.79 kcal/mol, respectively. The human bioavailability of the compounds ranged from 87.1-98.8% with no AMES toxicity.

Conclusions: The study has highlighted the anti-inflammatory potential of *M. lutea* compounds in drug development for cancer management.

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ANTIMALARIAL ACTIVITY OF SAPONIN FRACTIONATED FROM TERMINALIA AVICENNIOIDES LEAF AND ITS EFFECTS ON SOME ORGANS DYSFUNCTION IN PLASMODIUM BERGHEI INFECTED MICE

Olusegun Akanbi
Adekunle Ajasin University, Animal And Environmental Biology, Akungba-Akoko, Ondo State, Nigeria

Background and Aims: The resistance of *Plasmodium* to conventional antimalarial drugs calls for alternative therapy. This study investigated the antimalarial activity of methanolic extract of saponin from *Terminalia avicennioides* leaves and its effects on selected biochemical parameters.

Methods: Forty mice used in this study were divided into eight groups. Groups 1 and 2 not infected with *Plasmodium berghei* (normal and treatment control). Group 3 was infected but not treated (negative control). Group 4 was infected and treated with 5.0mg/kg of 'Lonart®' (positive control). Groups 5, 6, 7 and 8 were infected and treated with 50, 100, 150 and 200mg/kg of saponin, respectively. Treatments were administered by oral intubation for five days. Blood was collected daily from the tail of the mice before treatment to assess parasitaemia.

Results: The results showed that parasitaemia was significantly lower ($p<0.05$) in all treated groups compared with the negative control. The parasite inhibition rate was highest in the group treated with 200mg/kg of saponin. There was increase in serum Alanine aminotransferase, alkaline phosphatase, and Aspartate aminotransferase levels in the negative control. Urea and creatinine levels were significantly reduced ($P<0.05$) in the treated groups compared with the negative control. The mean serum malondialdehyde (MDA) was significantly higher ($p<0.05$) in the negative control than in all other groups. The serum superoxide dismutase was significantly higher ($p<0.05$) in the mice treated with 150 and 200 mg/kg saponin than in the other groups.

Conclusions: This study concluded that the antimalarial activity of the saponin from *T. avicennioides* is comparable with lonart at 200 mg/kg.

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TARGETING HELMINTH ENERGY METABOLISM: TRICLABENDAZOLE IMPAIRS SUCCINATE DEHYDROGENASE ACTIVITY IN VITRO

Tobias Kämpfer[1,2], Maria Eugenia Ancarola[1], Pascal Zumstein[1,2], Alice Bernal[1,2], Trix Zumkehr[1], Seraina Mühlemann[1], Natalie Wiedemar[1,3], Britta Lundström-Stadelmann[1,3]
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Background and Aims: Mitochondrial energy metabolism in parasitic helminths exhibits variations throughout their life cycle. While under aerobic conditions the mitochondrial complex II functions as succinate dehydrogenase (SDH), when oxygen availability is limited, it can act as a fumarate reductase. This reverse reaction is essential for parasite survival but is largely absent in host organisms, highlighting SDH as a promising target for anthelmintic therapy. Here, we screened a benzimidazole library for *in vitro* activity on SDH, in the search for novel treatments against the cestode *Echinococcus multilocularis*.

Methods: A set of 20 benzimidazoles (40µM) were tested for inhibition on SDH activity on mitochondrial fractions of the disease-causing stage of *E. multilocularis*, using a fluorometric assay. Selected benzimidazoles from the library were further tested *in vitro* on mitochondrial fractions from other helminths (trematodes and nematodes), and mammalian organisms.

Results: Triclabendazole and its metabolites (triclabendazole-sulfoxide and -sulfone), markedly inhibited SDH activity in mitochondria from *E. multilocularis* metacestode vesicles, whereas activity remained above 80% for all other compounds. Furthermore, inhibition by triclabendazole and its metabolites was consistently observed across all other tested trematode, nematode, and mammalian species, in contrast to albendazole and its metabolites, which showed minimal to no inhibition. Sequence alignments indicated that SDH subunits are largely conserved among species, and minor variations at the quinone-binding site reflect the use of an alternative quinone (such as rhodoquinone) in helminths.

Conclusions: These findings support the anthelmintic potential of triclabendazole as an inhibitor of SDH activity; however, possible cross-reactivity with mammalian mitochondria requires careful evaluation.

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SCHISTOSOMIASIS PREVENTION IN RURAL COMMUNITIES , THE NIGERIAN EXPERIENCE.

Dennis Anthony Musango
University of ABUJA, AIRPORT ROAD ABUJA, Nigeria

Background and Aims: Schistosomiasis remains one of the most significant neglected tropical diseases affecting rural populations in Nigeria and other parts of sub-Saharan Africa. Despite ongoing national and international control efforts, the disease continues to pose a major public health burden, particularly among school-aged children, farmers, fishermen, and communities with inadequate access to safe water and sanitation facilities. This paper examines the challenges, interventions, and outcomes associated with schistosomiasis prevention in rural Nigerian communities.

Methods: This study employed a descriptive narrative review and community-based public health assessment focusing on schistosomiasis prevention strategies implemented in rural communities in Nigeria The assessment focused on rural and semi-rural communities located in schistosomiasis-endemic regions of Nigeria, particularly areas with limited access to potable water, poor sanitation infrastructure, and frequent human contact with freshwater bodies such as rivers, ponds, streams, and irrigation canal Ethical Consideration The study adhered to ethical principles of public health research.

Results: The study revealed that schistosomiasis remains highly prevalent in many rural communities in Nigeria, particularly among school-aged children, farmers, fishermen, and individuals with frequent contact with contaminated freshwater sources. Poor sanitation, inadequate access to potable water, and low awareness levels were identified as major contributors to continued transmission.

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IDENTIFICATION OF MULTI-STAGE ACTIVE COMPOUNDS AGAINST TOXOPLASMA GONDII

Keigo Nagashima[1], Md. Forhad Hossain[1], Tatsuki Sugi[2], Mioko Saeki[1], Ryosuke Mikami[1], Ryoma Mita[1], Itsuki Muro[1], Hironori Bando[1]
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Background and Aims: Toxoplasmosis is a zoonotic disease caused by the obligate intracellular protozoan parasite *Toxoplasma gondii*. While acute infection can be treated by targeting tachyzoites, the parasite establishes latent infection in immunocompetent hosts by differentiating into bradyzoites. Reactivation of latent parasites under immunosuppression leads to severe diseases such as encephalitis, hepatitis, and myocarditis. Moreover, primary infection during pregnancy can result in congenital toxoplasmosis.
Currently, no approved drugs effectively target bradyzoites or reactivation stages. Therefore, this study aimed to identify compounds with anti-*T. gondii* activity against multiple parasite stages, including tachyzoites, bradyzoites, and reactivation, with particular attention to blood–brain barrier permeability.

Methods: Genetically engineered *T. gondii* strains expressing stage-specific luciferases for tachyzoites and bradyzoites were established. Using these reporter parasites, a total of 759 compounds with blood–brain barrier permeability were screened. Compound efficacy was evaluated against tachyzoite proliferation, bradyzoite persistence, and parasite reactivation using luminescence-based assays.

Results: The screening identified several compounds that significantly inhibited tachyzoite growth and reduced bradyzoite viability. Importantly, a subset of compounds showed inhibitory effects on parasite reactivation, suggesting potential activity across multiple life stages of *T. gondii*. These candidates exhibited favorable profiles for further validation as multi-stage anti-toxoplasmosis agents.

Conclusions: This study established a comprehensive screening platform targeting multiple developmental stages of *T. gondii*. Our findings provide promising candidate compounds with potential efficacy against acute, chronic, and reactivation phases of toxoplasmosis. These results contribute to the development of novel therapeutic strategies for the complete eradication of *T. gondii* infection.

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A NEW AUTOMATED, MOTILITY-BASED SCREENING ASSAY FOR DISCOVERY OF COMPOUNDS WITH ACTIVITY AGAINST THE JUVENILE STAGE OF FASCIOLA HEPATICA

Alice Bernal[1,2], Diana Gliga[2], Matias Preza[2], Caroline F. Frey[2], Andrew Hemphill[2], Britta Lundström-Stadelmann[2,3,4], Natalie Wiedemar[2,4]
[1]Graduate School for Cellular and Biomedical Sciences (GCB), University of Bern, Bern, Switzerland, [2]Institute of Parasitology, Department Of Infectious Diseases, Bern, Switzerland, [3]University of Bern; Echinococcus Reference Laboratory of the Swiss Federal Office for Food Safety and Veterinary Affairs, Bern, Switzerland, [4]University of Bern, Multidisciplinary Center For Infectious Diseases, Bern, Switzerland

Background and Aims: *Fasciola hepatica* is a trematode of the Fasciolidae family responsible for a parasitic liver disease called fasciolosis affecting humans and livestock[1]. Fasciolosis is responsible for substantial economic losses in cattle farming[2] and is classified as a neglected tropical disease by the World Health Organization[3]. As no vaccine is available, control relies on prevention and on drug treatment, particularly with triclabendazole[4]. However, *F. hepatica* became resistant to triclabendazole leading to treatment failure globally[5].Screening for new therapeutic options is therefore a critical priority.

Methods: We developed a semi-automated, standardized screening assay based on motility monitoring of newly excysted juveniles using microscopic live imaging. Ten compounds with known anthelmintic properties were tested at 10 and 40µM, including triclabendzole and the new *F. hepatica* TRPM channel activator benzamidoquinazolinone.Active hits were further characterized by dose-response assays and scanning electron microscopy.

Results: Four compounds showed strong activity with low EC_{50} values: benzamidoquinazolinone (0.69µM), the salicylanilide MMV665807 (40nM), niclosamide (30nM), and niclosamide ethanolamine (10nM). Scanning electron microscopy revealed no major morphological alterations at EC_{50} concentrations, while higher doses induced parasite shrinkage and tegumental damage. Complementary live/dead staining demonstrated that only triclabendazole exhibited parasiticidal activity, whereas the other compounds induced paralysis without parasite death within 72hours.

Conclusions: Overall, this assay provides a robust platform for the identification of compounds against fasciolosis. 1. Fairweather Triclabendazole progress report, 2005–2009 (2009) 2. Fairweather Drug resistance in liver flukes (2020) 3. Knubben-Schweizer Estimating the financial losses due to

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bovine fasciolosis in Switzerland (2005) 4. Mas-Coma Fascioliasis in *Digenetic Trematodes* (2019) 5. World Health Organization (2013)

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MULTI-TARGETING LIGANDS WITH POTENT ANTI-TRYPANOSOMIASIS ACTIVITY

Kajumee Bora Bhowal[1], Anh Minh Thao Nguyen[1], Ana Victoria Ibarra-Meneses[2], Andressa Brito Lira[3], K. Shanmugha Rajan[4], Anat Bashan[4], Jesus D Castaño[5], Francis Beaudry[5], Christopher Fernandez-Prada[2], Martin Olivier[6], Ada Yonath[4], William D. Lubell[1]
[1]Université de Montréal, Chemistry, Montreal, Canada, [2]Université de Montréal, Pathology And Microbiology, Saint-Hyacinthe, Canada, [3]McGill University, Medicine, And Of Microbiology And Immunology, Montreal, Canada, [4]Weizmann Institute of Science, Chemical And Structural Biology, Rehovot, Israel, [5]Université de Montréal, Biomédecine Vétérinaire, Montréal, Canada, [6]McGill University, Montreal, Canada

Background and Aims: Neglected tropical diseases (NTDs) caused by trypanosomatid parasites (*Leishmania*, *Trypanosoma* (*T.*) *brucei* and *T. cruzi*) infect over 20 million people globally causing long-term disability and death [1]. Need for novel safe effective and inexpensive antiparasitic medications has accentuated as efficacious vaccines remain elusive, and current chemotherapies encounter risk of resistance and host toxicity [2, 3]. References 1. Oryan, A.; Akbari, M. Asian Pac. *J. Trop. Med*. **2016***, 9*, 925–932. 2. Dujardin, J. C. *et al*. Trends Parasitol. **2010***, 26*(8), 395–403. 3. Alalaiwe, A. *et al*. *Front Microbiol*. **2018***, 9*, 342989.

Methods: Multiple targeting ligands offer means to enhance therapeutic efficacy [4]. Exploring anisomycin analogs conjugated to alkyne chains characteristic of the lipid tails of antiparasitic peptides, potent agents were identified with expanded activity especially against resistant *Leishmania* strains [4, 5]. References 4. Bora Bhowal, K. *et al*. *J. Med*. *Chem*. **2025***, 68*(19), 20264–20282. 5. Nguyen, A. M. T. *et al*. *Molecules* ***2021****, 26*(12)*, 3606*.

Results: For example, *O*-propargyl anisomycin was shown by single-particle cryo-electron microscopy to bind the conserved peptidyl transferase center of the ribosome, and revealed by thermal proteomic profiling and gene ontology analysis to exhibit a broader mode of action associated with pathways linked to glycosomal proteins [4]. References 4. Bora Bhowal, K. *et al*. *J. Med*. *Chem*. **2025***, 68*(19), 20264–20282.

Conclusions: Progressing to develop novel therapy to treat NTDs caused by trypanosomatid parasites, the presented dual targeting ligands offer interesting promise for targeting *Leishmania*, *T. brucei*, and *T. cruzi*.

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ANTILEISHMANIAL AMINOPYRAZOLES: FROM TARGETING BONE MARROW NICHES TO IDENTIFICATION OF THE MOLECULAR TARGET

Rokaya Ahmad[1], Magali Van Den Kerkhof[1], Philippe Leprohon[2], Natalia Smiejkowska[3], Ellen Wuyts[3], Pieter Van Wielendaele[3], Alvaro Baeza Garcia[1], Stéphanie Braillard[4], Charles Mowbray[4], Louis Maes[1], Yann Sterckx[3], Marc Ouellette[2], Guy Caljon[1]
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Background and Aims: Visceral *Leishmania* spp. persist in protected niches within the bone marrow, where parasites adopt drug-tolerant states such as quiescence. These sanctuary sites represent a major obstacle for current therapies and the development of new chemical entities. Remarkably, DNDi aminopyrazoles efficiently clear *Leishmania* from all tissues, including the bone marrow, yet their mechanism of action remained unknown, as resistance could not be readily selected for target deconvolution.

Methods: To elucidate the mechanism of action, chemical mutagenesis combined with aminopyrazole selective pressure and whole-genome sequencing was employed. Candidate resistance determinants were validated by gene overexpression and CRISPR-Cas9 gene editing. Genetic fusions with fluorescent protein tags enabled studies of subcellular localization and *in situ* target engagement, while drug–target interactions were further examined using biophysical assays.

Results: Analysis of 28 resistant mutant lines revealed a strong association between high-level aminopyrazole resistance (10–49-fold) and multiple independent heterozygous mutations in the C-terminal FYVE zinc-finger domain of LINF_180011100. Overexpression and genome editing confirmed its role as a resistance determinant. Fluorescent fusion studies demonstrated predominant localization to endocytic vesicles, with partial colocalization with the small GTPase Rab5. *In situ* target engagement assays demonstrated selective aminopyrazole binding that was abolished by resistance-conferring mutations. Finally, a direct interaction between the lead compound and the FYVE zinc-finger domain of LINF_180011100 was demonstrated biophysically.

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Conclusions: This work formally validates LINF_180011100 as the molecular target and a resistance determinant for several aminopyrazole leads and provides a mechanistic basis for their selective activity, including against parasites residing in bone marrow sanctuary niches.

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ACTIVITY OF DULOXETINE AGAINST TRYPANOSOMA CRUZI AND IDENTIFICATION OF CRUZIPAIN AS ITS POTENTIAL MOLECULAR TARGET VIA IN SILICO DOCKING

Mildred Camacho-Canto[1], Roberto Cuevas-Hernández[2], Sarai Martínez-Cerón[1]
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Background and Aims: Treatment for Chagas disease, caused by Trypanosoma cruzi, is limited. Neuroactive drugs such as memantine show trypanocidal activity; however, the potential of duloxetine had not been explored. This study evaluated the efficacy of duloxetine against T. cruziand identified its possible molecular target in the parasite.

Methods: We assessed the in vitro activity of duloxetine and other reference drugs, such as memantine, riluzole, and sertraline, among others, against T. cruzi epimastigotes using MTT assay, calculating the IC50. Through systematic bioinformatic screening using SwissTargetPrediction, KEGG, and UniProt, homologous targets were identified. The interaction with the selected protein was modeled using AutoDock Vina/Tools 1.5.6 and Discovery Studio 4.0.

Results: Duloxetine showed the highest trypanocidal potency, with an IC50 of 31.5 µM, surpassing the other neuroactive drugs. The in silico analysis predicted the essential enzyme cruzipain (PDB ID: 2AIM) as the most relevant target. Molecular docking revealed an energetically favorable binding (ΔG = -5.9 kcal/mol) at the active site, involving key interactions with residues Cys25, Trp177, Met142, Gln19, Gly23, Cys22, and Gln21.

Conclusions: This study identifies duloxetine as a potent in vitro trypanocidal agent and proposes, for the first time for this class of compounds, cruzipain as its most probable molecular target in T. cruzi. These findings provide a solid foundation for the repurposing of duloxetine and future chemotherapy development against Chagas disease.

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ASSESSMENT OF NOVEL HETEROCYCLIC COMPOUNDS WITH ANTIMALARIAL ACTIVITY AGAINST THE BLOOD STAGE DEVELOPMENT OF PLASMODIUM FALCIPARUM

Maria Del Pilar Crespo-Ortiz[1], Valentina Meneses-Castañeda[2], Jairo Quiroga-Puello[3], Rodrigo Abonia-Gonzalez[3], Alberto Insuasty-Chamorro[3]
[1]Universidad del Valle, Microbiology, Cali, Colombia, [2]Universidad del Valle, Biology, Cali, Colombia, [3]Universidad del Valle, Chemistry, Cali, Colombia

Background and Aims: The emerging resistance of *P. falciparum* underscores the need for novel antimalarials to support control and eradication. Using *in silico* and *in vitro* approaches, we identified 10 hits (50% growth inhibition, IC_{50} <1 µM), including a series of novel diaminopyrimido-diazepine derivatives with antimalarial activity. This work aimed to validate the antimalarial effects of the most potent novel compounds on specific asexual stages of *P. falciparum* cultures.

Methods: Highly synchronized cultures at the ring or trophozoite stage of 3D7 *P. falciparum* parasites (1% parasitemia) were exposed to 5 times the IC_{50} concentration of known antimalarials or the novel molecules. Parasitemia and morphology were monitored, and quantification was performed after 3, 6, and 24 h of treatment and compared with a culture control. Images were acquired using an Axio Imager microscope (ZEN software) and further analyzed using ImageJ. Assays were performed in duplicate, and controls were normalized. The Mann-Whitney U test was used to compare groups using GraphPad Prism software.

Results: Chloroquine and artemisinin-treated parasites exhibited early alterations (3 h) in chromatin morphology, growth arrest, and vacuolation. A pyridyl-diaminopyridine-diazepine (802UV IC_{50} = 0.178 µM) showed vacuolation and morphology disruption of trophozoites (70%) after 3 h and 53% decrease in parasitemia when compared to controls, which was similar to that induced by CQ treatment at the same time period (p=0.54). No red blood cell alterations were observed after 802UV treatment.

Conclusions: This reinforces the antimalarial activity of compound 802UV and warrants further in-depth analysis of its pharmacophore and the mechanism of action.

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CTLA-4 IG PREVENTS T CELL EXHAUSTION AND PROMOTES CONTROL OF LEISHMANIA AMAZONENSIS INFECTION.

Alessandra Marcia Da Fonseca Martins[1], Eric D. Carlsen[2], Yuejin Liang[3], Lynn Soong[4], Herbert Leonel De Matos Guedes[5]
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Background and Aims: Leishmania amazonensis causes diffuse cutaneous leishmaniasis. Using murine models, we examine how CD4+ T cells and B cells shape parasite persistence or clearance during infection.

Methods: Mice were infected, treated, lesion size and parasite load measured, with qRT-PCR, flow cytometry and ELISA to assess immune responses.

Results: Through real-time PCR analysis, we identified a notable upregulation of LAG3 and CTLA4 expression in the infected footpad, with a corresponding increase in CD4+ T cells. Interestingly, the surge in Lag3+ expression was more pronounced in CD4+ FoxP3- T cells, whereas CTLA-4+ expression showed a higher increase in CD4+ FoxP3+ T cells. To investigate potential therapeutic avenues, we treated mice with monoclonal antibodies (mAbs) CTLA-4 Ig or anti-LAG3. Our findings revealed that CTLA-4 Ig treatment effectively curbed lesion development in both susceptible and resistant mouse models, although a reduction in parasite load was only observed in the resistant mouse background.

Conclusions: CTLA-4 Ig treatment exhibited a dual benefit by preventing T cell exhaustion (as evidenced by a reduction in the population of $CD4^+$ $PD\text{-}1^+$ $Lag3^+$ T cells) and by increasing the expression of IFNg at the lesion site. Treatment with anti-Lag3 did not control the lesion or parasite load, but it did induce higher levels of IFNg by $CD4^+$ and $CD8^+$ T cells. Consequently, CTLA-4 Ig treatment emerged as a promising strategy to control infection by promoting an efficient immune response.

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POSTER ON BOARD SHIFT 01
RESISTANCE AND DEVELOPMENT OF DRUGS AND VACCINES / DRUG DISCOVERY AND DEVELOPMENT PIPELINES - PART I

CROSS SPECIES ANNOTATION IN PARASITIC NEMATODES TRANSCRIPTOME ATLASES AND ITS CURRENT LIMITATIONS AND PITFALLS

Edouard Delanoe[1], James Wasmuth[2], Constance Finney[3], David Cruces-Gonzalez[4]
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Background and Aims: Parasitic nematodes are a significant risk to the global livestock industries and approximately 3 billion people living in endemic areas. The rapid spread of drug-resistant parasitic nematodes motivates the search for new anti-nematode drugs (anthelmintics). This depends on a better understanding of nematode biology. To meet this challenge, we are developing a comprehensive gene expression atlas using single-cell-RNA for the parasitic nematode *Heligmosomoides bakeri*, a mice parasite which serves as a model organism for parasites of livestock and humans. Our goal is to characterize the intestinal tract of *H. bakeri* as it is a source of potential new drug targets. The atlas will also deepen our knowledge of parasite-derived molecules that interfere with the host's immune defenses.

Methods: We are integrating cell type markers from *C. elegans* as well as parasite specific markers to assign *H. bakeri* clusters to the nematode anatomy. We used the Scanpy toolkit, along with Scanorama, Harmony, and other tools to integrate data and cirrocumulus for visualization. We then validate our annotations by using *in situ* hybridization with RNA-scope.

Results: We have been able to identify clusters belonging to neurons, muscles and secretory cells and validated these with *in situ* hybridization. However, to date, many of the other nematode tissue types have evaded identification, motivating us to investigate the reliability of cross-species annotation tools.

Conclusions: In our hands, standard single-cell-RNA analysis methods are insufficient to fully annotate atlases through cross-species knowledge transfer relying on orthologs, and must be expanded with specific approaches, namely lesser reliance on arbitrary clustering.

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POSTER ON BOARD SHIFT 01
RESISTANCE AND DEVELOPMENT OF DRUGS AND VACCINES / DRUG DISCOVERY AND DEVELOPMENT PIPELINES - PART I

TITLE: ALTERNATIVE OPTIONS IN LINE FOR CONTROL OF MALARIA PARASITE; EXPLORING INDIGENOUS NIGERIAN PLANTS.

Mercy Ezeunala[1], Samaili Ayuba[2], Kasim Izebe[1], Thomas Schaefer[3], Margareth Ekpenyong[4], Moses Ikegbunam[5], Ephraim Ezeaka[6], Lais De Carvalho[3], Jana Held[3]
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Background and Aims: BACKGROUND and AIMS: Malaria continues to pose a significant public health challenge in sub-Saharan Africa, compounded by the high cost of orthodox antimalarial drugs and the growing resistance of *Plasmodium falciparum* to existing drugs. In many endemic communities, indigenous medicinal plants remain an important component of malaria management, yet scientific validation of their antiplasmodial efficacy remains limited. This study evaluated the *in vitro* antiplasmodial activity of aqueous extracts of *Ximenia americana* and ake apple against the chloroquine-sensitive *P. falciparum* 3D7 strain.

Methods: METHODS: Plant materials were air-dried, pulverized, and extracted using aqueous solvents at graded concentrations. Parasite cultures were maintained in human erythrocytes and exposed to the extracts for 72 hours under standard in-vitro laboratory conditions. Parasite growth inhibition was quantified using the histidine-rich protein 2 (HRP2) enzyme-linked immunosorbent assay (ELISA), with chloroquine, untreated cultures, and solvent controls included for comparison. The half-maximal inhibitory concentration (IC_{50}) values were determined to assess extract potency.

Results: RESULTS: Showed that *Ximenia americana* exhibited moderate, dose-dependent antiplasmodial activity, indicating a measurable suppression of parasite growth. In contrast, ake apple demonstrated weak inhibitory effects, with an IC_{50} value of 20.47.

Conclusions: CONCLUSIONS: These findings provide experimental support for the ethnomedicinal use of *Ximenia americana* in malaria treatment and underscore its potential as a source of bioactive antimalarial compounds. Further phytochemical characterization and *in vivo* studies are recommended to elucidate its active constituents and therapeutic relevance.

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RESISTANCE AND DEVELOPMENT OF DRUGS AND VACCINES / DRUG DISCOVERY AND DEVELOPMENT PIPELINES - PART I

DEVELOPMENT AND OPTIMIZATION OF NOVEL FLUORIDE-RELEASING BIOACTIVE GLASS AS AN EFFECTIVE AND VIABLE ANTI-LEISHMANIA AGENT

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Background and Aims: The leishmaniases are complex of neglected tropical diseases. Pentavalent antimonials (SbV) are currently the first-line pharmacologic therapy for leishmaniasis worldwide. However, the increasing resistance to these compounds is reported. Therefore, always effort to find a new and effective treatment is desired. In this study, the effect of novel fluoride-doped bioactive glass (FBG) composites on *Leishmania major* promastigotes and amastigotes was determined.

Methods: An Iranian standard strain *Leishmania major*, MRHO/IR/75/ER, was used in this study. The infectivity of the parasites was maintained by regular passage in susceptible BALB/c mice. The release of fluoride ion from FBG was evaluated. The viability percentage of promastigotes after exposing of parasite to different concentrations of fluoride-doped bioactive glass composites (0/25, 0/5, 1 and 2 µg/ml) was evaluated. Cyto/biocompatibility of the same contents of FBG was determined for NIH 3T3 fibroblast cells, human adipose tissue derived stem cells and rat bone marrow derived mesenchymal stem cells.

Results: Fluoride-doped bioactive glass demonstrated sustained fluoride release over 14 days with associated changes in Ca, Si and P levels, supporting the formation of apatite structures. All compositions showed excellent biocompatibility with HF2 and G292 cells, while only minimal cytotoxicity was observed at the highest concentration (2 mg/ml) in hAT-MSCs. Promastigote assays demonstrated a dose- and time-dependent reduction in viability, with 2 mg/ml F-BG causing maximal mortality at 72 h. intracellular amastigotes in macrophages significantly decreased from 4.8 to 0.8 parasites at 48 h ($p \leq 0.05$).

Conclusions: Fluoride and silver compounds showed to be promising as an anti-Leishmania drug, in vitro studies.

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RESISTANCE AND DEVELOPMENT OF DRUGS AND VACCINES / DRUG DISCOVERY AND DEVELOPMENT PIPELINES - PART I

NEW NATURAL COMPOUNDS FOR THE CONTROL OF HAEMONCHUS CONTORTUS.

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Background and Aims: Background and aims: Given the substantial economic and health impacts of *Haemonchus contortus* infections, there is increasing interest in identifying alternative control strategies. This study aimed to evaluate the in vitro anthelmintic potential of selected natural compounds against *H. contortus*.

Methods: Methods:.A susceptible *H. contortus* RS-HCO1-8 isolate was used in the egg hatch test to generate dose–response curves and calculate the effective concentration inhibiting 50% of egg hatching (EC_{50}) of 29 natural compounds.

Results: Results: The EC_{50} values (µM) and their 95% confidence intervals (95% CI) were as follows: lapachol 0.935 (0.4689–1.864); stigmasterol 5.634 (1.197–26.52); gibberellic acid 5.711 (3.892–8.379); glycyrrhizic acid 7.002 (6.606–7.421); chlorogenic acid 11.1 (9.715–12.68); tannic acid 14.0 (12.86–15.23); aescin 18.64 (15.87–21.89); gallic acid 23.79 (21.93–25.81); caffeic acid 28.88 (26.82–31.10); podophyllin 109.2 (103.8–114.8); thymol 534.3 (520.0–548.9); pyrogallol 694.7 (494.7–975.6); specioside 10,110 (9,702–10,534); and caffeine 713,961 (506,448–1,006,000). Conversely, the compounds emodin, hesperidin, catechin, arbutin, α-amyrin, boldine, saponin, allantoin, and pyrocatechol showed no ovicidal activity. The compounds chrysin, ellagic acid, 2OH torosaol, and alizarin yielded inconclusive results due to the presence of pigments in the solution or insolubility.

Conclusions: Conclusions: Natural compounds belonging to phenolic acids, naphthoquinones, diterpenes, triterpenoid saponins, and steroid classes demonstrated in vitro ovicidal activity against *H. contortus*.

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RESISTANCE AND DEVELOPMENT OF DRUGS AND VACCINES / DRUG DISCOVERY AND DEVELOPMENT PIPELINES - PART I

CYS-LOOP RECEPTORS AS POSSIBLE DRUG TARGETS IN DIROFILARIA IMMITIS AND HAEMONCHUS CONTORTUS

Sean Forrester
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Background and Aims: Cys-loop ligand gated chloride channels are important receptors for parasitic nematodes such as *Haemonchus contortus* and *Dirofilaria immits*. In these parasites these receptors have unique functions and pharmacology and may represent important new drug targets. Our lab has been identifying and investigating these novel receptors and have uncovered unique features that separate them from similar receptors found in the mammalian host

Methods: We used molecular cloning techniques to identify and isolate several cys-loop receptor genes which were subsequently expressed in *Xenopus laevis* oocytes for electrophysiological and pharmacological characterization. We have also investigated these receptors using immunolocalization and RNAi to determine the possible functional roles that cys-loop receptors play in parasitic nematodes.

Results: We have identified several cys-loop receptors that respond to various cholinergic and GABAinergic compounds and exhibit unique pharmacological properties. These receptors may play various roles such as pharyngeal pumping. In addition, RNAi knockdown revealed that these receptors may also play a role in worm movement.

Conclusions: Nematodes exhibit unique cys-loop ligand gated chloride channels that are not found in the mammalian host. Not only are these receptors exclusive to nematodes but they also have unique pharmacological properties that may allow them to be utilized for drug discovery. This is also highlighted by observations that receptor gene knockdown shows significant reduction in worm movement. These results highlight the potential of nematode cys-loop receptors as potential targets for novel antiparasitics.

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SMOLCATHL2 OF THE ANCIENT ENDOPARASITE SPHAEROSPORA MOLNARI IS A CRUCIAL HEMOGLOBINOLYTIC PEPTIDASE AND PROMISING THERAPEUTIC TARGET.

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Background and Aims: Cathepsins are key in parasite pathogenesis and promising antiparasitic drug targets. *Sphaerospora molnari*, an ancient myxozoan endoparasite of common carp (*Cyprinus carpio*), proliferates in blood, causing severe anaemia. This study aimed to elucidate its hemoglobinolytic cascade and identify druggable enzymatic targets.

Methods: Combination of LC-MS with class- and enzyme-specific substrates/inhibitors profiled key peptidases and mapped hemoglobinolytic cascade. Using qPCR-based expressional profiling several cathepsin L-like enzymes were identified. Recombinant SmolCathL2 (recSmolCathL2) was produced in ArcticExpress *E. coli* cells and purified. Polyclonal antibodies raised against recSmolCathL2 enabled confocal immunofluorescence and TEM immunogold labelling. The 3D zymogen crystal structure was solved using sitting drop X-ray crystallography and compared to carp cathepsin Ls. In silico docking and molecular dynamics simulations allowed for identification of *S. molnari* specific inhibitors.

Results: Cysteine (cathepsin L) and aspartic (cathepsin D) peptidases drive hemoglobinolysis of *S. molnari*; single-class inhibition partially block it while combined inhibition fully abolish hemoglobin cleavage revealing parallels to other blood-feeding parasites. SmolCathL2 was the most abundant transcript in parasitic blood stages. RecSmolCathL2 was biochemically characterized and showed to cleave hemoglobin in vitro. Antibodies localized enzyme specifically to vesicles within parasite blood stages and lysosome-like structures. The 3D structure solved at 2Å resolution showed high host-parasite similarity. A unique Ser-206 have been identified in proximity of the active site and exploited for identification of myxozoan-specific inhibitor.

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Conclusions: We have mapped the hemoglobinolytic cascade of *S. molnari* and identified its key enzymes. RecSmolCathL2 was characterized and the 3D structure resolved offering targets for selective inhibitors against this anaemia-causing parasite.

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PEPTIDOMIMETIC INHIBITORS OF PHOSPHATIDYLINOSITOL-3 KINASE AS A DRUG FOR TRYPANOSOMES

Mengjin Gao[1], Lissa Cruz-Saavedra[1], Mulate Zerihun[2], Mahesh Kumar[2], Hayelom Berhe[2], Nir Qvit[2], Igor Cestari[1]
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Background and Aims: *Trypanosoma brucei* causes African trypanosomiasis and is transmitted by tsetse flies, resulting in health impacts in Africa. The parasite evades host immunity through periodic switching of variant surface glycoprotein (VSG) expression. Our laboratory identified phosphatidylinositol (PI) signalling enzymes involved in VSG switching. This process depends on the synthesis of phosphatidylinositol (3,4,5)-trisphosphate (PI(3,4,5)P3), which controls VSG gene silencing, and disruption of this pathway is lethal. We aimed to determine whether PI(3,4,5)P3 synthesis is essential for *T. brucei* survival and represents a drug target.

Methods: Metabolomic analyses characterized phosphoinositide species produced by *T. brucei*. Recombinant *T. brucei* phosphatidylinositol 3-kinase (PI3K) was expressed and purified from *Escherichia coli*, and activity was assessed using PI and phosphatidylinositol (4,5)-bisphosphate (PI(4,5)P2). Peptidomimetics were screened for inhibition of bloodstream-form parasite growth, and inhibition assays assessed target engagement and inhibition mechanisms.

Results: *T. brucei* produced phosphoinositides, including PI and phosphoinositide phosphates (PI3P, PI4P, PI5P), with lower abundance of PI(4,5)P2 and PI(3,4,5)P3. Recombinant PI3K phosphorylated PI and PI(4,5)P2, generating PI3P and PI(3,4,5)P3, respectively. Enzyme kinetics revealed a K_M of 96.6 µM and a V_{max} of 0.78 µmol·min^{-1} for PI, whereas PI(4,5)P2 exhibited a K_M of 216 µM and a V_{max} of 0.44 µmol·min^{-1}. IPP-102 and IPP-103 inhibited bloodstream-form parasite growth with IC_{50} values of 5 and 10 µM, respectively. PI3K overexpression increased resistance to both compounds, and they inhibited recombinant PI3K activity as competitive (K_i 4.4 µM) and uncompetitive (K_i 3.2 µM) inhibitors.

Conclusions: PI3K-dependent PI(3,4,5)P3 synthesis is essential for *T. brucei* viability and identifies PI3K as a promising therapeutic target.

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RESISTANCE AND DEVELOPMENT OF DRUGS AND VACCINES / DRUG DISCOVERY AND DEVELOPMENT PIPELINES - PART I

EFFECT OF PSYCHOTROPIC MEDICATIONS WITH ANTI-TOXOPLASMIC ACTIVITY IN BIPOLAR DISORDER: A CASE-CONTROL STUDY IN COLOMBIA

Laura Cardozo[1], German Moreno[2], Jairo Franco[1], Juliana Arias-Ramirez[1], Elizabeth Torres-Morales[3], Jorge Gomez-Marin[3,4]
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Background and Aims: *Toxoplasma gondii* is neurotropic parasite linked to psychiatric disorders like schizophrenia and bipolar disorder (BD). Colombia has high *T. gondii* and BD prevalence, warranting investigation into their association.The objetive of present study was to evaluate the association between latent *T. gondii* infection and BD in Colombian adults**.**

Methods: A matched case–control study in Pereira, Colombia, included 69 BD patients, 67 genetic controls (relatives), and 68 environmental controls. Seropositivity was assessed using IgG and IgM ELISA. Sociodemographic, clinical, and environmental data were collected. Logistic regression models estimated odds ratios (OR) and 95% confidence intervals (CI). Clinical severity was assessed using YMRS and MADRS scales. The impact of psychotropic medications with anti-toxoplasmic activity (TATA) on clinical outcomes was analyzed.

Results: Overall IgG seroprevalence was 49.5%. BD cases showed higher seropositivity (55.1%) than environmental controls (38.2%; OR=1.98, 95% CI 1.05–3.73), but similar to genetic controls (53.7%). Manic polarity was significantly associated with higher *T. gondii* seroprevalence (60.0%; OR=2.42, 95% CI 1.09–5.39). Multivariate analysis identified age and low socioeconomic status as independent predictors of seropositivity. TATA+ medications (valproate/divalproex) in seropositive manic patients linked to 66% reduction in median hospitalization days (20 vs. 60 days; p=0.041) and higher rates of good clinical response (34.6% vs. 9.1%; p=0.038).

Conclusions: Latent *T. gondii* infection was associated with manic polarity. TATA drugs improved clinical outcomes in seropositive patients. Environmental exposure and socioeconomic vulnerability are key drivers of infection risk. These findings support targeted pharmacotherapy in BD management.

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RESISTANCE AND DEVELOPMENT OF DRUGS AND VACCINES / DRUG DISCOVERY AND DEVELOPMENT PIPELINES - PART I

ESTIMATING THE TRUE EFFICACY OF NEW RIVER BLINDNESS TREATMENTS: INFORMING THE TESTING ABILITY OF ONCHOCERA DRUG TREATMENTS USING PARASITE OFFSPRING GENOTYPES.

Sam Fenton[1], Lucy Henshall[1], William Sands[2], Wilfried Kalonji[3], Eric Chatelin[3], Ivan Scandale[4], Sabine Specht[4], Eileen Devaney[1], Lisa Randford Cartwright[1], Michael Barrett[1], James Cotton[1], Arne Jacobs[1], Poppy Lamberton[1]
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Background and Aims: The nematode infection and neglected tropical disease Onchocerciasis (river blindness) is the second-leading cause of infectious blindness globally, with c.25million estimated infections. Current treatments, such as invermectin, are effective for removing microfilariae (the larval stage produced by adult worms inside humans) but fail to eliminate adult worms and are therefore not very effective for long-term treatment. In recent years, drugs have been developed with the potential capacity to kill or irreversibly sterilise adult worms.

Methods: This study explored the efficacy of two drugs in treating Onchocerciasis, Tyrosine and Emodepside, using whole genome analysis techniques. Parasite genomes from treated individuals were generated from pre and post-treatment microfilariae samples and patterns of kinship used to infer adult worm genotypes and determine the relatedness of microfilariae between pre- and post-treatment, thus determining the efficacy of the drugs in killing the adult worms.

Results: indicate a strong ability to differentiate siblings and half siblings from microfilariae and indicate that Tyrosine was not successful in killing the adult worms, with high numbers of siblings and half siblings between pre and post-treatment timepoints. However, Emodepside was successful in killing (or longterm sterilising) adult *Onchocera* worms. We also present the complexities of carrying out whole genome sequencing on microfilariae.

Conclusions: From these results we hope to add to the growing literature on *Onchocera* treatment as well as present a method of exploring drug efficacy in this – and similar – helminth disease systems where adult worms are not readily accessible.

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RESISTANCE AND DEVELOPMENT OF DRUGS AND VACCINES / DRUG DISCOVERY AND DEVELOPMENT PIPELINES - PART I

INVESTIGATION OF NOVEL CYS-LOOP LIGAND-GATED CHLORIDE CHANNELS FROM DIROFILARIA IMMITIS

Mozhdeh Heydari, Sean Forrester
Ontario Tech University, Oshawa, Canada

Background and Aims: *Dirofilaria immitis* is a mosquito-borne parasitic nematode causing canine heartworm disease, with occasional zoonotic infections in humans. Current treatments are limited and threatened by drug resistance, highlighting the need for novel molecular targets. In *D. immitis*, the neuromuscular system is essential for movement, feeding, mating, and development, with Cys-loop ligand-gated ion channels mediating rapid synaptic transmission. This study investigates the presence, developmental expression, and ligand responsiveness of selected Cys-loop chloride channels (Cys-loop LGCCs) in *D. immitis* to identify therapeutic targets.

Methods: RNA from *D. immitis* microfilariae and adult females was reverse-transcribed into cDNA. Expression of selected Cys-loop LGCCs was measured by qPCR. Full-length receptor sequences were amplified via RACE, cloned, sequenced, and confirmed by alignment with reference sequences. Initial functional studies assessed effects of small-molecule modulators on microfilarial motility using Worminator analysis, providing preliminary pharmacological insights. Receptors are currently heterologously expressed in *Xenopus laevis* oocytes for electrophysiological recordings to characterize channel activity and validate findings.

Results: Expression analysis of multiple Cys-loop LGCCs in microfilariae revealed transcript variability, suggesting differential gene regulation. Sequence alignment of the *lgc-46* gene identified several nucleotide polymorphisms, indicating possible receptor variants in adult females. Exposure of microfilariae to lindane and α-endosulfan, known blockers of the GABA receptor UNC-49, produced differential effects on motility.

Conclusions: This study provides preliminary evidence of variable Cys-loop LGCC expression in *D. immitis* and suggests they contribute to microfilarial motility.

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RESISTANCE AND DEVELOPMENT OF DRUGS AND VACCINES / DRUG DISCOVERY AND DEVELOPMENT PIPELINES - PART I

A NOVEL 2-PIPERAZINO-PYRIMIDINE COMPOUND EXHIBITS ASEXUAL ANTIMALARIAL ACTIVITY BY TARGETING PLASMODIUM FALCIPARUM PLASMEPSIN X

Jing Hong[1,2], Takaya Sakura[1,2,3], Naoya Asada[4], Kouki Fuchino[4], Daiki Kuwana[4], Rina Kaki[4], Ryuta Ishii[5], Teruhisa Kato[5], Kiyoshi Kita[1,3,6], Kenichi Matsumura[4], Daniel Ken Inaoka[1,2,3,6]
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Background and Aims: Although the number of cases of malaria has declined since the introduction of artemisinin-based combination therapy in endemic countries, the emergence of resistant parasites in the field has led to an alarming increase in treatment failure, making the search for novel drug candidates with precise targets critical. We identified a novel antimalarial compound X from SHIONOGI's chemical library through phenotypic screening in asexual blood stage of *Plasmodium falciparum* and aimed to elucidate its molecular target.

Methods: An *in vitro* evolution approach combined with whole-genome sequencing (WGS) analysis was used to identify resistance-associated mutations to compound X. Further docking simulation, along with genetic and biochemical analyses were performed to validate the target candidate.

Results: Parasites treated with Compound X exhibited a defect in the egress process. Compound X–resistant parasites were generated and five clones exhibiting modest EC_{50} shifts were isolated for WGS analysis. WGS of these isolates identified 2 clones harboring distinct single-nucleotide variants (SNVs) in plasmepsin X (PMX)-encoding gene (PfDd2_080013400) that were predicted to result in I252V or E55G substitutions; another 3 clones harbored duplications of the genomic region spanning PfDd2_080013400. Computer-assisted 3D docking predicted that compound X was docked into the PMX binding pocket well. Mutant parasites generated with the identified SNVs using CRISPR-Cas9 demonstrated similar EC_{50} fold changes to compound X-resistant strains.

Conclusions: These results indicate that Compound X inhibits merozoite egress by targeting *P. falciparum* plasmepsin X. Therefore, this work identifies a novel class of antimalarial compounds that target PMX and may contribute to drug development for malaria.

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IN VITRO ANTIMALARIAL ACTIVITY OF MANGIFERA INDICA L. EXTRACTS AGAINST FRESH ISOLATES OF PLASMODIUM FALCIPARUM FROM CHILDREN UNDER FIFTEEN YEARS ATTENDING FEDERAL MEDICAL CENTER JALINGO, TARABA STATE, NIGERIA

Eniola Kolawole[1], Kiliobas Sha'A[1], Elkanah Sambo[1], Tajudeen Lamidi[2]
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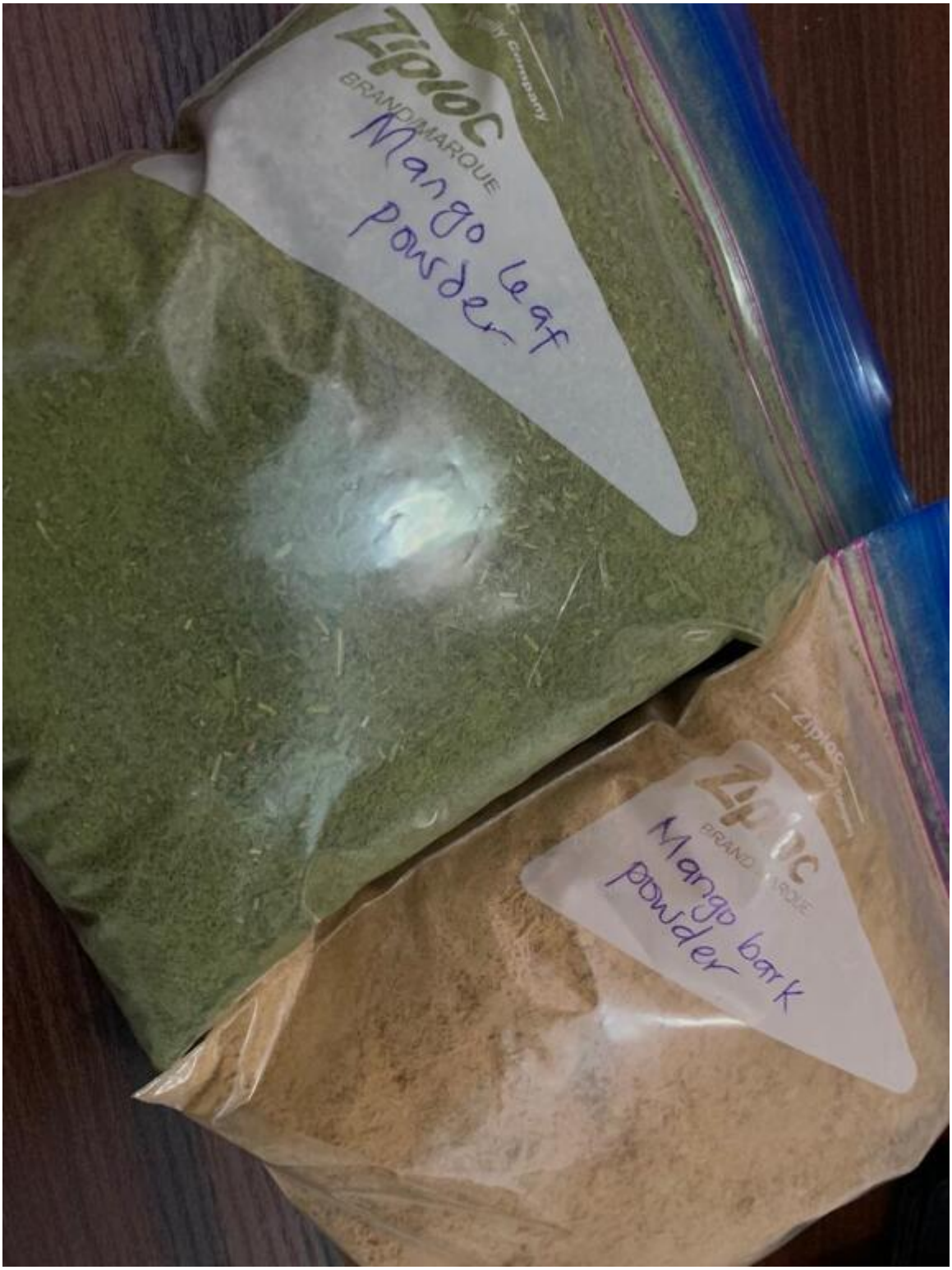
Background and Aims: Malaria continues to be a leading cause of morbidity and mortality among children under fifteen years in Nigeria. This study investigated the antimalarial activity of extracts of Mangifera indica L obtained from children

Methods: Leaves and stem bark were air-dried and extracted using distilled water and ethanol. Qualitative phytochemical screening was conducted to identify secondary metabolites, while quantitative analyses determined concentrations. In vitro activity was assessed using parasitized red blood cells, measuring mean parasitized cell counts, percentage inhibition, and IC_{50} values across concentrations of 1.56–100 µg/mL. Data were expressed as mean ± standard error of means (SEM), with statistical significance determined at $P \leq 0.05$.

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Results: Phytochemical analysis revealed high saponin content in aqueous extracts of both leaves and stem bark (+++), and elevated alkaloid levels in ethanolic bark extract (9.66 ± 0.27 mg/100 g). Flavonoids were most abundant in leaf ethanolic extract (11.24 ± 0.39 mg/100 g), whereas phenols were highest in aqueous bark extract (0.75 ± 0.03 mg/100 g). All extracts demonstrated concentration-

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dependent reductions in parasitized cells, with ethanolic bark and leaf extracts achieving lowest counts of 50.00 ± 1.96 and 40.16 ± 4.36, respectively, at 3.13 µg/mL, corresponding to high percentage inhibition (90.44 ± 2.57% and 93.22 ± 2.60%). IC_{50} values confirmed potent antiplasmodial activity, with ethanolic bark and leaf extracts exhibiting 2.63µg/mL and 2.93 µg/mL, respectively. Combination extracts showed consistent synergistic inhibition across concentrations.

Conclusions: These findings demonstrate that M. indica leaves and stem bark possess potent, solvent-dependent antimalarial activity, supporting their traditional use and potential for development as natural antiplasmodial agents.

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SHIFT01-396

POSTER ON BOARD SHIFT 01
RESISTANCE AND DEVELOPMENT OF DRUGS AND VACCINES / DRUG DISCOVERY AND DEVELOPMENT PIPELINES - PART I

CHEMOPROTEOMIC TARGET DECONVOLUTION IN GIARDIA DUODENALIS

Alex Lam[1], Louise Baker[1,2], Mengbo Li[3], Guillaume Lessene[4], Toby Dite[5], Dylan Multari[6], Isabelle Lucet[4], Andrew Thompson[4], Aaron Jex[1,2], Samantha Emery-Corbin[6]
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Background and Aims: The gastrointestinal parasite *Giardia duodenalis* continues to drive significant global health burdens due to its limited chemotherapeutic interventions. Sustained use of nitroheterocyclic antibiotics has led to frequent drug-resistant treatment failures. Alongside its noted toxicity, sustained global calls for novel and safer chemotherapeutics remain unmet. To meet this gap, we target the disproportionate kinome of *G. duodenalis* with small-molecule inhibitors revealing novel antigiardials and their molecular targets for next generation antiparasitics.

Methods: We immobilised a potent kinase inhibitor (IC_{50}=114nM) onto azide-agarose supports through “Click” chemistry to “pulldown” likely protein targets for this compound utilising mass spectrometry. Using competitive elutions as a negative control alongside stringent *in silico* filtering, we excluded non-specific binding proteins and reveal high-affinity targets in *G. duodenalis* for this compound. Compound engagment was validated through multiple orthogonal target engagement assays of the recombinant protein including differential scanning fluorimetry (DSF), native mass spectrometry and fluorescent assays.

Results: We experimentally demonstrate the likely primary target for this compound as a protein kinase “GiK5” and biochemically validated compound engagement *in vitro*. We probed and revealed compound-induced stabilisation of GiK5 through DSF and a subsequent shift in mass for the compound-GiK5 complex through native mass spectrometry. Lastly, we demonstrated GiK5 was an active kinase *in vitro* through the ADP-Glo platform, including inhibition of activity by the compound

Conclusions: We reveal a druggable kinase in *G. duodenalis*, including implementable workflows for drug-target identification in this non-model parasite. Our work incentivises high-throughput, target-centric screening campaigns for repurposing clinically-approved kinase inhibitors for chemotherapeutic interventions against this parasite.

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RESISTANCE AND DEVELOPMENT OF DRUGS AND VACCINES / DRUG DISCOVERY AND DEVELOPMENT PIPELINES - PART I

IMPROVED IVERMECTIN USE IN PEDIATRICS: DEVELOPMENT OF A NOVEL DRUG FORMULATION

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Background and Aims: Background: Ivermectin (IVM) is a broad-spectrum antiparasitic extensively used in human medicine, whose repurposing to other therapeutic fields is currently investigated. The lack of IVM pediatric formulations may compromise treatment efficacy and adherence. Consistently, WHO estimates that 50% of drugs prescribed to children are not available in suitable dosage forms. Orally disintegrating tablets (ODT) represent a promising strategy to improve pediatric drug administration. **Aims**: To develop and characterize IVM-ODT and to assess their systemic availability in comparison to a commercial IVM oral solution in a rat model.

Methods: Methods: IVM-ODT were prepared by direct compression: Their critical quality pharmaceutical attributes were evaluated. The *in vivo* study included sixteen healthy rats randomly assigned to receive either IVM-ODT or an IVM oral solution (2 mg). Blood samples were collected between 15 min and 48 h post-treatment. IVM plasma concentrations were measured by HPLC.

Results: The ODT exhibited excellent pharmaceutical properties (rapid disintegration, suitable mechanical strength, satisfactory content uniformity and *in vitro* dissolution) and good tolerance. IVM was rapidly detected in the bloodstream (15 min) in 50% (ODT) and 40% (Solution) of the animals. The plasma Cmax was slightly higher for the oral solution (121 ± 39 ng/mL) compared to the ODT (112 ± 36 ng/mL). No statistically significant differences were observed between formulations in terms of Cmax, Tmax or systemic exposure (AUC).

Conclusions: Although further characterization is required, the developed IVM-ODT represent a promising formulation for pediatric use as an antiparasitic agent or potentially if the drug is repurposed to other therapeutic indications

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RESISTANCE AND DEVELOPMENT OF DRUGS AND VACCINES / DRUG DISCOVERY AND DEVELOPMENT PIPELINES - PART I

HISTONE DEACETYLASE INHIBITORS POTENTIATE THE AMOEBICIDAL ACTIVITY OF AMPHOTERICIN B AGAINST NAEGLERIA FOWLERI

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Background and Aims: *Naegleria fowleri* infection causes primary amoebic meningoencephalitis (PAM), a rapidly progressive and highly lethal disease with increasing global incidence. Current treatment options are limited, and although amphotericin B (AmB) remains the most effective available agent, its clinical use is restricted by severe toxicity. This study aimed to evaluate the synergistic amoebicidal effects of histone deacetylase inhibitors (HDACis) in combination with AmB against *N. fowleri*.

Methods: Anti-proliferative and cytotoxic activities were assessed using the cell counting kit and lactate dehydrogenase assays, respectively. Apoptosis induction was analyzed via fluorescence-activated cell sorting (FACS). The impact of HDACis on *N. fowleri* encystation was assessed by quantifying intact cysts resistant to dissolution in 0.5% SDS using light microscopy.

Results: Class-specific HDACis (MS275, MGCD0103, and MC1568) showed minimal effects on trophozoite viability. In contrast, the pan-HDACis suberoylanilide hydroxamic acid (SAHA) and JNJ-26481585 (JNJ) significantly reduced viability in a dose- and time-dependent manner, consistent with apoptosis induction confirmed by FACS. Combination treatment with low-dose HDACis and AmB demonstrated stronger inhibitory and pro-apoptotic effects than either agent alone. Additionally, SAHA and JNJ effectively suppressed encystation of *N. fowleri*. Single treatments with each HDACi and the combination treatment with AmB showed minimal effects on human neuronal cell lines.

Conclusions: SAHA and JNJ exhibit strong potential as therapeutic candidates, particularly in combination with AmB, offering enhanced amoebicidal activity while potentially reducing AmB-associated toxicity. These findings support further investigation of HDACi-based combination strategies for the treatment of PAM.

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RESISTANCE AND DEVELOPMENT OF DRUGS AND VACCINES / DRUG DISCOVERY AND DEVELOPMENT PIPELINES - PART I

KAEMPFEROL ALTERS AMEBIC PROTEIN EXPRESSION AND MYELOPEROXIDASE ACTIVITY IN NEUTROPHILS AND ENTAMOEBA HISTOLYTICA INTERACTIONS

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Background and Aims: Background and aims: *Entamoeba histolytica (E. histolytica*) is a parasite that causes amebiasis on humans. The most employed drug for its treatment is metronidazole (MTZ); however, adverse effects have been reported, and furthermore some ameba strains are resistant to MTZ. Due to this, it is necessary to explore new compounds without toxicity. Flavonoids are polyphenolic natural compounds which have demonstrated inhibition of amebic growth, dysregulation of amebic proteins and regulation of antioxidants enzymes of host. Despite the knowledge acquired, its target and action mechanisms are not completely dilucidated. The present study evaluates the effect of kaempferol on *E. histolytica* proteins and neutrophil's myeloperoxidase (MPO) in amebas-neutrophils interactions.

Methods: Trophozoites were incubated with kaempferol or MTZ (reference drug) at 150 µM for 90 min and the protein expression was determined by 10% SDS-PAGE. The MPO activity was analyzed in supernatants from ameba-neutrophils interactions incubated with kaempferol or MTZ at the same concentration and time.

Results: Trophozoites incubated with kaempferol showed an overexpression of different molecular bands of 67, 59, 27, and 24 kDa compared with the amebas incubated with MTZ or the control group. Interactions of neutrophils-amebas incubated with kaempferol showed a decrease of MPO activity compared with the control groups.

Conclusions: Our study confirms that kaempferol alters some *E. histolytica* protein and in ameba-neutrophil interactions, kaempferol regulate of neutrophil MPO activity.

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RESISTANCE AND DEVELOPMENT OF DRUGS AND VACCINES / DRUG DISCOVERY AND DEVELOPMENT PIPELINES - PART I

PHARMACOKINETICS OF THE ANTIMALARIAL, MK-7602, CO-ADMINISTERED WITH EFAVIRENZ AND FOLLOWING LOW- AND MODERATE-FAT MEALS

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Background and Aims: MK-7602 is a dual-plasmepsin (PMIX/PMX) inhibitor in development for the treatment of malaria. This phase 1 study evaluated the effects of multiple doses of efavirenz, an inducer of the Cytochrome P450 3A (CYP3A) enzyme, and the effects of moderate- and low-fat meals on single-dose pharmacokinetics (PK) of MK-7602 in healthy adults.

Methods: MK-7602-005 was an open label, 2-part study. Part 1 was a nonrandomized, fixed-sequence, 2-period drug–drug interaction study with efavirenz (pre-dosed to reach steady state). Part 2 was a randomized, 3-period, complete crossover study in which participants received single-dose MK-7602 under fasted conditions, following a moderate-fat meal, or following a low-fat meal. Plasma sampling was performed for 144 hours post dose. Plasma PK parameters were estimated using a linear mixed-effects model. Safety was monitored through clinical and laboratory evaluations.

Results: Thirty-four participants (n=16 in part 1, n=18 in part 2) were enrolled and treated. In part 1, following coadministration of MK-7602 with efavirenz, exposures ($AUC_{0\text{-}inf}$) decreased ~46% (geometric mean ratio [GMR] [90% CI] 0.541 [0.459-0.638]) compared with MK-7602 alone. In part 2, exposures ($AUC_{0\text{-}inf}$) were similar following a moderate-fat meal (GMR [90% CI] 1.03 [0.824-1.28]) and decreased ~25% (GMR [90% CI] 0.746 [0.633-0.880]) following a low-fat meal when compared with fasting conditions. No serious adverse events, deaths, or discontinuations due to adverse events were reported.

Conclusions: Efavirenz coadministration resulted in a decrease in MK-7602 exposure. No clinically meaningful impact on exposure is anticipated when MK-7602 is administered with a moderate- or low-fat meal.

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RESISTANCE AND DEVELOPMENT OF DRUGS AND VACCINES / DRUG DISCOVERY AND DEVELOPMENT PIPELINES - PART I

ROCAGLATE AS A NOVEL CLASS OF ANTI-INFLAMMATORY MOLECULES TO ABROGATE THE NEUROINFLAMMATION AND PARASITEMIA ASSOCIATED WITH CEREBRAL MALARIA

Catherine Lyu[1], Albena Pramatarova[2], John Porco[3], Lauren Brown[3], David Langlais[2,4]
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Background and Aims: Malaria is a life-threatening parasitic disease that affects millions of people each year. *Plasmodium falciparum* causes the deadliest complication, cerebral malaria (CM), mostly affecting young children. In CM, infected red-blood-cells trapping in the brain vessels leads to rapid neuroinflammation and irreversible neurological damages, causing death of about half a million yearly. The designated first-line treatment is the anti-malarial drug artesunate, but it doesn't resolve neuroinflammation and faces drug resistance. Hence, there is an urgent need for new medications that can act alone or in combination with artesunate to minimize neurological symptoms and increase survival. Our lab has demonstrated that CR-1-31B, a small rocaglate molecule, has a dual-targeting effect on the parasite and the neuroinflammation by blocking the protein eIF4A, thereby inhibiting translation for both *Plasmodium* and mammalian cells. We hypothesize that other rocaglate molecules could have dual functions, but with more specificity towards either neuroprotection or anti-malaria.

Methods: We have synthesized 100 new rocaglate molecules followed by series of in vitro screening and in vivo screening.

Results: The new compounds have varying degrees of efficacy to block translation in vitro. From the most effective tested in vivo to date, 1 novel rocaglate can limit parasitemia and enhance survival. After in vivo screening, top molecules will be selected for complete preclinical characterization, including different treatment dosing and regimens, combined with deep immunological profiling and assessment of blood-brain barrier permeability.

Conclusions: This project will deliver new rocaglate compounds with increased species specificity and with complete pre-clinical characterization in mouse models of blood-stage malaria and CM.

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RESISTANCE AND DEVELOPMENT OF DRUGS AND VACCINES / DRUG DISCOVERY AND DEVELOPMENT PIPELINES - PART I

INVESTIGATING NEUOTRANSMITTER RECEPTORS IN BRUGIA MALAYI

Sean Malkin, Sean Forrester
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Background and Aims: *Brugia malayi* is a parasitic nematode that affects over 120 million people worldwide. Infection with this parasite can lead to lymphatic filariasis—a painful disease that disrupts the lymphatic system function and causes lymphoedema. Although treatment options are available, their effectiveness and ability to control the disease remain limited. This research aims to investigate the function of possible new drug targets from the ligand-gated chloride channel family from in *Brugia malayi*. Additionally, the study examines the effects of specific small molecules on parasite motility.

Methods: To achieve this, cloning techniques will be used to isolate the gene sequences of selected ligand-gated chloride channels. These sequences will eventually help localize the channels within the worm, to provide information on potential function. Future experiments will include expression of these channels in *Xenopus* oocytes to determine which molecules they respond to. The microfilarial stage of this parasite were also exposed to different concentrations of various biogenic amines and cholinergic compounds, and observed for 5 days at 24-hour intervals to assess changes in motility which was measured by a software program called Worminator.

Results: Partial gene sequences of, LGC-41,46&53 were isolated from *B. malayi*. We found that in general biogenic amines have more prominent effects on movement compared to cholinergic molecules. We found that biogenic amines, tyramine, inhibited movement by 100% after 5 days.

Conclusions: The findings from this research could have significant implications, including identifying specific ligand-gated ion channels as novel drug targets and discovering compounds that may be used to develop new therapeutic treatments for lymphatic filariasis.

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SHIFT01-403

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RESISTANCE AND DEVELOPMENT OF DRUGS AND VACCINES / DRUG DISCOVERY AND DEVELOPMENT PIPELINES - PART I

ENOLASE INHIBITORS AS THERAPEUTIC AGENTS FOR NAEGLERIA FOWLERI INFECTION

Jillian Mckeon[1], Samuel Kwain[2], Caroline Palmentiero[1], Colm Roster[1], Daniel Whitehead[2], James Morris[1]
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Background and Aims: Current treatments for *Naegleria fowleri* infections are inefficacious, with mortality rates exceeding 95%. Human enolase 2 (ENO2) phosphonate inhibitors were recently developed as lead agents to treat glioblastoma. Given the importance of glycolysis to the amoebae (Milanes, 2018), these inhibitors were evaluated as potential therapeutics against *N. fowleri*.

Methods: Phosphonate compounds were tested for inhibition of recombinant *N. fowleri* enolase (*Nf*ENO) and amoebicidal activity in culture. The most potent compound, (1-hydroxy-2-oxopiperidin-3-yl) phosphonic acid (HEX), was evaluated in an *in vivo* study using amoebae-infected rodents. HEX was also tested in combination with standard of care drugs to evaluate drug synergism. HEX-refractory amoebae were generated by chronic growth in low concentrations of the agent and were subject to -omics-based approaches to resolve targets and potential cellular responses to HEX.

Results: HEX potently inhibited recombinant *Nf*ENO (IC_{50} of 0.14 ± 0.04 µM) and was a potent amebicide in culture (EC_{50} of 0.21 ± 0.02 µM), while being minimally toxic to human cells (CC_{50} of >300 µM). HEX increased longevity of treated rodents but was not curative, indicating infection suppression without complete cure (Milanes, 2024). Tolerance to HEX was difficult to achieve and reversible in the absence of selection. Through -omics evaluation, HEX tolerant cells were found to upregulate genes that could impact levels of accumulated glycolytic intermediates.

Conclusions: Phosphonate ENO inhibitors target *Nf*ENO, are toxic to *N. fowleri* in culture, and demonstrate *in vivo* activity. These promising findings suggest that HEX may become a useful addition to existing treatment cocktails.

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RESISTANCE AND DEVELOPMENT OF DRUGS AND VACCINES / DRUG DISCOVERY AND DEVELOPMENT PIPELINES - PART I

DISCOVERY OF INHIBITORS OF BABESIA BOVIS THROUGH SYBR GREEN I-BASED HIGH-THROUGHPUT SCREENING

Silviane Miruka, Nanang Ariefta, Atefeh Fathi, Jae Seung Lee, Kota Komatsu, Masahito Asada
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Background and Aims: Background and Aims Bovine babesiosis, caused by *Babesia bovis*, is a major limitation to cattle production in endemic areas. Control options are restricted due to limited chemotherapeutics, potential drug resistance, and concerns over drug residues. This study aimed to identify novel anti-babesial compounds using a fluorescence-based high-throughput screening method and to evaluate their potential in combination therapy.

Methods: A library of 9,600 chemically diverse compounds from the Drug Discovery Initiative, University of Tokyo, was screened against *B. bovis* using a SYBR Green I fluorescence assay optimized for a 96-well microplate format. Assay robustness was assessed using standard parameters. Primary screening was performed at 1 µM, followed by dose–response assays (10 µM to 0.000128 µM) to determine IC_{50} values. Cytotoxicity was evaluated in Madin–Darby bovine kidney cells to assess selectivity. Combination assays with diminazene aceturate were performed and analyzed using combination index methods.

Results: Eight compounds inhibited parasite growth by more than 50% in the primary screen. The assay demonstrated high robustness (Z'= 0.85 ± 0.11; coefficient of variation <10%). Four compounds showed sub-micromolar activity (IC_{50} = 0.2–0.6 µM). Three compounds, T-049592, T-090118 and T-712926 exhibited acceptable selectivity indices (20–31). Combination assays were performed on these three compounds and revealed strong synergistic interactions, with combination index values ranging from 0.1 to 0.2.

Conclusions: Three chemically diverse compounds with *in vitro* anti-*B. bovis* activity were identified, supporting further evaluation of combination-based approaches.

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RESISTANCE AND DEVELOPMENT OF DRUGS AND VACCINES / DRUG DISCOVERY AND DEVELOPMENT PIPELINES - PART I

COMBINATORIAL ANTIMALARIAL STRATEGIES USING INDOLE-BASED HYBRIDS REVEAL POTENT SYNERGY WITH LUMEFANTRINE

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Background and Aims: Malaria remains a major global health burden, increasingly threatened by resistance to frontline artemisinin-based combination therapies. This highlights the urgent need for novel chemotypes with distinct mechanisms of action and improved resistance profiles. Indole-based scaffolds represent privileged structures in medicinal chemistry, offering versatile pharmacological properties and multitarget potential. In *Plasmodium*, indole derivatives have shown promise in modulating parasite signaling, cell-cycle regulation, and calcium homeostasis. This study aimed to evaluate the antimalarial potential of a focused library of indole-based hybrids and assess their suitability for combination therapy.

Methods: Twenty-four synthetically designed indole-based hybrids were evaluated in vitro against asexual blood stages of *Plasmodium falciparum* 3D7 and chloroquine-resistant Dd2 strains using flow cytometry-based assays. Gametocytocidal activity was assessed in late-stage gametocytes using a luciferase-expressing NF54 reporter line. Cytotoxicity was evaluated in HEK293T mammalian cells using MTT assays. Drug–drug interactions between the lead compound ARN53 and chloroquine, lumefantrine, and primaquine were analyzed using a fixed-ratio isobologram method, and fractional inhibitory concentration (FIC) indices were calculated.

Results: Several compounds exhibited potent antiplasmodial activity, with ARN53 showing the strongest inhibition of asexual parasite growth ($IC_{50} \approx 685$ nM) and high selectivity. ARN53 retained efficacy against chloroquine-resistant parasites. While showing limited gametocidal activity, ARN53 displayed pronounced and reproducible synergy with lumefantrine, whereas interactions with chloroquine and primaquine were additive to antagonistic.

Conclusions: Indole-based hybrids represent promising antimalarial candidates. The potent activity and strong synergistic interaction of ARN53 with lumefantrine support its potential for rational combination therapy and further preclinical development.

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RESISTANCE AND DEVELOPMENT OF DRUGS AND VACCINES / DRUG DISCOVERY AND DEVELOPMENT PIPELINES - PART I

CHARACTERIZATION OF NANOPARTICLES FROM AN ENTAMOEBA HISTOLYTICA NOSODE

Maritza Velasquez Torres[1], Brando Morales Barreda[2], Guadalupe Velázquez Martínez[2], Sirenia González Pozos[1], Issis Claudette Romero Ibarra[3], Virginia Sánchez Monroy[2], Edgar Fernando Cortés Martínez[4], Judith Pacheco-Yépez[2], José De Jesús Serrano Luna[1]
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Background and Aims: Amebiasis, caused by *Entamoeba histolytica*, represents a global health problem due to its high incidence in cases of colitis and Amebic Liver Abscess. Although metronidazole is the most widely used drug, its efficacy is compromised by the emergence of resistant strains and serious adverse effects, such as neurotoxicity and carcinogenic potential. Given this problem, interest has arisen in nosodes: biological preparations made from microorganisms. Therefore, our research group prepared a nosode derived from *Entamoeba histolytica* trophozoites. The aim of this study was to characterize the nanoparticles present in its formulation and determine its biological efficacy as an innovative therapeutic alternative for this disease.

Methods: For the structural characterization of the *E. histolytica* nosode in potencies from 6C to 200C, particle size was determined by Dynamic Light Scattering (DLS) using Anton Paar Litesizer equipment. These results were compared with Transmission Electron Microscopy (TEM) to validate the morphology of the structures present in both the aqueous and hydroalcoholic phases.

Results: Analysis of the nosode revealed the presence of nanoparticles, whose morphology and dimensions depend critically on the solvent used. In the aqueous medium, the particles are characterized by being small, well defined, and uniformly organized in space. In contrast, in alcoholic dilutions, a transition to larger and more heterogeneous structures is observed.

Conclusions: The stability of these nanoparticles depends on the polarity of the chemical environment. The use of water as a solvent ensures less aggregation thanks to the formation of hydrogen bonds and robust electrostatic stabilization on the surface of the particles.

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RESISTANCE AND DEVELOPMENT OF DRUGS AND VACCINES / DRUG DISCOVERY AND DEVELOPMENT PIPELINES - PART I

A HIGH-THROUGHPUT RATE-OF-KILL PROFILING EFFICIENTLY IDENTIFIES NOVEL ANTI-CHAGAS COMPOUNDS WITH TRYPANOCIDAL ACTIVITY

Japheth Ng'Etich[1,2,3,4], Michele Furaya[2,5], Mizuki Hayashishita[5,6], Tagod Suliman[2], Jean-Robert Ioset[7], Daisuke Maki[8], Lucas Silva Franco[7], Luiza Cruz[7], Takeshi Nara[9], Takaya Sakura[2,5,10], Daniel Ken Inaoka[2,5,10,11]

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Background and Aims: Chagas disease (CD), caused by *Trypanosoma cruzi*, affects at least 6 million people worldwide. Current therapies, nifurtimox and benznidazole, are limited by toxicity and reduced efficacy in chronic infection, highlighting the urgent need for new anti-Chagas drugs with improved efficacy and distinct modes of action.

Methods: A phenotypic high-throughput screening campaign was performed to identify compounds active against intracellular *T. cruzi* amastigotes from the Osaka University Chemical Library. A luminescence-based 384-well assay was used to screen 63,680 compounds at a single concentration (10 µM) in HeLa cells infected with CL Brener-Luc2 parasites. Cytotoxic compounds were then excluded by counter-screening against naive HeLa cells, followed by dose-response evaluation across multiple *T. cruzi* strains representing 4 discrete typing units (DTUs I, II, and VI). Hits were clustered based on chemical fingerprint similarity and structural novelty. Representative chemotypes were evaluated using a newly developed in-house high-throughput rate-of-kill (HT-RoK) assay to distinguish trypanocidal from trypanostatic profiles.

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Results: Primary screening identified 1,986 active compounds, which were reduced to 890 following host-cell viability filtering (>90%). Cross-strain dose-response profiling prioritized 403 hits, which were further refined to 388 after exclusion of false positives. Chemical clustering revealed 15 compounds comprising seven distinct chemical classes (four clusters and three singletons), selected for further evaluation. HT-RoK analysis identified three compounds from a single cluster that displayed unambiguous trypanocidal killing profiles against intracellular amastigotes.

Conclusions: The HT-RoK assay is a powerful approach for early prioritization of truly trypanocidal hits over trypanostatic compounds, thereby accelerating drug development to combat Chagas disease.

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RESISTANCE AND DEVELOPMENT OF DRUGS AND VACCINES / DRUG DISCOVERY AND DEVELOPMENT PIPELINES - PART I

IMPLEMENTATION OF AN ADVANCED IN VITRO GIARDIA DUODENALIS/PIG ODM CO-CULTURE SYSTEM TO EVALUATE ANTIGIARDIAL DRUG(S) EFFICACY AND SAFETY

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Background and Aims: Giardiasis, an enteric disease caused by the protozoan *Giardia duodenalis*, affects mammals, including humans. Infection symptoms range from asymptomatic to acute to chronic. In livestock, infection links to reduced feed efficiency, impaired weight gain, and intestinal dysfunction, resulting in economic losses. Current chemotherapeutic treatments in veterinary rely almost entirely on the same drug classes used in human medicine, increasing the risk of drug-resistant isolates. This study aimed to evaluate porcine intestinal organoid-derived monolayers (ODMs) as a physiologically relevant *in vitro* model for livestock giardiasis.

Methods: Porcine intestinal ODMs were produced and infected with *Giardia duodenalis* trophozoites. Culture conditions were optimised to sustain both the parasite and the epithelium during co-culture. Infection experiments were performed using different *G. duodenalis* isolates, and epithelial barrier function was monitored by measuring transepithelial electrical resistance and paracellular permeability. In parallel, selected antigiardial compounds were tested, and parasite proliferation and epithelial viability were quantified.

Results: A boron-based compound showed a strong inhibitory effect on *G. duodenalis* trophozoites proliferation without compromising epithelial viability. In addition, infection with different *G. duodenalis* isolates induced variable levels of epithelial barrier disruption, indicating strain-specific pathogenic effects.

Conclusions: Porcine intestinal ODMs represent a robust and physiologically relevant *in vitro* model for giardiasis. This platform enabled both the identification of a promising antigiardial compound and the evaluation of isolate-dependent epithelial damage, supporting its use as an animal-free tool for drug screening and host–parasite interaction studies.

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FRACTION OF SEX-INDUCING SUBSTANCES FACILITATES GROWTH AND BODY SHAPE CHANGE IN FASCIOLA FLUKES: A NOVEL IN VITRO RESEARCH PLATFORM FOR STUDYING LIVER-STAGE JUVENILES DERIVED FROM MICE

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Background and Aims: *Fasciola* flukes cause zoonotic infections that primarily affect the liver and the bile ducts. Infected juveniles can cause severe liver damage in animals, occasionally leading to sudden death. Previous studies have been unable to reproduce liver-stage development *in vitro*, hindering research on this stage. This study aimed to provide a novel *in vitro* research platform using a laboratory strain of *Fasciola hepatica/gigantica* hybrid.

Methods: Juveniles derived from the livers of mice at 7 and 11 days post-infection (dpi) survived for nearly 100 days in the basic medium consisting of Roswell Park Memorial Institute (RPMI) 1640 supplemented with 50% fetal bovine serum. Bovine red blood cells (RBC) and sex-inducing substances (SIS) that induce sexualization in a free-living flatworm (planarian) were supplemented to examine their effects on the developmental processes in the liver stage, including growth, body shape change, and reproductive development.

Results: SIS induced all three processes, although the last was incomplete, suggesting that the sex-inducing ability of SIS is conserved between free-living and parasitic flatworms. However, RBC was somewhat toxic and less effective than SIS for both growth and reproductive development and could not alter body shape. Furthermore, the combined effects of the two supplements were not observed.

Conclusions: In this study, the reproducibility of the development was carefully confirmed, and it was shown that a single SIS supplementation is currently the best condition and more closely mimics liver-stage development. This study provides a preliminary but outstanding *in vitro* research platform for liver-stage juveniles and will facilitate further drug development.

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ANTIPLASMODIAL ACTIVITY AND IMMUNO-MODULATORY PROPERTIES OF EXTRACTS OF PICRALIMA NITIDA FRUIT-RIND AND LEAVES IN PLASMODIUM BERGHEI-INFECTED MICE

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Background and Aims: Malaria has remained a public health challenge. Notwithstanding the significant reduction in mortality and morbidity recorded in recent years, decreased susceptibility of *Plasmodium falciparum* to the current first-line antimalarial drug, artemisinin-based combination therapy, has been reported. This further complicates the disease severity especially in low-income countries like Nigeria. One of the various strategies employed to combat artemisinin resistance is focus on discovery of novel drugs from plant sources especially in low/middle income countries. This study therefore evaluated antiplasmodial and immuno-modulatory effects of chloroform-methanol extracts of *Pacralima nitida* fruit-rind and leaf in *Plasmodium berghei* infected mice.

Methods: The bioactive constituents of the plant were first extracted using chloroform-methanol as solvent. Extracts obtained were assessed for antiplasmodial activity by the standard four-day suppressive test on *P. berghei* (ANKA) infected mice (Swiss strain).

Results: The absorption spectra from the HPLC chemical fingerprints of the extracts revealed several peaks representing bioactive phytochemicals while the results from the animal study showed that the extracts were significantly ($p < 0.05$) active against *P. berghei* in a dose-dependent manner compared to the untreated infected mice (negative control). Dose-dependent decreases ($p < 0.05$) in some oxidative stress indices of the extracts-treated groups as against the infected control were observed. The pro-inflammatory cytokines levels were found to be significantly ($p < 0.05$) low in the extracts-treated groups relative to the infected control.

Conclusions: Findings from the study recommend that methanol extracts of *P. nitida* fruit-rind and leaves possess appreciable antiplasmodial property with some immuno-modulatory effect against the *P. berghei* parasite.

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ANTICOCCIDIAL POTENTIAL OF ALSTONIA BOONEI STEM-BARK IN BROILER CHICKS EXPERIMENTALLY INFECTED WITH SPORULATED EIMERIA OOCYST

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Background and Aims: This study evaluated the anticoccidial property of methanol extract of *Alstonia boonei* stem-bark (MEABSB) in broiler chicks experimentally infected with sporulated *Eimeria* oocyst.

Methods: The *A. boonei* stem-bark were collected, authenticated, dried, pulverized and extracted using hydromethanol. Fifty (50) broilers were randomly assigned to 6 groups (1-6) (n = 8). Group 1 was the normal control (uninfected-untreated), while groups 2-6 were infected with *Eimeria* oocyst. Group 2 was untreated, group 3 received amprolium (125 mg/L) and groups 4, 5 and 6 received MEABSB at 250, 500 and 1000 mg/L of water, respectively, for 7 consecutive days. Thereafter, Faecal oocyst counts (FOC), haematological, and biochemical profiles were evaluated.

Results: The FOC of MEABSB (250, 500 and 1000 mg/L) and amprolium (125 mg/L) treated groups were significantly ($p < 0.05$) lower when compared with the FOC of the infected-untreated group on days 10, 13, 15 and 18 post infection. The MEABSB (250, 500 and 1000 mg/L) and amprolium (125 mg/L) treatment caused a significant ($p < 0.05$) increase in the HB, PCV, RBC, AST, ALT, total cholesterol and HDL-C levels of the treated broiler groups when compared with the infected-untreated broiler group. The leucocyte profile of the MEABSB (250, 500 and 1000 mg/L) and amprolium (125 mg/L) treated groups were significantly ($p<0.05$) reduced when compared with the infected-untreated group.

Conclusions: This study validates the folkloric use of *Alstonia boonei* stem-bark in treatment of *Eimeria* infections in broilers.

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RESISTANCE AND DEVELOPMENT OF DRUGS AND VACCINES / DRUG DISCOVERY AND DEVELOPMENT PIPELINES - PART I

AMOEBICIDAL EFFECT OF ARTEMISININ ON ENTAMOEBA HISTOLYTICA TROPHOZOITES

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Background and Aims: *Entamoeba histolytica* is the intestinal parasite responsible for dysentery, which affects millions of people worldwide.Despite the effectiveness of conventional treatment, there are reports that its use is associated with adverse effects which has led to the search for safe therapeutic alternatives. Artemisinin is recognized for its antiparasitic activity against malaria and has attracted interest as a new treatment. We evaluated the amoebicidal activity of artemisinin on *Entamoeba histolytica* trophozoites.

Methods: *In vitro* viability assays were conducted to determine amoebic damage using the WST compound and the median inhibitory concentration (IC_{50}) of artemisinin on *Entamoeba histolytica* was obtained. The cytotoxic effect of artemisinin on the VERO cell line was also evaluated using the compound mentioned above. In addition, alterations in the protein expression of the Gal/GalNAc lectin were analyzed using western immunoblot assays**.**

Results: Artemisinin showed a statistically significant amoebicidal effect on *Entamoeba histolytica* trophozoites with an IC_{50} of 600 µM. Artemisinin showed no cytotoxic effect on the VERO cell line. Protein expression studies revealed alterations in the Gal/GalNAc lectin, which is responsible for amoebic adhesion.

Conclusions: Artemisinin has amoebicidal effect on *Entamoeba histolytica* trophozoites. Furthermore, artemisinin is not cytotoxic to a cell line. An amoebic molecular target for artemisinin is the Gal/GalNAc lectin. Our results support the promising future use of artemisinin as a candidate for the treatment of amoebiasis.

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HERBAL MEDICAL PRACTICES FOR THE TREATMENT OF HELMINTHIASIS IN THE SOUTH SOUTH GEOPOLITICAL ZONE OF NIGERIA.

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Background and Aims: Intestinal parasitic worm infections, commonly referred to as helminthiasis, constitute a public health challenge in low-resource settings worldwide. Flooding, poor sanitation, poverty, and peculiar cultural practices, in the South - South Geopolitical Zone of Nigeria sustain the transmission of Helminths, contributing to the high incidence of helminthiasis. Herbal medicine continues to play an important role in disease management in the rural areas, yet scientific documentation of commonly used remedies, user perceptions, and integration challenges remains limited.This study aimed to assess the herbal medicinal practices for the treatment of helminthiasis across the six South-South States of Nigeria.

Methods: The study was a descriptively cross-sectional, mixed method. Data were collected using structured in-depth interviewer questionnaires on 2300 respondents, 30 key informant interviews (KIIs) and 12 Focus Group Discussions (FGDs), involving community members and traditional healers in the six South - South States of Nigeria.

Results: Respondents were predominantly females 51%, with 49% males. About 32% had tertiary education. Up to 31% of respondents earned above ₦100,000 monthly. High preference for herbal treatment was recorded (34%). The commonly used herbal remedies for helminthiasis were *Vernonia amygdalina* (bitter leaf) and *Mentha* spp. (mint leaf), across all six South-South States.Practitioners expressed strong beliefs in the efficacy of these herbs, with recorded symptom relief (97%) and no side effect (83%). Rural dwellers relied predominantly on herbal remedies.

Conclusions: Bitter leaf and mint leaf, remain widespread for helminthiasis treatment in South-South Nigeria. Integration of validated herbal remedies into National helminth control programmes may contribute to NTD elimination.

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RESISTANCE AND DEVELOPMENT OF DRUGS AND VACCINES / DRUG DISCOVERY AND DEVELOPMENT PIPELINES - PART I

INVESTIGATING THE CHEMICAL MODIFICATIONS OF SIRNAS AND THEIR IMPACT ON GENE SILENCING IN THE BRUGIA MALAYI

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Background and Aims: Lymphatic filariasis is a neglected tropical disease caused by the parasitic nematode *Brugia malayi* and remains a major public health concern in tropical and subtropical regions of Asia and Africa. The disease is characterized by chronic manifestations such as elephantiasis and lymphedema, which severely impair quality of life. Current antifilarial drugs, including albendazole, ivermectin, and diethylcarbamazine, primarily target the microfilarial stage and show limited efficacy against adult worms. Furthermore, prolonged mass drug administration programs raise concerns regarding the development of drug resistance. These limitations highlight the urgent need for alternative therapeutic strategies capable of targeting multiple life stages of *B. malayi*. RNA interference (RNAi), a gene-silencing pathway mediated by small interfering RNAs (siRNAs), offers a promising approach for functional genomics and novel therapeutic development. Building on this foundation, the present research aims to design, chemically synthesize, and biologically evaluate siRNAs targeting nicotinic acetylcholine receptor (nAChR) subunit genes (*unc-38*, *unc-29*, and *acr-16*) in *B. malayi*. These genes are essential for neuromuscular function, parasite motility, and muscle contraction, making them validated anthelmintic targets

Methods: Target-specific siRNA strands were synthesized using solid-phase oligonucleotide chemistry, followed by deprotection, HPLC purification, and duplex annealing. *B. malayi* microfilariae were cultured in 24-well plates at 37 °C with 5% CO_2, and motility was monitored over five days.

Results: Preliminary results demonstrate that siRNAs targeting *acr-16* and *gapdh* significantly reduce parasite motility, indicating effective gene knockdown.

Conclusions: Overall, this study enhances understanding of RNAi-mediated gene silencing in *B. malayi* and supports the development of more precise antiparasitic therapies.

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DESIGN AND SYNTHESIS OF NOVEL 3-NITRO-1H-1,2,4-TRIAZOLE-1,2,3-TRIAZOLE-1,4-DISUBSTITUTED ANALOGS AS POTENTIAL ANTITRYPANOSOMATID AGENTS

Bruno Pelizaro[1], Gisele Portapilla[1], Talícia Silva[1], Cristiana Rotta[1], Cristiane Shiguemoto[2], Diego Carvalho[2], Adriano Baroni[2], Sérgio De Albuquerque[1]
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Background and Aims: Neglected diseases are a type of sickness that primarily affects developing tropical countries due to severe economic disparity and misinformation. The pharmacological therapy for these disorders is limited to a tiny percentage of the population. Still, it does not pique the pharmaceutical sector's interest due to low purchasing power. Chagas disease and leishmaniasis are two of Brazil's most common neglected diseases, and they pose a severe public health threat. As a result, research focused on advancements in new drug candidates for a variety of illnesses is crucial.

Methods: Right here, a series of novel 3-nitro-1*H*-1,2,4-triazole-containing phenoxy and thiol rings with differing substitution patterns was designed and synthesized using click-chemistry approaches, employing classical medicinal-chemistry methods such as bioisosterism, hybridization molecular, Craig-plot, and the Topliss set

Results: Out of the fifteen analogues tested, ten 3-nitro-1*H*-1,2,4-triazole analogues demonstrated relevant in vitro antichagasic activity. Analogues **15 am** and **15aq** (R_1 = Ph and R_1 = 4-F-Ph, IC_{50} = 0.30 µM, SI = > 166.66, and IC_{50} = 0.20 µM, SI = > 250.00, respectively), exhibited a significant antichagasic activity (*Trypanosoma cruzi*, Tulahuen LacZ strain) up to 30-fold more active than benznidazole (BZN, IC_{50} = 6.15 µM, SI = > 8.13) with excellent selectivity index (SI), and moderate LipE (3.80).

Conclusions: Thus, 3-nitro-1*H*-1,2,4-triazole **15 am** and **15aq** are interesting antitrypanosomatid candidates for in vivo studies

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RESISTANCE AND DEVELOPMENT OF DRUGS AND VACCINES / DRUG DISCOVERY AND DEVELOPMENT PIPELINES - PART I

HELMINTH-DERIVED IMMUNOMODULATORS FROM TRICHURIS TRICHIURA AS INNOVATIVE THERAPEUTIC CANDIDATES FOR ALLERGIC DISEASES

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Background and Aims: Allergic diseases represent a growing global health burden and remain insufficiently controlled by current therapeutic strategies, underscoring the need for innovative immunomodulatory interventions. Helminth-derived molecules have gained increasing attention as a source of biologically active compounds capable of reshaping dysregulated immune responses. Omics-based investigations conducted by our group identified *Trichuris trichiura* proteins with marked immunoregulatory potential. This study aimed to evaluate the translational potential of the recombinant proteins rTtC4299 and rTtMIF as innovative immunomodulatory therapeutics and immunological adjuvants.

Methods: Recombinant rTtC4299 and rTtMIF were heterologously expressed in *Escherichia coli*, purified by chromatographic methods, and structurally validated by SDS-PAGE and immunoblotting. Cytotoxicity was assessed in human macrophage-like cells. Immunomodulatory activity was evaluated through cytokine profiling in vitro and in a murine model of *Blomia tropicalis*–induced allergic inflammation.

Results: Both recombinant proteins exhibited no detectable cytotoxic effects, supporting their biological safety. In vitro stimulation induced a regulatory and Th1-associated cytokine profile, characterized by increased IL-10 and IFN-γ levels and reduced pro-inflammatory and Th2-associated mediators. In vivo, rTtC4299 treatment significantly modulated leukocyte recruitment, antibody isotypes, and cytokine responses, while enhancing regulatory T-cell–associated immune signatures. rTtMIF showed complementary immunomodulatory effects, contributing to the redirection of Th2-skewed immune responses.

Conclusions: These findings identify rTtC4299 and rTtMIF as innovative helminth-derived immunomodulators with strong potential as bioactive therapeutics and immunological adjuvants. This work highlights the translational value of parasite-derived molecules and reinforces the relevance of helminth-based biotechnology within the *Innovative Therapeutics* framework

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RESISTANCE AND DEVELOPMENT OF DRUGS AND VACCINES / DRUG DISCOVERY AND DEVELOPMENT PIPELINES - PART I

BALANCING BENEFITS AND HAZARDS OF ANTIPARASITICS IN A CHANGING ECOLOGICAL LANDSCAPE.

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Background and Aims: Endo- and ectoparasites including helminths, protozoa, ticks, fleas, and mosquitoes pose major threats to human, livestock, companion-animal, and plant health. Driven by medical needs for human and animal health, antiparasitic agents are widely used and highly effective, but their extensive application has created two major concerns: the emergence of drug resistance and the contamination of ecosystems, with harmful consequences for non-target organisms and biodiversity. This work aims to highlight the need to balance therapeutic benefits with ecological risks and to evaluate the environmental impact of antiparasitic use, particularly in companion animals.

Methods: Current scientific literature was examined, with a focus on antiparasitic compounds known for high toxicity and environmental persistence. Special attention was given to the neonicotinoid imidacloprid and the phenylpyrazole fipronil, including their usage patterns, and documented ecological effects. Emerging studies on environmental contamination linked to companion-animal treatments were reviewed.

Results: Several antiparasitic compounds have been banned for agricultural and food-producing animal use in many regions due to their ecological hazards. Despite these restrictions, the same substances remain widely used in companion animals. Recent research increasingly demonstrates that this ongoing use contributes to environmental contamination especially of aquatic ecosystems where these compounds negatively affect non-target species and may exacerbate biodiversity decline.

Conclusions: The continued ecological impact of antiparasitic agents, even after agricultural bans, underscores the urgent need to balance therapeutic efficacy with environmental safety. Developing and implementing antiparasitic drugs with reduced environmental persistence, alongside integrating non-chemical parasite-control strategies, will be essential for sustainable parasite management in a changing ecological landscape.

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RESISTANCE AND DEVELOPMENT OF DRUGS AND VACCINES / DRUG DISCOVERY AND DEVELOPMENT PIPELINES - PART I

WHAT CAN GPCRS DO FOR YOU?: CHARACTERIZATION OF A NOVEL SEROTONIN-RESPONSIVE GPCR FROM SCHISTOSOMA MANSONI

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Background and Aims: Schistosomiasis is a neglected tropical disease affecting over 250 million people worldwide. Praziquantel (PZQ) remains the only FDA-approved treatment, and there is an urgent need for alternative therapeutic targets. G protein-coupled receptors (GPCRs) regulate critical aspects of schistosome biology, including neuromuscular function and reproduction, yet most remain uncharacterized. As part of a broader effort to deorphanize flatworm GPCRs, we conducted the first comprehensive functional analysis of a previously unstudied serotonin-responsive receptor, Smp_245850 (*Sm*.5HT$_{7b}$).

Methods: Knockdown was performed using RNAi targeting *Sm*.5HT$_{7b}$. For functional assays, *Sm*.5HT$_{7b}$ was cloned from *S. mansoni* and expressed in CHO cells, and activity was assessed using a real-time cAMP biosensor. Computational modeling employed the Molecular Operating Environment (MOE) and Schrodinger software suites.

Results: We achieved ~75% knockdown of *Sm*.5HT$_{7b}$ achieved in adult schistosomes resulting in unpairing of worms and paralysis. Knockdown also abolished egg production demonstrating a critical role for *Sm*.5HT$_{7b}$ in reproduction. Functionally, *Sm*.5HT$_{7b}$ exhibited a robust and selective response to nanomolar concentrations of serotonin. A targeted screen of GPCR ligands identified new *Sm*.5HT$_{7b}$ agonists, including FDA-approved triptans. Treatment of schistosomes with triptans caused hypermotility and contractile paralysis, consistent with modulation of serotonergic neuromuscular pathways.

Conclusions: We identified Smp_245850 as a previously uncharacterized serotonin receptor with essential roles in neuromuscular regulation and reproduction. Discovering that the receptor responds to triptans positions *Sm*.5HT$_{7b}$ as a compelling therapeutic target. The possibility of repurposing safe, approved human drugs for schistosomiasis offers a translational pathway for expanding treatment

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options, especially in low-resource communities.Ongoing structural and in vivo studies of *Sm*.5HT$_{7b}$ will advance the development of new antiparasitic interventions.

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SHIFT02-001

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RESISTANCE AND DEVELOPMENT OF DRUGS AND VACCINES / DRUG DISCOVERY AND DEVELOPMENT PIPELINES - PART II

CHELATION OF MITOCHONDRIAL IRON AS AN ANTIPARASITIC STRATEGY

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Background and Aims: Iron is an essential micronutrient but can also be toxic, making it a promising target for antimicrobial intervention. As part of nutritional immunity, the host restricts iron availability to inhibit parasitic growth, while parasites and pathogenic fungi deploy efficient iron-scavenging strategies. Because mitochondria are the cellular hub of iron metabolism, mitochondrial targeting of iron chelators (mitochelators) represents an attractive therapeutic approach.

Methods: We designed and evaluated a series of mitochondrially targeted derivatives of the clinically approved iron chelators deferoxamine and deferasirox for antiparasitic activity. These compounds consist of the chelator moiety, an alkyl linker, and a lipophilic cation derived from triphenylphosphonium.

Results: Mitochelators exhibited potent activity against a broad spectrum of pathogens, including *Trypanosoma* spp., *Toxoplasma gondii*, *Leishmania* spp., *Plasmodium falciparum*, *Babesia divergens*, as well as the pathogenic yeasts *Candida* spp. and *Cryptococcus neoformans*. In several cases, compounds were effective at low nanomolar concentrations and displayed up to several hundred-fold selectivity over host cells. Structure–activity relationship studies and mechanistic analyses demonstrated that mitochondrial accumulation of lipophilic, redox-active compounds—rather than iron chelation alone—is the primary driver of antimicrobial activity. In both protozoan parasites and pathogenic yeasts, mitochelators disrupted mitochondrial membrane potential and induced reactive oxygen species production, with minimal global iron deprivation.

Conclusions: Mitochelators show remarkable potency and selectivity against unicellular eukaryotic pathogens and benefit from prior development as anticancer agents, highlighting opportunities for drug repurposing.

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SHIFT02-002

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RESISTANCE AND DEVELOPMENT OF DRUGS AND VACCINES / DRUG DISCOVERY AND DEVELOPMENT PIPELINES - PART II

ZINC AS A DIETARY SUPPLEMENT IN EXPERIMENTAL HAEMONCHUS CONTORTUS INFECTION IN LAMBS

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Background and Aims: Chemoprophylaxis against the gastrointestinal parasitic nematode *Haemonchus contortus* by the repeated application of anthelmintics increases the risk of residues in food products and the development of anthelmintic resistance. The trace element zinc is essential for the proper functioning of both unspecific and specific immune defensive mechanisms and reduces the impact of many diseases by preventing disassembly of the immune system. This study includes three different experiments in which the efficacy of organic form of zinc and zinc nanoparticles (ZnO-NPs) have been evaluated on the parasitological status of lambs experimentally infected with *H. contortus parasite*.

Methods: Lambs were experimentally infected with 5,000 infective H. contortus larvae. The three experiments varied in housing (indoor vs. pasture), dietary supplementation (organic zinc vs. ZnO nanoparticles), and duration (38–107 days post-infection). Faecal egg counts (EPG) were quantified using the modified McMaster method.

Results: Regardless of the type of experiment, all three studies recorded significant reductions in faecal egg counts, as measured by the FECR test, with reductions ranging from 53% to 98%.

Conclusions: In conclusion, zinc supplementation clearly reduced parasitic infection dynamics. Key questions for discussion include whether zinc influenced the parasite prepatent period, whether faecal egg count reductions were comparable across the three experiments, and whether zinc acts through a rapid, direct effect on parasites or an indirect, longer-term effect on host resistance. *Acknowledgment:* The study was supported by funds from the NextGenerationEU of the Recovery and Resilience Plan for Slovakia under project No. 09I03-03-V04-00200/2024/VA.

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SHIFT02-003

POSTER ON BOARD SHIFT 02
RESISTANCE AND DEVELOPMENT OF DRUGS AND VACCINES / DRUG DISCOVERY AND DEVELOPMENT PIPELINES - PART II

DETECTION OF GENOMIC TRACES OF ENTAMOEBA HISTOLYTICA IN A NOSODE PREPARATION

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Background and Aims: Amebiasis is a parasitic disease with a global impact, and its main treatment, metronidazole, has critical limitations due to toxicity and resistance. As an alternative, nosodes are being studied, biological preparations derived from the causative agent that have demonstrated antimicrobial, anticancer, and antiparasitic activity. Currently, no nosode has been prepared or characterized from *Entamoeba histolytica*trophozoites, and it is necessary to investigate whether these preparations contain genetic material from the amoeba.

Methods: *E. histolytica* trophozoites (HM1:IMSS) cultured in TYI-S-33 medium were used to prepare the nosode. The dynamization process followed the 7th rule of the Mexican Homeopathic Pharmacopoeia, moving from a solid trituration phase (1X-6X) to a liquid dilution up to 200C. Subsequently, the DNA of the ultradilutions was quantified and the 18S ribosomal gene was amplified by qPCR. After purifying the products with ExoSAP-IT, sequencing was performed (ABI PRISM BigDye Terminator v.3.1) and the identity of the sequences was validated in GenBank using the BLAST algorithm.

Results: Using qPCR and sequencing of the 18S subunit, the biological identity of the DNA present in potencies ranging from 6 to 200C of the nosode was detected and validated, with Ct kinetics consistent with the dilution gradient.

Conclusions: DNA detection confirms the authenticity of the *E. histolytica* nosode. This demonstrates that the dynamization process preserves the genetic fingerprint of the original pathogen, validating the biological identity of the biotherapeutic and providing a scientific basis for its potential therapeutic effect.

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SHIFT02-004

POSTER ON BOARD SHIFT 02
RESISTANCE AND DEVELOPMENT OF DRUGS AND VACCINES / DRUG DISCOVERY AND DEVELOPMENT PIPELINES - PART II

THE DISCOVERY OF NEW RESISTANCE BREAKER ANTHELMINTICS USING THE MIGRATION TRAP ASSAY

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Background and Aims: Gastrointestinal nematodes are developing increasing levels of resistance to anthelmintics (AH) worldwide, and while the research community is still searching for a novel anthelmintic that could break resistances. Monitoring and managing these resistance levels in field isolates is of fundamental interest. Here, we present the results of an *in vitro* assay, namely the Migration Trap Assay (MTA), which can be used for high-throughput screening of endoparasites and to manage resistance in the field by diagnosing susceptibility and resistance to AH.

Methods: The main assay used in this study, the MTA, allowed the detection of the macrocyclic-lactone effect in the nanomolar range after only 24h of exposure to the compound. This assay can also be used to detect a small proportion as low as 5% of resistant *Haemonchus contortus* L3 larvae among sensitive larvae, and it can detect resistance factors from resistant field isolates up to 50 fold compared to the Weybridge-susceptible isolate.

Results: Here, we present the results of *in vitro* assays for several promising molecules developed by Animol, Inc. (Animol Discovery), which showed high activity against both sensitive and resistant strains of a wide range of species, including *Trichostrongylus colubriformis, Teladorsagia circumcinta, Haemonchus contortus, and Cooperia oncophora*.

Conclusions: Several of these compounds, particularly the compound named ANM-0008409, showed better potency against the resistant isolate Kokstad than against the susceptible isolate Weybridge, indicating a putative resistance-breaking compound.

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SHIFT02-005

POSTER ON BOARD SHIFT 02
RESISTANCE AND DEVELOPMENT OF DRUGS AND VACCINES / DRUG DISCOVERY AND DEVELOPMENT PIPELINES - PART II

A PRACTICAL MIRACIDIAL MOTILITY ASSAY FOR ASSESSING FASCIOLA HEPATICA SENSITIVITY TO COMPOUNDS IN VITRO

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Background and Aims: *Fasciola hepatica* causes fasciolosis in livestock and humans worldwide, yet reliable tools to assess drug efficacy against the early developmental stages of this parasite are lacking.

Methods: Here, we developed an automated miracidial motility assay (MMA) using the WMicroTracker ONE infrared detection system to quantify the sensitivity of *F. hepatica* miracidia to anthelmintic compounds including clorsulon (CLORS), closantel (CLOS), triclabendazole (TCBZ) and triclabendazole-sulphoxide (TCBZ-SO). Systematic optimisation of assay conditions, including inoculum size, observation window and solvent concentration yielded a reliable platform for evaluating the sensitivity of *F. hepatica* miracidia from diverse geographic isolates to these compounds.

Results: Our results demonstrated that three compounds (CLOS, TCBZ and TCBZ-SO) produced concentration-dependent motility inhibition, whereas CLORS had no effect. CLOS displayed the highest potency across New South Wales (NSW), Tasmania (TAS) and Victoria (VIC) isolates, whereas TCBZ and TCBZ-SO exhibited isolate-specific sensitivity patterns. Miracidial responses of the NSW and TAS isolates to TBZ, TBZ-SO and CLOS were also compared *in vitro* with those of newly excysted juveniles (NEJs) produced from the same isolates.

Conclusions: Overall, the findings show that MMA provides a reproducible, host-independent and high-throughput phenotypic platform for assessing miracidial sensitivity to compounds.

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SHIFT02-006

POSTER ON BOARD SHIFT 02
RESISTANCE AND DEVELOPMENT OF DRUGS AND VACCINES / VACCINE DEVELOPMENT: CHALLENGES AND BREAKTHROUGHS

IDENTIFICATION OF NOVEL IMMUNOGENS AND DRUG TARGETS AS CARGO OF LEISHMANIA INFANTUM EXTRACELLULAR VESICLES.

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Background and Aims: Over the last two decades, the DNA vaccine Neoleish® against canine leishmaniasis, based on the activated protein kinase C receptor analog (LACK) antigen gene from *Leishmania infantum*, was developed in the PMV lab at CIB-CSIC. Based on the positive opinion of the European Medicines Agency, it was authorized by the European Commission (Reg. No. 2/22/290). By integrating omics approaches and following our own selection criteria based on differential gene expression, low homology with host orthologs, immunoinformatics, and functional relevance, we identified and validated a library of vaccine candidates and potential drug targets. This study aimed to characterize the protein cargo of *Leishmania infantum* extracellular vesicles (EVs).

Methods: EVs were purified from promastigotes and intracellular amastigotes (U937 model) and protein cargo characterization was performed using LC-MS/MS proteomics analysis. Immunogenicity and efficacy of vaccine candidate assays were performed in murine models. Molecular docking and drug repurposing strategies were employed to identify novel therapeutic targets.

Results: EV markers such as gp63 and HSPs were found in the study samples. Surprisingly, the validated vaccine candidates and potential drug targets we had independently identified were consistently present in the EV samples. Furthermore, these components, actively exported via

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vesicles, were confirmed to exhibit a synergistic potential with the LACK gene, significantly enhancing the immunogenic response and protective efficacy in experimental models.

Conclusions: Potential drug targets and validated immunogens to be combined with LACK in an updated Neoleish® formulation have been identified in *L. infantum* EVs. These findings highlight the role of EVs in pathogenesis. Funding: 20172680 (Fundación Ramón Areces)

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SHIFT02-007

POSTER ON BOARD SHIFT 02
RESISTANCE AND DEVELOPMENT OF DRUGS AND VACCINES / VACCINE DEVELOPMENT: CHALLENGES AND BREAKTHROUGHS

FROM BENCH TO CLINIC: DEVELOPMENT HISTORY OF NEOLEISH®, THE FIRST DNA VACCINE AGAINST CANINE LEISHMANIASIS.

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Background and Aims: Canine leishmaniasis (CanL) is a major zoonosis requiring innovative prevention. Using the activated protein kinase C receptor analogue (LACK) gene from *Leishmania infantum* and the pPAL mammalian expression plasmid vector, the DNA vaccine Neoleish® was formulated and evaluated.

Methods: Safety, immunogenicity, and efficacy of the LACK-based DNA vaccine prototypes and the final pPAL-LACK formulation were evaluated through preclinical tests in Beagle dogs. Experimental infections were performed by the intravenous route with 10^8 highly-infective stationary phase promastigotes at early passages. Following preclinical trials, a double-blind, randomized field trial was conducted in three areas of Spain according to the distribution of the sand fly, involving multiple breeds naturally exposed to the parasite over a two-year period.

Results: Neoleish® was safe and immunogenic. A LACK-specific Th1 cell response predominated over Th2 in PBMC, spleen, and lymph nodes. Circulating anti-LACK IgG2 levels predominated over IgG1. In field trials, vaccinated dogs showed a 2-fold lower risk of active infection and a 3-fold lower risk of clinical disease. qPCR confirmed that the vaccine significantly reduced both parasitemia and bone marrow parasite burden by an order of magnitude. To date, it is the only canine vaccine demonstrated to decrease these specific parameters. European Commission authorization was granted in 2022 (Reg. No. EU/2/22/290/001-002).

Conclusions: Neoleish® is the first authorized CanL DNA vaccine. It lacks antibiotic resistance marker and eukaryotic replicons, contains a long CpG motifs, and ensures unprecedented safety and efficacy, by significantly reducing infection risk, clinical disease, and parasite load. Funding: IPT-2011-1019-900000 and RTC-2014-1767-1 (AEI). CZ Veterinaria.

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SHIFT02-008

POSTER ON BOARD SHIFT 02
RESISTANCE AND DEVELOPMENT OF DRUGS AND VACCINES / VACCINE DEVELOPMENT: CHALLENGES AND BREAKTHROUGHS

ASSESSING THE EFFICACY OF C. PARVUM ANTIGENS AS VACCINE CANDIDATES FOR CRYPTOSPORIDIOSIS

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Background and Aims: Cryptosporidiosis is the second most common cause (after rotavirus) of moderate to severe diarrhea in children and immunocompromised individuals. *C. parvum* oocysts release sporozoites when ingested, infecting and replicating in the intestinal epithelial cells. The only drug approved by the US Food and Drug Administration for the treatment of Cryptosporidiosis is nitazoxanide. This drug is not effective in treating malnourished children and immunocompromised individuals. Surface-expressed antigens represent promising vaccine targets due to their accessibility to host immune recognition. This study aimed to assess the immunogenic potential of three C. parvum surface protein (GP45, GP900, and P23) administered individually and in combination as vaccine candidates in C57BL/6 mice.

Methods: Recombinant GP45, GP900, and P23 proteins were expressed and purified, and administered to C57BL/6 mice using a standard immunization schedule. Following vaccination, immune responses were evaluated by cytokine quantification assays to assess antigen-induced cellular and ELISA to assess humoral immune activation.

Results: All recombinant proteins elicited detectable immune responses when administered individually and in combined formulations. Increased production of interferon-gamma (IFN-γ) and interleukin-12 (IL-12) was observed, indicating induction of Th1-type cellular immunity. In addition, elevated levels of interleukin-4 (IL-4) were detected, suggesting activation of Th2-associated immune responses. Combined antigen formulations demonstrated comparable or enhanced immunogenicity relative to single-antigen groups, with exception of P23.

Conclusions: GP45, GP900, and P23 induced robust immune responses and promoted a balanced Th1/Th2 cytokine profile in immunized mice. These findings support their potential as promising vaccine candidates against *C. parvum* and warrant further evaluation of protective efficacy.

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SHIFT02-009

POSTER ON BOARD SHIFT 02
RESISTANCE AND DEVELOPMENT OF DRUGS AND VACCINES / VACCINE DEVELOPMENT: CHALLENGES AND BREAKTHROUGHS

DEVELOPMENT OF A SCHISTOSOMIASIS VACCINE: PRIMING WITH AN ADENOVIRUS-BASED VACCINE AND BOOSTING WITH RECOMBINANT SCHISTOSOMA MANSONI CATHEPSIN B DISPERSED IN ADDAVAX ADJUVANT

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Background and Aims: Schistosomiasis, also known as bilharzia, is a parasitic disease caused by blood flukes of the genus Schistosoma. It is a neglected tropical disease (NTD) infecting over 250 million people, mainly in sub-Saharan Africa, where poor and rural communities suffer disproportionately. According to the WHO, over 800 million people worldwide are at risk of infection. Chronic morbidity results from parasite eggs trapped in tissue, leading to granulomas and fibrosis. Praziquantel is currently the only FDA-approved drug, which acts on the adult worm's tegument and disrupts calcium permeability, paralyzing it, however this drug does not protect against re-infection and a prophylactic vaccine is of utmost importance.

Methods: Our lab has developed a heterologous prime and boost vaccine formulation delivered intramuscularly. It consists of a priming immunization using a low dose human adenovirus serotype 5 expressing Schistosoma mansoni Cathepsin B (SmCB) at week 0, followed by two boosting doses of recombinant SmCB protein adjuvanted with AddaVax at weeks 3 and 6. At week 9, all groups were infected with 150 cercariae and will be assessed for parasite burden at week 16. Humoral responses to SmCB are measured by ELISAs.

Results: This vaccine schedule resulted in significant humoral responses and the production of antigen specific IgG1, IgG2c and high avidity IgGs, while IgE was kept low. Assessment of parasite burden is ongoing.

Conclusions: So far, the AdSmCB group seems to show a Th1 skewed response and high antibody titers against SmCB. The development of a vaccine for schistosomiasis would be a highly important public health control measure.

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SHIFT02-010

POSTER ON BOARD SHIFT 02
RESISTANCE AND DEVELOPMENT OF DRUGS AND VACCINES / VACCINE DEVELOPMENT: CHALLENGES AND BREAKTHROUGHS

EVALUATION OF THE PROTECTIVE EFFICACY OF NOVEL ORNITHODOROS MOUBATA (ACARI: ARGASIDAE) ANTIGENS USING AN MRNA-LNP VACCINE PLATFORM IN RABBITS

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Background and Aims: *Ornithodoros moubata* is a vector of *Borrelia duttonii*, the causative agent of human tick-borne relapsing fever (TBRF), and African swine fever virus (ASFV) in sub-Saharan Africa. The development of effective anti-*O. moubata* vaccines could reduce tick infestations and contribute to disease control. Recent vaccinomics pipelines have enabled the prediction of novel vaccine targets from the salivary glands and midguts of *O. moubata*, while the mRNA–LNP platform has proved suitable for inducing robust anti-*Ornithodoros* immune responses in rabbits. Here, we evaluated the protective efficacy of six predicted *O. moubata* vaccine candidates using the mRNA–LNP platform.

Methods: Three salivary gland and three midgut antigens were selected as vaccine targets, with firefly luciferase used as a control. Corresponding mRNAs were synthesised by *in vitro* transcription and encapsulated in LNP. Rabbits received three monthly immunisations, followed by challenge with *O. moubata* and *O. erraticus*—the Iberian vector of TBRF and ASF—15 days after the final dose.

Results: Salivary antigens elicited stronger humoral responses than midgut antigens, with IgG titres ranging from 1:12,000 to 1:200. All six antigens conferred moderate protection (12–38%) against at least one *Ornithodoros* species.

Conclusions: The mRNA–LNP platform is a suitable tool for experimental screening and validation of *O. moubata* vaccine candidates. Three of the six candidates showed sufficient promise to warrant further development for anti-*Ornithodoros* vaccine design. *Funding: Grant "PID2022-136644OB-I00" (MCIN/AEI, Spain); Grant "CLU-2019–05–IRNASA/CSIC Unit of Excellence" (Junta de Castilla y León, Spain), and Grant MMT24-IRNASA-01 (CSIC, Spain).*

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SHIFT02-011

POSTER ON BOARD SHIFT 02
RESISTANCE AND DEVELOPMENT OF DRUGS AND VACCINES / VACCINE DEVELOPMENT: CHALLENGES AND BREAKTHROUGHS

NIFURTIMOX TREATMENT SHAPES TC24-SPECIFIC TH1 IMMUNE RESPONSES IN CHRONIC HUMAN CHAGAS DISEASE

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Background and Aims: Chagas disease, caused by *Trypanosoma cruzi*, affects approximately 6–7 million people worldwide. An estimated 20–30% of chronically infected individuals develop Chronic Chagas Cardiomyopathy (CCC), a severe and progressive condition for which current treatments fail to halt disease progression. Therapeutic vaccines have therefore emerged as a promising strategy. Although Tc24-C4 formulated with TLR-4 agonists has shown efficacy during the acute and early chronic phases, its effects during the late chronic phase—when cardiac damage is already established—remain unknown.

Methods: This study evaluated the therapeutic efficacy and immune response induced by rTc24-C4 + GLA-SE in a murine model of chronic *T. cruzi* infection. Forty BALB/c mice were infected with 5,000 trypomastigotes and immunized on days 128, 156, and 184 post-infection. On day 210 post-infection, samples were collected to assess parasite load, cardiac inflammatory infiltrate and fibrosis, as well as cellular and humoral immune responses. Statistical analyses were performed using one-way ANOVA with Tukey's post hoc test or Kruskal–Wallis with Dunn's correction, as appropriate ($p < 0.05$).

Results: No significant differences in parasite load were observed between groups (Fig. 1). However, vaccinated mice showed a significant reduction in cardiac inflammatory infiltrate ($p < 0.0001$) and fibrosis ($p < 0.05$) compared with untreated infected controls (Fig. 2). Vaccination also increased the IFN-γ^+/IL-4^+ ratio in $CD4^+$ T cells following rTc24-C4 restimulation ($p < 0.05$) (Fig. 3) and significantly elevated rTc24-C4–specific IgG levels ($p < 0.05$) (Fig. 4).

Conclusions: rTc24-C4 formulated with GLA-SE demonstrated therapeutic potential by reducing cardiac pathology and inducing a robust immune response during late chronic *T. cruzi* infection.

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SHIFT02-012

POSTER ON BOARD SHIFT 02
RESISTANCE AND DEVELOPMENT OF DRUGS AND VACCINES / VACCINE DEVELOPMENT: CHALLENGES AND BREAKTHROUGHS

VAXLEISH, A THERAPEUTIC VACCINE FOR HUMAN VISCERAL LEISHMANIASIS BASED ON A CHIMERIC ANTIGEN WITH MULTIPLE TANDEM REPETITIVE UNITS

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Background and Aims: Visceral leishmaniasis (VL) lacks a licensed vaccine. Many antigens stall because discovery does not align with translational requirements. To address this gap, we identified and characterized a novel *Leishmania* antigen, LinKAP. We then engineered rKAPA2, a chimeric protein combining LinKAP with the amastigote-specific A2 antigen. Adjuvant screening with rKAPA2 in VL models established the VaxLeish formulation as a therapeutic vaccine candidate within a GLP-aligned development package.

Methods: Immunoproteomics of *Leishmania* extracts using sera from VL-protected mice identified LinKAP. Localization and function were assessed by bioinformatics, subcellular fractionation, CRISPR–Cas9 tagging, confocal microscopy, and knockout phenotyping. Recombinant LinKAP was evaluated as a vaccine antigen, then used to generate the rKAPA2 chimera (LinKAP–A2), which was produced under GLP conditions. Adjuvants were screened in mouse, hamster, and dog VL models to define VaxLeish; the formulation was evaluated for immunogenicity, prophylactic/therapeutic efficacy, stability, and repeat-dose toxicology.

Results: LinKAP contains conserved tandem repeats and showed mitochondrial-associated localization. Parasite knockout did not affect in vitro growth, differentiation, or macrophage infectivity. Vaccination with recombinant LinKAP induced partial protection. This motivated the engineering of the chimera rKAPA2 with A2 domains. Systematic adjuvant screening across VL models identified a human-use dsRNA-based TLR3 agonist as optimal, defining VaxLeish (rKAPA2 plus adjuvant). Therapeutic VaxLeish elicited antigen-specific Th1 responses and significantly reduced parasite burden in target organs. GLP-aligned batches met predefined release specifications. Stability was confirmed for ≥9 months (−20°C, ongoing). VaxLeish was well-tolerated in repeat-dose rat toxicology.

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Conclusions: The integrated discovery-to-development strategy advanced VaxLeish as a promising therapeutic VL vaccine candidate supporting clinical translation.

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SHIFT02-013

POSTER ON BOARD SHIFT 02
RESISTANCE AND DEVELOPMENT OF DRUGS AND VACCINES / VACCINE DEVELOPMENT: CHALLENGES AND BREAKTHROUGHS

EVALUATING EXPOSURE TO LEISHMANIA USING A GLP-GRADE LEISHMANIA ANTIGEN PREPARATION

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Background and Aims: We have recently shown that a phenol-free, lyophilized Leishmania antigen formulation developed from *L. donovani* and produced under GLP conditions (GLP-LST) can successfully identify exposure to Leishmania using Interferon gamma release assays, an essential tool towards vaccination efforts and identification of biomarkers of protection. Here, we explored the reactivity of peripheral blood mononuclear cells (PBMCs) to GLP-LST in a cohort of individuals representing the spectrum of Leishmania exposure.

Methods: Individuals with: i) a history of LCL (healed) (n=17), ii) active disease (n=15), or iii) subclinical infection (n=7) were recruited in an area endemic for Cutaneous Leishmaniasis in Brazil. Controls consisted of naïve individuals (n=10), residing in a non-endemic area. PBMCs were stimulated with GLP-LST or with in-house Soluble Leishmania Antigen (SLA) at 5µg/ml for 72h. Supernatants were collected and evaluated for cytokine production.

Results: Both SLA and GLP-LST induced significantly higher levels of TNF and IFN-g in comparison to unstimulated cells in active CL patients and in healed individuals. Only GLP-LST induced significantly higher IL-10 levels in these individuals. In individuals with subclinical infection, GLP-LST also stimulated the production of IFN-g and TNF. Further experiments are underway to identify novel immune mediators induced by GLP-LST stimulation.

Conclusions: These findings indicate that GLP-LST is a sensitive tool for detecting and quantifying specific cellular immune responses in individuals previously exposed to a distinct *Leishmania sp.* Studies are underway to identify biomarkers of exposure to Leishmania using GLP-LST.

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SHIFT02-014

POSTER ON BOARD SHIFT 02
RESISTANCE AND DEVELOPMENT OF DRUGS AND VACCINES / VACCINE DEVELOPMENT: CHALLENGES AND BREAKTHROUGHS

IMMUNOPEPTIDOMICS OF LEISHMANIASIS PATIENTS REVEALS THE NATURAL ANTIGENIC LANDSCAPE

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Background and Aims: Despite the global burden of leishmaniasis, no licensed human vaccine exists. Vaccine antigens are typically based on in silico predictions or their immunogenicity in animal models and cured patients. Protective immunity is largely T cell–mediated and depends on antigen presentation by MHC molecules, yet T cell epitopes have only been mapped for fewer than 2% of over 8,000 Leishmania proteins. Whether infected phagocytes naturally present such antigens during human disease, and prior to the onset of parasite-induced regulatory mechanisms, is missing.

Methods: To partly address this gap, we performed mass spectrometry–based immunopeptidomics on spleen/bone marrow aspirates from 10 VL patients and lesional biopsies from 27 Ethiopian CL patients spanning the full clinical spectrum.

Results: In CL patients, we newly identified 333 MHC-I and 247 MHC-II epitopes from 398 *L. aethiopica* proteins, including 19 peptides and 51 antigens recurrently presented across patients of which several were also epitope-rich. Some peptides were also detected during early infection in a *L. aethiopica*–infected THP-1 monocyte model, highlighting their relevance from disease onset. Notably, despite the broad predictive coverage of NetMHCpan and NetMHCIIpan, these tools missed 20–70% of the naturally presented epitopes while predicting millions of candidates, underscoring the limitations of prediction-only vaccine pipelines. Analyses on VL patients are ongoing and ready for presentation by August.

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Conclusions: This first comprehensive map of the *Leishmania* immunopeptidome in human disease reveals conserved and prevalent antigens that can inform rational vaccine design and deepen our understanding of protective T cell responses in leishmaniasis.

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SHIFT02-015

POSTER ON BOARD SHIFT 02
RESISTANCE AND DEVELOPMENT OF DRUGS AND VACCINES / VACCINE DEVELOPMENT: CHALLENGES AND BREAKTHROUGHS

MCP-1 AND PVS47 PROTEINS AS POTENTIAL VACCINE TARGETS AGAINST PLASMODIUM VIVAX

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Background and Aims: Malaria caused by *Plasmodium vivax* remains a public health challenge, demanding novel control strategies. MCP-1, a reticulocyte-binding protein, and Pvs47, a gametocyte surface protein, are potential targets for vaccine development. This study evaluated the ability of antibodies against MCP-1 and Pvs47 to interfere with the parasite life cycle.

Methods: To assess anti-MCP-1 activity, *P. vivax* isolates containing ≥60% trophozoites were cultured for 24–48 hours. Schizonts (enriched with 45% Percoll) were then co-cultured with enriched reticulocytes (70% Percoll). Invasion was evaluated by light microscopy (trophozoites in 1,000 cells) and calculated using delta (Δ = 24h – 0h), where a positive Δ indicates invasion and a negative Δ indicates inhibition. To evaluate anti-Pvs47, reconstituted infected blood (hematocrit 50%) was used to feed *Anopheles darlingi* females (control vs. 1:10). Slides with parasites were processed for IFI using primary antibodies, conjugated secondary antibodies, and DAPI.

Results: Anti-MCP-1 (1:10) showed a significant reduction in invasion compared to control (Δ 8.0 ± 3.46 vs. Δ –10.0 ± 0.28; p = 0.03). After 7 days of *P. vivax* infection, anti-Pvs47 did not block transmission but reduced infection intensity (43.2 ± 37.8 vs. 26.0 ± 26.5 oocysts; p = 0.045). IFI images showed antibody binding: Pvs47 in gametocytes and MCP-1 in young erythrocytic forms, although not confirmed in schizonts.

Conclusions: These findings demonstrate the potential of MCP-1 to inhibit merozoite invasion and suggest a partial role for Pvs47 in reducing oocyst burden. Further studies with additional isolates and optimized conditions are ongoing to confirm the vaccine potential of both targets.

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SHIFT02-016

POSTER ON BOARD SHIFT 02
RESISTANCE AND DEVELOPMENT OF DRUGS AND VACCINES / VACCINE DEVELOPMENT: CHALLENGES AND BREAKTHROUGHS

CHIMERIC ANTIGEN SMQUIMERA01 AS A VACCINE CANDIDATE FOR SCHISTOSOMA MANSONI INTESTINAL SCHISTOSOMIASIS: PROTECTIVE EFFICACY IN MICE ACROSS VACCINE FORMULATIONS

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Background and Aims: Mathematical models predict that a schistosomiasis vaccine could reduce morbidity and transmission, especially if it induces strong parasite-burden reductions. Because *S. mansoni is a* complex parasite, multiepitope chimeric recombinant proteins may elicit stronger protection. Our group developed SmQuimera01, a chimeric protein containing five epitopes from distinct antigens, in this study we assessed its immunogenicity and formulation-dependent protective efficacy in mice.

Methods: The synthetic gene (*smquimera01*) was cloned into pET21a, expressed in *E. coli* BL21 (DE3) Star, and purified by nickel affinity chromatography. C57BL/6 mice received three doses of rSmQuimera01 (25 µg/animal) or saline at 15-day intervals formulated with either Alhydrogel (alum), MPLA-SM + Alhydrogel or CT-VAD1. Challenge infection occurred 21 days (alum) or 28 days (MPLA-SM + alum or CT-VAD1) after the third dose. Worms were recovered 50 days later by mesenteric perfusion. Egg burdens in the liver and intestine were quantified, hepatic granuloma areas were measured and anti-rSmQuimera01antibodies were assessed by ELISA

Results: All formulations were imunogenic, inducing high antibodies titers. Protective outcomes were formulation-dependent. Alum-rSmQuimera01 did not significantly reduce worm or egg burdens but significantly decreased hepatic granuloma area, suggesting an anti-pathology effect. MPLA-SM + alum-rSmQuimera01 significantly reduced worm burden, lowered egg loads in liver and intestine, and reduced worm fertility, without a significant change in granuloma area. In contrast, the SmQuimera01/CT-VAD1 formulation did not confer significant protection or anti-pathology effects.

Conclusions: SmQuimera01 is a promising vaccine candidate with distinct, formulation-driven outcomes: anti-pathology and anti-parasitic. These results support further mechanistic studies to optimize protective immunity. **Financial Support:** Fapemig, CNPq and CAPES

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SHIFT02-017

POSTER ON BOARD SHIFT 02
RESISTANCE AND DEVELOPMENT OF DRUGS AND VACCINES / VACCINE DEVELOPMENT: CHALLENGES AND BREAKTHROUGHS

VIVAXIN, A MULTI-EPITOPE PAN-VACCINE SPECIFIC FOR SPOROZOITE SURFACE PROTEIN FROM PLASMODIUM VIVAX

Ana Clara Gazzinelli[1], Ana Luiza Chaves[1], Flávia Bagno[1], Julia Castro[1], Gregório Almeida[1], Isabela Gomes[1], Natalia Salazar[1], Diana Gomez[1], Pablo Reis[1], Bianca De Oliveira[2], Renata Salgado[1], Graziella Rivelli[1], Rodolfo Marques[3], Xiomara Alexandra Gaitán[4], Rogerio Armino[5], Maísa Araújo[6], Helton Santiago[1], Ana Paula Fernandes[1], Santuza Teixeira[1], Irene Soares[3], Ricardo Gazzinelli[1]
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Background and Aims: Malaria remains a major global health burden, and *P. vivax* is responsible for ~9.87 million cases, particularly in Southeast Asia and the Americas. In contrast to *P. falciparum*—for which the RTS,S/AS01E (Mosquirix®) and R21/Matrix-M® vaccines are WHO-recommended—there are no licensed vaccines targeting *P. vivax*. Here, we describe the preclinical development of Vivaxin, a recombinant vaccine (PvCSP-*All epitopes*) comprising the repeat domains representing the three PvCSP variants (VK210, VK247, and *P. vivax*-like), produced in *Pichia pastoris*. This study aimed to (i) manufacturability and stability of API, (ii) characterize humoral and cellular immunogenicity (iii) evaluate protective efficacy in challenge model, (iv) assess safety and toxicity studies.

Methods: The lyophilized product was prepared and manufactured under GMP conditions. The lyophilized formulation was assessed in QC and stability programs using physicochemical, biochemical, microbiological, and functional assays. Humoral responses were quantified by ELISA against the chimera and individual variants. Cellular immunity was evaluated and IFN-γ measurement. Protective efficacy was tested by challenge with sporozoites. Safety and toxicity were assessed in both rodents and non-human primates.

Results: Vivaxin showed robust manufacturability and stability supporting product integrity for advanced development. The vaccination induced high titers of antigen-specific total IgG and subclass responses to all three PvCSP repeat variants. Cellular assays demonstrated vaccine-induced antigen-specific IFN-γ production. Following challenge conferred sterilly protection. Safety and toxicity of vaccine proved to be safe in both models.

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Conclusions: These findings position Vivaxin as a stable, safe, and protective pre-erythrocytic *P. vivax* vaccine and provide a strong rationale for continued translational development toward early-phase clinical evaluation.

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SHIFT02-018

POSTER ON BOARD SHIFT 02
RESISTANCE AND DEVELOPMENT OF DRUGS AND VACCINES / VACCINE DEVELOPMENT: CHALLENGES AND BREAKTHROUGHS

SELECTION OF SUITABLE CATIONIC LIPIDS FOR A CELLULAR IMMUNITY-INDUCING MESSENGER RNA MALARIA VACCINE

Jiun-Yu Jian[1], Atsushi Hayata[2], Yujiro Kidani[3], Odsuren Sukhbaatar[1], Mayumi Taniguchi[1,4], Shun Kitahata[2], Daisuke Suzuki[2], Megumi Tanaka[1,5], Kanoko Iki[1,6], Sayuri Nakamae[1,7], Rina Iwamoto[8], Makoto Matsumoto[9], Norikazu Kuroda[8], Shigeru Kawakami[9], Takeshi Annoura[10], Akira Kugimiya[2], Katsuyuki Yui[11], Kenji Hirayama[4,12], Shinya Omoto[3], Shusaku Mizukami[1,4]
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Background and Aims: Despite the recent historic milestone of the first malaria vaccine receiving World Health Organization (WHO) prequalification, the urgent development of highly effective vaccines is still required. We previously reported that an mRNA lipid nanoparticle (mRNA-LNP) vaccine induced liver-resident memory T cells (T_{RM}) and elicited robust protection against parasite challenges in a mouse model. Nevertheless, optimizing the mRNA-LNP formulation remains challenging. Thus, we first focused on optimizing cationic lipids to further develop a promising mRNA-LNP-based malaria vaccine.

Methods: BALB/c mice received two doses of mRNA-LNP coding *Plasmodium berghei* circumsporozoite protein (PbCSP) with a three-week interval. At week seven, liver and spleen samples were harvested for immunological analyses, or mice were challenged with Pb sporozoites expressing luciferase to monitor the parasites. To elucidate immune mechanisms of protection, *in vivo* cell-depletion assays for total $CD8^+$ cells and their subsets were conducted.

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Results: Our candidate, Lipid S-containing mRNA-LNP, elicited robust antigen-specific $CD8^+$ T cells as well as T_{RM} in the liver and spleen. Additionally, Lipid S induced high titers of PbCSP-specific IgG in the serum. Furthermore, the vaccine formulated with Lipid S induced complete sterilizing immunity against sporozoite challenge up to 14 days post-challenge, in a $CD8^+$ T cell-dependent manner.

Conclusions: In this study, we demonstrate that Lipid S formulated mRNA-LNP-based vaccine is a potent vaccine candidate that elicits both cellular and humoral immunity. Further studies are underway to elucidate the detailed mechanisms underlying this robust protection, thereby facilitating next-generation vaccine development.

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SHIFT02-019

POSTER ON BOARD SHIFT 02
RESISTANCE AND DEVELOPMENT OF DRUGS AND VACCINES / VACCINE DEVELOPMENT: CHALLENGES AND BREAKTHROUGHS

FROM CHALLENGE TO IMPACT: MALARIA VACCINES - PROVING THAT PARASITES CAN BE DEFEATED

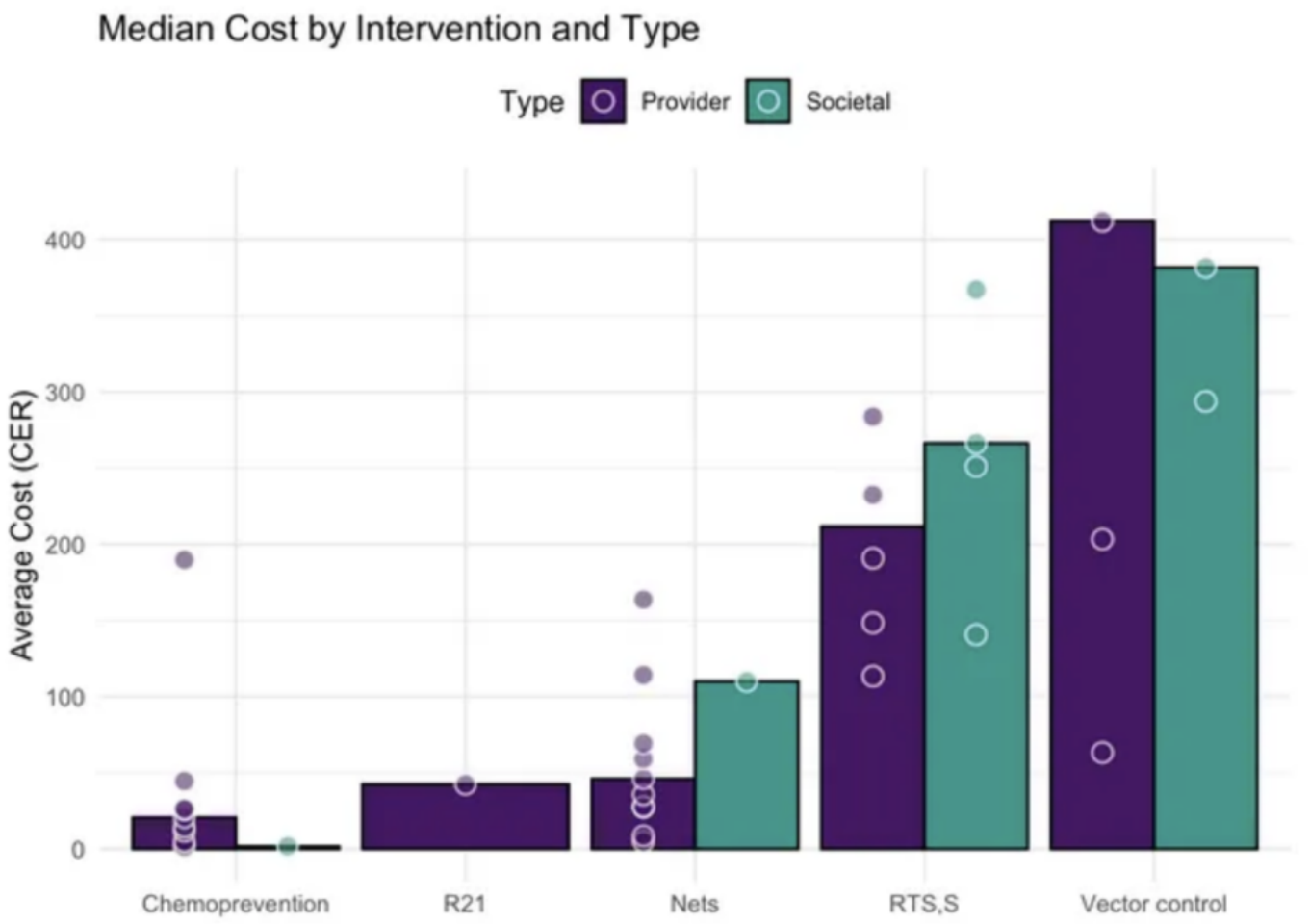
Zacharia Kafuko
1Day Sooner, 1day Africa, Lusaka, Zambia

Background and Aims: Background Malaria remains a leading killer in Sub-Saharan Africa. With more than 282 million cases and more than 600,000 deaths annually. This, despite decades of efforts through vector control, chemoprophylaxis, and improved diagnostic tools. The development and prequalification of malaria vaccines RTS,S/AS01 (Mosquirix) and R21/Matrix-M have introduced a significant advancement in the malaria control toolkit. Aim This presentation seeks to highlight the current scope of impact of interventions against the malaria parasite as a result of the introduction of the malaria vaccines.

Methods: This project was launched in 2023 to track and provide advocacy for the regulatory proccesses and the vaccine deliveries. We tracked (ongoing) all sub-Saharan African countries with a Malaria burden to generate data on the efforts, challenges and successes. We also used this data to create a realtime Malaria impact tracker for the continent.

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Results:



RTS,S, the first WHO recommended and prequalified vaccine showed efficacy of between 36 to 63%. It also demonstrated a 13% reduction in all cause mortality through the studies and the pilot rollout in the Malaria Vaccines Implementation Programme in Kenya, Ghana and Malawi. The newer vaccine R21 showed efficacy above 75% and is projected to have similar or better immunity and durability of protection as RTS,S. An estimated 40 million doses have so far been delivered. The project also found that the R21 Malaria vaccine was the second most cost effective intervention in the fight against Malaria.

Conclusions: 40 million doses delivered, prove that even for parasites complicated as the Malaria, it is possible to defeat them.

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SHIFT02-020

POSTER ON BOARD SHIFT 02
RESISTANCE AND DEVELOPMENT OF DRUGS AND VACCINES / VACCINE DEVELOPMENT: CHALLENGES AND BREAKTHROUGHS

IDENTIFYING VACCINE TARGETS FOR CHAGAS DISEASE USING A YEAST SURFACE DISPLAY SCREEN

Mira Loock, Lissa Cruz-Saavedra, Igor Cestari
McGill University, Institute Of Parasitology, Ste Anne de Bellevue, Canada

Background and Aims: Chagas disease (ChD) affects ~7 million people and is caused by infection with the protozoan parasite *Trypanosoma cruzi,* resulting in 12,000 annual deaths. There is an urgent need for new therapeutic interventions as existing drugs - nifurtimox and benznidazole - are highly toxic and not effective at the chronic stage, with no vaccine currently available. We aim to identify and validate new vaccine targets for ChD.

Methods: We developed a *T. cruzi* genome-wide library for yeast surface display (YSD). To identify antigenic polypeptides, we screened the YSD library against antibodies from patients with chronic ChD or healthy donors using magnetic-activated cell sorting (MACS). A selected antigen was validated in murine models via recombinant protein or whole yeast vaccine strategies in conjunction with the IL-12/GM-CSF adjuvant.

Results: Our YSD library demonstrated a 30-fold coverage of the parasite genome, expressing over 240,000 unique polypeptides. After MACS, we identified ~1600 antigens ($p<0.05$; fold-change > 2) and defined immunogenic regions at nucleotide resolution. Of the 829 antigens exclusively enriched with antibodies from ChD patient serum compared to non-Chagas individuals, candidates were identified by conservation among *T. cruzi* DTUs and among infectious stages of the disease to identify 70 candidates. Following immunization with an antigen, we identified a decrease in parasite burden in the muscle tissue of infected mice, which correlates with an increase in CD8+ T-cell populations from blood and stimulated splenocytes.

Conclusions: The YSD screen provides a viable approach for identifying vaccine targets to stimulate antibody and T-cell responses. Further analysis of immunization is in progress.

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SHIFT02-021

POSTER ON BOARD SHIFT 02
RESISTANCE AND DEVELOPMENT OF DRUGS AND VACCINES / VACCINE DEVELOPMENT: CHALLENGES AND BREAKTHROUGHS

A STUDY ON HOMOLOGY AND HETEROLOGY BETWEEN PANASIA-II & PAK- 98 LINEAGES OF FOOT AND MOUTH DISEASE VIRUS SEROTYPE-O

Hafza Zahira Manzoor
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Background and Aims: To understand the dynamics of epidemics, it is crucial to evaluate prior exposure to infectious diseases as a possible defense against newly developing strains.The cross-protection prediction is unresolved matter that holds great importance, particularly in the field of FMDV.new methods for controlling FMD involve the implementation of vaccine, limited movement, containment and elimination of cattle that are either affected or vulnerable to disease.The primary objective of this study was aimed to explain the genetic homologous and heterologous association among two ancestries of FMDV serotype O found in Pakistan and to find a suitable strain from field for selection of vaccinated seed that provide cross-protection against both ancestries.

Methods: Virus neutralization tests (VNTs) were used to evaluate an antigenic association among the two groups of viruses, indicates a significant degree of antigenic similarity between stains within a particular cluster. Conversely, r1 values ranging from 0.07-0.2 were obtained from crosscombinations among clusters, suggesting antigenic variation amongst virus strains.

Results: These results were further supported by in vitro tests, which showed that heterologous combinations of PanAsia-II and PAK-98 isolates showed antigenic variation, except for 25-06-PK-19, SDG-43-FSD-PK, and TANWL-34PK, which showed a 0.3 (r1 value). In contrast, when considering homologous combination of strains from both lineages showed antigenic similarity. Furthermore, it was projected that these combinations would provide protective titers during in-vivo.

Conclusions: As a conclusion, it is recommended that all strains, except 25-06-PK-19, SDG-43-FSD-PK, and TANWL-34PK considered as potential vaccine candidates for effectively managing outbreaks of FMDV serotype-O, due to their ability to confer broad-spectrum immunity.

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SHIFT02-022

POSTER ON BOARD SHIFT 02
RESISTANCE AND DEVELOPMENT OF DRUGS AND VACCINES / VACCINE DEVELOPMENT: CHALLENGES AND BREAKTHROUGHS

IMMUNE RESPONSE DYNAMICS AND EFFICACY OF NOVEL ANTIGEN AND ADJUVANT VACCINES FOR RHIPICEPHALUS MICROPLUS

Christine Maritz-Olivier, Laura Bishop, Christian Stutzer
University of Pretoria, Biochemistry, Genetics And Microbiology, Pretoria, South Africa

Background and Aims: Resistance of *Rhipicephalus microplus* tick populations to acaricides necessitates alternative control strategies. Immunological control represents a critical complementary approach; however, the efficacy of the Bm86-based vaccine varies widely (0–100%) and remains suboptimal. This variability highlights the need for improved and sustainable vaccines through the identification of novel antigens, optimisation of adjuvant formulations, and insight into correlates of protection. In this study, novel antigens and four adjuvant formulations were evaluated in bovines to assess vaccine efficacy, epitope recognition, innate and adaptive immune responses, and immune dynamics at tick feeding sites.

Methods: Bovine vaccination trials followed by homologous tick challenge were conducted. Antibody kinetics were assessed using ELISA, while cellular immune responses were evaluated by flow cytometry and multiplex cytokine profiling. The effects of vaccine formulations on host immune responses at tick attachment sites were investigated using high-throughput transcriptomic analyses.

Results: The findings demonstrated that adjuvant selection significantly modulates antibody profiles, enhances vaccine efficacy, and influences linear B-cell epitope recognition, underscoring the critical role of adjuvants in improving tick vaccines. Analyses revealed activation of key immune pathways in vaccinated cattle compared with controls, including toll-like receptor signalling, cytokine–cytokine receptor interactions, natural killer cell–mediated cytotoxicity, and complement cascade pathways. These results advance understanding of local immune responses at tick feeding sites and identify immune mechanisms associated with protective vaccination.

Conclusions: This study addresses critical gaps in the development of ick vaccines. By elucidating the role of adjuvants in immune responses, optimising antigen–adjuvant combinations, and host immune dynamics, it provides a strategic framework for next-generation tick vaccines.

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SHIFT02-023

POSTER ON BOARD SHIFT 02
RESISTANCE AND DEVELOPMENT OF DRUGS AND VACCINES / VACCINE DEVELOPMENT: CHALLENGES AND BREAKTHROUGHS

REGULAR MASS DRUG ADMINISTRATION OF PRAZIQUANTEL TO BOOST ANTI-SCHISTOSOME IMMUNITY: PRECLINICAL AND CLINICAL EVIDENCES

Bernard Marie Bitye Zambo[1], Mireille Kameni[2], Leonel Meyo Kamguia[1], Thabo Mpotje[3], Marie Madeleine Noubissi Fotseu[1,4], Frank Brombacher[3], Claudia Demarta-Gatsi[5], Thomas Spangenberg[5], Justin Komguep Nono[1]
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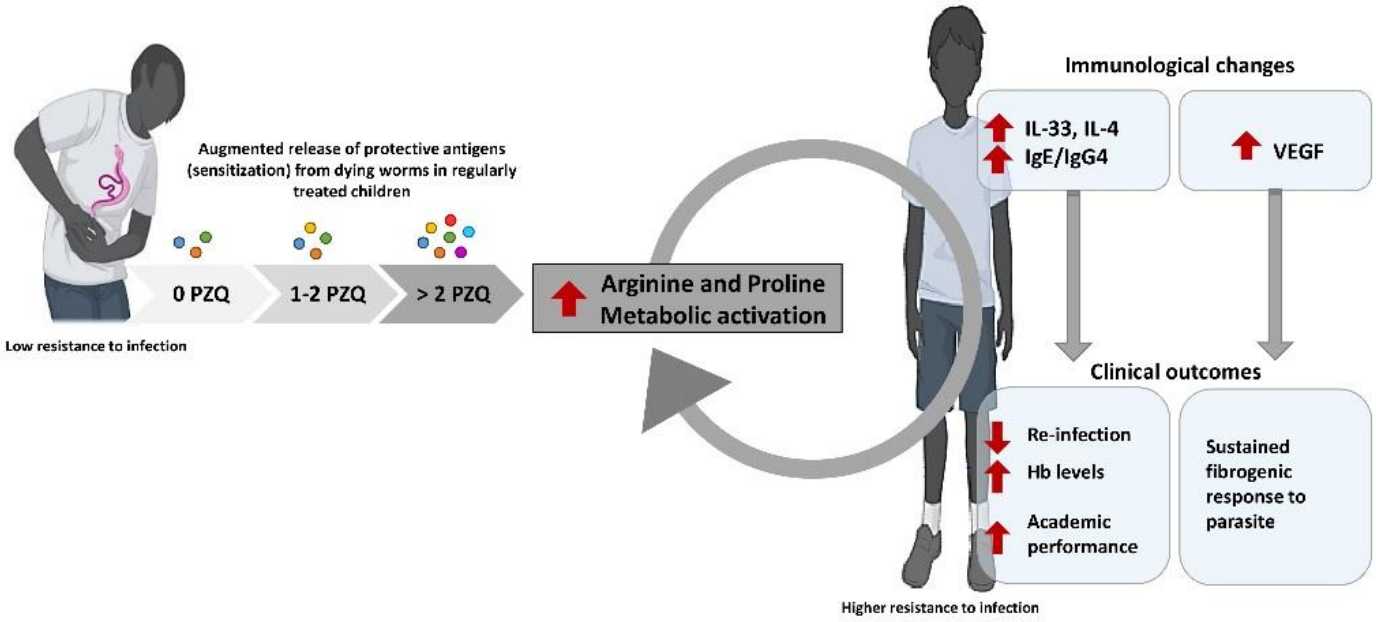
Background and Aims: Schistosomiasis remains a significant public health challenge in endemic regions, leading to substantial morbidity, in the absence of a functional vaccine until date. While regular mass drug administration (MDA) of praziquantel (PZQ) is a cornerstone of schistosomiasis control programs in endemic areas, emerging evidence suggests that its benefits, not fully harnessed, may extend beyond mere parasite killing, orchestrating a chemotherapeutically-induced immunity in treated hosts. We aimed to address this possibility.

Methods: We examined schistosomiasis in school-aged children from affected areas who received different cumulative numbers of annual PZQ treatments. We monitored reinfection rates and health effects, like anemia, cognitive impairment and liver fibrosis. A mouse model was also used to analyse, beyond association, the likelihood of causality in how the numbers of infection-PZQ treatment cycles might affect susceptibility to reinfection, pathologies and immune responses.

Results: Analysis of data and samples from SAC showed that regular use of PZQ in endemic area activated more rapidly arginine/proline metabolism and increased protective IgE levels and type-2 cytokines in these SAC. This lowered the chance of subsequent high parasite levels (AOR = 0.16) and improved hemoglobin (AOR = 2.58) and academic performance (AOR = 2.39). Similarly, In mouse models, repeated cycle of infection/treatments boosted immune responsiveness, particularly of the type-2 arm (parasite-directed IgE and IgG1, Arginase-1) and translated into increased resistance to reinfection.

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Conclusions: The study shows that regular treatment with PZQ can improve protective immune responses in children from schistosomiasis-endemic areas, reducing the risk of reinfection and associated sequelae.



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SHIFT02-024

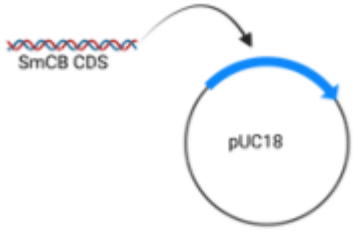
POSTER ON BOARD SHIFT 02
RESISTANCE AND DEVELOPMENT OF DRUGS AND VACCINES / VACCINE DEVELOPMENT: CHALLENGES AND BREAKTHROUGHS

DEVELOPING AN MRNA VACCINE AGAINST SCHISTOSOMIASIS BASED ON S. MANSONI CATHEPSIN B PROTEIN

May O'Connell[1,2], Momar Ndao[3]
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Background and Aims: Schistosomiasis is a disease that affects nearly one billion people around the world. It is caused by a parasitic blood worm of the genus *Schistosoma*, which infects those who contact freshwater in endemic regions. These worms produce eggs which end up lodged in various bodily organs. These eggs are the cause of chronic pathology that can lead to serious health concerns later in life, resulting in over 200,000 deaths per year. Praziquantel is the only drug effective for schistosomiasis; however, it does not protect against reinfection. Our lab has been studying an *S. mansoni* gut protein, which is essential for parasite growth and nutrient acquisition. It has been shown that worms lacking this protein lay fewer eggs due to a lack of nutrients, breaking the transmission cycle. We have experimented with multiple forms of protein delivery for mouse vaccination, and after challenging these mice with *s. mansoni* infection, we observe with striking results of up to 75% protection. We believe that an mRNA vaccine based on this protein will result in high protection levels with an excellent safety profile.

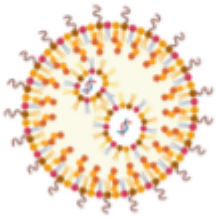
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1. Ligate SmCB coding sequence into plasmid



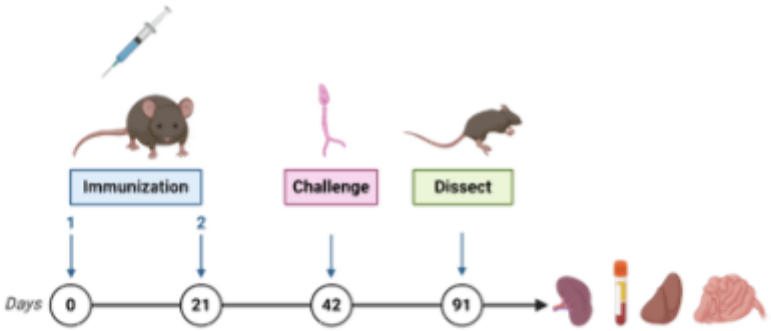
2. Polyadenylation, capping and *in vitro* synthesis



3. SmCB mRNA encapsulation using Sm102 ionizable lipid particles



4. *In vitro* transfection of mammalian cells to confirm protein expression



5. Intramuscular injection of C57BL/6 mice with 2 doses, 21 days apart, followed by challenge with infection on day 42. N = 5



Recomb SmCB + Addavax | PBS | 10 ug SmCB mRNA | 1 ug SmCB mRNA | 10 ug Ova mRNA

Methods: We have developed an mRNA-LNP vaccine based on this protein and are testing its immunogenicity and protection against challenge in mice.

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Results:

High encapsulation efficiency of >96% confirmed by Ribogreen Assay and optimal LNP size confirmed by Dynamic Light Scattering

Encapsulated mRNA (ug/mL)	Total mRNA (ug/mL)	Encapsulation efficiency %	Avg Z-avg (nm)	Polydispersity index (PDI)
348.7	362.4	96.2	93.9	0.034

Figure 2. Encapsulation efficiency and size of SmCB mRNA encapsulated with Sm102 ionizable lipid particles. Encapsulation evaluated using a Ribogreen Assay. Fluorescence measured at excitation/emission 480/520 nm against a curve of mRNA standards ranging from 0.1 ug/mL to 2.5 µg/mL. Z-avg and PDI determined via dynamic light scattering (DLS).

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Figure 3. Western blot performed on cell lysate demonstrating *in vitro* SmCB expression in HEK293 cells. (-) Untransfected cells 1) 2 ug SmCB mRNA 2-4) + control recombinant SmCB protein. Expected band at 37 kDa. Probed using crude anti-SmCB mouse serum antibodies and detected via chemiluminescence.

Preliminary results indicate over 96% encapsulation efficiency, optimal LNP sizes, and protein expression *in vitro*.

Conclusions: If successful, not only would this vaccine change the lives of millions, but it could also pave the way as a model for future parasitic vaccine development and the potential of mRNA as a suitable vector.

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SHIFT02-026

POSTER ON BOARD SHIFT 02
RESISTANCE AND DEVELOPMENT OF DRUGS AND VACCINES / VACCINE DEVELOPMENT: CHALLENGES AND BREAKTHROUGHS

"OPISTHORCHIS VIVERRINI" ANTI-CANCER VACCINE TARGETING THE LIVER FLUKE HOST-PARASITE INTERFACE

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Background and Aims: Infection with the food-borne liver fluke *Opisthorchis viverrini* infects ~10 million people and is the principal risk factor for bile duct liver cancer (cholangiocarcinoma, CCA) in SE-Asia. A vaccine is needed to reduce future infections and the many thousands of annual deaths. But flukes are difficult foes, and an efficacious vaccine has eluded researchers so far. We believe an effective vaccine will need to target a number of key parasitism pathways to be sufficiently efficacious.

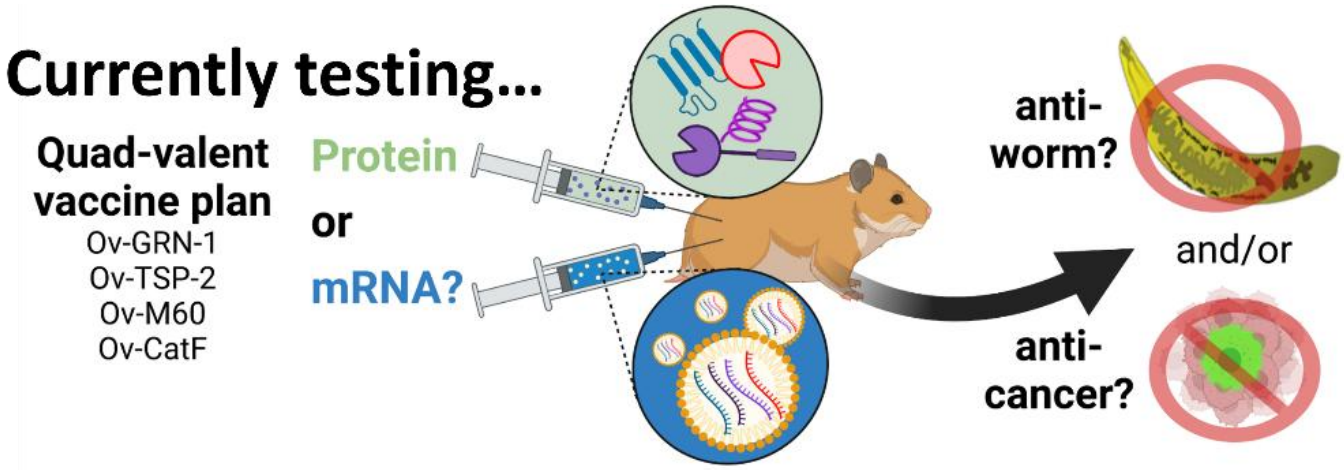
Methods: We are exploring the efficacy of *Ov*-GRN-1, *Ov*-TSP-2, and other nutrient acquisition antigen combinations as CRISPR/Cas9 gene knockout flukes. Then by combining this understanding we evaluate both protein antigens and mRNA-lipid nanoparticle formulations as potential vaccines in the hamster model of fluke infection and CCA.

Results: Flukes with the carcinogenic fluke growth factor *Ov-grn-1* knocked-out survived but showed less hepatobiliary tract disease and high-grade CCA manifested during chronic infection. *Ov*-TSP-2 is a critical tetraspanin abundant on the surface of *O. viverrini* secreted extracellular vesicles (EVs) and *Ov-tsp-2* gene knockout is lethal for flukes *in vivo*. Vaccine results currently underway will be presented.

Conclusions: We believe that a quad-valent formulation targeting fluke-host communication in combination with nutrient acquisition pathways will ultimately combat this liver fluke infection associated malignancy in the form of a novel anti-fluke/anti-cancer vaccine. We hope this research will lead to a vaccine with the potential to benefit millions of impoverished residents of endemic

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regions.



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SHIFT02-027

POSTER ON BOARD SHIFT 02
RESISTANCE AND DEVELOPMENT OF DRUGS AND VACCINES / VACCINE DEVELOPMENT: CHALLENGES AND BREAKTHROUGHS

A MODEL OF CRYPTOSPORIDIOSIS USING INTERFERON GAMMA RECEPTOR KNOCK-OUT MICE TO EVALUATE THE EFFICACY OF THE GP45 VACCINE CANDIDATE

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Background and Aims: Cryptosporidiosis causes moderate-to-severe diarrhea in infants in developing countries, and mortality in newborn dairy calves worldwide. Vaccination to prevent the disease would be an excellent approach. Developing a model to study the efficacy of vaccine candidates to prevent cryptosporidiosis becomes a necessity. Interferon gamma receptor knock-out (IFNgammaR-KO) mice are a good model because they are susceptible to *Cryptosporidium parvum* (*C. parvum*) infection. Few proteins showed a potential as vaccine candidates; gp45 protein, also called gp40, is among the most promising antigens.

Methods: IFNgammaR-KO mice were Immunized with gp45 protein and then challenged with *C. parvum* oocysts to study the potential of this vaccine candidate. Flow cytometry and quantitative polymerase chain reaction (qPCR) were useful to quantify the number of oocysts in the intestine of immunized mice challenged with *C. parvum*.

Results: IFNgammaR-KO adult mice immunized with gp45 showed lower intestinal parasite burdens when challenged with *C. parvum* oocysts. Both flow cytometry and qPCR analysis were used to quantify the significant reductions in parasite burdens. Interestingly, a male/female difference in susceptibility to infection has been revealed. The goal being to develop a vaccine to immunize moms to protect infants or newborn dairy calves, we also established a mother/pups model. Immunizing IFNgammaR-KO mother mice successfully transmitted passive immunity to newborn pups and reduced parasite burdens in the intestine of pups.

Conclusions: Immunization and challenge of IFNgammaR-KO mice is an excellent mouse model to determine the best combination of *C. parvum* gp45 protein with different adjuvants for a vaccine against cryptosporidiosis.

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SHIFT02-028

POSTER ON BOARD SHIFT 02
RESISTANCE AND DEVELOPMENT OF DRUGS AND VACCINES / VACCINE DEVELOPMENT: CHALLENGES AND BREAKTHROUGHS

THE EFFECT OF DASATINIB IN THE EXPERIMENTAL MODEL OF CHAGAS DISEASE

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Background and Aims: Chagas disease (CD) caused by protozoan *Trypanosoma cruzi* is a neglected tropical disease.Benznidazole (BZ) and Nifurtimox (NIF) are the only drugs used to treat CD. These drugs have side effects that impact treatment adherence by individuals infected with CD. Aim of our project was to verify the effect of Dasatinib (DAS) to the treatment of CD. DAS is a tyrosine kinase inhibitor used in the treatment of chronic myeloid leukemia (CML) and acute lymphoblastic leukemia (ALL) that acts by regulating the proliferation of tumor cells.

Methods: BALB/cmice were infected with *T. cruzi* strain CL-Brener Luc/Neon and treated with DAS, and the parasite load and survival curve of the experimental animals. Viability assay were performed with different cell (LLC- MK2, THP-1 and RAW 264.7) and on the parasite. In addition, we verified the effect of DAS on the release of EVs from the trypomastigote forms of T. cruzi and parasite invasion assays.

Results: The in vivo results showed an increase in the parasitemia in mice treated with DAS and reduction in the survival of animals compared to control. In in vitro cell viability assays, we observed that DAS did not alter the viability of LLC-MK2 cells, human monocytes (THP-1) and *T. cruzi*

Conclusions: In conclusion, our results may help in the search for alternatives for the treatment of CD, corroborating the findings of other studies on the potential of DAS to modulate the immune system, and also reinforcing the importance of understanding the molecular mechanisms of action of this compound in CD. Support by FAPESP and CNPq

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SHIFT02-029

POSTER ON BOARD SHIFT 02
RESISTANCE AND DEVELOPMENT OF DRUGS AND VACCINES / VACCINE DEVELOPMENT: CHALLENGES AND BREAKTHROUGHS

EVALUATION OF INTRADERMAL INJECTION SYSTEM (IMMUCISE) FOR THE LIVE ATTENUATED VACCINE, CENTRIN-DEFICIENT LEISHMANIA MAJOR (LMCEN-/-), AGAINST LEISHMANIASIS

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Background and Aims: *Leishmaniasis* remains challenging due to limited treatment options in endemic regions. A live attenuated, *centrin*-deficient *Leishmania major* vaccine shows promise in preclinical studies. This study aimed to show if *LmCen*$^{-/-}$ can elicit a protective immune response by FDA-approved TERUMO needle, comparable to insulin needle used in preclinical studies, and to evaluate the applicability of its use in clinical trials.

Methods: In vitro *LmCen*$^{-/-}$ viability: Cultured *LmCen*$^{-/-}$ parasites were passed through needles of different gauge sizes, and viability was evaluated by live–dead staining using flow cytometry and microscopy. **In vitro *LmCen*$^{-/-}$ infectivity:** Bone marrow–derived macrophages were infected with *LmCen*$^{-/-}$ parasites after passing through TERUMO needles; parasite burden per macrophage was assessed at 24 and 72 hours. **CD4^{+} T cell response to soluble Leishmania antigen (SLA):** Mice were immunized with *LmCen*$^{-/-}$ parasites, and CD4^{+} T cells were isolated from lymph nodes at 6 weeks post-immunization. Cytokine production in response to SLA was measured by flow cytometry and ELISA. **Protective efficacy:** Mice were vaccinated with *LmCen*$^{-/-}$ parasites using a TERUMO or an insulin needle and were challenged with wild-type *Leishmania major*. Parasite burden in infected ear was quantified at 12 weeks post-challenge.

Results: Parasite viability, infectivity, and cytokine production were maintained after passing through TERUMO needle, comparable to insulin needle. Mice vaccinated with *LmCen*$^{-/-}$ passing through TERUMO needles showed protective efficacy similar to control group.

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Conclusions: These findings demonstrated that *LmCen*$^{-/-}$ vaccination with Terumo needles elicited an immune response and protection like insulin needles, supporting the *LmCen*$^{-/-}$ vaccine with Terumo needles to go to clinical stages.

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SHIFT02-030

POSTER ON BOARD SHIFT 02
RESISTANCE AND DEVELOPMENT OF DRUGS AND VACCINES / VACCINE DEVELOPMENT: CHALLENGES AND BREAKTHROUGHS

COMPARATIVE POPULATION GENETIC RESOLUTION BETWEEN MITOCHONDRIAL, MICROSATELLITE, AND RNA-SEQ DATA IN RHIPICEPHALUS MICROPLUS

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Background and Aims: Quantifying genetic diversity in *Rhipicephalus microplus* is essential for understanding its evolutionary potential across geographic regions. The rate at which acaricide resistance becomes fixed depends on existing diversity, as smaller effective population sizes with increased homozygosity facilitate the rapid spread of resistance alleles through asymmetric mating and stronger genetic drift. We therefore compared mitochondrial, microsatellite, and RNA-seq markers to assess their relative power in accurately capturing population genetic diversity.

Methods: DNA and mRNA were extracted from individual ticks collected from farms in three provinces of South Africa. The nuclear ITS2 and mitochondrial COI genes were sequenced and used for phylogenetic haplotype analyses, while six microsatellite markers were genotyped to assess population structure. mRNA was sequenced, and SNPs within exons of the octopamine/tyramine receptor genes were identified and analysed to evaluate resistance-associated genetic diversity across different tick life stages.

Results: ITS2 and COI sequences grouped *R. microplus* into three non-geographic clades, but provided limited resolution of population substructure. Microsatellite markers indicated a largely panmictic population with high homozygosity and low fixation, with limited geographic substructure. RNA-seq SNP data revealed life-stage specific expression patterns and polymorphic variants associated with amitraz resistance.

Conclusions: While ITS2, COI, and microsatellite analyses identified broad population subdivision, they lacked sufficient resolution to quantify population-level diversity. In contrast, SNP data linked exon-associated polymorphisms to acaricide resistance, and revealed the genotypic diversity in these genes. Future work will focus on transcriptome-wide SNPs to enable robust analyses of population structure and substructure.

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SHIFT02-031

POSTER ON BOARD SHIFT 02
RESISTANCE AND DEVELOPMENT OF DRUGS AND VACCINES / VACCINE DEVELOPMENT: CHALLENGES AND BREAKTHROUGHS

PROGRAMMING ANTIGEN-SPECIFIC IMMUNITY AGAINST TRYPANOSOMA CRUZI THROUGH THERAPEUTIC MRNA VACCINATION WITH TCJ2

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Background and Aims: Chagas disease, caused by *Trypanosoma cruzi,* remains a neglected tropical disease with limited and toxic therapeutic options beyond the acute phase. Messenger RNA (mRNA)-based vaccines represent a promising immunotherapeutic approach. This study evaluated the therapeutic effect of an mRNA vaccine encoding the *T. cruzi* antigen TcJ2 during acute experimental infection.

Methods: BALB/c mice were infected with *T. cruzi* and treated with an mRNA vaccine encoding TcJ2. Control groups included non-infected mice, infected untreated mice, and infected mice receiving lipid nanoparticle-formulated mRNA encoding an unrelated antigen (eGFP). Parasitemia and survival were monitored. Cardiac inflammation and fibrosis were assessed histologically. Cellular immune responses were analyzed by flow cytometry (CD4+, CD8+, IFN-gamma, perforin), and humoral responses were evaluated by indirect ELISA for total IgG.

Results: mRNA-TcJ2 vaccination did not significantly alter parasitemia kinetics or cardiac parasite load compared with infected controls. However, vaccination induced an antigen-specific immune response, with increased frequencies of IFN-gamma–producing CD8+ T cells compared with the mRNA-eGFP control, reaching levels comparable to natural infection, while perforin-expressing CD8+ T cells were not significantly increased. Notably, mRNA-TcJ2 elicited a strong TcJ2-specific IgG response, significantly higher than that observed in all control groups, including naturally infected mice.

Conclusions: Therapeutic vaccination with mRNA-TcJ2 induced a robust TcJ2-specific IgG response and an antigen-specific CD8+ IFN-gamma response without affecting parasitemia during the acute phase. These findings demonstrate the high immunogenicity of the mRNA-TcJ2 platform and support its further evaluation as a therapeutic strategy against *T. cruzi* infection.

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SHIFT02-032

POSTER ON BOARD SHIFT 02
RESISTANCE AND DEVELOPMENT OF DRUGS AND VACCINES / VACCINE DEVELOPMENT: CHALLENGES AND BREAKTHROUGHS

A NEXT-GENERATION MRNA VACCINE ENCODING TC24 INDUCES POTENT HUMORAL AND CD8-POSITIVE T-CELL RESPONSES DURING ACUTE TRYPANOSOMA CRUZI INFECTION.

Erika Arisai Alvarez Dominguez[1], Eilynn Alejandra Velazquez Alvarez[1], Victor Dzul-Huchim[1], Ana Castro-Díaz[1], Christian Teh Poot[1], Leroy Versteeg[2], Jeroen Pollet[2], Julio Cruz Chan[1], Liliana Villanueva-Lizama[1]
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Background and Aims: Chagas disease, caused by the protozoan *Trypanosoma cruzi*, remains a major public health problem in Latin America and is increasingly detected worldwide. Current drug treatments are most effective during the acute phase, while therapeutic options for chronic infection are limited and often associated with toxicity. This study aimed to evaluate the immunogenicity and protective effect of a therapeutic mRNA vaccine encoding the *T. cruzi* Tc24 antigen during acute infection in BALB/c mice.

Methods: The experimental group consisted of infected mice treated with a Tc24-encoding vaccine. Control groups included uninfected mice, untreated infected mice, and infected mice treated with an mRNA vaccine encoding eGFP. Parasitemia and survival were monitored. Cellular immune responses were analyzed by flow cytometry ($CD4^+$, $CD8^+$, IFN-γ, perforin). Humoral responses were evaluated by indirect ELISA for total IgG.

Results: Tc24 mRNA vaccination significantly reduced parasitemia at the peak of infection compared with untreated infected controls, although no significant differences in cardiac parasite burden were observed. Vaccinated mice exhibited increased frequencies of perforin-producing $CD8^+$ T cells, whereas the frequency of IFN-γ–producing T cells did not differ significantly among infected groups. A strong Tc24-specific IgG response was detected in vaccinated animals, with significantly higher antibody levels than in all control groups.

Conclusions: The Tc24 mRNA vaccine induced robust antigen-specific humoral responses and enhanced cytotoxic $CD8^+$ T-cell activity during acute *T. cruzi* infection. The immunogenic profile supports the potential of this mRNA platform as a therapeutic vaccination strategy and warrants further studies to optimize protective efficacy and long term protection.

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POSTER ON BOARD SHIFT 02
RESISTANCE AND DEVELOPMENT OF DRUGS AND VACCINES / VACCINE DEVELOPMENT: CHALLENGES AND BREAKTHROUGHS

COMPUTATIONAL EVALUATION OF ROP13 AS A VACCINE CANDIDATE AGAINST TOXOPLASMA GONDII

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Background and Aims: Toxoplasma gondii, an intracellular parasite, utilizes a variety of rhoptry proteins (ROPs) to facilitate invasion and interaction with host cells. Among these ROPs, Rhoptry Protein 13 (ROP13) stands out for its expression in both bradyzoite and tachyzoite forms of T. gondii and its ability to engage with various host cytoplasmic compartments.

Methods: In this bioinformatics study, we employed a range of tools to predict the fundamental characteristics of the ROP13 protein.

Results: Our analysis revealed that the ROP13 protein consists of 400 amino acid residues with an average molecular weight (MW) of 44,714.15 daltons. The grand average of hydropathicity (GRAVY) was determined to be -0.311, indicating the protein's hydrophilic nature, while the aliphatic index scored 84.40, highlighting its hydrophobic character. Furthermore, we identified 43 post-translationally modified sites within the ROP13 sequence. When examining the secondary structure, the ROP13 protein was predicted to have a composition of 40% alpha helix, 9.25% extended strand, and 50.75% random coil using the GOR4 method, suggesting a diverse structural organization that may contribute to its functional versatility. Additionally, our analysis identified several potential B- and T-cell epitopes within the ROP13 sequence, indicating regions that could be targeted for immune responses.

Conclusions: The bioinformatics analysis of ROP13 provides valuable insights into its structural, immunogenic, and antigenic properties, highlighting its potential as a target for vaccine development against toxoplasmosis. By leveraging the predicted characteristics of ROP13, researchers can explore various vaccine strategies to enhance host immunity and combat T. gondii infection effectively.

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SHIFT02-035

POSTER ON BOARD SHIFT 02
RESISTANCE AND DEVELOPMENT OF DRUGS AND VACCINES / DRUG RESISTANCE AND MOLECULAR BIOLOGY

IN VITRO SUSCEPTIBILITY OF PLASMODIUM FALCIPARUM KINSHASA CLINICAL ISOLATES TO ANTIMALARIAL DRUGS AND ASSOCIATED RESISTANCE GENES POLYMORPHISMS

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Background and Aims: Artemisinin derivatives remain the frontline treatment for malaria in Africa, while sulfadoxine–pyrimethamine (SP) is used for prevention. Although SP resistance has long been widespread, artemisinin resistance (ART-R) was historically absent. Recently, distinct Kelch 13 (K13) mutations such as A675V, C469Y, and R561H, different from the typical Southeast Asian mutations C580Y and R539T have emerged, suggesting local ART-R development. Integrating molecular surveillance with in vitro drug sensitivity testing is essential to guide malaria control strategies.

Methods: We analysed drug resistance genes and *in vitro* drug sensitivity profiles of *P. falciparum* isolates collected from malaria patients in Kinshasa, the Democratic Republic of the Congo (DRC). From 2022 to 2023, 303 patients diagnosed with *P. falciparum* malaria via rapid diagnostic tests (RDT) were enrolled.

Results: Blood samples were collected, and 177 malaria cases were confirmed by microscopy and analyzed by PCR amplification and sequencing of the K13 gene. One isolate carried the A578S substitution, previously shown not to confer artemisinin resistance (ART-R). Thirty-one *P. falciparum* isolates were adapted to in vitro culture for whole-genome sequencing. Key resistance loci (K13, *dhps*, *dhfr*, and K13-interacting genes) were screened for SNPs, and 20 isolates underwent drug susceptibility testing. All showed pyrimethamine resistance. The A578S isolate was sensitive to DHA in the Ring-stage Survival Assay (RSA), while five isolates exhibited RSA survival rates >1%, suggesting partial DHA tolerance.

Conclusions: These results suggest early signs of emerging artemisinin resistance in the DRC and highlight the importance of integrated surveillance to monitor resistance spread and inform timely updates to national treatment guidelines.

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SHIFT02-036

POSTER ON BOARD SHIFT 02
RESISTANCE AND DEVELOPMENT OF DRUGS AND VACCINES / DRUG RESISTANCE AND MOLECULAR BIOLOGY

FIRST SUSPICION OF PYRANTEL RESISTANCE IN PARASCARIS SPP. IN FRENCH FOALS

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Background and Aims: *Parascaris* spp. are common and pathogenic parasites in foals. Repeated use of the four available anthelmintic (AH) molecules has led to resistance. In France, studies from 2012–2013 reported resistance to ivermectin, but no data exist for other molecules. This study evaluated in 2025 the efficacy of AHs against *Parascaris* spp. in horses ≤2 years old.

Methods: In each group, individual fecal egg counts (FEC) were performed before treatment (D0) and 14 days post-treatment (D14). A FEC reduction test (FECRT) was performed following the World Association for the Advancement of Veterinary Parasitology (WAAVP) recommendations. If the FEC at D0 was sufficient to have a sample size large enough to achieve a statistical power ≥80%, the upper limit (UL) of the 90% confidence interval (CI) of the mean FECR was calculated and compared with the WAAVP efficacy threshold (99.9%). Otherwise, the percentage of shedders on D0 was compared with that on D14.

Results: Eleven groups from ten farms were recruited (72 horses). No resistance to fenbendazole was detected in 3/3 groups, while resistance to pyrantel was observed in 1/2 groups (UL[CI90%] = 99,1%) and to ivermectin in 1/1 group (UL[CI90%] = 81,8%). Semi-quantitatively, pyrantel resistance was suspected in 1/1 group (100% of shedders on D14), moxidectin resistance in 1/3 groups (20% of shedders on D14) and no resistance to fenbendazole in 1/1 group.

Conclusions: This study highlighted the first report of pyrantel's failure to control *Parascaris* spp. Besides, the persistence of eggs after a treatment with moxidectin underlines the importance of monitoring this molecule.

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SHIFT02-037

POSTER ON BOARD SHIFT 02
RESISTANCE AND DEVELOPMENT OF DRUGS AND VACCINES / DRUG RESISTANCE AND MOLECULAR BIOLOGY

DIFFERENTIAL DHA SUSCEPTIBILITY OF P. FALCIPARUM PARASITES GROWN UNDER STATIC AND SUSPENSION CULTURE CONDITIONS

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Background and Aims: The *in vitro* cultivation of *Plasmodium falciparum* is a cornerstone of malaria research, yet the impact of different microenvironments, especially static versus suspension cultures, on drug susceptibility remains poorly understood. This study investigates how suspension culture conditions influence the response of *P. falciparum* to dihydroartemisinin (DHA) and explores the potential epigenetic mechanisms driving these shifts.

Methods: We first optimized a Chromatin Immunoprecipitation Sequencing (ChIP-Seq) protocol for *P. falciparum*, achieving precise DNA fragmentation (150–500 bp) and robust enrichment of histone marks. Using this optimized protocol, we generated high-quality libraries for epigenetic profiling. Simultaneously, we assessed the drug susceptibility of 3D7, Dd2, and W2mef strains under varying culture conditions using *in vitro* growth inhibition and Ring-stage Survival Assays (RSA).

Results: Our results demonstrate that suspension culture significantly alters DHA susceptibility in a strain-specific and time-dependent manner. Initially, 3D7 exhibited increased survival in suspension, whereas Dd2 and W2mef showed decreased survival. By week six, W2mef survival increased, while the survival of 3D7 and Dd2 declined. Notably, returning the Dd2 parasites to static conditions did not fully reverse these phenotypic changes, suggesting the induction of lasting alterations.

Conclusions: The optimized ChIP-Seq protocol is currently being utilized to map the epigenetic landscapes associated with these differential responses. This research provides critical insights into how environmental stress in culture may promote artemisinin resistance. Understanding these mechanisms is essential for informing public health interventions and preventing the spread of resistant malaria across Africa.

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SHIFT02-038

POSTER ON BOARD SHIFT 02
RESISTANCE AND DEVELOPMENT OF DRUGS AND VACCINES / DRUG RESISTANCE AND MOLECULAR BIOLOGY

BEYOND ASEXUAL GROWTH AND ARTEMISININ RESISTANCE: INVESTIGATING KELCH-13 FUNCTION DURING SEXUAL AND MOSQUITO STAGES IN PLASMODIUM FALCIPARUM ** AUTHOR REQUEST

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Background and Aims: *Plasmodium falciparum* has a complex life cycle with stages in both human and *Anopheles* mosquito hosts. Primary treatment for malaria depends on artemisinin-based combination therapies, however, the emergence of artemisinin resistance threatens the control of this disease. The most prevalent mutations associated with artemisinin resistance in the field are encoded within the parasites' *Kelch13* (K13) gene. During asexual development, K13 protein plays an important role in the formation, stability and function of a double-membraned invagination called the cytostome, whose role is to facilitate uptake of haemoglobin from the host red blood cell into the parasite. Whilst K13 and it's role in haemoglobin uptake is well studied during asexual development, it's role during sexual blood, mosquito and liver stages of development are yet to be investigated.

Methods: Fluorescence, super resolution and lattice light-sheet microscopy were used to observe the location of K13-GFP across gametocytogenesis and mosquito stages of development. We used a knock-sideways approach to investigate the biological consequences of K13 mislocalisation across these stages.

Results: K13-GFP displays a dynamic localisation pattern throughout gametocytogenesis, with several K13-GFP puncta often converging to form a unique rod-shaped structure in late-stage gametocytes. These K13-GFP puncta/rods are not packaged into male microgametes, but remain present in activated female gametes. Knock-sideways studies revealed that K13 is essential during early gametocyte development, but appears to be dispensible beyond stage III.

Conclusions: This work helps to elucidate the biology of cytostomes and determine, for the first time, whether they serve a function other than haemoglobin uptake during other life cycle stages.

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SHIFT02-039

POSTER ON BOARD SHIFT 02
RESISTANCE AND DEVELOPMENT OF DRUGS AND VACCINES / DRUG RESISTANCE AND MOLECULAR BIOLOGY

A DOUBLE THREAT TO MALARIA ELIMINATION: HRP2/3 DIAGNOSTIC ESCAPE & KELCH13-INTERACTOME MEDIATED ARTEMISININ RESISTANCE

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Background and Aims: Artemisinin-based combination therapies (ACTs) are the frontline treatment for *Plasmodium falciparum* globally. Artemisinin resistance, associated with *Pfkelch13* mutations, disrupts haemoglobin uptake. Genetic deletions in histidine-rich protein 2/3 (*hrp2* /3) loci cause false negatives, compromising RDT reliability, which leads to misdiagnosis. This prompt WHO to recommend revise ongoing monitoring if it exceed 5%. The study compares RDTs, microscopy, and PCR to assess *Pfhrp2*/3 gene deletions in *RDT-negative and microscopically positive P. falciparum* samples and investigates the prevalence of *Pfkelch13* mutations, identify associated interactomes and co-existing genetic loci contributing to artemisinin resistance to understand the *hrp2/3* deletion and *K13* mutation of dynamics in India.

Methods: Based on microscopy & RDT results dried blood samples were amplified targeting 18S rRNA, population genetic marker merozoite surface protein 1/2 (*Pfmsp1/2*). After genotyping and DNA quality assessment, *Pfkelch13* propeller fragment was amplified and sequenced. To confirm and characterize sub telomeric deletions, amplification of *exon1-2,* upstream and downstream flanking genes of *Pfhrp2/3* were done. Protein-protein interaction networks were built using STRING matrix. Whole- genome and transcriptomic datasets were analysed, with functional clustering via k-means.

Results: The deletion rate of *hrp2/3* in various endemic areas of India ranged from 0.02% to 0.09%. DNA alignment revealed nonsynonymous *Pfkelch13* mutations, especially in the Northeast, indicating emerging multidrug resistance. Epistatic networks disrupted PfK13 ubiquitination, elevating PI3K/PI3P signalling and artemisinin survival.

Conclusions: This study reveals low-level Pfhrp2/3 deletions and multigenic Pfkelch13-mediated resistance in India, highlighting the need for improved diagnostics, effective RDTs, extended ACTs, long-acting therapies, and strengthened malaria surveillance.

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SHIFT02-040

POSTER ON BOARD SHIFT 02
RESISTANCE AND DEVELOPMENT OF DRUGS AND VACCINES / DRUG RESISTANCE AND MOLECULAR BIOLOGY

CHALLENGES IN APPLYING W.A.A.V.P. CRITERIA TO DIAGNOSING TRICLABENDAZOLE RESISTANCE IN FASCIOLA HEPATICA, AN EXAMPLE FROM THE SOUTHERN TABLELANDS OF NEW SOUTH WALES, AUTRALIA.

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Background and Aims: *Fasciola hepatica* (liver fluke) is a zoonotic parasite of global concern. In Australia, it is the 13th most important cause of economic loss in the sheep meat industry alone. Resistance to triclabendazole (TCBZ), was first recorded in Australia in 1995 and has since emerged globally. In 2023, producers from the New South Wales (NSW) Southern Tablelands raised concerns over a reported 230% increase in liver fluke, which they suspected was due to drug resistance.

Methods: We co-designed a diagnostic field investigation aligned with guidelines from the World Association for the Advancement of Veterinary Parasitology (W.A.A.V.P.) to evaluate the prevalence and susceptibility of *F. hepatica* on naturally infected sheep, cattle, and goat properties. Nine mobs (seven sheep, one goat, one cattle) across eight farms were split into three treatment groups (15 animals/group) and treated with either TCBZ, closantel/abamectin (CLOS/AVM, positive control – sheep), albendazole (ABZ, positive control – goats), or water (H_2O; negative control). Prevalence was determined by sedimentation and faecal egg count (FEC), alongside a commercial coproantigen ELISA (cELISA) and in-house qPCR. Drug efficacy was assessed using faecal egg count reduction tests (FECRT) and coproantigen reduction tests (CRT).

Results: TCBZ resistance was confirmed on one sheep property (86–89% efficacy). The goat property demonstrated susceptibility to TCBZ (97–98% efficacy), but reduced efficacy of ABZ (79%), representing the first potential report of ABZ resistance in *F. hepatica* infecting goats.

Conclusions: These results highlight the complexity of diagnosing and managing drug resistance in naturally infected populations and reinforce the need for *Fasciola*-specific W.A.A.V.P. guidelines.

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SHIFT02-041

POSTER ON BOARD SHIFT 02
RESISTANCE AND DEVELOPMENT OF DRUGS AND VACCINES / DRUG RESISTANCE AND MOLECULAR BIOLOGY

LOSS-OF-FUNCTION MUTATIONS IN PUTATIVE CHLORIDE PUMPS KCC-2 AND ABTS-1 CONFER IVERMECTIN RESISTANCE IN THE MODEL NEMATODE CAENORHABDITIS ELEGANS.

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Background and Aims: Ivermectin has been a foundational de-worming drug for decades, yet its efficacy is increasingly compromised by the evolution of resistance in treated parasite populations. An understanding of the mechanisms of resistance would allow us to better track the spread of resistance and use available drug classes more effectively. Because molecular genetics in nematode parasites is challenging, we study the model free-living roundworm *C. elegans*.

Methods: Ivermectin activates neurotransmitter receptor glutamate-gated chloride channels (GluCls), which suppresses neuronal and muscle activity. In C. elegans two GluCls, AVR-14 and AVR-15, each confer sensitivity to ivermectin and a double knock-out mutant is highly resistant. However, mutations in parasite GluCls do not appear to contribute to resistance. Therefore, we performed a mutagenesis screen to identify novel resistance genes that act in the AVR-14 or AVR-15 sensitivity pathways.

Results: We found alleles of two genes predicted to affect chloride transport, *kcc-2* and *abts-1*, that act genetically in the AVR-14 pathway to confer over 50-fold ivermectin resistance. No alleles affected the AVR-15 pathway. Alleles are recessive missense mutations except *kcc-2(vu315)*, which is a dominant nonsense allele. Absent ivermectin, recessive single and double *abts-1; kcc-2* mutants have mildly uncoordinated locomotion, whereas a double mutant of the dominant allele with *abts-1* results in severe developmental/locomotion phenotypes. Compromised chloride transport could confer resistance by reducing neuronal inhibition, increasing excitatory tone. Post-synaptic potentials of the M3 motor neuron showed an increase in neuronal activity, consistent with increased excitation.

Conclusions: We propose that *kcc-2* and *abts-1* are candidate ivermectin resistance genes in nematode parasites.

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SHIFT02-042

POSTER ON BOARD SHIFT 02
RESISTANCE AND DEVELOPMENT OF DRUGS AND VACCINES / DRUG RESISTANCE AND MOLECULAR BIOLOGY

MOLECULAR SURVEILLANCE OF CANINE HOOKWORMS AND BENZIMIDAZOLE RESISTANCE IN AUSTRALIAN DOG PARK SAMPLES

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Background and Aims: The detection of benzimidazole resistance mutations in *Ancylostoma caninum* from racing greyhounds, and pet dog cases, suggests anthelmintic resistance is emerging in Australia. However, its prevalence and distribution in pet dogs is unclear. Consequently, we have surveyed canine fecal samples from several Australian dog parks for benzimidazole resistance single nucleotide polymorphisms (SNPs) in this parasite.

Methods: The KeyScreen™ GI Parasite PCR Test as well as ITS2 and iso-1 b-tubulin Illumina deep amplicon sequencing (scanning codons 134 and 167 and codons 198 and 200) was applied, in duplicate, to 113 environmental fecal samples from Australian dog parks.

Results: 87 samples met minimum read-depth thresholds for downstream analyses. ITS2 profiling identified *A. caninum* mono-infections in all these samples except for two, one with *Uncinaria stenocephala* and one with *Ancylostoma tubaeforme*. PCR amplification of mammalian cytochrome B detected canine DNA in the latter sample suggested this was due to coprophagic behaviour and not parasitism. Macropod strongylid and free-living rhabditid DNA was also detected in some samples which again likely reflects environmental contamination through coprophagia. No benzimidazole resistance SNPs were detected at codons 134, 167 , 198 and 200 in any sample by either Illumina sequencing or the KeyScreen assay.

Conclusions: This work suggests that benzimidazole resistance may still be relatively uncommon in the Australian resident pet dogs population but more surveillance across broader host populations and regions will be important to further understand the current situation.

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SHIFT02-043

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RESISTANCE AND DEVELOPMENT OF DRUGS AND VACCINES / DRUG RESISTANCE AND MOLECULAR BIOLOGY

GLOBAL MOLECULAR EPIDEMIOLOGY OF BENZIMIDAZOLE RESISTANCE IN THE CANINE HOOKWORM ANCYLOSTOMA CANINUM

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Background and Aims: Anthelmintic resistance in the canine hookworm *Ancylostoma caninum* is an emerging concern. Benzimidazole resistance Single Nucleotide Polymorphisms (SNPs) at codons 134 and 167 Isotype-1 b-tubulin are widespread in North America and recently reported, predominantly in Greyhounds, in Australia. However, their global distribution is unknown . We are undertaking a global survey of *A. caninum* BZ resistance mutations and resistance alleles to understand their distribution and infer patterns of spread.

Methods: Fecal DNA has been prepared from more than 2,000 hookworm-positive fecal samples from 16 countries spanning North America, South America, Europe, Africa, Asia, and Oceania for screening by the KeyScreen™ GI Parasite PCR panel, Illumina short read, and Oxford Nanopore long-read deep amplicon sequencing on the near full-length b-tubulin sequences for novel variants and investigate patterns of haplotype distribution.

Results: We have started with a set of samples across Canada and Australia. ITS2 profiling identified *A. caninum* in almost all typed samples. No canonical benzimidazole resistance SNPs were detected in any samples from Australian dog parks, by Illumina sequencing or KeyScreen. Both Q134H and F167Y were detected in the Canadian samples at a prevalence and overall frequency of 16% and 18.1% respectively which is lower than USA.

Conclusions: The profile of resistant alleles in Canada is similar to the USA as expected from the geographical proximity and connectedness of their domestic canine populations. Data will be presented from the additional countries currently being analyzed to produce a global map of resistance alleles to inform diagnostics and resistance management, and ongoing baseline monitoring.

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SHIFT02-044

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RESISTANCE AND DEVELOPMENT OF DRUGS AND VACCINES / DRUG RESISTANCE AND MOLECULAR BIOLOGY

REVEALING THE PERSISTENCE OF LEISHMANIA INFANTUM IN THE HOST: A PHOTOCONVERTIBLE BIOSENSOR TO TRACK REPLICATION AND QUIESCENCE

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Background and Aims: The persistence of *Leishmania infantum* within the host poses a major challenge to disease control, as parasites survive by dynamically modulating replication and entering quiescence states. However, tools capable of monitoring these processes with high temporal resolution remain scarce. This study aimed to develop a photoconvertible biosensor to monitor parasite replication, persistence, and quiescence, including across drug-sensitive and drug-resistant lines.

Methods: We engineered *L. infantum* lines expressing the photoconvertible fluorescent protein mKikGR (Kikume), stably integrated into the ribosomal SSU *locus*. Upon photoconversion at 405 nm, green fluorescence irreversibly shifts to red, enabling dilution of the red signal and re-emergence of green fluorescence to serve as quantitative readouts of cell proliferation. From drug-sensitive parasites, we selected lines resistant to trivalent antimony (SbIII), miltefosine (MTF), and amphotericin B (AmB). Parasite fluorescence, viability, and growth were assessed *in vitro*. Intracellular dynamics were examined using murine bone marrow-derived macrophages (BMDMs) infected with photoconverted or non-photoconverted parasites and monitored for up to 72 h.

Results: All parasite lines exhibited strong and stable Kikume expression, with a highly linear correlation between parasite number and green/red fluorescence intensities ($R^2 > 0.99$). Photoconversion did not impair parasite viability, growth, or infectivity, thereby enabling longitudinal tracking. Single-cell analysis of infected macrophages revealed heterogeneous replication behaviors, allowing quantitative estimation of infectivity, intracellular replication, persistence, and potential entry into quiescence.

Conclusions: Collectively, this photoconvertible biosensor constitutes a robust and sensitive platform for real-time analysis of *L. infantum* replication and persistence, and provides a powerful framework for comparing drug-sensitive and drug-resistant phenotypes within host cells.

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SHIFT02-045

POSTER ON BOARD SHIFT 02
RESISTANCE AND DEVELOPMENT OF DRUGS AND VACCINES / DRUG RESISTANCE AND MOLECULAR BIOLOGY

"HISTATIN 5 : A POTENTIAL PEPTIDE IN THE FIGHT AGAINST CHAGAS DISEASE."

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Background and Aims: Chagas disease is a neglected tropical disease that affect millions of people around the world., 25 million people worldwide are at risk of Chagas disease. Antimicrobial peptides represent a possible solution to this challenge. Peptide-based drugs are attractive due to their high selectiveness, effectiveness, low toxicity, and ease of production. Several studies have demonstrated that peptides are effective against Chagas . The objective of this study was to evaluate the antimicrobial activity of histatin 5 and its effect on nitric oxide (NO) release of reactive oxygen species (ROS) production, and programmed cell death in human macrophages infected with *Trypanosoma cruzi* epimastigotes alternative.

Methods: Damage in macrophages infected with *T.cruzi* epimastigotes and stimulated with histatin 5 (61.5%) for 72h were determined by transmission electron microscopy (TEM). Additionally, nitrites and reactive oxygen species (ROS) production were evaluated. The production of nitrites and ROS was analyzed by Griess reaction and incubation with 2',7'-dichlorodihydrofluorescein diacetate (H_2DCFDA), respectively. Programmed cell death was assessed with the TUNEL assay and with Annexin-V.

Results: Histatin 5 induced extensive damage in *T.cruzi* epimastigotes and increase the ROS production and reduces NO.

Conclusions: Histatin 5 exhibits an important antimicrobial activity with *T.cruz*i epimastigotes .

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SHIFT02-047

POSTER ON BOARD SHIFT 02
RESISTANCE AND DEVELOPMENT OF DRUGS AND VACCINES / DRUG RESISTANCE AND MOLECULAR BIOLOGY

EFFICACY OF THREE ARTEMISININ-BASED COMBINATIONS THERAPIES FOR THE TREATMENT OF UNCOMPLICATED MALARIA IN CHILDREN IN BURKINA FASO : 2/3 AND 3/3 PCR-CORRECTED RESULTS

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Background and Aims: Burkina Faso has conducted regular therapeutic efficacy monitoring of artemisinin-based combination therapies (ACTs) since their introduction in the early 2000s. Since 2008, artemether-lumefantrine (AL), the most widely used ACT, has shown reduced efficacy in Burkina Faso. To inform national malaria programme, we conducted a therapeutic efficacy study in 2020-2021.

Methods: Children aged 6 months to 12 years with uncomplicated *Plasmodium falciparum* malaria were enrolled in three sites, treated with either AL, dihydroartemisinin-piperaquine (DP), or artesunate-pyronaridine (AS-PYR), and monitored for 28 or 42 days. Dried blood spots were collected to determine recrudescence from new infection in late treatment failures and presence of known molecular resistance markers. Samples collected on day-7 in the AL arm were analyzed for blood lumefantrine concentrations.

Results: A total of 1,078 participants were enroled. Uncorrected cumulative 28-day efficacy ranged from 57.1% (95% confidence intervals (CI) 49.4-64.0) to 64.4% (95% CI 56.8-71.0) for AL, from 96.6% (95% CI 89.8-98.9) to 98.9% (95% CI 92.2-99.8) for DP, and from 82.2% (95% CI 72.2-88.9) to 90.7% (95% CI 82.3-95.2) for AS-PYR. Using WHO 3/3 method, PCR-corrected efficacy was above 90% for all drugs and sites except for AL in Nanoro where the efficacy was 89.4% (95% CI 82.2-94.4). No molecular markers associated with artemisinin resistance were found and there was no statistically significant association between treatment failure and sub-therapeutic blood lumefantrine levels.

Conclusions: There is evidence of continued poor suitability of the use of AL in Burkina Faso given the low uncorrected efficacy in a highly endemic setting with high multiplicity of infection.

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SHIFT02-048

POSTER ON BOARD SHIFT 02
RESISTANCE AND DEVELOPMENT OF DRUGS AND VACCINES / DRUG RESISTANCE AND MOLECULAR BIOLOGY

A TRANSCRIPTOMIC APPROACH TO UNDERSTANDING MACROCYCLIC LACTONE RESISTANCE IN THE SHEEP SCAB MITE, PSOROPTES OVIS

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Background and Aims: Sheep scab, caused by the ectoparasitic mite *Psoroptes ovis*, is a major welfare problem in sheep with infested animals suffering from intense pruritus and wool loss. In the UK alone, sheep scab causes £80-£200 million economic losses annually. Resistance to macrocyclic lactones (MLs), one of only two available treatments, is now well reported and threatens effective control. Therefore, this study aimed to identify potential mechanisms of ML resistance using transcriptomic methods to evaluate changes in constitutive and inducible gene expression between susceptible and resistant *P. ovis*.

Methods: To evaluate *in vivo* expression, six sheep were infested with ML susceptible (ML-SUSC) and twelve sheep with ML resistant (ML-R) *P. ovis*. Seven days antemortem a half therapeutic dose of moxidectin was administered to six ML-R animals, and mites retained for RNAseq analysis. To evaluate *in vitro* expression, six replicates of 200 mites per isolate (ML-R & ML-SUSC) were exposed to an EC_{99} (for ML-SUSC) of moxidectin *in vitro*. After 48 hours, surviving mites were retained for RNAseq analysis.

Results: Gene expression data revealed significant over-expression, both *in vivo* and *in vitro,* of a novel fusion UDP-glucuronosyltransferase gene (log2foldchange=6.19-6.33, $p<0.0001$) which was the most highly upregulated transcript in all resistant populations. The ML-R populations also observed significant over-expression of carboxylesterases *in vivo* ($p<0.001$), with some constitutive over-expression of cuticular genes *in vitro* ($p<0.001$).

Conclusions: These findings highlight key molecular changes associated with ML resistance and will support improved sheep scab management, including future diagnostic tools to detect and monitor resistance.

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SHIFT02-049

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RESISTANCE AND DEVELOPMENT OF DRUGS AND VACCINES / DRUG RESISTANCE AND MOLECULAR BIOLOGY

COPROLOGICAL AND MOLECULAR ANALYSES OF RUMINANT FARMS IN QC, CANADA, SHOW A VARIABLE EFFICACY OF IVERMECTIN AGAINST GASTRO-INTESTINAL NEMATODES

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Background and Aims: Gastro-intestinal nematodes (GINs) are of greater concern in grazing ruminants, such as camelids, ovines and caprines, affecting animal health and productivity. The development of anthelmintic resistance (AR) to the compounds used to control GINs has not been recently evaluated in Québec, including ivermectin (IVM), the most used anthelmintic drug in livestock. The aims of this study were to determine the efficacy of IVM and identify which GIN species are affecting different ruminant farms in QC, Canada.

Methods: We collected fecal samples from six farms with different ruminant species(camelids, goats and sheep) before and after IVM treatment, analyzing them by Fecal Egg Count (FEC) and further assessments on IVM efficacy through the Fecal Egg Count Reduction Test (FECRT). In addition, molecular analyses were conducted using PCR, targeting the ITS-2 and COX-1 genes to identify GIN species.

Results: FECRT was applied only in three farms, showing a variable results with optimal IVM efficacy in only one farm, whereas on the other two farms, FECRT exhibited reduced efficacy, suggesting the development of IVM resistance. 13 GIN species were found, comprising Haemonchus contortus and Trichostrongylus vitrinus as the most prevalent GIN species across ruminant farms.

Conclusions: Our work reports evidence of an uneven efficacy of IVM against GINs including the most likely emergence of IVM resistance, along with the diversity of GIN species found in ruminant farms. This is helpful information for veterinarians and animal producers in setting an optimal parasite management program, including the proper use of IVM and alternative anthelmintic drugs and methods to control these pathogens in grazing livestock.

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SHIFT02-050

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RESISTANCE AND DEVELOPMENT OF DRUGS AND VACCINES / DRUG RESISTANCE AND MOLECULAR BIOLOGY

COMPARING THE EFFICACY OF BENZIMIDAZOLES AGAINST ANCYLOSTOMA CANINUM IN NATIVE AND IMPORTED DOGS IN QUEBEC, CANADA, REVEALS THE PRESENCE OF SENSITIVE AND RESISTANT HOOKWORM ISOLATES.

Pablo Godoy
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Background and Aims: Anthelmintic resistance (AR) in the dog hookworm *Ancylostoma caninum* has emerged in the last years, spreading globally. We aimed to evaluate the efficacy of the benzimidazoles against *A. caninum* isolates from native dogs and adopted dogs, coupled with genetic analyses on the β-tubulin isotype-1 gene linked to BZ resistance in *A. caninum*.

Methods: We included 52 native dogs and 3 imported greyhounds from the US with persistent hookworm-like ova releasing. Native dogs were treated with fenbendazole, and imported dogs with febantel plus pyrantel. Furthermore, the Mini-Flotac method was used for fecal egg counts, applying the fecal egg count reduction test (FECRT). Nematode eggs were used for DNA extraction and PCR to identify hookworm species and SNPs on the β-tubulin isotype-1 gene

Results: *A. caninum* was found in 30.7% (15/52) of native dogs and 100% (3/3) of the imported dogs. The FECRT reported a variable fenbendazole efficacy, with most of the native dogs showing a full efficiency. In the imported dogs, the efficacy of febantel and pyrantel was variable with good and low efficacies, indicating one *A. caninum* isolate resistant to BZs. Further genetic analyses demonstrated that *A. caninum* isolates from native dogs carried no SNPs (167, 200 codons) at the β-tubulin isotype one gene, whereas one *A. caninum* isolate from the imported dog was positive for the SNP at position 167.

Conclusions: We found variable efficacies of two benzimidazoles against *A. caninum* from different canine origins, in line with the reports of AR in *A. caninum* in North America. This is important for veterinarians in the control of hookworms in companion animals through anthelmintic drugs.

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SHIFT02-051

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RESISTANCE AND DEVELOPMENT OF DRUGS AND VACCINES / DRUG RESISTANCE AND MOLECULAR BIOLOGY

FIELD EVIDENCE OF MULTIPLE ANTHELMINTIC RESISTANCE IN GASTROINTESTINAL NEMATODES OF SMALL RUMINANTS WITH COMPARATIVE EVALUATION OF MOLECULAR DIAGNOSTIC TOOLS FOR BENZIMIDAZOLE RESISTANCE IN HAEMONCHUS CONTORTUS

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Background and Aims: Anthelmintic resistance in gastrointestinal nematodes (GINs) poses a significant threat to the health and productivity of livestock worldwide. This study assessed resistance against three major anthelmintic classes; benzimidazoles (BZ), macrocyclic lactones, and salicylanilides with emphasis on molecular diagnostic approaches for detecting benzimidazole raesistnce in *Haemonchus contortus*.

Methods: A four groups, 10 goats each, with GINs infection (>150 EPG) were selected for FECRT. Three molecular diagnostic approaches; Allele-specific PCR (AS-PCR), restriction fragment length polymorphism PCR (RFLP-PCR), and direct sequencing were compared for their sensitivity and practical applicability in identifying the F200Y mutation of the β-tubulin isotype 1 gene associated with BZ resistance in *H. contortus*.

Results: The FECRT results demonstrated widespread multiple anthelmintic resistance, with fecal egg reductions fell below the 95% efficacy threshold to only 69.93%, 75.38%, and 79.32% following treatment with albendazole, ivermectin, and closantel, respectively. Post-treatment larval cultures confirmed *H. contortus* as the predominant surviving species across all drug treatments and the primary resistant parasite. Molecular analysis revealed a high prevalence of BZ-resistant alleles, with 47% detected by AS-PCR and 48% by RFLP-PCR. Importantly, while AS-PCR detected heterozygous resistant genotypes in only two of three samples tested, both RFLP-PCR and direct sequencing successfully identified heterozygosity across all samples, indicating superior sensitivity for detecting mixed populations.

Conclusions: This study provides field evidence of multiple anthelmintic resistance in small ruminant GINs, predominantly attributed to *H. contortus*. The findings demonstrate that RFLP-PCR offers a reliable molecular diagnostic tool for BZ resistance, making it particularly suitable compared to AS-PCR and direct sequencing approaches in low-resource setting.

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RESISTANCE AND DEVELOPMENT OF DRUGS AND VACCINES / DRUG RESISTANCE AND MOLECULAR BIOLOGY

UNLOCKING THE REGULATORS OF ANTHELMINTIC RESISTANCE IN HAEMONCHUS CONTORTUS

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Background and Aims: Drug resistance is a major cause of ineffective pharmacotherapy across many diseases, including helminthiases. In resistant helminth strains, enhanced drug deactivation and efflux driven by the induced expression of drug-metabolising enzymes (DMEs) have been repeatedly observed. However, the molecular mechanisms underlying DME up-regulation remain poorly understood. We hypothesise that DME expression is regulated at the transcriptional level by specific transcription factors (TFs) and is further modulated by epigenetic mechanisms, particularly DNA methylation.

Methods: This project focuses on selected UDP-glycosyltransferases and P-glycoproteins in *Haemonchus contortus*, a globally important haematophagous gastrointestinal parasite of small ruminants. One anthelmintic-sensitive strain (ISE) and one resistant strain (IRE) were used. To enrich for relevant regulatory proteins, albendazole-treated worms were homogenised, and nuclear proteins were isolated by differential centrifugation. Cytoplasmic and nuclear fractions were subsequently analysed by tandem mass spectrometry. In parallel, the contribution of epigenetic regulation was investigated using nanopore sequencing.

Results: We present preliminary mass spectrometry data identifying proteins in each fraction with a particular focus on transcription factors and nuclear hormone receptors that may interact with albendazole and regulate DME expression. Although DNA methylation is considered rare or absent in many nematodes, nanopore sequencing data from the IRE strain indicate the presence of modified bases (6mA and 5mC) across nuclear chromosomes, the mitochondrial genome, and coding sequences.

Conclusions: Ongoing work will extend this analysis to identify DNA methylation patterns associated with anthelmintic resistance. This study is supported by the Czech Science Foundation (GA25-16530S).

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SHIFT02-053

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RESISTANCE AND DEVELOPMENT OF DRUGS AND VACCINES / DRUG RESISTANCE AND MOLECULAR BIOLOGY

TRADITIONAL TRANSHUMANT SHEEP FARMING SYSTEMS ARE NOW FACING MULTI-RESISTANCE TO ANTHELMINTICS IN THE FRENCH PYRÉNÉES MOUNTAINS.

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Background and Aims: The frequency of anthelmintic treatments is considered to be the main risk factor for multi-resistance in sheep farming. Therefore, transhumant mountain farming systems, which use few treatments per year, should be less affected. However, in 2023, a preliminary study showed a decrease in the effectiveness of ivermectin in five farms located in the French Pyrenees. For this reason, a study was conducted in 2024 to evaluate the effectiveness of fenbendazole, levamisole, ivermectin, moxidectin, closantel and monepantel in 36 farms practising transhumance, distributed across seven summer pastures in the Hautes-Pyrénées (south-western France).

Methods: Fecal egg count reduction tests were carried out in the farms to evaluate the efficacy of anthelmintics. Faecal cultures were performed to identify the species of nematodes that had resisted the different treatments.

Results: Fenbendazole has never shown efficacy. Out of 36 farms, ivermectin remained effective on seven farms and moxidectin on 12 only. Levamisole remained effective on 25 / 32 tested farms. These resistances were mainly due to *Haemonchus contortus* and *Teladorsagia circumcincta* species. No resistance has been reported for monepantel, and closantel was effective everywhere on *Haemonchus contortus* species. Results vary sometimes between farms of the same summer pasture, depending on the degree of mixing of herds during summertime.

Conclusions: This study has shown that multi-drug resistance is no longer confined to intensive sheep farming in lowland areas, but that traditional systems in mountains are now affected. The role of summer pastures in the transmission of resistant parasites between sheep flocks deserves further study.

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RESISTANCE AND DEVELOPMENT OF DRUGS AND VACCINES / DRUG RESISTANCE AND MOLECULAR BIOLOGY

BENZIMIDAZOLE RESISTANCE IN CATTLE NEMATODES: REDUCED EFFICACY AGAINST COOPERIA PUNTACTA CARRYING SUSCEPTIBLE ALLELES

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Background and Aims: The convergence of our understanding of the molecular mechanisms underlying anthelmintic resistance with advances in sequencing technologies has enabled the development of molecular approaches for resistance diagnosis.

Methods: The aim of this study was to evaluate the benzimidazole (BZD) resistance status of gastrointestinal nematode (GIN) species infecting cattle across six large scale commercial farms in Argentina, using ITS-2 and β-tubulin isotype-1 genes deep amplicon sequencing

Results: Seven GIN species were identified: *H. placei (64.1%), C. punctata (26.6%), O. radiatum (3.6%), O. ostertagi (3.5%), H. contortus (1.1%), C. oncophora (0.9%) and T. axei (0.2%)*. Screening for SNPs identified 4 BZD-resistant polymorphisms: F167Y, E198A, E198L and F200Y. *C. punctata*, *C. oncophora*, *H. contortus*, and *O. ostertagi* harbored one or more of BZD resistant alleles, whereas *H. placei* and *T. axei* have only susceptible alleles. Treatment failures after BZD administration were mainly associated with *C. punctata* and, to a lesser extent, *O. ostertagi*. Surprisingly, *C. punctata* survivor specimens frequently carried susceptible alleles, suggesting additional factors—such as other mechanisms of resistance, anatomical niche or a drug pharmacokinetic-based limitation— may be involved in the efficacy failure where BZD susceptible alleles were predominant.

Conclusions: While this work focuses on cattle nematode parasites, the emergence of anthelmintic resistance across a wide range of host species underscores the broader relevance of this problem. Moreover, the increasing risk of drug resistance in soil-transmitted helminth parasites in humans highlights the urgent need for further work on molecular surveillance for early detection of resistance.

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POSTER ON BOARD SHIFT 02
RESISTANCE AND DEVELOPMENT OF DRUGS AND VACCINES / DRUG RESISTANCE AND MOLECULAR BIOLOGY

SCREENING OF PEPTIDE-BASED INHIBITORS AGAINST TRYPANOSOMA CRUZI FOR CHAGAS CARDIOMYOPATHY: ADVANCES IN DRUG DISCOVERY AND CHARACTERIZATION OF NOVEL DRUGGABLE TARGETS

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Background and Aims: Chagas disease is a debilitating chronic illness caused by the protozoan parasite Trypanosoma cruzi. It affects an estimated 6-7 million people mainly in developing countries and causes around 12,000 deaths every year. Only two drug treatments are available: either Nifurtimox or Benznidazole, with variable efficacy.
In a collaborative effort with groups in Canada and Israel, we screened a series of 34 peptide inhibitors developed using T. cruzi's PI3K, PI4K, NMT, RACK, and IPMK as design scaffolds. In parallel, we are studying the role of the TcRACK1 protein in its biology and as a drug target.

Methods: Peptides were tested against trypomastigotes of different strains (Tulahuen β-galactosidase and DM28c β-galactosidase) in 96-well plates, assessed in Vero cells for cytotoxicity, and in infection assays performed over a 3-day period.

Results: 18 peptides were selected, based on their activity against infective forms and selectivity, to attach a hydrophobic peptide to facilitate cell penetration and repeat testing. Around 40% of candidates showed trypanocidal activity and no effect in host cell viability at concentrations up to 100 µM, indicating a good selectivity index. TcRACK1 overexpression experiments show that the HA-tagged versions of this protein localize to the cytoplasm and increase protein synthesis as measured by a puromycin incorporation assay. Yeast complementation in a ASC1-null strain shows partial rescue.

Conclusions: Selected drug candidates will be further investigated in their mode of action and therapeutic potential. Furthermore, TcRACK1 appears to be an important player in protein synthesis with probable involvement in ribosomal function.

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RESISTANCE AND DEVELOPMENT OF DRUGS AND VACCINES / DRUG RESISTANCE AND MOLECULAR BIOLOGY

UNRAVELING MACROCYCLIC LACTONE RESISTANCE: LINKING GENETIC ADAPTATION WITH DRUG EFFLUX AND SENSING

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Background and Aims: Gastrointestinal nematodes remain a major constraint on livestock health and productivity. *Haemonchus contortus*, a highly pathogenic blood-feeding parasite of small ruminants, is particularly challenging due to its strong adaptive capacity under anthelmintic and environmental selection pressures. Macrocyclic lactones (MLs) constitute a cornerstone of parasite control; however, the rapid global rise in ML resistance now jeopardizes their long-term efficacy.

Methods: In this review, we synthesize recent advances from ML-selected strains of *Caenorhabditis elegans* and from naturally resistant parasitic nematodes..

Results: Resistance in nematodes is multifactorial, involving changes in drug efflux, neuronal signaling, and transcriptional regulation. Recent findings from ML-selected *Caenorhabditis elegans* strains and naturally resistant parasites highlight the central contribution of ATP-binding cassette transporters, especially P-glycoproteins. In parallel, structural and functional impairments of amphid sensory neurons—often reflected by dye-filling (Dyf) phenotypes—have emerged as important determinants of ML susceptibility. Increasing evidence also implicates nuclear hormone receptors and associated regulatory networks in coordinating xenobiotic response, PGP expression, and sensory system integrity.. Recent population genomics, genome-wide association studies, and transcriptomic profiling in *H. contortus* have identified genomic regions and expression signatures strongly associated with ML resistance.

Conclusions: The major challenge now lies in functionally characterizing these variants and defining their causal roles in resistance phenotypes. Establishing these genotype–phenotype relationships is essential for developing robust molecular diagnostics and refining sustainable parasite control strategies in the face of accelerating anthelmintic resistance.

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ARE PYRETHROIDS FAILING? FIELD TRIAL ON FLIES FROM DAIRY SHEEP FARMS, SOUTHERN FRANCE

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Background and Aims: Flies cause major welfare and economic losses in livestock systems, with control relying heavily on insecticides that select for resistance. Dairy sheep farms of southern France represent a high-value production system where fly control is critical, particularly during lambing. This study evaluated adulticide treatment efficacy, lethal concentrations, and resistance in wild *Musca domestica* (sucking fly), laboratory and wild *Stomoxys calcitrans* (biting fly).

Methods: Seven farms were treated with a pyrethroid commercial formulation containing permethrin (250 g/L), prallethrin (10 g/L), and piperonyl butoxide (150 g/L). Professional technicians applied treatments at double-dose (500 mg/m^2 permethrin) via sheepfold spraying. Operational efficacy was assessed by measuring knockdown and 24-hour mortality using cage bioassays. Residual efficacy was assessed weekly for three months by cone tests on laboratory strains of *S. calcitrans*. WHO tube bioassays were used to determine lethal doses (LD_{50}/LD_{95}) in wild *M. domestica* and *S. calcitrans*.

Results: Spraying achieved 100% knockdown on both species. Residual efficacy against wild houseflies was low (3–29% mortality at D1), while laboratory susceptible stable flies showed >95% mortality up to 60 days. LD_{50} assays revealed high resistance in wild houseflies and laboratory resistant stable flies (up to 78-fold higher than laboratory susceptible *S. calcitrans*). Wild collected stable flies showed heterogeneous mortalities (5–69%) at the applied dose at D1.

Conclusions: These results highlight the resistance concern in wild flies and question about the continued pyrethroid use. Widespread, heterogeneous resistance necessitates integrated pest management combining environmental, biological, and physical tools against flies.

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HIGH PREVALENCE OF CYATHOSTOMINS AND REDUCED DORAMECTIN EFFICACY IN HORSES FROM SÃO PAULO STATE, BRAZIL

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Background and Aims: Brazil holds the fourth largest equine population worldwide, with approximately 5.8 million horses, including 353,000 in São Paulo State, a major breeding and competition hub. Despite this relevance, equine production faces significant sanitary challenges, particularly gastrointestinal nematode (GIN) infections that compromise animal health. This study aimed to determine the prevalence and intensity of GIN infections in equine breeding farms in São Paulo and to investigate evidence of anthelmintic resistance.

Methods: A cross-sectional survey was conducted on eight farms in the Botucatu region, evaluating 130 adult horses, predominantly Quarter Horses. Individual fecal egg counts (FEC) were performed using the McMaster technique and classified as light (≤200 eggs per gram, EPG), moderate (200–500 EPG), or heavy (>500 EPG) infections. Pooled coprocultures, each per farm, were prepared to identify third-stage larvae (L3). On a farm where only cyathostomins were detected, a Faecal Egg Count Reduction Test (FECRT) using doramectin 1% was performed in 20 horses according to WAAVP guidelines, with calculations perfomed using Shiny-eggCounts platform.

Results: Overall, 18.5% of horses had zero-EPG. Light, moderate, and heavy infections occurred in 32.3%, 16.1%, and 33.1% of animals, respectively, with counts ranging from 550 to 3,200 EPG. Cyathostomin L3 were identified on all farms, while *Strongylus edentatus* and *Strongylus equinus* were each detected on 37.5% of farms. Many heavily infected horses had been dewormed within the previous three months.

Conclusions: The FECRT showed only 57% efficacy of doramectin, indicating anthelmintic resistance and reinforcing the need for resistance monitoring to support evidence-based parasite control strategies in Brazilian equine herds.

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RESISTANCE AND DEVELOPMENT OF DRUGS AND VACCINES / DRUG RESISTANCE AND MOLECULAR BIOLOGY

DUAL TARGETING OF PARASITE AND HOST SPHINGOSINE KINASE-1 REVEALS THE S1P AXIS AS A DRUGGABLE VULNERABILITY IN LEISHMANIA DONOVANI INFECTION

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Background and Aims: Background and aims Sphingosine-1-phosphate (S1P) is a bioactive lipid regulator of apoptosis, autophagy, inflammation, and intracellular pathogen survival. Although S1P signalling has been implicated in *Leishmania donovani* infection, the coordinated roles of parasite-encoded and host-derived sphingosine kinase-1 (SphK1) remain poorly defined. This study aimed to delineate the dual contribution of SphK1 in parasite survival and host immune modulation, and to evaluate its therapeutic potential.

Methods: Methods Recombinant *L. donovani* SphK1 (rLdSphK1) and human SphK1 (rhSphK1) were cloned, expressed, and biochemically characterized. Enzyme inhibition by PF-543 was assessed using activity assays, microscale thermophoresis, and in silico docking. Parasite infectivity was evaluated following LdSphK1 overexpression or pharmacological inhibition. Host SphK1 was disrupted in THP-1 macrophages using CRISPR/Cas9. Cytokine responses, autophagy, apoptosis, and intracellular parasite burden were analysed. Therapeutic efficacy was assessed using PF-543 alone and in combination with Amphotericin B in vitro and in *L. donovani*-infected mice.

Results: Results Both rLdSphK1 and rhSphK1 were catalytically active and effectively inhibited by PF-543. LdSphK1 overexpression enhanced parasite infectivity, while inhibition compromised parasite survival; S1P supplementation rescued ceramide-induced parasite death. Host SphK1 knockout significantly reduced intracellular amastigote burden, induced a pro-inflammatory cytokine shift (↑TNF-α, ↑IL-12, ↓IL-10), enhanced autophagy, and increased apoptosis. PF-543 phenocopied these effects, whereas SphK2 inhibition showed minimal impact. PF-543 demonstrated synergistic activity with Amphotericin B, achieving >90% parasite reduction in vivo.

Conclusions: Conclusions The SphK1–S1P axis represents a convergent host–parasite survival pathway. Targeting SphK1 offers a promising host-directed and parasite-targeted therapeutic strategy for visceral leishmaniasis.

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RESISTANCE AND DEVELOPMENT OF DRUGS AND VACCINES / DRUG RESISTANCE AND MOLECULAR BIOLOGY

ANTIBIOTIC RESISTANCE AND MOLECULAR DETECTION OF NUC AND MECA GENES OF STAPHYLOCOCCUS AUREUS ISOLATED FROM MASTITIC MILK

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Background and Aims: The emergence of methicillin resistant Staphylococcus aureus (MRSA) is mainly due to the presence of resistant genes. The objective of this study is to perform antibiotic susceptibility profiling and molecular detection of nuc and mecA genes of Staphylococcus aureus isolated from mastitic milk.

Methods: A total of 105 milk samples were collected from dairy farms in the Faisalabad region. They were cultured on Staph-110 media and mannitol salt agar. Biochemical tests included catalase and coagulase, were also performed for the identification of Staphylococcus aureus. Antibiotic susceptibility profiling of MRSA was done by disc diffusion method and molecular screening of Staphylococcus aureus for the detection of nuc and mecA genes was done by using PCR. Selected 8 antibiotics based on previous history being commonly used in dairy practices.

Results: The prevalence of Staphylococcus aureus observed by PCR was 58.09%. Oxacillin (1µg) and vancomycin (30µg) showed 100% resistance, while enrofloxacin (5µg), amoxicillin (25µg), ampicillin (10µg), oxytetracycline (1µg), gentamycin (10µg) and tylosin (30µg) showed 1.64, 55.74, 73.77, 13.11, 1.64 and 11.74% resistance, respectively. Susceptibility percentages of enrofloxacin (5µg), amoxicillin (25µg), ampicillin (10µg), oxytetracycline (1µg), gentamycin (10µg) and tylosin (30µg) were 27.87, 37.70, 14.75, 19.67, 98.36 and 9.84%, respectively. Enrofloxacin (5µg), amoxicillin (25µg), ampicillin (10µg), oxytetracycline (1µg) and tylosin (30µg) showed 70.49, 6.56, 11.47, 67.21 and 78.69% intermediate resistance against MRSA.

Conclusions: Among the mastitic cases, 58.09% were positive for Staphylococcus aureus. These isolates were susceptible to gentamycin in 98.36% and tylosin in 78.69% cases, so these antibiotics can be used for the treatment of mastitis.

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CHARACTERIZATION OF THE INTERGENIC REGION BETWEEN TWO SINGLE NUCLEOTIDE POLYMORPHISMS ASSOCIATED WITH MACROCYCLIC LACTONE RESISTANCE IN DIROFILARIA IMMITIS

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Background and Aims: *Dirofilaria immitis* is the filarial nematode that causes canine heartworm disease. Prevention relies almost exclusively on macrocyclic lactones (MLs). Increasing reports of ML resistance have emerged in the United States. Previous studies identified two single nucleotide polymorphisms (SNP1 and SNP2) strongly associated with resistance. Our lab has developed a digital droplet PCR (ddPCR) assay that distinguishes susceptible and resistant isolates. However, isolates sometimes display a resistant genotype at SNP1 but not SNP2, and vice versa, suggesting the presence of variants within the region separating these SNPs. This project aims to investigate whether this region contains genetic features contributing to the resistant phenotype.

Methods: High-molecular-weight (HMW) genomic DNA (gDNA) was extracted from adult *D. immitis* male worms of resistant and susceptible strains whose genotypes were initially assessed by ddPCR. Then, the gDNA was sequenced using PacBio HiFi long-read technology. Genome assemblies are ongoing, and variants will be identified through genome alignments and comparisons.

Results: HMW gDNA extraction was optimized, leading to high-quality DNA from adult worms. The ddPCR genotyping of resistant isolates showed mostly concordant resistant SNP profiles. However, some isolates showed discordant SNP1 and SNP2 profiles, and two isolates showed susceptible SNP1 and SNP2 profiles, supporting a susceptible genotype despite being resistant isolates.

Conclusions: This study aims to characterize the intergenic region and identify variants associated with ML resistance in *D. immitis*. **Acknowledgments:** *D. immitis* adult worms were kindly provided by the Prichard lab. Louise McDonald was supported by the McGill Graduate Excellence Fellowship Award and the National Center for Veterinary Parasitology.

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APPARENT REDUCED EFFICACY OF PRAZIQUANTEL AND DOUBLE-DOSE PYRANTEL AGAINST ANOPLOCEPHALA SPP. IN FRENCH EQUIDS

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Background and Aims: Little information is available regarding the efficacy of the anthelmintic (AH) molecules commonly used against *Anoplocephala* spp. in equids (praziquantel and pyrantel). This study aimed to evaluate their efficacy in French equine facilities.

Methods: In each group (n≥5), individual fecal egg counts (FEC) were performed before treatment (D0) and 14 days after treatment (D14). Based on FECs, groups were classified into four categories: i) **strong suspicion of resistance**: ≥ 50% of shedders on D0 and N shedders on D14/N shedders on D0 ≥ 50%; ii) **moderate suspicion**: < 50% of shedders on D0 and N shedders on D14/ N shedders on D0 ≥ 50%; iii) **low suspicion**: ≥ 50% of shedders on D0 and N shedders on D14/ N shedders on D0 between 0-50%, and iv) **no suspicion**: ≥ one shedder on D0 and none on D14.

Results: Thirteen groups (94 horses) from 8 breeding farms were recruited. For praziquantel, strong suspicion of resistance was observed in 6/9 groups (57-100% shedders on D0 and 67-150% shedders on D14 compared to D0), while no suspicion was noted in the 3 others. For pyrantel, moderate suspicion of resistance was noted in 1/4 groups (38% shedders on D0 and 67% shedders on D14 compared to D0), and low suspicion in the others (67-75% shedders on D0 and 17% shedders on D14 compared to D0).

Conclusions: The identification of groups with a strong suspicion of praziquantel resistance supports veterinarians'field concerns. This study highlights the urgent need to rationalize the use of AH treatments to control *Anoplocephala* spp..

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IN VITRO EFFICACY AND CHEMICAL–NUTRITIONAL CHARACTERIZATION OF THREE AQUEOUS PLANT EXTRACTS AGAINST EGGS OF GASTROINTESTINAL NEMATODES IN SHEEP

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Background and Aims: Gastrointestinal nematode (GIN) infections are a major constraint in small ruminant production worldwide. This study evaluated the in vitro anthelmintic activity and nutritional profile of aqueous extracts from *Kyllinga odorata*, *Artemisia absinthium*, and *Verbena litoralis*, selected for their ethnobotanical relevance.

Methods: Egg hatch assay (EHA) was conducted using eggs from naturally infected sheep. Extracts were analyzed for proximate composition (crude protein, fiber, ash, minerals) and bioactive compounds, including phenols and tannins (colorimetric assays) and polyphenols (LC-MS).

Results: Parasitological identification revealed *Haemonchus contortus* (60%), *Trichostrongylus* spp. (20%) and *Strongyloides* spp. (20%). All extracts inhibited egg hatching at concentrations of 150–2400 µg/mL. *K. odorata* achieved ~96% inhibition at the lowest dose. *A. absinthium* maintained >90% inhibition across all concentrations, while *V. litoralis* showed lower efficacy (33% at 150 µg/mL). Positive controls (ivermectin and levamisole) yielded 91.8% and 85.3% inhibition, respectively. Extracts contained relevant levels of protein, fiber, and polyphenols, supporting potential nutraceutical use.

Conclusions: Aqueous extracts of *K. odorata*, *A. absinthium*, and *V. litoralis* demonstrated significant *in vitro* anthelmintic activity against sheep GIN eggs. Their chemical–nutritional profiles highlight potential as nutraceuticals. Further *in vivo* validation is recommended.

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RESISTANCE AND DEVELOPMENT OF DRUGS AND VACCINES / DRUG RESISTANCE AND MOLECULAR BIOLOGY

POSSIBLE INFLUENCE OF SEASONAL MALARIA CHEMOPREVENTION (SMC) ON THE GENETIC DIVERSITY OF THE MEROZOITE SURFACE PROTEIN-1 (MSP1) OF PLASMODIUM FALCIPARUM IN SENEGAL

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Background and Aims: Seasonal malaria chemoprevention (SMC) has been scaled up in Senegal since 2014 for children under 10 years old. The monitoring and evaluation of this strategy is a strong recommendation from the WHO for its sustainability. The objective of this study was to examine the polymorphism of the *Pfmsp1* gene in a SMC area before and after its implementation.

Methods: Genotyping of the *Pfmsp1* gene was performed from *Plasmodium falciparum*-positive samples selected from cross-sectional studies conducted on asymptomatic subjects one year before (2013) and one year after (2015) the scaling up of SMC in Southeastern Senegal. A Nested PCR was performed to determine the allelic variability of the *Pfmsp1* gene.

Results: The K1 allelic family was predominant both in 2013 (41.4%) and in 2015 (85.7%). Polyclonal infections K1/RO33 and K1/Mad20/Ro33 were only noted in 2015. The allelic polymorphism increased from 13 to 15 alleles in 2013 and 2015 respectively. The minor alleles $K1_{260}$, $K1_{280}$, and $Mad20_{200}$ were noted only in 2015. On the other hand, the $Mad20_{100}$ allele was only observed in 2013. Our results showed a significant increase in the frequency of multiple infections between 2013 (10.3%) and 2015 (60.3%) ($p < 0.001$). Furthermore, we noted an increase in the multiplicity of infection from 1.1 to 1.9 respectively in 2013 and 2015.

Conclusions: This study showed a high genetic diversity of Plasmodial strains circulating in the SMC area. However, long-term monitoring would allow for evaluation of the long-term evolution of this diversity and the lasting impact of SMC.

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EXPLORING THE RELATIONSHIP BETWEEN PLASMODIUM FALCIPARUM GENETIC DIVERSITY AND ANTIMALARIAL DRUGS RESISTANCE MARKERS IN A MALARIA-ENDEMIC REGION OF BURKINA FASO

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Background and Aims: The diversity of *Plasmodium falciparum* genotypes affects the dynamics of malaria transmission and is thought to be one of the factors hampering malaria control efforts. This study aimed to investigate the relationship between *Plasmodium falciparum* genetic diversity and chloroquine and sulfadoxine-pyrimethamine resistance markers in malaria endemic areas of Burkina Faso.

Methods: In a cross-sectional study, populations residing in Nouna health district were randomly recruited. Blood samples were used for microscopic malaria diagnosis, and genetic polymorphism alleles of *msp*-1 and *msp*-2 genotyping by nested PCR. Restricted fragment length polymorphism analysis was used to identify antimalarial resistance markers. Logistic regression analysis explored the association between *msp*-1/*msp*-2 alleles and antimalarial drug resistance markers. ANOVA was used to explore the association between the mean complexity of infection (mCOI) and prevalence of resistance markers.

Results: The overall prevalence of *Plasmodium falciparum* infection was 27.1%. The proportions of K1, MAD20, RO33, FC27, 3D7 individuals with mutations in the pfcrt76T gene were 4.3%, 6.9%, 7.0%, 6.8% and 7.1% respectively. Those with mutations in pfmdr1 were 2.7%, 2%, 2.3%, 6.8% and 7.1%. No significant associations were detected between msp-1/msp-2 alleles and chloroquine or sulfadoxine-pyrimethamine resistance markers. However, the mean complexity of infection (mCOI) was significantly higher in individuals with the pfcrt76T mutation.

Conclusions: Overall, this study showed that the genetic diversity of *Plasmodium falciparum* does not significantly affect the presence of antimalarial drug resistance genes. The competition between different strains (polyclonality) of the parasite within the host was probably unfavorable for mutant strains.

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RESISTANCE AND DEVELOPMENT OF DRUGS AND VACCINES / DRUG RESISTANCE AND MOLECULAR BIOLOGY

CHARACTERISATION OF GIARDIA INTESTINALIS FROM PATIENTS WITH METRONIDAZOLE-REFRACTORY GIARDIASIS IN THE CZECH REPUBLIC.

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Background and Aims: Giardiasis is a worldwide-spread parasitic disease caused by the single-celled eukaryotic pathogen *Giardia intestinalis* and is treated with metronidazole (MTZ). This study investigates the epidemiology of MTZ treatment failure (MTZ-TF), which is increasing worldwide, in a cohort of patients in the Czech Republic. Additionally, it explores parasite-specific genetic factors potentially driving drug resistance, including genotypes, sequence polymorphisms, and copy-number variation (CNV) in MTZ-related genes.

Methods: A total of 77 *Giardia* DNA isolates collected between 2020 and 2024 were analysed, along with clinical data, treatment outcome, and travel history. Multilocus genotyping was applied to determine assemblages/sub-assemblages of the isolates. Sequence polymorphisms in genes involved in MTZ metabolism and detoxification (e.g. *NR1, NR2, prx1*, *hmp*) were examined by Sanger sequencing, while CNV of the *hmp* gene was assessed by digital PCR.

Results: MTZ-TF was observed in 33.8% (26/77) of cases, with 96.2% of the infections imported to the Czech Republic, predominantly from the Indian subcontinent (65.4%). Only assemblages A and B were detected, with a predominance of assemblage B (80.5%). MTZ-TF was associated with sub-assemblage BIII ($p = 0.016$) and with an Indian subcontinent origin of infection ($p = 0.0001$). Shared amino acid substitutions in nitroreductases were identified among MTZ-TF isolates. Digital PCR revealed differences in *hmp* copy number between sub-assemblages; however, no association with MTZ-TF was observed.

Conclusions: Our results highlight the importance of assemblage B, particularly sub-assemblage BIII, and the Indian subcontinent origin in MTZ-TF giardiasis. Shared non-synonymous polymorphisms in MTZ-metabolism genes suggest parasite-specific adaptations that may contribute to reduced MTZ susceptibility.

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RESISTANCE AND DEVELOPMENT OF DRUGS AND VACCINES / DRUG RESISTANCE AND MOLECULAR BIOLOGY

LIFE TRAITS OF AN EPRINOMECTIN-RESISTANT HAEMONCHUS CONTORTUS ISOLATE – IMPACT OF DRUG EXPOSURE

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Background and Aims: Anthelmintics resistance in *Haemonchus contortus* is a growing problem in small ruminant farming. Mechanisms of resistance to macrocyclic lactones are partly based on the export of molecules via ATP-dependent efflux pumps like P-glycoproteins. Several studies have shown absence of fitness cost for resistant nematodes compared with susceptible ones in absence of compound. But is there an energy cost when the resistant worm is exposed to the treatment?

Methods: We compared life traits of a resistant isolate either without exposure to eprinomectin or exposed throughout its life-cycle to eprinomectin. Thirty nine-month-old ewes were infected with 5,000 L3 of the same eprinomectin-resistant isolate. Of these, fifteen ewes received injections of eprinomectin at a dose of 0.2 mg.kg-1 LBW every five days, from the day before infection until the end of the experiment, 49 days after infection. Life traits of *H. contortus* such as establishment rate of worms within the host, fertility and size of females and the ability of eggs to develop into L3 were monitored. Eprinomectin concentrations in serum and faeces were also measured.

Results: The only difference observed between the worms exposed to the compound and the others was a sharp decrease of development rate of eggs into larvae for parasites settled in treated animals. However, the assays showed a concentration 50 times higher in the faeces than in the serum which could explain this difference.

Conclusions: These observations show that, with the exception of larval development in faeces, the life traits didn't seem to be affected by the presence of the compound.

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SHIFT02-068

POSTER ON BOARD SHIFT 02
RESISTANCE AND DEVELOPMENT OF DRUGS AND VACCINES / DRUG RESISTANCE AND MOLECULAR BIOLOGY

THE ROLE OF THE KYNURENINE PATHWAY IN MACROCYCLIC LACTONE RESISTANCE IN DIROFILARIA IMMITIS

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Background and Aims: Macrocyclic lactones (MLs), including ivermectin (IVM), are preventative drugs used against the filarial nematode *Dirofilaria immitis*, which causes canine heartworm disease. However, ML resistance is increasingly reported in North America, and the underlying mechanisms remain unclear. Unlike other nematodes, filarial species show limited paralysis at therapeutic IVM concentrations, suggesting alternative mechanisms involving immunomodulatory pathways. Comparative metabolomic analyses of susceptible and resistant isolates identified alterations in several pathways, including the kynurenine pathway (KP). The KP is involved in tryptophan metabolism and immune regulation. Following IVM treatment in susceptible isolates, several KP metabolites were more abundant. KP genes also showed differential expression between isolates. This work aims to functionally characterize the role of KP genes, particularly *kynureninase (kynu)*, in ML sensitivity.

Methods: We are investigating *D. immitis* KP gene function in *Caenorhabditis elegans* using transgenic complementation. *D. immitis* KP genes were cloned with *C. elegans* promoters for expression in *C. elegans* loss-of-function mutants. Resulting transgenic lines will be used to assess IVM sensitivity using larval development assays.

Results: Larval development assays showed that deletions in KP genes alter IVM sensitivity in *C. elegans*. A deletion in *kynu* resulted in reduced IVM sensitivity. To assess whether *D. immitis* KP genes contribute similarly to ML response, the *C. elegans kynu* promoter was successfully cloned for transgenic expression studies.

Conclusions: These findings lay the foundation for functional characterization of KP genes in ML response. Ongoing transgenic studies in *C. elegans* will clarify the contribution of *kynu* and tryptophan metabolism to IVM sensitivity and ML resistance in filarial nematodes.

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SHIFT02-069

POSTER ON BOARD SHIFT 02
RESISTANCE AND DEVELOPMENT OF DRUGS AND VACCINES / DRUG RESISTANCE AND MOLECULAR BIOLOGY

DEEP AMPLICON SEQUENCING REVEALS GEOGRAPHICAL VARIATION IN THE RELATIONSHIP BETWEEN THE LEVAMISOLE RESISTANCE PHENOTYPE OF HAEMONCHUS CONTORTUS AND ACR-8 (S168T) SNP FREQUENCY

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Background and Aims: A non-synonymous Single Nucleotide Polymorphism (SNP) in (S168T) in the *acr-8* gene has been shown to be associated with levamisole resistance in *H. contortus*. The aim was to develop a deep amplicon sequencing assay for this mutation in multiple GIN species to investigate how the frequency of this SNP correlates with the levamisole resistance phenotype of field isolates across the USA.

Methods: Primers designed to amplify *acr-8* from the three main ovine GIN species, *H. contortus*, *T. circumcincta* and *T. colubriformis*, were developed and Illimuna deep amplicon sequencing performed. Amplicon Sequence Variants (ASVs) were taxonomically assigned to species and the frequency of the SNP (S168T) determined for each species.

Results: A total of 59 USA sheep farm samples submitted to the University of Georgia (2015 and 2019) for LDA analysis were phenotypically categorised as either levamisole susceptible (n=18), suspected-resistance (n=12), low-resistance (n=15) and high-resistance (n=14). The mean frequency of the *acr-8* S168T SNP was 2.5% and 66.6% in the susceptible and high-resistance groups, respectively. For the suspected and low-levamisole resistance groups there were two discrete populations; those from western USA and those from central/eastern USA with mean *acr-8* S168T SNP frequencies of 0.3% and 26.9% respectively.

Conclusions: The *acr-8* codon 168 genotype was very predictive of high levamisole resistance versus the susceptible phenotype in *H. contortus*. However, it was less predictive of lower resistance levels and the data suggests that an additional resistance mutation(s), conferring lower levels of levamisole resistance, may predominate in western USA.

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SHIFT02-070

POSTER ON BOARD SHIFT 02
RESISTANCE AND DEVELOPMENT OF DRUGS AND VACCINES / DRUG RESISTANCE AND MOLECULAR BIOLOGY

MONITORING MOLECULAR MARKERS ASSOCIATED WITH ANTI-MALARIA DRUG RESISTANCE IN A POPULATION OF PREGNANT WOMEN IN KINSHASHA, DEMOCRATIC REPUBLIC OF CONGO

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Background and Aims: Malaria in pregnancy (MiP) remains a major public health problem in sub-Saharan Africa, where around 30 million pregnant women are exposed to *Plasmodium* infection each year. MiP is associated with serious adverse outcomes including abortion, stillbirth, intra-uterine growth restriction, premature delivery, and low birthweight. These complications contribute to approximately 10,000 maternal deaths and 100,000 neonatal deaths annually. Preventive strategies include intermittent preventive treatment with sulfadoxine-pyrimethamine (IPTp-SP) administered three to four times during antenatal care (ANC) visits. Treatment of malaria infections during pregnancy relies on artemisinin-based combination therapies (ACTs), particularly artemether-lumefantrine. Since malaria control depends heavily on effective drugs, monitoring the emergence of parasite resistance markers is essential.

Methods: This study investigated the presence of molecular markers associated with resistance to SP and ACTs in *P. falciparum* parasites isolated from pregnant women. Peripheral blood samples were collected from 497 pregnant women attending routine ANC care. *P. falciparum* infections were detected using qPCR, and 156 women (31.4%) tested positive for malaria. Resistance markers in the *pfdhfr* and *pfdhps* genes were analysed using PCR-RFLP, while mutations in the PfK13 gene associated with ACT resistance were assessed by Sanger sequencing.

Results: showed a high prevalence of SP resistance-associated mutations, including the **I**51**R**59**N**108-**G**437**E**540 quintuple mutant and the *pfdhps* **G**437**E**540**G**581 triple mutant. However, key mutation frequencies remained below WHO thresholds for discontinuing IPTp-SP. PfK13 mutations were detected in 5.1% of samples, with A578S—linked to ACT resistance—identified three times.

Conclusions: Artemether-lumefantrine remains effective for treating malaria in pregnancy in DRC, though continued molecular surveillance is recommended.

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SHIFT02-071

POSTER ON BOARD SHIFT 02
RESISTANCE AND DEVELOPMENT OF DRUGS AND VACCINES / DRUG RESISTANCE AND MOLECULAR BIOLOGY

MOLECULAR SURVEILLANCE OF ARTEMISININ RESISTANCE MARKERS AND TREATMENT OUTCOMES AMONG CHILDREN WITH MALARIA IN ILORIN, NIGERIA.

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Background and Aims: Malaria remains a major public health challenge in sub-Saharan Africa, particularly among children, pregnant women, and immuno-compromised individuals. *Plasmodium falciparum*, the most virulent species, is highly polymorphic and contributes to rising antimalarial drug resistance. Although artemisinin-based combination therapies (ACTs) remain the WHO-recommended first-line treatment, mutations in the *pfkelch-13* propeller domain have been associated with artemisinin resistance, yet data from Ilorin, Kwara State, remain limited.This study evaluated parasite clearance after artemether–lumefantrine (AL) treatment, assessed *P. falciparum* genetic diversity, and screened for *pfkelch-13* mutations in infected children.

Methods: Fifty-one children aged 1–12 years with confirmed *P. falciparum* infection and parasitaemia ≥2,000/µL were recruited for a 28-day therapeutic efficacy study at Ajikobi General Hospital, Ilorin. Blood samples were analysed using microscopy, 18S rRNA amplification, MSP-2 genotyping, and bi-directional sequencing of the *pfkelch-13* propeller domain.

Results: Parasite clearance was rapid: slide positivity fell from 96.1% on Day 0 to 3.9% by Day 3, confirming AL's continued efficacy. Four recurrent infections (7.8%) occurred. The mean multiplicity of infection was 1.95, with allele recurrence indicating possible recrudescence or reinfection. One rare, non-validated *pfkelch-13* mutation (A359D) was detected outside recognised artemisinin-resistance loci, and no significant demographic associations were observed. Notably, WHO-validated resistance mutations including F446I, R539T, R561H, and C580Y, were absent, aligning with favourable treatment outcomes.

Conclusions: These results show that malaria remains a significant burden among children in Ilorin. Younger children carried higher parasite loads but cleared infections quickly with AL. While ACTs remain effective, the rare variant detected highlights the need for ongoing molecular surveillance.

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SHIFT02-072

POSTER ON BOARD SHIFT 02
RESISTANCE AND DEVELOPMENT OF DRUGS AND VACCINES / DRUG RESISTANCE AND MOLECULAR BIOLOGY

EFFECTS OF GOLGI INHIBITORS ON SURVIVAL AND INTRACELLULAR STRUCTURE OF TRYPANOSOMA BRUCEI

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Background and Aims: *Trypanosoma brucei* is a protozoan parasite of mammals that causes African trypanosomiasis. As in other eukaryotes, the Golgi apparatus in this protozoan is involved in the glycosylation and sorting/trafficking of membrane proteins and lipids, and is important for its normal proliferation. However, it remains to be determined which of the multiple functions served by the Golgi apparatus critically impacts the survival of *T. brucei*. This study aimed to reveal which Golgi function is critical for *T. brucei* survival and what occurs within *T. brucei* when Golgi function is impaired.

Methods: Bloodstream form *T. brucei* derived from Lister strain 427 were grown in HMI-9 medium and treated with Golgi inhibitors. Changes in Golgi apparatus morphology and membrane proteins distribution were analyzed.

Results: An ionophore known as a Golgi inhibitor exhibited potent cell proliferation inhibition, accompanied by the disappearance of GPI-anchored cell membrane receptors and changes in the intracellular localization of the Golgi apparatus itself. However, the absence of the *N*-linked glycan remodeling system in the Golgi apparatus alone did not result in such changes. Therefore, it was considered that inhibition of the sorting/trafficking pathway caused these changes.

Conclusions: In *T. brucei*, the sorting/trafficking pathway of the Golgi apparatus is more critical for survival, and its inhibition leads to the loss of a cell membrane receptor and changes in the intracellular location of the Golgi apparatus. These results suggest that the sorting/trafficking pathway is an attractive target for drug development.

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SHIFT02-073

POSTER ON BOARD SHIFT 02
RESISTANCE AND DEVELOPMENT OF DRUGS AND VACCINES / DRUG RESISTANCE AND MOLECULAR BIOLOGY

GENOMIC ADAPTATION TO ANTI-TRYPANOSOMATID DRUGS IN TRYPANOSOMA CRUZI

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Background and Aims: *Trypanosoma cruzi*, the causative agent of Chagas disease, exhibits remarkable genome plasticity, a hallmark of trypanosomatids. This plasticity arises from aneuploidy, chromosomal rearrangements, and copy number variations (CNVs), generating large-scale genomic changes. We hypothesize that genomic plasticity contributes to the parasite's adaptation to environmental stress and drug pressure. Benznidazole (BNZ) is the first-line treatment for Chagas disease, but treatment often fails, particularly in later disease stages. Here, we investigate whether genome plasticity facilitates adaptation of *T. cruzi* epimastigotes to BNZ exposure.

Methods: Three *T. cruzi* (DTU1) strains underwent repeated *in vitro* cycles of BNZ selection and recovery. Each cycle included one week of BNZ exposure, enrichment of active parasites, and a one-week drug-free recovery. Cycles were repeated until BNZ-exposed parasites exhibited better growth under drug pressure compared to non-exposed parasites. Whole-genome short-read sequencing will be performed after each cycle, with long-read sequencing after the fourth and final rounds, accompanied by BNZ susceptibility and growth measurements.

Results: We present results on the role of genome plasticity in *T. cruzi* adaptation to BNZ, integrating growth, drug susceptibility, and genome-wide changes in aneuploidy and CNVs. Preliminary analyses revealed reproducible somy alterations following BNZ exposure. Chromosome 23 consistently showed reduced somy across biological replicates and strains, alongside additional chromosome-specific changes, suggesting early genome remodeling may contribute to adaptation under drug pressure.

Conclusions: Preliminary findings support a role for genome plasticity in *T. cruzi* adaptation to BNZ stress. Ongoing multi-round selection experiments will elucidate the stability and extent of these genomic changes under sustained drug pressure.

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SHIFT02-074

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RESISTANCE AND DEVELOPMENT OF DRUGS AND VACCINES / DRUG RESISTANCE AND MOLECULAR BIOLOGY

COMBINATION THERAPY WITH A METHYLTRANSFERASE INHIBITOR AND POLYHEXAMETHYLENE BIGUANIDE: A NOVEL STRATEGY FOR ENHANCED ANTIAMOEBIC EFFECTS

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Background and Aims: Acanthamoeba keratitis (AK) is a severe ocular infection where standard treatment with polyhexamethylene biguanide (PHMB) is often limited by the high drug resistance of the cyst form. This study investigated the role of DNA methylation in the encystation process and the potential efficacy of combining the methyltransferase inhibitor 5-azacytidine (5-AzaC) with PHMB treatment.

Methods: Encystation was induced in *Acanthamoeba castellanii* using an encystation buffer. The expression levels of methyltransferase and encystation-related genes were assessed via quantitative PCR. The impact of 5-AzaC on the percentage of encystation and its synergistic effect with PHMB on parasite survival were also evaluated.

Results: Methyltransferase expression was found to be upregulated during the encystation process. Treatment with 5-AzaC reduced cyst formation and induced transcriptional changes in genes associated with the encystation pathway.Furthermore, combining PHMB with 5-AzaC led to a significant decrease in *A. castellanii* survival compared to PHMB monotherapy.

Conclusions: Inhibiting DNA methylation disrupts the formation of resistant cysts, thereby enhancing the antiamoebic activity of PHMB. This combination approach represents a promising novel therapeutic strategy for treating AK by targeting the parasite's ability to transition into its protective life stage.

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RESISTANCE AND DEVELOPMENT OF DRUGS AND VACCINES / DRUG RESISTANCE AND MOLECULAR BIOLOGY

INTEGRATING FECRT WITH ITS-2 AND ACR-8 ANALYSIS HINTS AT REGIONAL AND SPECIES-SPECIFIC LEVAMISOLE RESISTANCE MECHANISMS IN SHEEP GASTROINTESTINAL NEMATODES

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Background and Aims: Anthelmintic resistance in sheep gastrointestinal nematodes (GINs) is widespread and threatens sustainable parasite control. Faecal egg count reduction tests (FECRTs) provide phenotypic evidence of resistance but do not identify the species involved or the underlying genetic mechanisms. This study integrated FECRTs with ITS-2 metabarcoding and targeted analysis of the levamisole-associated acetylcholine receptor subunit gene (*acr-8*) to investigate species-specific resistance dynamics in field populations.

Methods: Eighteen sheep farms in southeast England were investigated using FECRTs following treatment with ivermectin, levamisole, or their combination. Faecal samples were collected at treatment and 14 days post-treatment, followed by ITS-2 metabarcoding to characterise species composition. Targeted amplicon sequencing of exon 4 of *acr-8* was performed to examine the resistance-associated substitution at codon 168 (S168T), previously reported in multiple GIN species.

Results: FECRTs revealed highly variable efficacy across farms (30–99% reduction), indicating widespread resistance. Post-treatment populations were consistently dominated by *Teladorsagia circumcincta* and *Trichostrongylus colubriformis*, while *Haemonchus contortus* was largely reduced. Despite strong phenotypic evidence of levamisole resistance in *T. circumcincta*, the resistance-associated *acr-8* substitution S168T was absent in the populations studied. In contrast, S168T was common in *T. colubriformis* and was not detected in *H. contortus*, as expected from their respective phenotypes.

Conclusions: These findings indicate marked species-specific differences in levamisole resistance mechanisms in the UK. The absence of S168T in phenotypically resistant *T. circumcincta* suggests alternative causal resistance mutation(s) in this region, highlighting the genetic heterogeneity of levamisole resistance and the need for broader approaches to fully resolve resistance mechanisms.

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SHIFT02-076

POSTER ON BOARD SHIFT 02
RESISTANCE AND DEVELOPMENT OF DRUGS AND VACCINES / PARASITE GENE REGULATION AND HOST ADAPTATION

TOXOPLASMA GONDII DDX6 RNA HELICASE: A MEDIATOR OF TRANSLATIONAL SUPPRESSION

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Background and Aims: *Toxoplasma gondii* is an obligate intracellular parasite of the phylum Apicomplexa that could cause severe disease in immunocompromised individuals and establish chronic infection in healthy hosts. This study focuses on RNA-dependent DEAD-box ATPases (DDXs), a highly conserved and abundant family of RNA helicases, which are increasingly recognized as potential therapeutic targets. Here, we characterize *Tg*HoDI, a *T. gondii* homolog of the *Plasmodium* DDX6-class RNA helicase DOZI. Our working hypothesis is that *Tg*HoDI functions as a translational regulator and represents a potential target for preventing *T. gondii* infection.

Methods: We used the MS2 RNA–protein tethering system, combined with deletion and site-directed mutagenesis, to investigate the structural determinants and functional role of *Tg*HoDI in translation suppression. To evaluate its biological importance, *Tg*HoDI expression was ablated through gene knockout, enabling assessment of its contribution to parasite stress adaptation. Differential transcript levels were identified through RNA-sequencing and validated by RT-qPCR.

Results: We demonstrate that *Tg*HoDI mediates translational suppression when recruited to RNA–protein complexes, and that its ATP-binding and ATP-hydrolysis activities are essential for this function. Unexpectedly, *Tg*HoDI knockout parasites exhibit enhanced host-cell invasion but reduced intracellular proliferation. Transcriptomic analysis reveals that *Tg*HoDI regulates stress-associated gene expression, including modulation of a dynein heavy chain transcript implicated as a negative regulator of invasion.

Conclusions: Our findings identify *Tg*HoDI as a molecular link between stress responses and invasion mechanisms in *T. gondii*. This work provides new insight into DDX-family helicases and highlights *Tg*HoDI as a promising target for therapeutic intervention against toxoplasmosis.

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SHIFT02-077

POSTER ON BOARD SHIFT 02
RESISTANCE AND DEVELOPMENT OF DRUGS AND VACCINES / PARASITE GENE REGULATION AND HOST ADAPTATION

CANONICAL WNT SIGNALING PATHWAY AND ANTERO-POSTERIOR SPECIFICATION DURING THE IN VITRO EVAGINATION OF TAENIA SOLIUM

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Background and Aims: The evagination of the *Taenia solium* scolex is a decisive step for its establishment in the human host and subsequent development into an adult tapeworm. This research aims to identify and characterize the expression of canonical Wnt signaling pathway elements and developmental markers during this larval-to-adult transition. Specifically, the study explores how these molecular pathways mediate the specification of the anterior and posterior poles, a crucial process for the parasite's morphogenesis.

Methods: An in vitro model was optimized using taurocholic acid (TA) to induce controlled scolex evagination. Cysticerci were collected from infected pigs, and groups were defined as recently collected (RC), non-cultivated (NC), pre-evaginated (PRE; up to 6 h in culture), and recently evaginated (EV; up to 40 h in culture). Gene expression was quantified via qPCR after identifying orthologs in the *T. solium* genome.

Results: The study provides the first record of canonical Wnt pathway expression in *T. solium*. Key genes such as *wnt1* and *wnt11a* showed significantly increased expression during the transition to define the posterior proglottid-forming zone, while *sfrp* delimited the anterior pole (as happens in model cestodes like *Hymenolepis*). An "anticipatory response" was observed: genes related to muscle formation (*myoD*) and cell migration (*snail-1*) were upregulated before scolex evagination.

Conclusions: The PRE and EV phases exhibit distinct molecular identities, both of which are regulated by the Wnt pathway, which acts as a master regulator of body axis specification. These findings should elucidate *T. solium* development and identify potential targets for pharmacological strategies to interrupt the parasite's life cycle.

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SHIFT02-078

POSTER ON BOARD SHIFT 02
RESISTANCE AND DEVELOPMENT OF DRUGS AND VACCINES / PARASITE GENE REGULATION AND HOST ADAPTATION

TAUROCHOLIC ACID–INDUCED TRANSCRIPTOMIC REMODELING DRIVES LARVAL-TO-ADULT TRANSITION IN TAENIA SOLIUM

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Background and Aims: The metamorphosis of *Taenia solium´* s cyst to adult tapeworm within the human intestine drives parasite dispersal and disease transmission. We published a high-resolution transcriptome generated via Next-Generation Sequencing, capturing three distinct morphological phases of in vitro cyst activation (DOI: 10.1038/s41597-025-05141-2). We used the raw dataset to identify differentially expressed genes and processes essential for parasite maturation.

Methods: Our in vitro activation model included *T. solium* cysts collected before (PRE; up to 6 h in culture) and right after evagination of the scolex (EV; upt to 40 h in culture), induced or not by 0.1% taurocholic acid (TA). Following Illumina RNA-Seq and genomic mapping, we performed an integrated functional re-annotation and differential expression analysis (DESeq2). Gene co-expression networks (WGCNA) and functional enrichment (GSEA/ORA) were used to characterize the signaling pathways and molecular modules driving the larval-to-adult transition.

Results: The comparison of PRE and EV phases showed that the evagination of the scolex in *T. solium* is a programmed biological process in which TA acts as a master ligand, activating the MAPK/ERK and JNK signaling pathways to coordinate metamorphosis. This host-derived chemical stimulus triggers a massive transcriptomic reprogramming of 706 unique genes, optimizing glucose metabolism, redox balance, and the splicing machinery for the transition to the adult stage.

Conclusions: TA triggers the transition of the cyst into an early adult via a transcriptomic reprogramming that modulates differentiation factors and increases cellular proliferative capacity, thereby preparing the parasite for establishment, growth, and reproduction within the human intestine.

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SHIFT02-079

POSTER ON BOARD SHIFT 02
RESISTANCE AND DEVELOPMENT OF DRUGS AND VACCINES / PARASITE GENE REGULATION AND HOST ADAPTATION

TRANSCRIPTOMIC AND ULTRASTRUCTURAL RESPONSES TO AMIODARONE–ITRACONAZOLE IN NATURALLY BENZNIDAZOLE-RESISTANT AND -SUSCEPTIBLE TRYPANOSOMA CRUZI STRAINS

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Background and Aims: Chagas disease, caused by *Trypanosoma cruzi*, remains a therapeutic challenge due to the limited efficacy of benznidazole and the presence of naturally resistant strains. Drug repurposing represents a promising strategy to identify novel trypanocidal therapies. Here, we evaluated the combinatorial effect of Amiodarone–Itraconazole (Amiozole) against two *T. cruzi* TcI strains: one benznidazole-sensitive (MG) and one naturally resistant (DA), aiming to elucidate its molecular and ultrastructural mechanisms of action.

Methods: Trypanocidal activity was assessed by MTT viability assays and IC_{50} determination in epimastigotes cultured in LIT medium. Transcriptomic profiling was performed by RNA sequencing, with reads mapped to the *T. cruzi* Dm28c reference genome. Differential expression analysis was conducted using DESeq2, followed by Gene Ontology enrichment and metabolic pathway reconstruction. Ultrastructural alterations were examined using transmission electron microscopy (TEM).

Results: Both strains were susceptible to Amiozole but displayed distinct transcriptional responses. The resistant DA strain showed 35 upregulated and 87 downregulated genes, with activation of purine metabolism and inhibition of surface renewal pathways. The sensitive MG strain exhibited 57 upregulated and 412 downregulated genes, including enhanced sphingolipid metabolism and broad suppression of energy and nucleotide biosynthesis pathways. TEM revealed severe cellular damage in both strains, including mitochondrial disruption, nuclear disorganization, autophagosome formation, and extensive membrane vesiculation.

Conclusions: Amiozole exerts a multifaceted mode of action disrupting essential metabolic and structural pathways in *T. cruzi*, supporting its potential as a combinatorial therapy against strains with distinct resistance profiles. Further studies in infective stages, dose-response models, and diverse intra-DTU lineages are required to validate its clinical applicability.

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SHIFT02-080

POSTER ON BOARD SHIFT 02
RESISTANCE AND DEVELOPMENT OF DRUGS AND VACCINES / PARASITE GENE REGULATION AND HOST ADAPTATION

EFFECTS OF IRON AVAILABILITY ON PROLIFERATION AND GENE EXPRESSION OF LEISHMANIA DONOVANI

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Background and Aims: *Leishmania* parasites, which survive and proliferate within parasitophorous vacuoles of host macrophages, require iron for intracellular survival, whereas excess iron can be toxic, necessitating mechanisms to appropriately regulate iron uptake and processing. This study aimed to elucidate the effect of iron availability on proliferation and gene expression in *Leishmania donovani*, while investigating a candidate protein potentially involved in iron retention.

Methods: First, differential expression of iron-related genes in two developmental stages were examined. Next, the effects of iron supplementation and depletion on *L. donovani* proliferation and survival in vitro, as well as iron-related gene expression, were examined. In addition, the effect of overexpression of a novel iron-binding gene on intracellular iron homeostasis and expression of other iron-related genes was analyzed.

Results: Both aberrant iron depletion and supplementation inhibited *L. donovani* proliferation, whereas intermediate levels of iron depletion or supplementation showed no apparent impact on the parasites. Through transcriptome analyses, we found gene expression patterns unique to iron-depleted or supplemented conditions as well as those unique to each developmental stage. When a novel iron-binding gene, which is one of the most upregulated in amastigotes, was overexpressed in promastigotes, enhanced intracellular iron retention and increased expression of other iron-related genes were observed.

Conclusions: These findings demonstrate that *L. donovani* possesses mechanisms to maintain intracellular iron homeostasis under varying extracellular iron concentrations. Furthermore, our results suggest the presence of master regulator(s) contributing to intracellular iron homeostasis by regulating other iron-related genes in *Leishmania*.

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SHIFT02-081

POSTER ON BOARD SHIFT 02
RESISTANCE AND DEVELOPMENT OF DRUGS AND VACCINES / PARASITE GENE REGULATION AND HOST ADAPTATION

EXPLORING THE ROLE OF LONG NON-CODING RNAS IN LEISHMANIA DEVELOPMENTAL GENE REGULATION AND PATHOGENESIS

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Background and Aims: Long non-coding RNAs (lncRNAs; >200 nt) are essential components of various biological processes across all domains of life, regulating transcription, pre-mRNA processing, RNA stability, and translation. Despite some recent progress, lncRNAs are still poorly characterized in *Leishmania*, and little is known about their molecular and regulatory functions throughout the parasite development. This study aims to characterize further the repertoire of developmentally regulated lncRNAs in *L. infantum* and to assess their mode of regulation and role in pathogenesis.

Methods: We employed Nanopore direct RNA sequencing to comprehensively interrogate the non-coding transcriptome of *L. infantum* developmental stages. To investigate further the role of selected lncRNAs in developmental gene expression and parasite infectivity, we combined CRISPR-Cas9 gene-editing, RNA-seq, qPCR, proteomics, and macrophage infections.

Results: We found that the *L. infantum* transcriptome expresses a rich repertoire of lncRNAs that are processed mostly from 3'UTRs through *trans*-spicing and polyadenylation, similarly to mRNAs. Generally, these lncRNAs exhibit distinct expression patterns from their upstream CDS, and several are developmentally regulated, representing 27% of the *L. infantum* stage-regulated transcriptome. The majority of these newly identified lncRNAs seem to be preferentially expressed in *L. infantum*, and less or not at all in *L. major*, which suggests they may be associated with parasite tropism.

Conclusions: This is the first comprehensive analysis of the *L. infantum* non-coding transcriptome using Nanopore DRS, providing new insights into the transcriptome complexity. Studies are underway to better understand the function of lncRNAs in developmental gene regulation and parasite infectivity or tropism.

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POSTER ON BOARD SHIFT 02
RESISTANCE AND DEVELOPMENT OF DRUGS AND VACCINES / PARASITE GENE REGULATION AND HOST ADAPTATION

RESAZURIN-BASED PROFILING REVEALS A NARROW ENVIRONMENTAL PH OPTIMUM IN MIGRATORY ASCARIS SUUM L3

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Background and Aims: Parasitic helminth larvae encounter rapidly shifting microenvironments during host invasion, requiring precise metabolic and physiological adaptation. The third-stage larva (L3) of *Ascaris suum* is central to the hepato-tracheal migration and experiences dynamic changes in pH, oxygen levels, and nutrient availability. However, the regulatory mechanisms underlying larval adaptation to host microenvironments remain poorly characterized. Building on our resazurin-based assay, we investigated whether pH represents a critical bottleneck shaping L3 metabolic fitness.

Methods: Mechanically hatched and sucrose-gradient–purified *A. suum* L3 were incubated in defined media adjusted to pH 5–7. Metabolic activity was quantified by resazurin reduction at multiple time points, complemented by motility and viability scoring. Parallel metabolomic and proteomic profiling is currently being conducted to identify pathways responsive to pH-dependent metabolic stress.

Results: L3 exhibited a narrow pH optimum centered around pH 6, with significantly reduced resazurin reduction at pH 5 and pH 7. This metabolic pattern was mirrored by decreased motility and viability outside the optimal pH range, highlighting that metabolic impairment translates into measurable loss of larval fitness. Fluorescence microscopy confirmed robust resorufin accumulation at pH 6, indicating maximal metabolic turnover.

Conclusions: Our findings demonstrate that *A. suum* L3, unlike many other parasitic helminths, possesses a remarkably narrow pH optimum, suggesting finely tuned metabolic regulation during host tissue migration. Further, we highlight that the resazurin assay provides a powerful platform for dissecting the environmental regulation of larval physiology in dynamically changing conditions. By defining pH limits of larval fitness, our study sets the stage for developing intervention strategies based on host-niche factors.

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POSTER ON BOARD SHIFT 02
RESISTANCE AND DEVELOPMENT OF DRUGS AND VACCINES / PARASITE GENE REGULATION AND HOST ADAPTATION

H3SER10P AND ITS POTENTIAL RELATIONSHIP WITH MITOSIS REGULATION IN TRYPANOSOMA CRUZI

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Background and Aims: *Trypanosoma cruzi* exhibits a complex life cycle with regulated changes. Post-translational modifications of histones are regulatory mechanisms crucial in cell cycle progression. In many eukaryotes, phosphorylation of Serine 10 in histone H3 (H3Ser10p) is key for chromosome condensation during mitosis. *T. cruzi* has a conserved H3Ser10, but its phosphorylation has not been previously reported. Despite the chromatin of this parasite does not condense into chromosomes during mitosis, we have previously described an Aurora B kinase homologue in *T. cruzi*, the enzyme in charge of H3Ser10p production in other eukaryotes. This work aims to evaluate the cell cycle-regulated occurrence of H3Ser10p and its potential relationship with cell cycle regulation in *T. cruzi*

Methods: Western blot analysis revealed the presence of this PTM in exponentially growing epimastigotes. We monitored the presence of H3Ser10p by fluorescence microscopy in epimastigotes, amastigotes, and trypomastigotes, and simultaneously registered the number and cell cycle-associated morphology of the kinetoplast, flagellum, and nucleus.

Results: The H3Ser10p signal was present in every 1N2K2F cell, indicative of mitosis. This result suggests a role for H3Ser10p, especially during mitosis. Furthermore, we analyzed cell cycle-synchronized parasites and observed an increment of H3Ser10p in the G2/M phase. The presence of this histone mark correlates with chromatin condensation.

Conclusions: H3Ser10p was detected during mitosis in replicative stages of *T.cruzi,* suggesting a regulatory role in cell division. We overexpressed wild-type histone H3, in adition mutant versions in which Ser10 was replaced by either a non-phosphorylatable or a phosphomimetic residue. This approach will facilitate the elucidation of the physiological role.

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POSTER ON BOARD SHIFT 02
RESISTANCE AND DEVELOPMENT OF DRUGS AND VACCINES / PARASITE GENE REGULATION AND HOST ADAPTATION

THE UNUSUAL PROPERTIES OF THE TBP TRANSCRIPTION FACTOR IN THE PROTOZOAN PARASITE, GIARDIA INTESTINALIS

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Background and Aims: *Giardia intestinalis* infects humans and over 40 animal species. Giardia has a compact (~12 Mb) genome containing a reduced complement of genes encoding proteins for essential cellular processes, including transcription. The TATA-box binding protein (TBP) is a universal eukaryotic transcription factor but Giardia TBP (gTBP) is highly divergent, featuring substitutions at residues critical for binding to double-stranded DNA and a markedly narrowed DNA-binding pocket. We aimed to characterize the DNA-binding properties of gTBP and the structural properties of the DNA it binds to.

Methods: Recombinant gTBP was used in electrophoretic mobility shift assays (EMSAs) to study its interaction with DNA. G-quadruplex formation in DNA was analyzed by circular dichroism spectroscopy and thioflavin T fluorescence assays.

Results: We determined that gTBP binds single-stranded DNA in two distinct modes. In **A mode**, gTBP multimers bind to ssDNA sequences with four or more consecutive guanine bases that can form secondary structures called G-quadruplexes, which have roles in gene regulation, genome stability, and telomere function in other eukaryotes. In contrast, **B mode** involves monomeric gTBP binding to more flexible DNA regions, measured by low base stacking energies. A search of the Giardia genome showed that regions of low base stacking energies are prevalent in the promoters of genes encoding proteins, tRNAs, and rRNAs, suggesting that this may be an important feature recognized by gTBP for gene regulation.

Conclusions: These findings offer a novel perspective on eukaryotic transcription regulation, highlighting an unconventional interaction between gTBP and ssDNA, including between gTBP and G4s.

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POSTER ON BOARD SHIFT 02
RESISTANCE AND DEVELOPMENT OF DRUGS AND VACCINES / PARASITE GENE REGULATION AND HOST ADAPTATION

DISTINCT REPEAT ARCHITECTURE LANDSCAPES IN THE PROTEOMES OF PROTOZOAN PARASITES

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Background and Aims: Protozoan parasites cause major infectious diseases and pose persistent challenges to global health, particularly the emergence of drug-resistant strains. Tandem repeats (TRs) and other repetitive architectures are widespread in proteomes, especially in protozoan proteins, where they have been implicated in host–parasite interactions, immune evasion, and antigenicity. However, repeat-containing proteins (RPs) exhibit highly diverse architectures that often extend beyond the simple reiteration of a single motif, making comprehensive and quantitative characterization challenging.

Methods: We performed bioinformatics analysis of repeat architectures in protozoan proteins. In addition to the established repeat-detection approaches, we developed a new algorithm, Drepper, which quantifies repeat-architecture complexity.

Results: By integrating diverse repeat-related features, we clustered RPs across species and identified distinct groups associated with parasite lineages. Notably, we detected a *Plasmodium*-specific RP cluster and a *Trypanosoma*/*Leishmania*-specific RP cluster; both were characterized by large repeat regions but exhibited contrasting repeat-structure complexity. The *Plasmodium*-specific RPs showed high complexity, whereas the *Trypanosoma*/*Leishmania*-specific RPs displayed significantly low complexity. Functional enrichment analyses indicated that these lineage-associated clusters were enriched in parasite-specific factors. Furthermore, evolutionary analyses suggested that low-complexity repeat architectures may be actively maintained through concerted evolution.

Conclusions: Taken together, our results reveal lineage-specific characteristics of protozoan repeat architectures and provide a quantitative framework for studying their biological and evolutionary roles.

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POSTER ON BOARD SHIFT 02
RESISTANCE AND DEVELOPMENT OF DRUGS AND VACCINES / PARASITE GENE REGULATION AND HOST ADAPTATION

IRON REGULATION OF CATHEPSIN B MRNAS FROM GIARDIA DUODENALIS

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Background and Aims: *Giardia duodenalis* colonizes the human small intestine and triggers cytotoxic mechanisms leading to symptoms of giardiasis. Cystein proteases (CPs) of *G. duodenalis* participate in alterations of cellular tight junctions, degradation of chemokines and pro-apoptotic processes of epithelial cells leading to disruption of the epithelial barrier. Although CPs are among the most secreted virulence factors by the parasite, the regulation processes by which these genes are expressed are not well understood. Iron responsive elements (IRE) are steem-loops structures found in mRNA and binding to iron responsive proteins (IRP) regulates gene expression depending on the iron status of the cell and position of the IRE. Our research group has previously found stem-loops structures in mRNAs of virulent iron-dependent genes from *G. duodenalis*. The aim of this work was to evaluate the impact of iron on the regulation of some *G. duodenalis* proteases.

Methods: Thus, *in silico* research was performed for possible stem-loops structures in mRNAs encoding cytotoxic proteases using ZuckerMfold. In addition, we performed molecular docking between a predicted stem-loop and IRP. To determine *in vitro* iron regulation, *G. duodenalis* trophozoites were cultured in low, normal, and high iron concentrations and RT-PCR assays were done.

Results: showed IRE-like structures predicted in the translated and untranslated regions (UTR) of the mRNA encoding Giardial CPs. Interaction of a predicted IRE-like structure to human IRP was predicted. Lastly, the analyzed CP gene is iron regulated as expected.

Conclusions: These results suggest the presence of IRE-like structures on both translated and UTR iron regulated cathepsin B mRNA.

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POSTER ON BOARD SHIFT 02
RESISTANCE AND DEVELOPMENT OF DRUGS AND VACCINES / PARASITE GENE REGULATION AND HOST ADAPTATION

IDENTIFICATION OF A PERCHLORIC-ACID-SOLUBLE PROTEIN-LIKE IN GIARDIA DUODENALIS

María Angélica Sánchez González[1], Gabriel Leon[2], Sarita Montaño-Valdez[3], José Garzón Tiznado[3], Hector López-Moreno[3], Jeanett Chávez-Ontiveros[3], Nidia León Sicairos[3], Claudia León Sicairos[3]
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Background and Aims: One of the main mechanisms by which eukaryotic cellular iron levels are regulated is through the IRE/IRP (Iron Responsive Element/Iron Regulatory Protein) system. IREs are stem-loops structures found in the untranslated regions of mRNAs that encode iron-regulated proteins. IRPs bind to these IREs depending on cellular iron levels to maintain homeostasis. Recently, this study has been extended to protozoa due iron is essential for their virulence. Our group has reported the presence of stem-loops structures in virulence mRNAs of *Giardia duodenalis*; however, the genome does not possess a canonical IRP. A Perchloric-Acid-Soluble protein (PSP) named Tv-PSP1 was previously reported in *T. vaginalis* , this protein binds to IREs suggesting multifunctional proteins as components of this mechanism. This study aimed to identify a PSP-like in *G. duodenalis*.

Methods: *In silico* search for PSP-like sequences was performed using Tv-PSP1 of *T. vaginalis* as probe. Molecular dockings were conducted to predict interactions between human and protozoan IRE-like structures and both IRP and PSP. Lastly, *Giardia* trophozoites were cultured under different iron conditions, and RT-PCR assays were performed to assess expression of the PSP-like encoding gene.

Results: Bioinformatic analysis identified a candidate PSP-like protein in the *G. duodenalis* genome, showing sequence similarity to Tv-PSP1. RT-PCR assays demonstrated iron-dependent regulation of the corresponding gene. Molecular docking analyses predicted interactions between the *G. duodenalis* PSP-like, and both human IRE and Giardial IRE-like structures.

Conclusions: Results suggest the presence of an iron regulated PSP-like in *G. duodenalis*. This protein could act as an IRP since it interacts with reported Giardial IRE-like structures.

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POSTER ON BOARD SHIFT 02
RESISTANCE AND DEVELOPMENT OF DRUGS AND VACCINES / PARASITE GENE REGULATION AND HOST ADAPTATION

THE EFFECT OF DNA DAMAGE ON GIARDIA INTESTINALIS WHEN THE EXPRESSION OF A NUCLEAR HEME PROTEIN IS REDUCED

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Background and Aims: *Giardia intestinalis* can cause severe and infectious diarrhea in humans. Class II cytochrome b5s are soluble heme proteins found only in anaerobic protozoa including *Giardia*, *Plasmodium*, *Toxoplasma*, *Leishmania*, and *Trichomonas* with unknown functions. *Giardia* encodes four paralogs of cytochrome b_5, each localized to a distinct cellular compartment. One paralog, gCYTB5-III, is localized to the nucleus, and prior work from our lab suggests it may play a role in the parasite's response to DNA damage. *Giardia* appears to lack orthologues of several key proteins involved in all DNA repair pathways, so investigating gCYTB5-III offers a novel entry point to explore the role of redox- or heme-responsive factors in mediating this streamlined DNA repair system.

Methods: CRISPR interference (CRISPRi) was used to generate a Giardia cell line with up to 50% reduction in gCYTB5-III. This culture was treated with methyl methanesulfonate (MMS) or metronidazole (MTZ) to trigger DNA repair. Comet assays were performed to measure the DNA damage in the Giardia cultures, which involves visualizing the extent of DNA fragmentation (comet tail) compared to intact DNA (comet head) in each cell.

Results: RT-qPCR analysis showed that MMS-treated gCYTB5-III KD cells exhibited reduced induction of Mre11 and Rad52, which are key proteins in DNA repair pathways, compared to control cells expressing a non-specific gRNA. RT-qPCR analysis with MTZ-treated cells and the comet assays are ongoing.

Conclusions: This work will shed light on the poorly understood Class II cytochrome b_5 proteins, providing new insights into their functions in *Giardia* and other parasitic protists.

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POSTER ON BOARD SHIFT 02
RESISTANCE AND DEVELOPMENT OF DRUGS AND VACCINES / PARASITE GENE REGULATION AND HOST ADAPTATION

TRYPANOSOMATID MITOCHONDRIAL RNA EDITING AND USE OF GUIDE RNAS – A CONSISTENT VULNERABLITY

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Background and Aims: Uridylate (U)-insertion/deletion RNA editing of kinetoplastids, is the massive, targeted insertion and deletion of Us to mitochondrial protein-coding transcripts, to generate the open reading frame. The overall machinery and mechanism for this process were determined mainly in the model *Trypanosoma brucei*. The life cycles of trypanosomatid parasites infecting mammals vary, and different parasite species likely regulate editing to suit their specific survival agendas. However, identifying what regulation is essential to what parasite, and why, is complex.

Methods: Trypanosomatid mitochondrial transcriptomes are highly heterogenous since 1) only a tiny portion of transcripts achieve a fully edited and thus translatable state, and 2) deep sequencing reads include U insertions or deletions differing from what will result in an open reading frame. The research community has developed different computational tools to analyze elements of U-insertion/deletion editing but lack the synthesis of these tools. We successively utilized these tools to analyze U-insertion/deletion editing regulation in a pathogen other than *T. brucei*.

Results: We obtained a repertoire of likely open reading frame sequences in three strains of the agent of Chagas disease, *Trypanosoma cruzi*, and used this output to analyze the processivity of editing of *RPS12* and *A6* transcripts.

Conclusions: To analyze the regulation of mRNA editing in specific context across the range of disease-causing trypanosomatids, a multistep high-throughput sequencing approach is required. We have created this overall analysis pipeline for use in any species or strain, and optimized library generation and computational analysis of each step.

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SHIFT02-090

POSTER ON BOARD SHIFT 02
RESISTANCE AND DEVELOPMENT OF DRUGS AND VACCINES / PARASITE EXTRACELLULAR VESICLES (EVS) FOR THERAPEUTICS AND DIAGNOSTICS

DIFFERENTIAL ANTIBODY PROFILES IN ASYMPTOMATIC AND SYMPTOMATIC MALARIA: IDENTIFYING PROTECTIVE TARGETS IN GHANAIAN CHILDREN

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Background and Aims: Background and Aims: Asymptomatic malaria is characterized by acquired immunity, resulting in low parasite loads and clinical silence. However, distinguishing between antibodies that signify **protection** (immunity) versus those that simply signify **infection** (exposure) remains a challenge for vaccine development. This study aimed to characterize antibody targets that correlate with clinical protection in a malaria-endemic region.

Methods: Methods: A cross-sectional study was conducted in Obom, Ghana, involving children categorized as symptomatic (n=27), asymptomatic (n=107), or uninfected (n=15). Utilizing a magnetic bead-based Luminex assay, we measured antibody titers against 19 *P. falciparum*, 6 *P. vivax*, and 1 *Anopheles gambiae* antigens. Associations between antibody levels and parasite load were analyzed to identify potential correlates of protection.

Results: Results: Asymptomatic children exhibited significantly higher antibody titers to EBA175, Etramp4, MSP2 (alleles CH150 and d2), Rh2.230, and Rh5.1 compared to symptomatic peers. Conversely, symptomatic individuals showed higher levels of antibodies to MSP1-19 and SBP1. Crucially, antibodies elevated in the asymptomatic group (EBA175, MSP2, and Rh2.230) were **inversely correlated** with parasite load, suggesting a protective role. In contrast, MSP1-19 and SBP1 titers correlated positively with parasite load, identifying them as markers of active exposure rather than protection.

Conclusions: Conclusions: Our findings demonstrate that specific antibody profiles dictate clinical outcomes. The inverse correlation of EBA175, MSP2, and Rh2.230 with parasite density supports their candidacy for inclusion in next-generation multi-antigen malaria vaccines.

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POSTER ON BOARD SHIFT 02
RESISTANCE AND DEVELOPMENT OF DRUGS AND VACCINES / PARASITE EXTRACELLULAR VESICLES (EVS) FOR THERAPEUTICS AND DIAGNOSTICS

HARNESSING NATIVE INTESTINAL MICROBIOTA FOR DELIVERY OF THERAPEUTICS AGAINST PARASITIC NEMATODES

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Background and Aims: Intestinal helminth infections impose a major global burden on humans and animals. Increasing drug resistance and limited efficacy of existing treatments call for therapeutic alternatives to control these parasites. This study aims to develop transgenic native gut *Escherichia coli* capable of producing and delivering anthelmintic molecules via outer membrane vesicles (OMVs) directly to the site of infection in the gastrointestinal tract following re-engraftment.

Methods: Transgenic native gut *E. coli* strains were generated by stable genomic integration of sequences encoding nematode-specific RNA- and protein-based therapeutics, including self-circularising RNA sponges designed to sequester essential nematode microRNAs. Noncoding RNAs known to impair nematode viability and the nematocidal protein Cry5B were included as controls and proof-of-concept effectors. Transgenic bacteria were characterized and production and extracellular release of the anthelmintic molecules were quantified by RT-qPCR. The non-parasitic model organism *Caenorhabditis elegans* and the parasitic nematode *Heligmosomoides polygyrus* were exposed to the engineered bacteria, and phenotypic outcomes were assessed.

Results: Engineered bacteria produced and secreted the intended therapeutic molecules, with extracellular localization observed in *E. coli* OMVs. Exposure of *C. elegans* to bacteria expressing different therapeutic products induced measurable changes in larval development and chemosensory behaviour compared to wildtype controls. Additionally, the transgenic bacteria were able to re-engraft in mice and persist for several weeks.

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Conclusions: These data indicate that transgenic native *E. coli* can serve as a platform for the production and delivery of anthelmintic therapeutics. While further validation is required, this approach shows promise as a novel strategy for nematode control.

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RESISTANCE AND DEVELOPMENT OF DRUGS AND VACCINES / PARASITE EXTRACELLULAR VESICLES (EVS) FOR THERAPEUTICS AND DIAGNOSTICS

PHAGE DISPLAY IDENTIFICATION OF PEPTIDES SPECIFIC TO EXTRACELLULAR VESICLES RELEASED BY LEISHMANIA AMAZONENSIS PROMASTIGOTES WITH DISTINCT VIRULENCE PROFILES

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Background and Aims: *Leishmania (Leishmania) amazonensis* releases extracellular vesicles (EVs), which participate in the host–pathogen relationship. Thus, EVs can be potential targets for therapeutic and vaccine strategies. This study aims to identify peptides targeting *L. (L.) amazonensis* EVs using Phage Display technology.

Methods: EVs were obtained from *L. (L.) amazonensis* promastigotes with distinct virulence profiles: virulent (EVs IVD-P) and attenuated (EVs LT-P). EVs were obtained using standard protocol and characterized by nanoparticle tracking analysis. EVs were used as a target to select phages from the library expressing 12 random amino acids, as follows: Biopanning 1 (B1) - both EVs as target; Biopanning 2 (B2) - phages binding EVs LT-P were eliminated before incubating with EVs IVD-P. Each biopanning was performed 5 times (rounds). The ligand phages were sequenced to identify the ligand sequences.

Results: Nanoparticle tracking analysis (NTA) revealed that EVs from IVD-P parasites exhibited a size of 188 nm, whereas EVs from LT-P parasites showed 184 nm. In B1, high phage titers were detected after round 1. A decrease in phage titers was detected in the next rounds, followed by a 16-fold reduction by round 5 (3.22×10^{11}pfu/mL). In B2, a reduction by approximately 100-fold relative to the phage input. In subsequent rounds, an increase was observed, achieving elevated titers in round 5 (4.23×10^{11} pfu/mL). The enrichment sequences were obtained and characterized.

Conclusions: Phage Display is efficient in identifying peptides with high affinity to molecules present in *Leishmania* EVs.

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RESISTANCE AND DEVELOPMENT OF DRUGS AND VACCINES / PARASITE EXTRACELLULAR VESICLES (EVS) FOR THERAPEUTICS AND DIAGNOSTICS

NAEGLERIA FOWLERI SECRETES EXTRACELLULAR VESICLES AFTER INTERACTION WITH NEUTROPHILS AND DURING THE INFECTION PROCESS IN THE MOUSE MODEL OF PAM

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Background and Aims: *Naegleria fowleri* causes Primary Amebic Meningoencephalitis (PAM), a fulminant infection of the central nervous system (CNS) with a mortality rate exceeding 95%. Furthermore, there are currently no timely diagnostic methods or vaccines available to treat or prevent this infection. In this regard, extracellular vesicles (EVs) released by parasites represent promising tools in the prevention and diagnosis of infectious diseases, since their specific molecular content allows their use as biomarkers, the design of epitopic vaccines, and the monitoring of infection progression.The objective of this work was to determine EVs secretion after the interaction of trophozoites with PMNs and the progression of PAM caused by *N. fowleri* in a mouse model.

Methods: In this work, extracellular vesicles were examined using fluorescence microscopy: in interactions of N. fowleri trophozoites with mouse bone marrow neutrophils or in the Balb/c mouse model of PAM infection.

Results: Our results show that *N. fowleri* releases a wide variety of EVs after interaction with mouse PMNs; furthermore, the content of these vesicles includes MP2CL5, Nfa1, and cathepsin B and that EVs release occurs both in the nasal cavity and olfactory bulb of the mouse. In addition, trophozoites were observed along with the presence secretion products in contact with the plexiform layers of the olfactory bulb.

Conclusions: These findings suggest that the plexiform layers function as critical points of pathological amplification, where the combined action of PMNs and EVs contributes decisively to the rapid progression of the disease in the murine model.

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RESISTANCE AND DEVELOPMENT OF DRUGS AND VACCINES / PARASITE EXTRACELLULAR VESICLES (EVS) FOR THERAPEUTICS AND DIAGNOSTICS

COMPOUNDS GPQF-815 AND GPQF-817 REGULATORY ROLE IN THE EVS FORMATION IN T. CRUZI

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Background and Aims: *Trypanosoma cruzi*, the etiologic agent of Chagas disease, releases EVs and containing surface molecules represented by glycoproteins anchored to the membrane via GPI (glycosylphosphatidylinositol). Our group previously showed that EVs released by infective *T. cruzi* forms modulate inflammatory responses in host cells and during *in vivo* infection. However, the biogenesis and mechanism of shedding of the epimastigotes EVs is poorly understood. Aiming to better understand EV release in this parasite, we studied the effects of GPQF-815 and GPQF-817, which are derivatives of N-acylhydrazones. GPQF-815 and 817 are synthetic compounds that do not yet have well-determined biological activities, but which have already shown anti-prion and antimicrobial actions**.**

Methods: Methods: Epimastigotes forms of the Y strain of *T. cruzi* were incubated with different concentrations of the compounds (250, 125 and 62.5, 31.25, 15.625 and 7.8125 µg/mL) during 2 h, at 28°C. Released EVs were characterized in size and concentration by NTA. In parallel, we measure the protein content.

Results: The results showed that released EVs concentration and size were not affected in any case. However, the amount of protein released with EVs increased for both compounds relative to the control, which indicated that the number of released particles is not dependent on the protein concentration.

Conclusions: Conclusion: Compounds GPQF-815 and 817 could have a possible regulatory role in the EVs formation in *T. cruzi* and consequently be used to control the inflammatory response during parasite infection. Support by FAPESP and CNPq

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SHIFT02-095

POSTER ON BOARD SHIFT 02
RESISTANCE AND DEVELOPMENT OF DRUGS AND VACCINES / TRANSLATIONAL RESEARCH IN AGRICULTURAL AND BIOMEDICINE

UNDERSTANDING ALBENDAZOLE LIMITED EFFICACY AGAINST TRICHURIS SPP.: DOSE DEPENDENT PATTERN OF GASTROINTESTINAL TISSUE DISTRIBUTION IN A PIG MODEL

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Background and Aims: Background: *Trichuris* spp. is frequently refractory to benzimidazole anthelmintic therapy in both humans and animals, attributable to insufficient drug exposure at the parasite's intestinal site of infection. There is only limited information concerning the albendazole (ABZ)/metabolites dose-exposure relationship within the gastrointestinal tract. **Aim**: This study evaluated the systemic and intestinal fluid/mucosa exposure of ABZ/metabolites following its oral administration in pigs (used as a translational animal model).

Methods: Two groups of pigs (n = 6) received ABZ orally at either 10 (ABZ_{10}) or 30 (ABZ_{30}) mg/kg. Blood samples were collected up-to 48 h post-treatment (p.t.). To investigate the gastrointestinal drug distribution pattern, two groups (n = 8) of animals were treated at the same doses. At 6, 10, 24, and 30 h p.t., two animals per group were euthanized and samples of blood, intestinal mucosa, and contents of the small and large intestine were collected. ABZ and its metabolites were quantified by HPLC.

Results: Albendazole sulfoxide (ABZSO) was the main analyte recovered in plasma. ABZSO plasma Cmax was significantly higher in the ABZ_{30} (5.27 µg/mL) than in the ABZ_{10} group (2.97 µg/mL). Similarly, systemic exposure (AUC) was higher in ABZ_{30} (78.9 ± 17 µg·h/mL) compared with ABZ_{10} (39.6 ± 10 µg·h/mL). This enhanced systemic exposure was reflected at the intestinal tract, although the drug/metabolites accumulation pattern differed among intestinal segments.

Conclusions: Increasing ABZ doses resulted in higher drug levels achieved in parasite-relevant intestinal tissues, which may account to enhance its efficacy against the *Trichuris* spp.

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SHIFT02-096

POSTER ON BOARD SHIFT 02
RESISTANCE AND DEVELOPMENT OF DRUGS AND VACCINES / TRANSLATIONAL RESEARCH IN AGRICULTURAL AND BIOMEDICINE

DESIGNING NANOFORMULATIONS FOR THE DELIVERY OF PEPTIDASE-SENSITIVE ANTIMICROBIAL AGAINST FISH BACTERIAL INFECTIONS

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Background and Aims: Anisaxin-2S (A-2S) is a cecropin antimicrobial peptide (AMP) isolated from the zoonotic nematodes Anisakis simplex and *A. pegreffii*. It shows high potency against Gram-negative bacteria from both marine and terrestrial environments, including multidrug-resistant strains, but exhibits low peptide retention in host serum. The latter is due to A-2S's sensitivity to blood peptidases, which reduces the peptide's availability for interaction with bacteria.

Methods: To improve its stability, we designed organic polymer nanocapsules aiming to protect A-2S within the host circulation and deliver a concentration sufficient to kill bacteria. Physical properties of the nanocapsules were tested, as well as the effect of formulation *in vitro* cell assays.

Results: The water-in-oil-in-water (WOW) emulsion was enveloped with chitosan to facilitate the attachment to the negatively charged bacterial cell wall. A-2S-filled nanocapsules were 180 nm on average, showing a dose- and time-dependent cytotoxic activity in human peripheral blood cells (HPBCs), but statistically significant only at the highest concentrations tested (>2.8 µg/ ml) at prolonged treatment (24-48 h). The formulation did not induce DNA strand breaks in HPBCs, suggesting a lack of genotoxicity. The release profile of the A-2S from nanocapsules showed that the maximum concentration (2.49 ng/ µl) was released between 8-12 h.

Conclusions: The calculated maximum concentration of released A-2S (0.72 µM) was just above the minimum inhibitory concentration for the Mediterranean *Vibrio* spp. and *Aeromonas* spp. we tested. While the proof of concept has been confirmed, the next step is to develop an optimised formulation with a higher initial concentration of A-2Sto be released over 24 h.

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POSTER ON BOARD SHIFT 02
RESISTANCE AND DEVELOPMENT OF DRUGS AND VACCINES / TRANSLATIONAL RESEARCH IN AGRICULTURAL AND BIOMEDICINE

BEYOND THE BENCH: THE IMPACT AND CLINICAL MANAGEMENT OF DRUG-RESISTANT COMPANION ANIMAL PARASITES IN THE SOUTHERN USA

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Background and Aims: The emergence of multiple anthelmintic drug resistant (MADR) parasitic infections in companion animals in the USA is a topic that should concern veterinary and human medical professionals worldwide. The prevalence of such cases is likely underreported, and with interstate animal relocations and the growing global pet trade, we will surely continue to see changes in the geographic range of these parasites.

Methods: This presentation will describe various cases of suspected drug-resistance in canine and feline parasites that have been submitted to the University of Georgia Diagnostic Parasitology Lab between 2020-2025, including information on patient signalment, clinical presentation, and treatment success.

Results: This case series includes hundreds of submissions for suspected drug-resistant *Ancylostoma caninum* and *Dipylidium caninum*.

Conclusions: Current management and prevention recommendations will be covered, along with the impacts these parasites can have on the human-animal bond.

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POSTER ON BOARD SHIFT 02
RESISTANCE AND DEVELOPMENT OF DRUGS AND VACCINES / TRANSLATIONAL RESEARCH IN AGRICULTURAL AND BIOMEDICINE

EVALUATION OF FIFTEEN ADJUVANTS FOR COMPATIBILITY WITH BEAUVERIA BASSIANA AND THEIR SYNERGISTIC EFFICACY AGAINST RHIPICEPHALUS MICROPLUS TICKS

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Background and Aims: The compatibility of adjuvants with entomopathogenic fungi (EPFs) is a critical determinant of biopesticide efficacy. This study evaluated the effects of fifteen commercial adjuvants on the viability and virulence of *Beauveria bassiana* isolate Bb-41, as a potential biological control agent against the cattle tick *Rhipicephalus microplus*.

Methods: This study evaluated the effects of fifteen commercial adjuvants on the viability and virulence of *Beauveria bassiana* isolate Bb-41, as a potential biological control agent against the cattle tick *Rhipicephalus microplus*. Using three concentrations (0.025 %, 0.05 %, and 0.1 %), colony-forming unit (CFU) counts were used to assess conidial viability at two inoculum levels (10^3 and 10^4 conidia·mL^{-1}). In addition larval mortality was measured at 10^6 conidia·mL^{-1}.

Results: The results demonstrated that adjuvant effects were both concentration- and dose-dependent. Adjuvants such as Designer®, Bond®, Summit Super®, Aquawet®, and Tronic® significantly inhibited fungal viability across all concentrations, likely due to the presence of fungitoxic components. In contrast, non-ionic surfactants like Tween® 80, Ballista®, Break-thru®, Silwet l-77®, and Tween® 20 maintained or enhanced conidial viability, particularly at 0.025 %. Virulence assays revealed that only a subset of adjuvants that promoted viability also enhanced pathogenicity. It was suggested that, Break-thru®, Tween® 20, and Ballista® enhanced conidial adhesion and penetration, as they improved larval mortality at 0.025 %.

Conclusions: These findings highlight the importance of comprehensive compatibility screening in EPFs formulation development and identify several promising adjuvants for field deployment against *R. microplus*.

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POSTER ON BOARD SHIFT 02
HOST IMMUNITY, MOLECULAR INTERACTIONS, AND MICROBIOME / INNATE AND ADAPTIVE IMMUNE RESPONSES TO PARASITES

ASSOCIATION OF BREED AND B-GLOBIN HAPLOTYPES WITH LOCAL MHC II+ IMMUNE RESPONSE IN LAMBS INFECTED WITH HAEMONCHUS CONTORTUS

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Background and Aims: Resistant or resilient animals exhibit a robust Th2 response to the blood-feeding *Haemonchus contortus* parasite. Under anemic conditions, these animals usually maintain homeostasis by compensatory mechanisms, including production of a high affinity for oxygen β-globin, which relies on their specific β-globin haplotypes (Hb-A or Hb-B). This study evaluated abomasal MHC II cells in White Dorper (DO), Texel (TX), and Santa Ines (SI) lambs infected with *H. contortus*, considering β-globin genotypes.

Methods: Thirty-two lambs (16 SI, 8 TX, 8 DO) were genotyped (AA=5, AB=7, BB=20) through blood DNA analysis. At six months of age, they were dewormed and infected with 4,000 *H. contortus* L3 larvae and slaughtered after 28 days. The abomasum tissue was formalin-fixed and paraffin-embedded for immunohistochemical analysis of MHC II+ cells. Positive cell quantification was performed on five randomly selected images taken at 400x magnification through QuPath software.

Results: A significant effect of breed was observed on MHC II+ counts (p=0.0028, DO: 272.7±21.8, SI: 1182.3±277.5, TX: 595.8±136.2), with SI showing the highest means. A trend toward differences among β-globin genotypes was observed, with AA animals presenting higher MHC II+ counts (p=0.0887, AA: 1836.4±821.6, AB: 671.4±171.7, and BB: 599.2±94.7). There was a significant difference for *H. contortus* burden among breeds (p=<0.0001, DO: 92.5±16.4, SI: 84.3±11.6, TX: 5721±2012), but it wasn't correlated with MHC II+ count.

Conclusions: The local MHC II+ cells are differentially regulated among the sheep breeds and seem to be also differentially regulated between β-globin haplotypes and consequently have an important role in the development of mechanisms against *H. contortus* infection.

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POSTER ON BOARD SHIFT 02
HOST IMMUNITY, MOLECULAR INTERACTIONS, AND MICROBIOME / INNATE AND ADAPTIVE IMMUNE RESPONSES TO PARASITES

HELMINTH-DERIVED IMMUNOMODULATION: SYPHACIA OBVELATA ANTIGENS RESTORE THE FOXP3/RORɣT BALANCE IN PBMCS OF IBD PATIENTS.

Abbas Amin[1], Seyyed Javad Seyyed-Tabaei[1], Niloofar Taghipour[1], Mohammad Rostami-Nejad[2], Foroogh Alborzi Avanaki[3], Reyhaneh Jafarshad[4], Zahra Arab-Mazar[5], Nariman Mosaffa[6]
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Background and Aims: Inflammatory Bowel Disease (IBD) is driven by a disrupted mucosal immune system, marked by a decreased ratio of FOXP3+ regulatory T cells to pro-inflammatory RORγt+ Th17 cells. The nematode *Syphacia obvelata* modulates immune tolerance and promotes Treg pathways. This study investigates the in vitro effects of *S. obvelata* antigens on the FOXP3/RORγt expression balance in peripheral blood mononuclear cells (PBMCs) from IBD patients.

Methods: PBMCs were isolated from six patients currently in the active phase of IBD. The cells were cultured and exposed to three formulations of *S. obvelata* adult worm antigens: excretory-secretory antigens (ES-Ag), somatic antigens (S-Ag), and a combination of both (ES/S-Ag) for an optimal duration of 24 hours. Following exposure, total RNA was extracted, and the gene expression levels of the master regulators FOXP3 and RORγt were quantified using RT-qPCR. Gene expression fold changes and FOXP3/RORγt ratios were subsequently calculated and compared to an untreated control group.

Results: Treatment with all three *S. obvelata* antigen formulations (ES-Ag, S-Ag, and ES/S-Ag) resulted in a statistically significant increase in FOXP3 gene expression. Concurrently, the expression of the pro-inflammatory marker RORγt was significantly downregulated following exposure to ES-Ag and the combined ES/S-Ag. Consequently, the FOXP3/RORγt gene expression ratio—a crucial indicator of inflammatory status—was significantly elevated after 24 hours of exposure across all antigen treatment groups when compared to the unexposed controls.

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Conclusions: *S. obvelata* antigens effectively shift the immune balance toward an anti-inflammatory state in human PBMCs by upregulating FOXP3 and suppressing RORγt. These findings offer a promising foundation for developing antigen-based therapeutic strategies for IBD.

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POSTER ON BOARD SHIFT 02
HOST IMMUNITY, MOLECULAR INTERACTIONS, AND MICROBIOME / INNATE AND ADAPTIVE IMMUNE RESPONSES TO PARASITES

LAMBS WITH HAEMONCHOSIS SHOW IMPROVED ANTIBODY RESPONSES TO HERBAL AND MINERAL SUPPLEMENTS.

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Background and Aims: *Haemonchus contortus* is a pathogenic gastrointestinal nematode of small ruminants, causing anaemia and production losses. Rising anthelmintic resistance highlights the need for sustainable parasite control, including improving host resistance through nutritional supplementation. This study evaluated the effects of dietary supplementation with medicinal plants (Herbmix) or organic selenium (Selplex) on immune responses, abomasal histopathology, and parasite burden in lambs infected with *H. contortus*.

Methods: Twenty-one lambs were infected and reinfected with ~11,000 third-stage larvae of *H. contortus* on days 0, 49, and 77. Animals were divided into three groups: Control (unsupplemented), Herbmix, and Selplex. Supplementation started on day 0 and continued throughout the study. Blood samples were taken for antibody analyses. On day 119, necropsies evaluated worm burden, IgA in abomasal mucus, and abomasal histopathology.

Results: Worm counts were lower in supplemented groups, with reductions of 51.3% (Herbmix) and 36.0% (Selplex) compared to the Control. IgG responses against adult worms varied significantly over time ($P < 0.001$). Serum and mucus IgA peaked in the Herbmix on day 15, while serum IgM was affected by treatment ($P = 0.048$) and time ($P < 0.001$). Histologically, Herbmix induced strong local inflammation with lymphoid aggregates, whereas Selplex increased eosinophils, globule leukocytes, and plasma cells.

Conclusions: Dietary supplementation with medicinal plants or organic selenium enhanced humoral and local immune responses and reduced parasite burden in *H. contortus*-infected lambs, suggesting their potential as natural alternatives for sustainable parasite control. The study was supported by funds from the Ministry of Education of the Slovak Republic and the SAS (VEGA 2/0007/25).

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HOST IMMUNITY, MOLECULAR INTERACTIONS, AND MICROBIOME / INNATE AND ADAPTIVE IMMUNE RESPONSES TO PARASITES

LIVING WITH PARASITES: EXPLORING TOLERANCE TO NEMATODES IN SHEEP

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Background and Aims: Gastrointestinal nematodes (GIN) cause significant productivity losses in the livestock industry. Individuals cope with infections in two ways: resistance, which is the reduction of parasite burden with an immune response, and tolerance, the maintenance of productivity despite increases in parasite burden. Whilst resistance has been well documented, less is known about the mechanisms underpinning tolerance. We need to establish that variation in tolerance to GIN exists and understand its associations with immunity before it can be exploited in sustainable parasite control.

Methods: Thirty lambs were trickle infected with L3 *Teladorsagia circumcincta* for 12 weeks and sampled weekly for faecal egg counts (FEC), bodyweight and blood. Blood samples were analysed for cytokines (IL-4, IL-10, IL-17a and IFNγ) and GIN-specific antibodies (IgA, IgE, IgG, IgG1 and IgG2). Random regression models were used to estimated tolerance as the slope of weight on FEC and quantified between-individual variation. Bivariate random regression models were used to estimate the covariance between tolerance and immune parameters.

Results: Weight was negatively associated with FEC, but this relationship varied by individual, suggesting lambs varied in their tolerance of GIN infection. IgG, IgE, and IFNγ were negatively associated with FEC, while IL-10 was positively associated with FEC. Immune parameters were weakly associated with individual variation in tolerance.

Conclusions: Individuals varied in tolerance to *Teladorsagia circumcincta*, suggesting a possible genetic basis that could be exploited by breeding, but associations between immune measures and individual-specific tolerance slopes were generally weak.

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HOST IMMUNITY, MOLECULAR INTERACTIONS, AND MICROBIOME / INNATE AND ADAPTIVE IMMUNE RESPONSES TO PARASITES

THE BITES BEFORE THE INFECTION: HOW EXPOSURE TO UNINFECTED SANDFLY BITES SHAPES SKIN HYPERSENSITIVITY

Myriam Beaulieu[1,2], Leon Melo[1,2], Camila Gaio[1,2], Matheus Carneiro[1,2], Gabi Gee[1,2], Rohit Arora[3], Nicole Rosin[3], Elodie Labit[3], Audrey Simon[1,2], Claudio Meneses[4], Jeff Biernaskie[3,5,6], Chukwunonso Nzelu[1,2], Nathan Peters[1,2]
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Background and Aims: In regions highly endemic for cutaneous leishmaniasis, only about 4% of sandflies are infected with *Leishmania*, implying hosts experience years of repeated uninfected bites before parasite exposure. Despite 80% of the global population at risk of insect-borne disease, vaccines that perform well in laboratory settings (including against leishmaniasis) often underperform in bite-exposed populations. This raises a key question: how does a lifetime of insect bites shape skin immunity, including vaccine-mediated immunity?

Methods: Mice will be exposed to uninfected sandflies to model acute, short-term, and long-term biting. Bite-induced delayed-type hypersensitivity (DTH) will be quantified by ear thickness. Local and systemic immune responses will be assessed in exposed and contralateral ears, blood, and spleen using flow cytometry, gene expression analysis, and serum antibody profiling. The roles of eosinophils and Innate Lymphoid Cells (ILC) will be tested using cell-deficient mouse models and serum-based approaches.

Results: Prior observations show repeated sandfly exposure drives robust ear DTH consistent with type-IVb (eosinophil- and Th2-associated) hypersensitivity. We hypothesize that contralateral challenge after unilateral exposure will elicit DTH responses in previously unexposed skin. Immune signatures across skin, blood, and spleen will support a recallable state extending beyond the initial bite site, with cell-deficient models defining contributions of eosinophils and ILCs.

Conclusions: This work may inform strategies to improve vaccine efficacy by targeting host responses to bites rather than the pathogen itself. Importantly, it is grounded in the lived reality of endemic

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populations, where sandfly bites are an everyday exposure and infection is the exception rather than the rule.

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HOST IMMUNITY, MOLECULAR INTERACTIONS, AND MICROBIOME / INNATE AND ADAPTIVE IMMUNE RESPONSES TO PARASITES

MAPPING THE EXPOSOME OF ZIMBABWEAN PRESCHOOL-AGED CHILDREN (PSAC) USING A NOVEL PEPTIDE MICROARRAY APPROACH

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Background and Aims: Prior exposures shape host immune responses to unrelated immunogens. Both immunomodulatory helminths and anthelminthic treatment have been shown to affect host responses to unrelated immunogens. With the widespread rollout of mass drug administration (MDA) across Africa, many populations have now received at least one round of deworming treatment. This raises critical questions about the nature, persistence, and significance of treatment-related immunological shifts in endemic settings with repeated exposures. In Africa, which bears a disproportionate share of the global helminth burden, there is growing interest in how these factors shape immune responses. Thus, the aim of this proof-of-concept study was to survey and define the exposome, or the collection of immunogens a host has been exposed to, of a helminth-endemic rural Zimbabwean population using a novel peptide microarray approach.

Methods: Using a bespoke peptide microarray, we determined antigen-specific serum IgM and IgG reactivities of 20 children under five years of age against a variety of B-cell epitopes from immunogens of interest to Zimbabwe, including pathogens, vaccines, allergens, autoantigens, and a variety of parasites.

Results: Data from this microarray revealed broad, heterogenous immunogen recognition early in life. The most frequently recognized exposome constituted viruses, bacteria, fungi, autoantigens, and both micro- and macro-parasites, including helminths, trypanosomes, and plasmodium species.

Conclusions: This diverse early-life infectome signature may contribute to differential susceptibility to infection severity, vaccine responsiveness, or immune-mediated pathology. Results from the present discovery study provide baseline proof of concept reference values for helminth-endemic under five immune ecologies and should be considered in future public health planning.

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HOST IMMUNITY, MOLECULAR INTERACTIONS, AND MICROBIOME / INNATE AND ADAPTIVE IMMUNE RESPONSES TO PARASITES

ROLE OF IL-33 IN ALVEOLAR ECHINOCOCCOSIS

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Background and Aims: Alveolar echinococcosis (AE) is a zoonotic disease caused by *Echinococcus multilocularis*. AE disease outcome is closely linked to host immune responses, with inflammation resulting in parasite remission and regulatory responses promoting parasite growth. Interleukin 33 (IL-33) is an alarmin released from damaged cells and has context-dependent effects during parasitic infections, ranging from protective to disease pathology exacerbation. This project aims to characterise the role of the IL-33 pathway in AE liver pathology and disease progression.

Methods: A secondary murine model of *E. multilocularis* infection has been used for *in vivo* quantification and identification of IL-33 and ST2-expressing cells. *In vitro* experiments were conducted using *E. multilocularis*-derived products to assess IL-33 activity induced by the parasite in fibroblasts. Additionally, mass spectrometry approaches have been utilised to identify specific parasite-derived molecules responsible for IL-33 expression and secretion in the host.

Results: Post *E. multilocularis* infection, there is a significant upregulation of the IL-33/ST2 pathway in multiple tissues. Structural liver cells were the main sources of IL-33 post infection. An overall increase in immune cell populations was observed, yet we uncovered a distinct lymphocyte subset that specifically upregulated ST2 expression upon infection. *In vitro* addition of *E. multilocularis*-derived products triggered IL-33 expression in a fibroblast cell line. Further analyses are ongoing to identify and characterise potential IL-33-inducing compounds from the parasite.

Conclusions: Our preliminary data indicate that the secretory component of the parasite promotes IL-33 expression in structural cells, which preferentially activated a specific lymphocyte population, which may contribute to immune regulation and parasite persistence.

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ANISAKIS-SPECIFIC IGE PRODUCTION IS ASSOCIATED WITH GASTRIC BACTERIA

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Background and Aims: There are four clinical manifestations of anisakiasis caused by Anisakidae: gastric anisakiasis, intestinal anisakiasis, extra-gastric anisakiasis, and anisakis allergy. Of these, anisakis allergy is most dangerous, because not only live anisakis but also dead anisakis can cause life-threatening anaphylactic shock including dyspnea within minutes to hours. Therefore, identification of allergens and elucidation of immune responses in anisakis allergy are urgent.

Methods: In this study, to evaluate IgE responses specific for anisakis, we established a mouse anisakis model by infecting mice with live anisakis using a gastric tube after intraperitoneal immunization with excretory and secretory (ES) or crude antigens (CA) prepared from anisakis collected from mackerels along the pacific coast.

Results: Unimmunized mice showed only a slight increase in specific IgE, whereas the immunized mice showed a marked increase in specific IgE after anisakis infection. This effect was stronger in mice immunized with ES than with CA. Furthermore, analysis of the gastric microbiota showed that more than 90% were *Lactobacillus* species in ES-immunized but not CA-immunized mice.

Conclusions: We are currently conducting a comprehensive analysis of the detecting in the components of ES and CA using LC/MS, and are analyzing which components in ES increase *Lactobacillus*.

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THYMIC ATROPHY DEVELOPMENT IN A NON-LETHAL PLASMODIUM INFECTION.

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Background and Aims: The thymus is a vital organ for T-cell development that undergoes significant changes during malaria infection, including atrophy and disrupted structure, which weaken immune responses.

Methods: This study examines the mechanisms behind thymic atrophy during *Plasmodium chabaudi chabaudi* AS infection, focusing on cellular and molecular changes across different parasitemia stages.

Results: Our results show that thymic atrophy is marked by a substantial decrease in thymus weight and cellularity, especially at 20–30% parasitemia levels. A significant loss of DP and SP thymocytes mainly causes this atrophy. Histological analysis revealed notable cortical shrinkage and DNA fragmentation in thymic cells, indicating increased apoptosis. Elevated serum cytokines and chemokines, including IFN-γ, TNF-α, IL-6, and CXCL9, were observed throughout the infection, with higher levels correlating with the degree of thymic atrophy. Also, early release of thymocytes into peripheral tissues, especially the spleen, worsened the decrease in thymic cellularity. However, after parasitemia resolved, the thymus recovered, restoring its structure and thymocyte populations.

Conclusions: These results highlight the complex interaction between parasite-induced inflammation and immune cell behavior, suggesting that even though *P. c. chabaudi* AS infection is not lethal, a prolonged parasitic burden can cause severe thymic dysfunction, possibly impairing immune system function.

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POSTER ON BOARD SHIFT 02
HOST IMMUNITY, MOLECULAR INTERACTIONS, AND MICROBIOME / INNATE AND ADAPTIVE IMMUNE RESPONSES TO PARASITES

SEROLOGICAL ANALYSIS OF ANTIGENS ASSOCIATED WITH PROTECTION FROM SCHISTOSOMA MANSONI INFECTION

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Background and Aims: Schistosomiasis is a major cause of morbidity and a significant socio-economic burden in endemic countries. In the absence of effective vaccines, prophylactic measures rely on the mass administration of praziquantel in populations at risk of infection. Unfortunately, high re-infection rates highlight the need for a vaccine conferring long-term protection. Animal models such as mice (after vaccination with radiation-attenuated cercariae) and rhesus macaques (who can self-cure) have shown antibody-mediated resistance to Schistosoma infection, likely targeting the somule, the larval form of the parasite. Yet, the identity of the antigens targetted by these protective antibodies remain elusive.

Methods: Using a library of recombinant extracellular Schistosoma mansoni somule proteins produced in mammalian cells, we have tested murine sera with different levels of protection: naïve (permissive) mice infected with wild-type cercariae, and mice vaccinated once or three times with irradiated cercariae before challenge with wild-type cercariae.

Results: Vaccinated group showed up to 75% protection as judged by worm recovery and liver egg burden. A significant increase in antibody reactivity against discrete antigens was observed in immunised groups, with a marked isotype shift from mostly IgG1 in naive animals to IgG2 in protected groups.

Conclusions: We aim to investigate whether immunisation with this set of antigens can recapitulate the protection observed in animals immunised with irradiated cercariae. This could lead to the development of new vaccine strategies.

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HOST IMMUNITY, MOLECULAR INTERACTIONS, AND MICROBIOME / INNATE AND ADAPTIVE IMMUNE RESPONSES TO PARASITES

CYTOKINE PROFILES OF PREGNANT WOMEN WITH ACUTE AND CHRONIC TOXOPLASMOSIS IN SOUTHEASTERN BRAZIL

Luiz De Mattos, Jessica Fernandes Da Silva Toscano, Fernando Henrique Murata, Danilo Donizete-Da-Silva, Maria Grigoli Pessarello Afonso, Fernanda Evangelista, Priscila Santana, Lygia Junqueira Franco Spegiorin, Lilian Castiglioni, Cinara Cássia Brandão, Christiane Maria Ayo
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Background and Aims: The main concern regarding gestational toxoplasmosis is vertical transmission of *Toxoplasma gondii*, which can cause serious fetal and neonatal damage. Modifications in immune regulation and cytokine expression at the maternal–fetal interface may enable the parasite to evade host defenses, consequently increasing the likelihood of congenital transmission and maternal injury due to infection. This study aimed to assess the immunological profile of cytokine expression in pregnant women from southeastern Brazil.

Methods: The investigation included 50 pregnant women diagnosed with chronic infection, 18 with acute infection, and 35 presenting negative serology for anti-*T. gondii* IgG and IgM. Of the total number of pregnant women with acute infection, 6 cases of vertical transmission were confirmed. IL-6, IL-8, IFN-γ, TNF-α, IL-21, IL-17, IL-23, IL-4, IL-10, and IL-13 were quantified in plasma samples using ELISA or multiplex assays. Student's t-test (for parametric data) or Mann-Whitney/Kruskal-Wallis tests (for non-parametric data) were applied to compare means or medians. A p-value<0.05 was considered statistically significant.

Results: The group of patients with acute toxoplasmosis exhibited lower plasma levels of IL-10 compared with both the group of women with chronic toxoplasmosis ($p<0.01$) and the group of uninfected women ($p<0.05$). Furthermore, pregnant women in whom transmission occurred showed higher levels of IL-17 compared with those in whom transmission did not occur ($p<0.05$).

Conclusions: The differential cytokine expression patterns suggest that reduced IL-10 and elevated IL-17 production may play a crucial role in compromising the maternal–fetal immune balance, thereby increasing the risk of fetal infection by Toxoplasma gondii.

"Acknowledgements: FAPESP 2020/14783-0; CAPES; INCT-CNPq"

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VACCINATION AND O. OSTERTAGI CHALLENGE REMODEL BOVINE T CELL LANDSCAPE, UNMASKING EXPANDING LINGO2+ CD8 T CELL SUBSET

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Background and Aims: *Ostertagia ostertagi* is a ubiquitous gastro-intestinal nematode (GIN) in grazing cattle causing diarrhoea, weight loss, and economic losses. Although vaccine development is a priority for sustainable control, protective immunity remains incompletely understood. In mice, helminth-induced IL-4 responses have been shown to drive the expansion of virtual memory T-cells (T_{vm}), potentially enhancing protection against unrelated infections. However, whether T_{vm} exist and play a similar role across species, including in cattle, remains unclear.

Methods: In a vaccine trial, three groups of four 4-5-month-old calves were vaccinated with two forms of adjuvanted activation-associated protein (ASP), either native (n-OoASP) or recombinant (r-OoASP), before being challenged via a trickle infection of 25,000 third-stage *O. ostertagi* larvae. Calves were euthanized 55 days after infection. PBMCs were collected at baseline, five days post-vaccination, and at endpoint. PBMCs were processed for single-cell RNA sequencing using SPLiT-seq and a total of 784,178 cells passed quality controls.

Results: Initial analysis on T-cells identified WC1.3$^+$PIK3AP1$^+$ γδ-T-cells representing 42.4% of all T lymphocytes, revealing a γδ-T-cell dominance. Vaccination remodelled CD8$^+$ T-cell states by contracting TOX$^+$ exhaustion (cluster 6) and NK-like cluster 12 (TRPM3$^+$GPC5$^+$KLRB1$^+$, calcium/NK-lysin axis). Notably, a rare LINGO2$^+$ CD8$^+$ T-cell subset, absent from public bovine immune datasets, was identified. This cluster was present at baseline but expanded after *O. ostertagi* infection, exhibiting a chronically activated, non-exhausted phenotype. This might parallel murine TFF3-LINGO2-dependent restraint of helminth-induced Th1 cell proliferation.

Conclusions: Initial analysis of this bovine T-cell atlas reveals γδ-dominance, vaccine-induced remodelling and identifies previously unrecognized LINGO2$^+$ CD8$^+$ T-cells.

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CYTOTOXIC RESPONSE OF CD8+ T CELLS STIMULATED BY TOXOPLASMA GONDII PEPTIDES IN COLOMBIAN INDIVIDUALS WITH OCULAR TOXOPLASMOSIS

Danilo Donizete-Da-Silva[1], Monica Vargas-Montes[2], Elizabeth Torres-Morales[2], Maria Antonia Restrepo-Duque[2], Jocelyn Alexandra-Arredondo[2], Sebastián Serna-Rivera[2], Cinara Cássia Brandão[1], Luiz De Mattos[1], Jorge Gomez-Marin[2], Christiane Maria Ayo[1]
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Background and Aims: Inflammation of the retina is a consequence of *Toxoplasma gondii* infection, resulting in ocular toxoplasmosis (OT). The host immune response involves immunogenetic and cellular factors during infection. The aim was to investigate the cytotoxic immune response mediated by $CD8^+$ T cells stimulated by *T. gondii* peptides binding to HLA class I molecules in Colombian individuals with OT.

Methods: The study included five individuals with inactive OT (IgG^+/IgM^-) and six seronegative individuals, all evaluated by fundus examination. DNA was extracted, and the *HLA-A*02* and *HLA-A*24* allelic groups were identified by PCR. PBMCs were stimulated for six hours with P1 and P8 peptides, with positive and negative controls included. The cytotoxic response was assessed by CD107a expression using flow cytometry in live $CD3^+CD8^+$ lymphocytes, stratified into memory and naïve subsets.

Results: HLA typing demonstrated a high frequency of the *HLA-A*02* and *HLA-A*24* alleles (73% and 91%, respectively). The P1 (p=0.0264) and P8 (p=0.0104) peptides induced significant activation of cytotoxic responses in total $CD8^+$ T lymphocytes from individuals with OT compared to the negative control. Increased activation was observed in memory $CD8^+$ T cells upon stimulation with P1 and P8 (p=0.0312), and in naïve $CD8^+$ T cells upon stimulation with P1 (p=0.0078) and P8 (p=0.0045). Individuals without OT showed activation only with the positive control (p=0.0156).

Conclusions: *T. gondii* peptides P1 and P8 induced a cytotoxic response and demonstrated immunogenic potential, suggesting that they are promising candidates for a multi-epitope vaccine against toxoplasmosis.

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THE IMPACT OF PROGENITOR-LIKE CD4 T CELLS IN REGULATING THE BONE MARROW ENVIRONMENT OF LEISHMANIA DONOVANI INFECTED MICE

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Background and Aims: Visceral Leishmaniasis (VL) represents the severe and fatal form of leishmaniasis. Persistent infection is largely driven by IL-10, predominantly produced by type 1 regulatory T cells (Tr1). In contrast, IFNγ produced by Th1 cells provide protection. However, sustained IFNγ signaling also triggers emergency hematopoiesis, diverting myeloid differentiation toward a regulatory monocyte phenotype. These monocytes are highly permissive to *Leishmania* replication and greatly contribute to disease exacerbation.

Methods: We recently characterized a subset of $CD4^+$ T cells with progenitor-like capacity, termed $TLCP_{lo/int}$, which expands during chronic VL. These cells give rise to multiple $CD4^+$ Tcell effector and regulatory lineages and display protective effects upon adoptive transfer. In this study, we examine the role of $TLCP_{lo/int}$ cells in shaping parasite driven emergency hematopoiesis and the development of regulatory monocytes in the bone marrow of *L. donovani* infected mice.

Results: Following adoptive transfer of $TLCP_{lo/int}$ cells into *Rag1* $^{-/-}$ mice, *L. donovani* infection drove a preferential differentiation of these cells into Tr1 cells within both the spleen and bone marrow. Moreover, the spleens of mice receiving $TLCP_{lo/int}$ cells were markedly smaller than those given naïve CD4 T cells, suggesting altered regulation of bone-marrow hematopoietic output. To investigate this further, we assessed hematopoiesis and observed that $TLCP_{lo/int}$ transfer led to a pronounced delay in the onset of emergency hematopoiesis, accompanied by reduced frequencies of regulatory phenotype monocytes. Future transcriptome analysis will help dissect pathways involved in regulating emergency myelopoiesis during experimental VL.

Conclusions: Our results underscore the critical role of myelopoiesis in shaping disease outcome in experimental VL.

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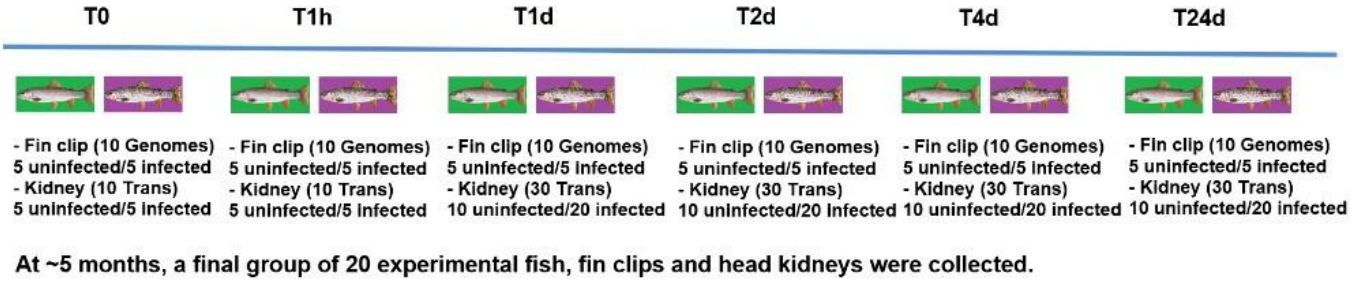
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HOST IMMUNITY, MOLECULAR INTERACTIONS, AND MICROBIOME / INNATE AND ADAPTIVE IMMUNE RESPONSES TO PARASITES

GENOMIC AND TRANSCRIPTOMIC ANALYSIS OF THE WHIRLING DISEASE-RESISTANT GUNNISON RIVER RAINBOW TROUT

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Background and Aims: Whirling disease is a debilitating disease of Rainbow Trout caused by *Myxobolus cerebralis*. The parasite invasion causes severe damage and high mortality. Here, we aim to elucidate the survival mechanisms of a Whirling disease–resistant Rainbow Trout strain developed in the Gunnison River, Colorado.

Methods: We analyzed the genomes and transcriptomes of this resistant strain at different time points after challenge with *M. cerebralis*.



Results: Signature selection analysis revealed several regions across the genome under selection, with the highest density on chromosome 23. Several genes under selection are associated with neuron differentiation and nervous system development. Also, several immuno-genes were under selection, related to the innate and adaptive immune response. The transcriptomic analysis revealed that the Gunnison River Rainbow Trout (GRR) develops a comprehensive immune response after exposure to *M. cerebralis*. This is supported by the significant enrichment of specific immune response pathways, including differentiation and activation of B-cells and T-cells. Also, more than half

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of the top 20 upregulated immune genes are components of the complement pathway. Notably, CD209 (DC-SIGN), a critical gene involved in antigen recognition and dendritic cell function, is among the most highly upregulated genes. The results also indicate the presence of a specific region on chromosome 9 in this strain, previously linked to resistance to this disease.

Conclusions: These results suggest that certain immune pathways are likely to participate in building the GRR's early, mid, and long-term immune response against *M. cerebralis*, while other pathways related to nervous system development help juvenile fish survive the effects of Whirling disease.

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ALTERED MACROPHAGE IMMUNE RESPONSES TO EXTRACELLULAR VESICLES FROM ANTIMONY-RESISTANT LEISHMANIA INFANTUM

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Background and Aims: The emergence of drug-resistant *Leishmania* strains poses a major challenge to leishmaniasis control and underscores the need to understand non-genetic mechanisms contributing to treatment failure. Parasite-derived extracellular vesicles (EVs) are key mediators of host–pathogen communication and can modulate macrophage immune responses. However, functional differences between EVs from antimony-resistant (drug-resistant, DR) and drug-sensitive (DS) *Leishmania infantum* strains remain poorly defined. This study aims to characterize how DR- and DS-derived EVs differentially influence macrophage immune activation.

Methods: EVs were isolated from DR and DS *L. infantum* strains and used to stimulate human THP-1–derived macrophages and mouse bone marrow–derived macrophages (BMDMs) in the absence of live parasites. Transcriptional responses were assessed at 6 and 24 hours post-exposure using transcriptome profiling (RNA-seq), followed by targeted quantitative PCR validation of key inflammatory and regulatory genes.

Results: Macrophage responses were time-dependent, with stronger transcriptional activation observed at 24 hours compared to 6 hours. EVs derived from drug-resistant parasites induced elevated expression of pro-inflammatory and interferon-associated genes, including IL6, TNF, IRF1, and STAT1, consistent with enhanced innate immune activation. In contrast, EVs from drug-sensitive strains preferentially upregulated the anti-inflammatory cytokine IL10, suggesting a more regulatory macrophage phenotype.

Conclusions: These findings indicate that EVs from drug-resistant *L. infantum* strains elicit a distinct, more pro-inflammatory macrophage transcriptional profile compared to EVs from drug-sensitive strains. This suggests an EV-mediated, non-genetic mechanism contributing to altered host immune responses and parasite persistence under drug pressure. Furthermore, parasite-derived EVs emerge as promising biomarkers of drug resistance and potential targets for host-directed therapeutic strategies in leishmaniasis.

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EXPRESSION OF INDUCIBLE NITRIC OXIDE SYNTHASE (INOS) AND ARGINASE-1 IS ASSOCIATED WITH INFECTION OF HUMAN LUNG TISSUE BY TOXOPLASMA GONDII

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University of Salford, Biomedical Research And Innovation Centre, Salford, United Kingdom

Background and Aims: *Toxoplasma gondii* infects around one third of the global human population and all warm-blooded animals. Previous studies in laboratory animals have shown that key enzymes associated with the immune system, Inducible Nitric Oxide Synthase (iNOS) and Arginase-1, may play a role in controlling *T. gondii* infections. However, their role in naturally infected animals and, particularly, in humans is less well understood. In a recent study of lung cancer patients in the UK, we observed a high prevalence of infection of 100% (95%CI [93.9, 100] (n=72). The aims of this study were to investigate the spatial distribution of *T. gondii* infection in lung tissues from infected subjects in relation to the expression of iNOS and Arginase-1.

Methods: Immunofluoresence assays (IFA) were conducted on tissue sections of human lung tissue (n=25) using antibodies against *T. gondii*, iNOS and Arginase-1.

Results: There were clear areas of the human lung tissue that were preferred sites of infection by the parasite. Significant associations in spatial distribution were found between the parasite and both iNOS and Arginase. Furthermore, expression of both of these proteins was associated with the cyst wall in dormant stages.

Conclusions: This suggests that both enzymes are expressed in response to infection with *T. gondii* in a naturally infected human cohort. Furthermore, it suggests the involvement of these enzymes in the regulation of infection and raises questions about the mechanism of enzyme induction by the parasite.

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HOST-DIRECTED PROPHYLAXIS FOR CANINE LEISHMANIOSIS: NUCLEOTIDES AND AHCC LIMIT EARLY LEISHMANIA INFANTUM ESTABLISHMENT IN VITRO

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Background and Aims: Canine leishmaniosis (CanL) caused by *Leishmania infantum* is a major zoonotic threat, with dogs as the main domestic reservoir. Control still depends largely on chemotherapy, which is limited by toxicity, lengthy treatment, and emerging resistances, underscoring the need for preventive approaches that block early parasite establishment. Yeast-derived nucleotides (NU) and AHCC, an extract of *Lentinula edodes* mycelia (LE), have shown clinical benefits in both sick and clinically healthy *Leishmania*-infected dogs. Since infection depends on macrophage permissiveness, we evaluated the host-directed activity of these compounds in therapeutic and preventive *in vitro* settings.

Methods: Murine bone marrow–derived macrophages were infected with *L. infantum* and treated with NU, LE or a 1.88:1 NU-LE combination (therapeutic arm), or pre-exposed for 24 h to the same compounds before infection (preventive arm). Intracellular parasite burden was quantified 48 h post-infection *versus* untreated controls. Direct leishmanicidal activity was also assessed.

Results: The compounds showed no direct leishmanicidal activity, supporting a host-mediated mode of action. All compounds reduced intracellular infection in both therapeutic and preventive experiments, with strongest effects in prevention: NU nearly abolished intracellular parasites, while LE and NU-LE significantly lowered infection rates and amastigote loads.

Conclusions: NU, LE, and NU–LE act as host-directed anti-*Leishmania* agents, with their strongest effects in preconditioned macrophages, indicating potential prophylactic value for healthy dogs in CanL-endemic areas. **Acknowledgements:** Work in the CFP-Lab was supported by NSERC Discovery Grant RGPIN-2024-04103 and CFI grants 37324 and 38858. CFP holds the Canada Research Chair in Sustainable Integrative Parasitology (CRC-2024-00026).

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GAMMA DELTA T CELLS REGULATE TH1-IMMUNE RESPONSE OF PLASMODIUM-SPECIFIC CD4+ T CELLS IN MALARIA

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Background and Aims: Malaria remains a major global health burden, and effective cellular immunity is essential for controlling blood-stage Plasmodium infection. The aim of this study was to elucidate how γδ T cells orchestrate cDC1-mediated Th1 priming during the infection, with a focus on reciprocal cellular activation, chemokine-dependent cell positioning, and the initiation of Th1 differentiation within the splenic white pulp.

Methods: To evaluate the response of *Plasmodium*-specific $CD4^+$ T cells during *Plasmodium*infection, we utilized the *Plasmodium*-specific T cell receptor (TCR)-transgenic (PbT-II) mouse line

Results: Our study revealed that γδ T cells promote the complete maturation and production of interleukin-12 and CXCR3-ligands specifically in type 1 cDCs (cDC1), with minimal impact on cDC2 and monocyte derived DCs (Mo-DCs). During the initial infection phase, γδ T cell activation and temporal accumulation in the splenic white pulp, alongside cDC1, occur via CCR7-signaling. Furthermore, cDC1/γδ T cell interactions in the white pulp are amplified through CXCR3 signaling in γδ T cells, optimizing Th1 cell priming by cDC1. We also demonstrated how transitional Th1 cells arise in the white pulp before establishing their presence in the red pulp as fully differentiated Th1 cells. Additionally, we elucidate the reciprocal activation between γδ T cells and cDC1s.

Conclusions: These findings suggest that Th1 cell priming is orchestrated by this reciprocal activation in the splenic white pulp during the early phase of blood-stage *Plasmodium* infection.

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MACROPHAGE POLARIZATION AND METABOLIC REPROGRAMMING IN EX-VIVO AND IN-VIVO MODELS OF VISCERAL LEISHMANIASIS

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Background and Aims: Metabolic adaptation to the surrounding microenvironment instructs activation status of macrophages. *Leishmania donovani*, the causative agent for visceral leishmaniasis (VL), induces M2 polarization of macrophages, but information regarding metabolic reprogramming is limited. Accordingly, this study correlated the status of metabolism-regulatory genes with the macrophage phenotype in *ex-vivo* and *in-vivo* models of VL.

Methods: In *L. donovani*-infected BALB/c mice and peritoneal macrophages, parasite burden was quantified by kDNA-qPCR and *A2* respectively. Splenic transcriptomic profiling was performed using poly-A RNA-seq along with expression of M2 and M1 markers. Macrophage metabolic genes associated with (i) glycolysis, (ii) mitochondrial respiration and (iii) AMPK signalling pathway were assessed by qPCR. The status of cytokines was measured by ELISA.

Results: Transcriptomic analysis of *L. donovani*-infected spleen showed an upregulation of mitochondrial metabolic genes and was substantiated by qPCR. In infected splenic and hepatic tissues, the glycolytic genes was downregulated, whereas mitochondrial metabolic genes were enhanced. Similarly, in infected macrophages, mitochondrial metabolic genes were upregulated, whereas glycolytic genes remained unchanged There was an elevated expression of *Ampk* and *Pgc1α*, with a concomitant downregulation of *Hif1α*. M2-associated cytokines (IL-10, TGF-β, IL-13) were elevated in infected mice. TNF-α was elevated in both models, whereas IL-1β, IL-12, and IFN-γ remained unchanged.

Conclusions: *Leishmania* infection promoted enhanced mitochondrial metabolism concomitant with M2 macrophage polarization. The anti-inflammatory cytokine milieu, upregulation of mitochondrial respiratory genes, and activation of the AMPK–PGC1α axis, indicated that mitochondrial metabolic reprogramming supported parasite survival and disease progression thus identifying potential metabolic targets for therapeutic intervention.

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DECIPHERING MACROPHAGE HETEROGENEITY WITH MASS CYTOMETRY IMMUNOPHENOTYPING IN A MOUSE MODEL OF ALVEOLAR ECHINOCOCCOSIS

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Background and Aims: *Echinococcus multilocularis* is a tapeworm whose larvae can infect the liver and cause a severe disease known as alveolar echinococcosis (AE). In AE, periparasitic granuloma formation is a key pathological feature, characterized by infiltration of immune cells, including macrophages. Macrophages are suspected to play dual role during the course of infection, however, their clear role remains poorly defined. Using complementary approaches we described the heterogeneity of macrophage populations during AE.

Methods: A murine peritoneal AE model was used to characterize macrophage populations by mass cytometry immunophenotyping, integrated with RTqPCR and immunofluorescence imaging. In parallel, we evaluated the effect of *E. multilocularis* antigen on human macrophages derived from Peripheral Blood Mononuclear Cells using RTqPCR and multiplex cytokine dosage, in order to compare to mouse findings.

Results: Mass cytometry immunophenotyping revealed F4/80^{Hi}CD64^{-} macrophages subtypes at early stages of infection, some expressing atypical markers CD31 and DC-SIGN. As infection progressed, these subtypes were replaced by F4/80$^{Lo/int}$CD64^{+} macrophages with classical M1 (Ly6C^{+}CD206^{-}) or M2 (Ly6C^{-}CD206^{+}) profile. Integrating these data with RTqPCR and immunofluorescence imaging suggested that early recruited macrophages are transforming into epithelioid cells (expression of E-cadherin) while macrophages present at late stages are transforming into fibrocyte-like cells (expression of COL1A1 and FN1). Antigen stimulation favored a pro-inflammatory macrophage profile rather than M2 like, epithelioid or fibrocytic phenotypes, but also stimulated production of VEGFA and Galectin-3.

Conclusions: These results highlight the heterogeneity of macrophages during AE and suggest that stage specific macrophage populations can affect disease progression.However, the precise role of these populations remain to be investigated.

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RESVERATROL AND MINOCYCLINE AMELIORATE NEUROINFLAMMATION AND COGNITIVE IMPAIRMENT INDUCED BY ANGIOSTRONGYLUS CANTONENSIS INFECTION VIA MODULATING MICROGLIAL POLARIZATION AND THE TREML2–NLRP3 PATHWAY

Wen-Zhen Lee
Chang Gung University, Department Of Medicine, Taoyuan County, Taiwan

Background and Aims: *Angiostrongylus cantonensis* infection induces neuroinflammation and cognitive dysfunction by infiltrating the central nervous system, yet the immune mechanisms linking microglial polarization to neuronal damage remain unclear. This study aimed to evaluate the cognitive, synaptic, and immunological alterations following infection and assess the therapeutic efficacy of albendazole combined with anti-inflammatory agents including Minocycline and Resveratrol.

Methods: A murine model was established using C57BL/6 mice infected with 50 third-stage larvae via esophageal intubation. Treatment with albendazole alone or combined with resveratrol or minocycline commenced on day 7 post-infection. Assessments included Morris Water Maze, Novel Object Recognition, and Y-maze for cognitive function; Golgi staining for synaptic density; and Western blotting/ELISA for analyzing microglial markers (iNOS, Arginase-1), inflammatroy-related markers (TREML2, NLRP3) and peripheral immunity.

Results: Behavioral tests revealed significant spatial memory impairments and reduced hippocampal dendritic spine density starting two weeks post-infection. Western blotting showed elevated hippocampal TREML2 and NLRP3 (M1 activation-related), while Arginase-1 increased later as a compensatory M2 response. Splenic analysis indicated an early Th2-dominant response (high Arginase-1/IL-4), suggesting peripheral L-arginine consumption restricts CNS availability, thereby delaying microglial M2 polarization. Therapeutically, the albendazole–resveratrol combination demonstrated the strongest effects, significantly improving behavior and restoring synaptic structure by reducing TREML2 and NLRP3 expression.

Conclusions: These findings demonstrate that *A. cantonensis* infection drives cognitive deficits through neuroinflammatory mechanisms mediated by microglial polarization, highlighting TREML2 as a potential amplifier of M1 activation and inflammasome signaling. Albendazole combined with resveratrol offers a promising therapeutic strategy for preserving synaptic integrity and mitigating neurodegeneration involving microglial dysregulation .

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SHIFT02-121

POSTER ON BOARD SHIFT 02
HOST IMMUNITY, MOLECULAR INTERACTIONS, AND MICROBIOME / INNATE AND ADAPTIVE IMMUNE RESPONSES TO PARASITES

PARALLEL IN VIVO AND IN SILICO ANALYSES REVEAL CD200 UPREGULATION ON MIGRATORY DENDRITIC CELLS AND CO-INHIBITORY CHECKPOINT NETWORKS IN NONHEALING LEISHMANIASIS

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Background and Aims: Cutaneous leishmaniasis, caused by *Leishmania* parasites, requires a strong Th1 response mediated by immune cells such as dendritic cells (DC) to control infection. However, nonhealing phenotypes actively induce inhibitory checkpoints, such as CD200, CTLA-4, and PD-1, as molecular evasion mechanisms promoting immune exhaustion and parasite persistence. This study aimed to elucidate draining lymph node (dLN) co-inhibitory checkpoint architecture and DC phenotypic shifts in nonhealing cutaneous leishmaniasis.

Methods: We integrated flow cytometry evaluation of cell-surface CD200 expression on leukocyte and DC subsets in dLNs of *Leishmania amazonensis*-infected C57BL/6 mice with *in silico* systemic analysis. Public RNA-seq data (GSE236962) from nonhealing *Leishmania major*-infected dLNs were analyzed in R using DESeq2, STRINGdb, and Enrichr to construct protein-protein interaction (PPI) networks centered on *Cd200*, *Pdcd1*, and *Ctla4* hubs (p-adj < 0.05).

Results: Flow cytometry demonstrated significant up-regulation of CD200 on $CD45^+$ leukocytes, predominantly within migratory subsets of $CD11c^+$ DC populations. PPI analysis mapped a core 38-gene signature, revealing that a pronounced pro-inflammatory Th1 response (↑*Ifng,* ↑*Tbx21,* ↑*Tnf,* ↑*Gzmb*) was systematically countered by a suppressive axis (↑*Ido1,* ↑*Cd274,* ↑*Pdcd1lg2,* ↑*Ctla4,* ↑*Ptpn22,* ↑*Klrc1*), alongside down-regulated myeloid sentinels (↓*Itgam,* ↓*Cd163*) and trafficking anchors (↓*Cx3cl1/Cx3cr1*). Gene Ontology Biological Process enrichment indicated that these interactors regulate T-cell receptor signaling, and negatively regulate T-cell activation and cell-cell adhesion, while *Cd200* was selectively associated with negative regulation of cytokine production.

Conclusions: Nonhealing progression is potentially mediated by an antigen-driven pathological reprogramming, in which co-inhibitory immune checkpoint networks functionally neutralize effector Th1 immunity within the infected microenvironment.

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POSTER ON BOARD SHIFT 02
HOST IMMUNITY, MOLECULAR INTERACTIONS, AND MICROBIOME / INNATE AND ADAPTIVE IMMUNE RESPONSES TO PARASITES

LOSS OF THE SLC15A4/TASL COMPLEX IMPEDES THE TH1-PROTECTIVE IMMUNE RESPONSE AGAINST LEISHMANIA MAJOR

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Background and Aims: Endosomal Toll-like receptors (TLR)7-9 signal through the immune adaptor complex SLC15A4/TASL (and its murine paralogue TASL2/*Gm6377*) to activate the transcription factor IRF5 and drive type I interferon (IFN), inflammatory cytokine and IL-12 production. In addition to sensing nucleic acids from viral and host sources, TLR7/9 can detect *Leishmania major* and trigger innate responses that initiate protective immunity. Because effective control of cutaneous leishmaniasis relies on robust IL-12–driven Th1 immunity, we investigated whether SLC15A4/TASL loss disrupts the development of protective responses during *L. major* infection.

Methods: SLC15A4-deficient *feeble* or double-knockout *Tasl* x *Gm6377* ($TASL^{DKO}$) mice of C57BL/6J background, naturally resistant to *L. major* infection, were intradermally inoculated with metacyclic promastigotes. Ear lesion score, parasite load, innate and adaptive immune responses were studied in the ear and draining lymph nodes. Additionally, their innate immune cells were tested after *ex vivo* infection with *L. major*.

Results: Macrophages and dendritic cells from $TASL^{DKO}$ and $SLC15A4^{feeble}$ mice showed impaired IL-12 induction upon *L. major* recognition as observed with TLR7/9 stimulation, while parasite infectivity was unaffected. $SLC15A4^{feeble}$ and even more severly $TASL^{DKO}$ mice failed to control *L. major* infection, with increased lesion size and parasite burden, decreased IL-12, IFNγ-producing T cells and impaired IgG2c switch, but produced elevated levels of IL-4, IL-10 and IgE, indicative of a Th2, *L. major*-permissive response.

Conclusions: Our findings demonstrate the crucial role of the SLC15A4/TASL complex in Th1-driven immune responses essential for pathogen control in *L. major* infection, underscoring its role in host resistance to intracellular pathogens.

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SHIFT02-123

POSTER ON BOARD SHIFT 02
HOST IMMUNITY, MOLECULAR INTERACTIONS, AND MICROBIOME / INNATE AND ADAPTIVE IMMUNE RESPONSES TO PARASITES

REACTIVE OXYGEN SPECIES PRODUCTION AND INTRACELLULAR BURDEN IN TOXOPLASMA GONDII-INFECTED MACROPHAGES FROM NATURALLY EXPOSED CATTLE

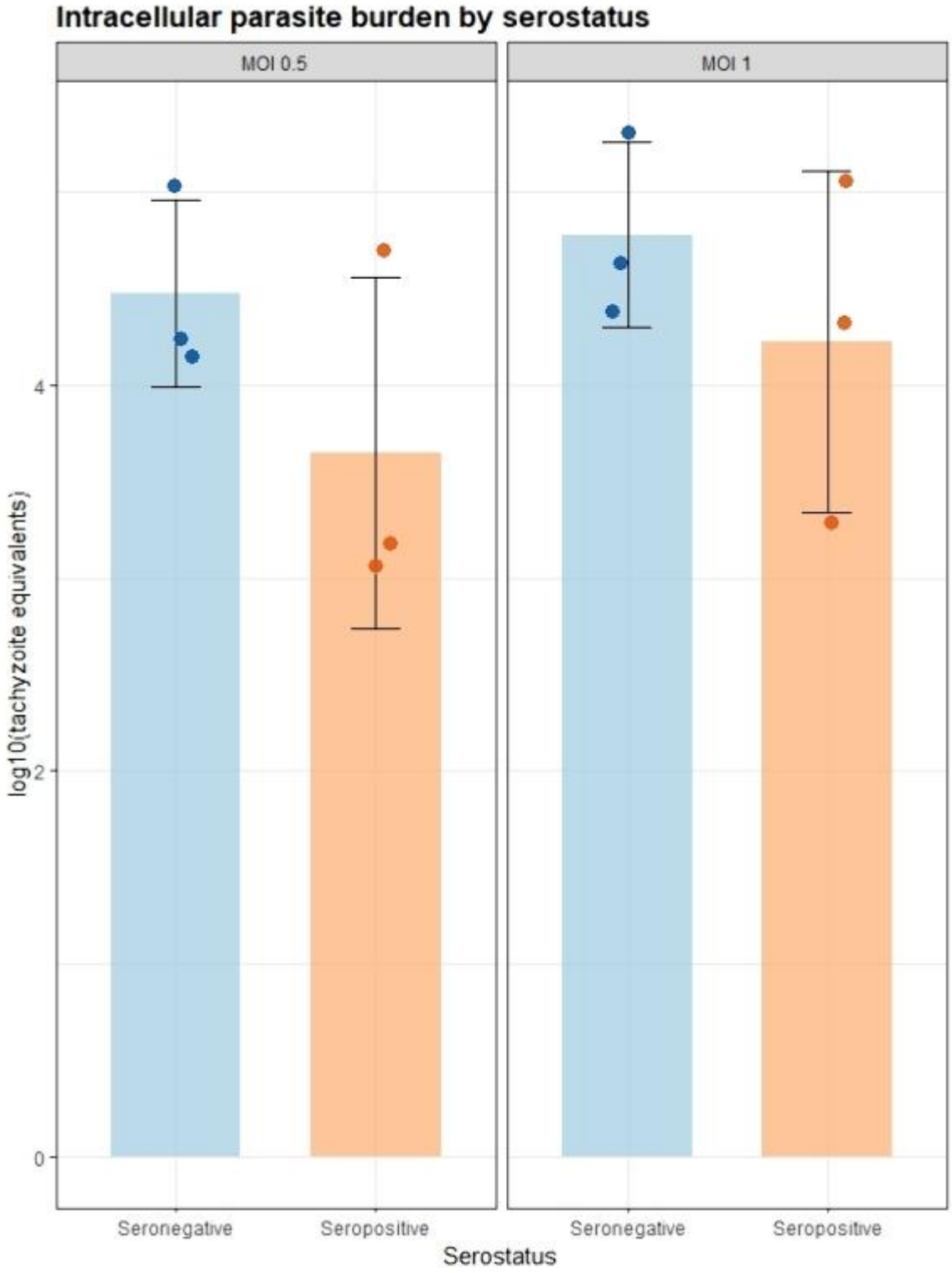
Camila Muñoz-Zanzi[1], Sofía Jara-Méndez[1], Juliette Brante-Bernier[1], Nikita Enciso[1], Anaís France-Calfueque[1], Catalina Alfaro-Godoy[1], Camila Varela[1], Galia Ramírez-Toloza[2]
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Background and Aims: *Toxoplasma gondii* is a widely distributed zoonotic parasite that infects several animal species. Cattle are considered relatively resistant to clinical toxoplasmosis, but the immunological mechanisms underlying this resistance remain unclear. This study aimed to determine whether prior natural exposure to *T. gondii* is associated with altered oxidative responses and intracellular parasite control in bovine macrophages.

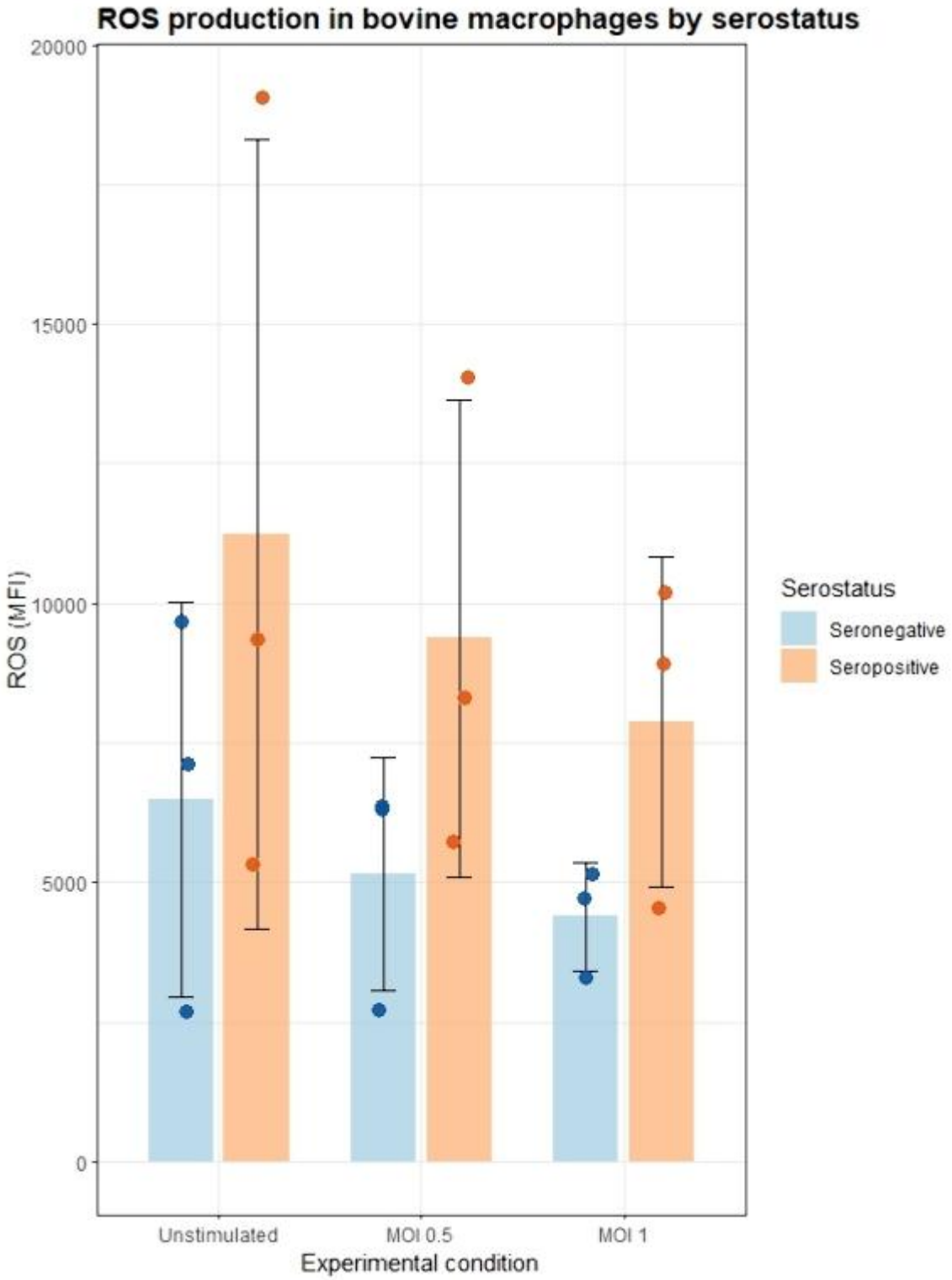
Methods: Peripheral blood monocyte-derived macrophages were obtained from Holstein cattle seropositive (n = 3) or seronegative (n = 3) for T. gondii. Cells were left unstimulated or infected in vitro with T. gondii tachyzoites at multiplicities of infection (MOI) of 0.5 and 1. Reactive oxygen species (ROS) production was quantified by flow cytometry. Intracellular parasite burden was quantified by qPCR and expressed as log10-transformed tachyzoite equivalents. A linear mixed-effects model was fitted with log10-transformed parasite burden as the response variable, standardized ROS production, serostatus, and their interaction as fixed effects, and animal ID as a random intercept.

Results: Macrophages from seropositive cattle showed higher ROS levels and lower median intracellular parasite burdens than those from seronegative cattle. In the mixed-effects model, standardized ROS production was negatively associated with parasite burden ($\beta \approx -0.82$), although no statistically significant effects of ROS, serostatus, or their interaction were detected.

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Conclusions: There is variation in oxidative responses and intracellular parasite burden in bovine macrophages depending on prior exposure to *T. gondii*. Acknowledgement: Fondecyt 1231686

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POSTER ON BOARD SHIFT 02
HOST IMMUNITY, MOLECULAR INTERACTIONS, AND MICROBIOME / INNATE AND ADAPTIVE IMMUNE RESPONSES TO PARASITES

UNRAVELING THE MIRNA LANDSCAPE IN TSETSE FLY SALIVA AND SALIVARY GLANDS DURING TRYPANOSOMA BRUCEI INFECTIONS

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Background and Aims: MicroRNAs are emerging as potential mediators of cross-species gene regulation in vector-borne diseases. Using small RNA sequencing, this study investigated the presence of miRNAs in the tsetse fly-trypanosome parasite system, focusing on the salivary glands and saliva during Trypanosoma brucei infections.

Methods: To ensure annotation accuracy, we implemented a robust pipeline incorporating de novo prediction, comparative analyses and manual curation to minimize false positives.

Results: Fifty-four (54) unique miRNAs were detected in tsetse saliva and salivary glands, among which 12 were novel to tsetse flies, 5 were putative novel miRNAs and none originated from T. brucei. Salivary gland samples contained most detected miRNAs, with a large subset also present in saliva, some exhibiting high relative abundance. Interestingly, two miRNAs appeared more represented in saliva as compared to salivary glands (let-7 and miR-8), and one miRNA (miR-184) was only detected in uninfected saliva. The presence of a T. brucei infection in the salivary glands significantly reduced miRNA diversity and abundance in saliva. Target analysis of tsetse salivary miRNAs identified numerous potential host targets, but functional enrichment was limited.

Conclusions: In conclusion, this is the first description of miRNAs in the salivary glands and saliva of a tsetse fly species, a blood-feeding vector of African trypanosomes. Our study emphasises the importance of a robust bioinformatics pipeline in de novo detection of miRNAs in non-model organisms and unconventional matrices like insect saliva. Our work highlights the challenges and limitations of miRNA target prediction, underscoring the need for rigorous experimental validation of miRNAs in host–vector–pathogen interactions.

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HOST IMMUNITY, MOLECULAR INTERACTIONS, AND MICROBIOME / INNATE AND ADAPTIVE IMMUNE RESPONSES TO PARASITES

INVESTIGATING THE ROLE OF INTERFERON-GAMMA DURING CHRONIC CRYPTOSPORIDIUM INFECTION

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National Institute of Scientific Research-Armand-Frappier Sante Biotechnologie Research Center, Department Of Virology And Immunology, Laval, Canada

Background and Aims: *Cryptosporidium spp.* are enteric protozoan parasites that infect intestinal epithelial cells (IEC) and are an important cause of diarrhoeal disease worldwide. Previous studies have found that chronically infected *Rag2*$^{-/-}$ mice, which lack T and B cells, require persistent IFN-γ signalling to limit parasite replication. We recently demonstrated that treating WT and IFNγ$^{-/-}$ mice with IFN-γ during infection transiently decreases *Cryptosporidium* burden. In contrast, our preliminary data show the same treatment has no effect in infected *Rag2*$^{-/-}$ mice. Importantly, infected IECs produce type I IFN, which has previously been found to subvert IFN-γ signalling during *Cryptosporidium* infection. Thus, persistent type I IFN production during chronic infection could reduce IEC responsiveness to IFN-γ. The aim of this study is to determine the relationship between each type of IFN and the balance between parasite control and barrier integrity.

Methods: We will quantify the presence of each type of IFN in the small intestine of *Rag2*$^{-/-}$ mice using ELISA. Subsequent experiments will determine the impact of blocking each type of IFN during chronic *Cryptosporidium* infection on parasite burden and enteric pathology and on IEC responses to IFN using mice with an IFN-responsive fluorescent reporter.

Results: The results of this study will demonstrate the dynamics of the IEC response to continuous signalling by multiple types of IFN throughout chronic infection.

Conclusions: This project will elucidate how IECs balance pathogen control and maintenance of the intestinal barrier during chronic infection and identify strategies to boost host immunity in patients with deficient T-cell responses.

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ALPHA-GAL SYNDROME: A CASE SERIES OF 35 PATIENTS WITH DIVERSE CLINICAL PRESENTATIONS.

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Background and Aims: Alpha-gal syndrome (AGS) is an IgE-mediated allergy to the carbohydrate galactose-α-1,3-galactose, typically associated with delayed allergic reactions following ingestion of red meat or other mammalian-derived products. Sensitization is most commonly linked to tick bites. Owing to its heterogeneous clinical presentation and variable latency to symptom onset, AGS remains underrecognized. This study aimed to describe the clinical spectrum and temporal characteristics of allergic reactions in patients evaluated for suspected AGS.

Methods: Thirty-five patients with a history of unexplained anaphylaxis, angioedema, and/or urticaria were retrospectively evaluated. Diagnostic assessment included detailed clinical history with particular attention to food-related triggers and symptom timing, as well as measurement of serum α-Gal–specific IgE antibodies. Sensitization to mammalian meat allergens was assessed when clinically indicated.

Results: Elevated α-Gal–specific IgE levels were detected in 8 patients. Six reported anaphylactic reactions following red meat consumption. In five cases, symptoms developed predominantly during the evening or nighttime, typically 3–6 hours after the last meal. Three patients experienced anaphylaxis within one hour of food intake. Additionally, three patients presented with delayed angioedema occurring up to 24 hours after meat consumption and showed sensitization to beef and pork allergens. Overall, substantial variability was observed in clinical manifestations and latency to reaction.

Conclusions: AGS exhibits a broad clinical and temporal spectrum that may obscure diagnosis. In patients presenting with idiopathic anaphylaxis or atypical allergic symptoms associated with red meat intake, AGS should be considered. Failure to recognize AGS may result in missed diagnoses of potentially life-threatening anaphylaxis; therefore, heightened clinical awareness is essential.

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CROSS-REACTIVE ALLERGENS SHARED BY DERMATOPHAGOIDES FARINAE, ASCARIS LUMBRICOIDES, AND TOXOCARA CANIS IN PEDIATRIC ASTHMA.

Wojciech Ozimek, Montserrat Gras Graupera
Centrum Dr Ozimek, Parasitology & Tick-borne Diseases, Warsaw, Poland

Background and Aims: IgE cross-reactivity between helminths and house dust mites may affect allergy diagnostics and contribute to asthma pathogenesis. Ascaris lumbricoides and Toxocara canis share structural similarities with mite allergens, including muscle proteins and glycoproteins. This study aimed to characterize IgE cross-reactivity between nematodes and domestic dust mites, with particular focus on tropomyosin and the nematode polyprotein ABA-1.

Methods: Sera from 100 children with asthma were analysed using ELISA, immunoblotting, and IgE inhibition assays. Excretory–secretory and somatic extracts of Ascaris spp. and T. canis, along with mite extracts, were tested. Recombinant allergens were used as competitors, and IgE-binding components were identified by LC-MS/MS.

Results: Strong IgE cross-reactivity was observed between Ascaris and mite extracts. Tropomyosin accounted for a major proportion of this cross-reactivity, while paramyosins also contributed. In contrast, ABA-1 showed no IgE cross-reactivity. Some interactions were glycan-mediated, whereas tropomyosin-related cross-reactivity was peptide-driven.

Conclusions: IgE cross-reactivity between nematodes and house dust mites may confound allergy diagnostics in pediatric asthma. ABA-1 appears to be a nematode-specific marker with potential diagnostic value.

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HOST IMMUNITY, MOLECULAR INTERACTIONS, AND MICROBIOME / INNATE AND ADAPTIVE IMMUNE RESPONSES TO PARASITES

PARASITE-INDUCED LY6G EXPRESSION DEFINES A DISTINCT INTESTINAL EOSINOPHIL SUBSET DURING HELIGMOSOMOIDES BAKERI INFECTION

Shashini Perera, Zuhaib Rana, Breton Fougere, David Cruces-Gonzalez, Naomi Chege, Aralia Leon-Coria, Constance Finney
University of Calgary, Department Of Biological Sciences, Calgary, Canada

Background and Aims: Eosinophils are considered terminally differentiated effector cells that damage tissue-invading nematodes. Emerging evidence reveals phenotypically distinct eosinophil subsets defined by surface marker expression. Chronic parasitic infection, such as *Trichuris muris,* induces Ly6G on eosinophils in peritoneal exudate. SiglecF+Ly6G+ eosinophils are enriched in IL5-rich conditions *in-vitro*. Since gastrointestinal nematodes induce eosinophilia and type-2 cytokines, *Heligmosomoides bakeri*(*Hb*) may also induce Ly6G+ on intestinal eosinophils. We aim to characterize the SiglecF+Ly6G+ intestinal eosinophils and define their function during parasitic infections.

Methods: C57Bl/6 and ΔdbGATA1-/- females were infected with *Hb* using a bolus(single-dose, chronic infection) and a trickle regimen(small doses over time, clear worms). Eosinophils were assessed at 3 time points: tissue entry(2dpi), tissue exit(10dpi) and after worm clearance(56dpi,70dpi). Single-cell suspensions of granulomas(infection sites), intestinal lamina propria and blood were used for flow cytometry. Small intestines were isolated for ELISA and immunofluorescence, and RNAscope on frozen sections.

Results: Unsurprisingly, at 10dpi, SiglecF+ eosinophils were increased in the blood of infected animals, with the majority being Ly6G-. A small subset of SiglecF+Ly6G+ was detected in both blood and intestinal tissue by flow cytometry. Immunofluorescence of granuloma and villi revealed SiglecF+Ly6G+ cells as the dominant eosinophil population, which declined proportionally following granuloma resolution. Interestingly, trickled-infected animals that naturally clear worms showed marked eosinophil accumulation in granulomas, but with fewer being Ly6G+. SiglecF+Ly6G+ cells were MBP+ and absent in the eosinophil-deficient

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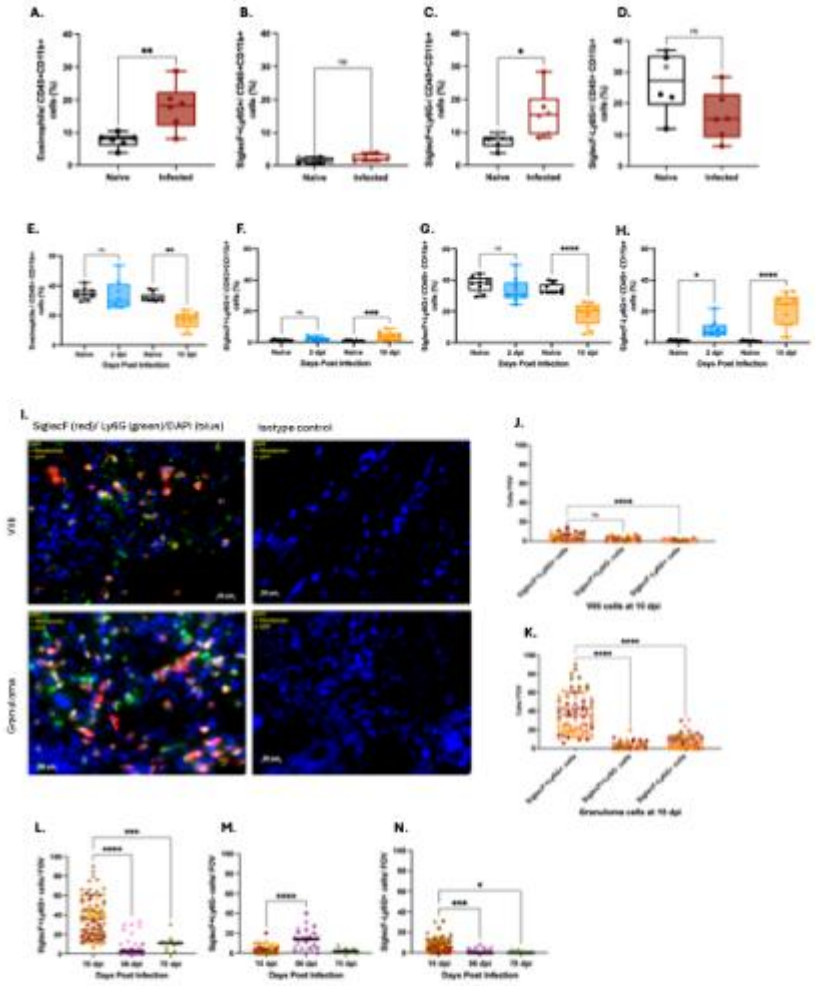


Figure 1: Blood and intestinal cells co-express SiglecF and Ly6G localization in the granuloma during *Hb* infection. 6-8 weeks female C57Bl/6 mice were infected with 200 L3 stage parasite larvae for 2, 10, 56 or 70 days. Whole blood and single-cell suspensions of small intestines were obtained for flow cytometry. Frozen sections of the first 5 cm of the small intestine were prepared for the immunofluorescent staining of SiglecF and Ly6G markers. Percentages of SiglecF+ eosinophils (A), SiglecF+Ly6G+ cells (B), SiglecF+Ly6G- cells (C) and SiglecF-Ly6G+ cells (D) in the blood as a proportion of CD45+CD11b+ cells by flow cytometry. Percentages of intestinal SiglecF+ eosinophils (E), SiglecF+Ly6G+ cells (F), SiglecF+Ly6G- cells (G) and SiglecF-Ly6G+ cells (H) as a proportion of CD45+CD11b+ cells by flow cytometry. Representative images of the immunofluorescent-stained frozen tissue sections showing SiglecF+ (red), Ly6G+ (green) and SiglecF+Ly6G+ (red-green colocalization) cells in the small intestinal villi and granuloma at 10 dpi (I). Microscopic images were taken from Axio Zoom 200M microscope at a total magnification of 630x. Number of SiglecF+/-Ly6G+/- cells in the granuloma in the villi (J) and the granuloma (K) at 10 dpi. Number of SiglecF+Ly6G+ cells (L), SiglecF+Ly6G- cells (M) and SiglecF-Ly6G+ cells (N) in the granuloma at 56 and 70 dpi (after worm clearance) compared to 10dpi by immunofluorescence staining. All experiments were repeated 2-3 times with a minimum of 3 mice per group. Normality test was performed (Shapiro-Wilk and/ or Kolmogorov-Smirnov test) following the unpaired t-test (A-D) and ANOVA by Kruskal-Wallis test with Dunn's multiple comparisons (E-H, J-N) to test the statistical significance between the groups. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$.

mice.

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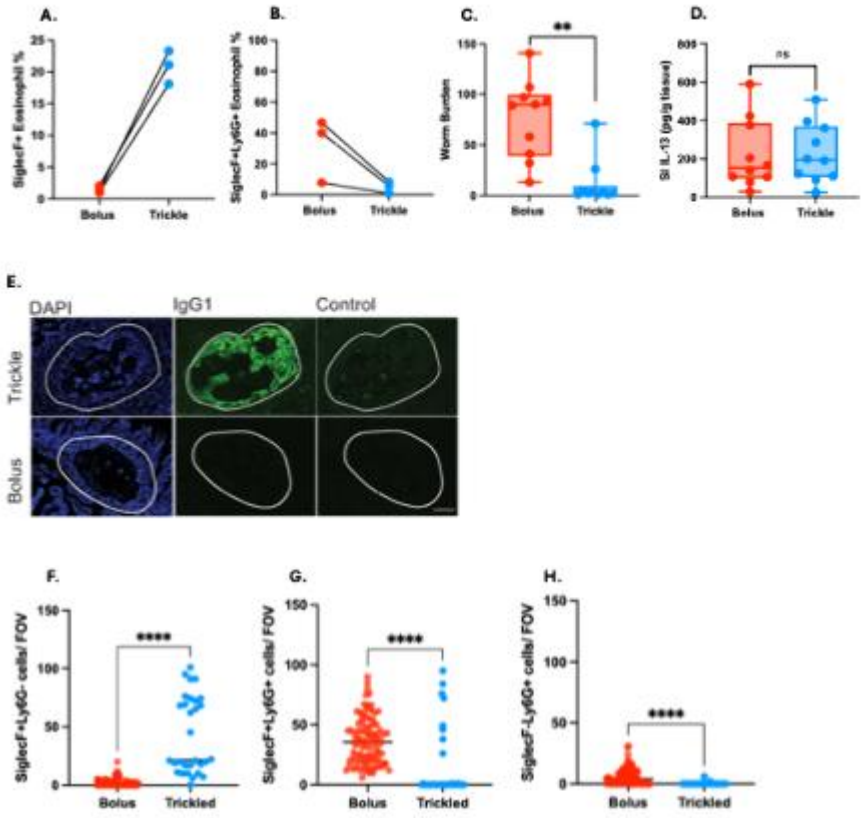


Figure 2: SiglecF+Ly6G+ cells are prominent in chronic infection but rare in more natural infection with better worm clearance. 6-8 weeks female C57Bl/6 mice were infected with 200 L3 stage parasite larvae for 10 and 21dpi to establish a chronic infection (bolus). To establish more natural infection with better worm clearance, mice were infected with 20 L3 stage parasite larvae every other day over a period of 18 days and euthanized at 21dpi. Single-cell suspensions of granuloma were obtained for flow cytometry. Percentages of SiglecF+ eosinophils (A) and SiglecF+Ly6G+ cells (B) in the acute granuloma (with worms) as a proportion of CD45+CD11b+ cells by flow cytometry. At 21dpi luminal worms were counted to assess the worm burden (C). IL13 levels in the tissue were compared by ELISA (D). Acute granulomas on FFPE whole small intestinal Swiss rolls from both bolus and trickled infections were stained for IgG1(E). Number of SiglecF+Ly6G- cells (F), SiglecF+Ly6G+ cells (G) and SiglecF-Ly6G+ cells (H) in the granulomas without worms were compared between the bolus and trickled-infection model by immunofluorescence staining. All experiments were repeated 2-3 times with a minimum of 3 mice per group. Normality test was performed (Shapiro-Wilk and/ or Kolmogorov-Smirnov test) following the unpaired t-test (A-D) to test the statistical significance between the percentages of cells between bolus and trickled-infected groups. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$.

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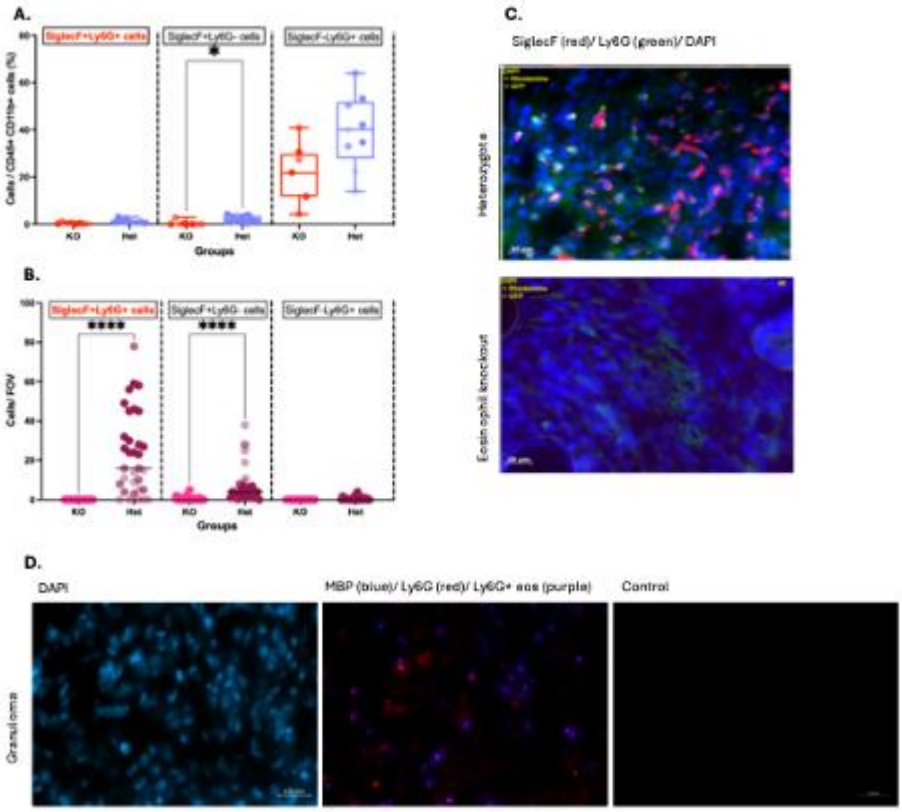


Figure 1: *Hb* induce Ly6G on small intestinal eosinophils. 6-8 weeks female C57Bl/6. ΔdbGATA1-/- mice littermate knockouts (KO) and heterozygotes (Het) were infected with 200 L3-stage parasite larvae. Whole blood was collected for flow cytometry. Percentages of SiglecF+/-Ly6G+/- cells in the blood as a proportion of CD45+CD11b+ cells between eosinophil KO and Het littermates were compared at 10dpi (A). Frozen sections of the first 5 cm small intestine from the mice were stained for SiglecF and Ly6G. Numbers of SiglecF+/- Ly6G+/- were compared between KO and Het littermates (B) in the granuloma and the representative images (C) at 10 dpi. Immunofluorescence staining for Major basic protein (MBP-eosinophil specific granule protein in blue) and Ly6G (red), demonstrating the majority of Ly6G+ cells in the granuloma at 10dpi are MBP+ (purple) (D). Microscopic images were taken from Axio Zoom 200M microscope at a total magnification of 630x. All experiments were repeated 1-2 times with a minimum of 3 mice per group. Normality test was performed (Shapiro-Wilk and/ or Kolmogorov-Smirnov test) following the ANOVA by Kruskal-Wallis test with Dunn's multiple comparisons to test the statistical significance of the percentage/ number of cells between infected KO and Het littermate. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$.

Conclusions: Small intestinal SiglecF+Ly6G+ cells are eosinophils, representing the majority of the eosinophilic response at the peak of *Hb*-infection. However, their role in worm clearance remains to be confirmed.

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HOST IMMUNITY, MOLECULAR INTERACTIONS, AND MICROBIOME / INNATE AND ADAPTIVE IMMUNE RESPONSES TO PARASITES

INFLUENCE OF LIPID METABOLISM ON IMMUNOPATHOGENESIS AND CLINICAL OUTCOME IN OBESE PATIENTS WITH CUTANEOUS LEISHMANIASIS

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Background and Aims: Cutaneous leishmaniasis (CL) patients due to *Leishmania braziliensis* develop inflammatory response which is associated with tissue damage and ulcer development. Obesity is an important public health problem worldwide, and its prevalence is increasing in developing countries. Our previous data show that obese patients with CL present more severe disease and higher rates of therapeutic failure to pentavalent antimonials compared to lean CL patients. **Aims:** To investigate the association between obesity and hypercholesterolemia with inflammation in CL patients.

Methods: Serum, peripheral blood mononuclear cells and biopsies were obtained from lean and obese CL patients, and functional studies were performed.

Results: : Our data show that CL patients present hypercholesterolemia and hypertriglyceridemia, along with increased apolipoproteins responsible for carrying LDL, chylomicrons, and triglycerides compared to healthy individuals. In addition, genes involved in LDL uptake, cholesterol efflux, and foam cell formation are upregulated in lesions and positively correlated. Functional assays using peripheral blood mononuclear cells from CL patients and macrophages infected with *L. braziliensis* demonstrate that the in vitro treatment with cholesterol, HDL, and LDL enhances inflammatory response. Interestingly, macrophages treated with cholesterol showed higher infection rates during the early stages. Finally, we found that obese patients exhibit higher levels of cholesterol, LDL, VLDL, triglycerides, APOE, and APOB100 compared to lean individuals with CL.

Conclusions: Obesity is associated with inflammation in CL patients.

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ANISAKID LARVAE IN SCOMBER SCOMBRUS FROM THE NORTH ADRIATIC SEA: A FORMIDABLE COMPROMISE BETWEEN THE HOST IMMUNE RESPONSE AND PARASITE SURVIVAL

Bahram Sayyaf Dezfuli[1], Martina Pinca[1], Emanuela Franchella[1], Luca Bellini[1], Giuseppe Castaldelli[2], Luisa Giari[2]
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Background and Aims: In spite of known health risks after consumption of raw or undercooked fish, the impact of Anisakid larvae on fish hosts is less understood. Scomber scombrus is among the commercially most important fish species in Adriatic Sea. We present a detailed account on the innate immune cells engaged in parasite encapsulation with aim of light and electron microscopies.

Methods: Samples of Atlantic mackerel (N= 45) were obtained from a commercial trawl fishing in the Scardovari lagoon. Pieces of infected/uninfected visceral organs were processed with routine methods for histopathology and ultrastructure purposes.

Results: Forty-two out of 45 (95.6%) mackerels were infected by anisakid third-stage larvae in visceral organs. The majority of the larvae were alive and co-occurred with calcified larvae encircled by host tissue. Intensity of infection ranged from 20 to 500 live larvae, and from 10 to 150 calcified larvae per fish. Histological sections of all infected visceral organs showed co-presence of two or more immune cell types around the larvae, namely mast cells, rodlet cells, macrophage aggregates and epithelioid cells.

Conclusions: This study suggests that the immune response of mackerel is capable of calcification of a small amount of anisakid larvae in all visceral organs. At the same time, numerous live nematode larvae are isolated and then encapsulated, mainly in the outer surface of the intestine. However, they do not appear to excessively compromise the function of the digestive tract. This is first absolute report of histopathological and ultrastructural assays on mackerel-Anisakid system.

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SHIFT02-131

POSTER ON BOARD SHIFT 02
HOST IMMUNITY, MOLECULAR INTERACTIONS, AND MICROBIOME / INNATE AND ADAPTIVE IMMUNE RESPONSES TO PARASITES

THE IMPACT OF PARASITIC INFECTIONS ON THE IMMUNE SYSTEM, ENERGY LEVELS, AND OVERALL WELL-BEING

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Background and Aims: Objective:
The aim of this study was to present the impact of parasitic infections on the immune system, energy levels, and overall human well-being.

Methods: A review of the scientific literature was conducted using the PubMed, Scopus, and Web of Science databases. The search included studies on parasitic infections caused by helminths and protozoa. The following keywords and their combinations were used: *parasitic infections*, *helminths*, *protozoa*, *immune response*, *immune modulation*, *immune evasion*, *energy metabolism*, *fatigue*, *chronic inflammation*, *malnutrition*, *anemia*, *cognitive function*, *neurological symptoms*, and *quality of life*. Publications concerning the host immune response, parasite immune evasion strategies, and the impact of infections on nutritional status, energy levels, cognitive function, and quality of life were analyzed.

Results: The analyzed literature indicates that parasitic infections exert multidimensional effects on the host organism, involving immune, metabolic, and neurological pathways. Parasites are capable of modulating the host immune response, leading to both immunosuppression and chronic inflammation, which facilitates their persistence. Immune activation is associated with increased energy expenditure, contributing to malnutrition, anemia, and reduced energy availability. These alterations are reflected in common clinical manifestations such as chronic fatigue, decreased physical performance, and impaired cognitive function. The reviewed studies also highlight that parasitic infections are frequently chronic and may result in long-term health consequences, particularly in children and immunocompromised individuals, ultimately leading to a significant reduction in overall quality of life.

Conclusions: Parasitic infections are more than just a biological invasion. They are complex, long-term disruptors of immunity and overall well-being. By reshaping the host's immune response they

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can silently impact quality of life. Understanding these hidden effects is key to improving diagnosis, treatment, and prevention strategies.

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SHIFT02-132

POSTER ON BOARD SHIFT 02
HOST IMMUNITY, MOLECULAR INTERACTIONS, AND MICROBIOME / INNATE AND ADAPTIVE IMMUNE RESPONSES TO PARASITES

CHRONIC TOXOPLASMA GONDII INFECTION INDUCES RESISTANCE TO ENCEPHALITIS-CAUSING VIRUSES IN MICE

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Background and Aims: Approximately one-third of the global population is estimated to be infected with *Toxoplasma gondii* (*T. gondii*). In general, chronic *T. gondii* infection is asymptomatic. In the 1960s, it was reported survival of chronically *T. gondii*-infected mice was improved following a lethal challenge with Mengo virus (*Picornaviridae,* positive-strand RNA virus; cause of encephalomyelitis). (Freshman *et al.*,1966) However, no follow-up studies have been reported to date, and the underlying mechanisms of the resistance induced in chronic *T. gondii* infection remain unclear. Ultimately, we aimed to define biological significance and mechanisms of this resistance. Here, we challenged chronically *T. gondii*-infected mice with viruses which have different genetic material and configurations.

Methods: Seven-week-old female C57BL/6 mice were infected intraperitoneally with 10^3 tachyzoites of *T. gondii* PLK strain. After forty days, the mice were challenged intracerebrally with Japanese encephalitis virus (JEV; positive-strand RNA virus), La Crosse virus (LACV; negative-strand RNA virus) or herpes simplex virus 1 (HSV-1; double-stranded DNA virus) at doses of 50 plaque-forming units (PFU), 100 PFU, or 10^6 PFU, respectively. Mice were monitored for 14 days post-virus challenge to assess survival.

Results: Following JEV challenge, *T. gondii*-uninfected mice died by day 7 post-virus infection, whereas 40% of *T. gondii*-infected mice survived beyond the observation period. Following LACV challenge, *T. gondii*-uninfected mice died by day 9, whereas *T. gondii*-infected mice survived by day 12. Following HSV-1 challenge, 14% of *T. gondii*-uninfected mice survived; however, all *T. gondii*-infected mice survived and remained largely asymptomatic.

Conclusions: Chronic *T. gondii* infection induces resistance in mice to various encephalitis-causing viruses.

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POSTER ON BOARD SHIFT 02
HOST IMMUNITY, MOLECULAR INTERACTIONS, AND MICROBIOME / INNATE AND ADAPTIVE IMMUNE RESPONSES TO PARASITES

THE IMMUNOMODULATORY ROLE OF THE THYMUS DURING EXPERIMENTAL TRYPANOSOME INFECTION.

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Background and Aims: Trypanosome infections cause significant immune regulation issues. However, their effects on thymic T cell development at the single-cell level remain unclear. In this study, we unravel the role of the thymus in regulating the immune response during *Trypanosoma brucei brucei (T. b. brucei)* infection.

Methods: Using a mouse model of *T. b. brucei* infection at different stages, we combined flow cytometry to identify cell populations in the thymus during the infection with single-cell RNA sequencing (scRNA-seq) to explore T cell differentiation, lineage tracing, and function throughout the infection. This method also facilitates the detection of rare thymus-derived cells at the mRNA and protein level.

Results: Our analysis showed that trypanosome infection rapidly disrupts thymic T cell development by reducing the number of double-positive (DP) T cells in the early stages. However, during the later chronic phases, DP cells regenerate in the thymus, suggesting mechanisms that restore their function. Notably, we found that the number of B cells in the thymus during infection is inversely related to T cell loss and decreases as DP T cells regenerate. These B cell accumulations demonstrate their function in thymus modulation in response to parasitic infection.

Conclusions: These findings highlight that trypanosome infection alters thymic immune cells and reveals new ways the immune system adapts and restores its functions. They also provide insights into how the thymus responds to infections and may apply to other infections that target the thymus.

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HOST IMMUNITY, MOLECULAR INTERACTIONS, AND MICROBIOME / INNATE AND ADAPTIVE IMMUNE RESPONSES TO PARASITES

HUMAN MONOCYTE-TO-MACROPHAGE DIFFERENTIATION AND NF-KB ACTIVATION BY EXTRACELLULAR VESICLES SHEDDING FROM TRYPANOSOMA CRUZI

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Background and Aims: Chagas disease (CD) is a neglected tropical disease, approximately 6 to 8 million individuals are infected in Latin American countries. Also increased these numbers in the US, Europe, and Asia. The investigation of EVs from *Trypanosoma cruzi* is central particle in the search for biomarkers, in the elucidation of immunopathogenesis, and in the development of new therapeutic approaches to combat CD. To analyze the modulation of the immune response on monocytes, mediated by EVs from the trypomastigote form of the Y strain of *T. cruzi*.

Methods: Peripheral blood mononuclear cells (PBMCs) were analyzed using an Amnis flow cytometer (PKH26-labeled *T. cruzi* EVs)during 60 minutes of interaction. Morphological changes in THP-1 cells were monitored using scanning EM and confocal microscopy (actin, vinculin, nucleus). Monocyte differentiation was assessed by the expression of CD80, CD86, HLA-DR, CD69, CD11b, CD209, and CD163 and cytokine production were evaluated by flow cytometry using the BD™ CBA Human Inflammatory Cytokines Kit.

Results: The results indicated the interaction between PBMCs and *T. cruzi* EVs resulted in increased phagocytic activity of monocytes and higher concentration of EVs in the peripheral region of the cells. Morphological analyses showed that EVs altered the cell surface by forming communication tubules, indicating a more dynamic cellular state and enhancing interactions between neighboring cells. Actin and vinculin cytoskeletal reorganization cells. Increased expression of CD11b, HLA-DR, and CD80 indicated monocyte activation.

Conclusions: Conclusion: *T. cruzi* EVs interact with monocytes, altering their cellular structure and promoting their differentiation into M1 macrophages.

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POSTER ON BOARD SHIFT 02
HOST IMMUNITY, MOLECULAR INTERACTIONS, AND MICROBIOME / INNATE AND ADAPTIVE IMMUNE RESPONSES TO PARASITES

PHOSPHOLIPASE C REGULATES PHAGOCYTIC CUP FORMATION AND LEISHMANIA AMAZONENSIS INTERNALIZATION BY MURINE MACROPHAGES

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Background and Aims: BACKGROUND: Leishmaniasis is a socially determined infectious disease and a major public health burden in numerous countries. Upon infection, *Leishmania* promastigotes are internalized by macrophages through phagocytosis, a process tightly regulated by phosphoinositide signaling and actin remodeling, central to the establishment of infection. **AIM:** To determine the mechanisms governing the early interaction of *L. amazonensis* with RAW 264.7 macrophages, focusing on the roles of PLC and AKT signaling.

Methods: METHODS: Cells were pre-treated with the PLC inhibitor U73122 or the AKT inhibitor GSK 690693 prior to infection. Binding and phagocytosis were dissected using Oregon Green 488-labeled promastigotes with F-actin staining. PIP_2 dynamics were tracked using the PLCΔPH-RFP biosensor. Infection kinetics were assessed at 2, 4, 8, 12, and 24 h post-infection by microscopy. Parasite viability was measured via SDS-mediated lysis and Alamar Blue assay. ROS production was quantified using H2DCFDA.

Results: RESULTS: Internalization occurred predominantly via the flagellum, driving elongated phagocytic cup formation; PLC inhibition disrupted this morphology, yielding rounded cups and premature PIP_2 accumulation. PLC inhibition robustly reduced infection rates across all time points, impaired parasite viability at 4 h, and suppressed ROS production. AKT inhibition had no significant effect. Combined inhibition reduced infection at 2 h and further decreased ROS levels.

Conclusions: CONCLUSION: PLC-driven PIP_2 regulation is essential for productive *L. amazonensis* infection, directly controlling phagocytic cup architecture and parasite internalization, establishing PLC as a key host-cell determinant of early *Leishmania* infection. **ACKNOWLEDGMENTS:** This study was supported by FAPESB, CNPq, and CAPES (Finance Code 001), INCT-DT.

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POSTER ON BOARD SHIFT 02
HOST IMMUNITY, MOLECULAR INTERACTIONS, AND MICROBIOME / INNATE AND ADAPTIVE IMMUNE RESPONSES TO PARASITES

SINGLE-CELL ANALYSIS OF PYRUVATE KINASE–INDUCED T CELL IMMUNOMODULATION DURING TRYPANOSOMA BRUCEI BRUCEI INFECTION

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Background and Aims: African trypanosomiasis, caused by *Trypanosoma brucei brucei*, is characterized by profound immune dysregulation that promotes parasite persistence. Parasite-derived molecules are increasingly recognized as modulators of host immunity, yet the mechanisms by which specific parasite antigens influence adaptive immune responses remain incompletely understood. Pyruvate kinase (PYK), a conserved metabolic enzyme expressed by *T. brucei*, has been implicated in host immune interaction, suggesting a potential immunomodulatory role.
This study aims to investigate how prior exposure to *T. brucei* pyruvate kinase modulates T cell-mediated immune responses during subsequent *T. brucei brucei* infection, using genetic mouse models and single-cell transcriptomic analyses.

Methods: Wild-type and genetically defined mice were pre-stimulated with recombinant *T. brucei* pyruvate kinase before experimental infection with *T. brucei brucei*. Parasitemia was monitored over time. Immune responses were assessed by flow cytometry and cytokine analysis. Single-cell RNA sequencing (scRNA-seq) data from immune cells were analyzed using Seurat to identify immune cell states associated with PYK-induced modulation.

Results: Preliminary data indicate that prior PYK exposure induces distinct immunomodulatory effects on adaptive immune responses during infection. Single-cell transcriptomic analysis reveals altered T cell states involving changes in activation, differentiation, and immune regulatory programs, suggesting heterogeneous T cell responses following PYK conditioning.

Conclusions: These findings highlight an immunomodulatory role for parasite-derived pyruvate kinase during *T. brucei brucei* infection. Integrating genetic mouse models with single-cell transcriptomics provides insight into how parasite antigens shape adaptive immune responses in African trypanosomiasis.

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POSTER ON BOARD SHIFT 02
HOST IMMUNITY, MOLECULAR INTERACTIONS, AND MICROBIOME / MECHANISMS OF EVASION

IDENTIFYING VSG GENE REGULATORS IN TRYPANOSOMA BRUCEI

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Background and Aims: *Trypanosoma brucei* is a single-celled, flagellated protozoan parasite that causes African trypanosomiasis in humans and animals. *T. brucei* evades the host antibody responses by periodically switching its Variant Surface Glycoprotein (VSGs) coat. VSGs are highly antigenic proteins, expressed in a monogenic manner from a repertoire of ~2,500 genes and pseudogenes. VSG genes are expressed from telomeric expression sites, but the mechanisms regulating their expression and periodic switching remain incompletely understood. Previous work identified that the phosphatidylinositol signaling pathway regulates VSG expression and switching in *T. brucei*. Immunoprecipitation coupled with cross-linking and mass spectrometry revealed that phosphatidylinositol 5-phosphatase (PIP5Pase) associates with the VSG silencer repressor-activator protein (RAP1) and several additional potential regulatory proteins. We aim to investigate the role of these candidate regulators in VSG switching.

Methods: To determine whether these interacting proteins play a role in VSG expression and switching, we constructed tetracycline-inducible RNA-interference (RNAi) cell lines in the bloodstream form of *T. brucei* SM427 for knockdown, followed by gene expression analysis.

Results: Five regulatory protein-encoding genes were cloned into the MC177VSG221RNAi vector, and two constructs were successfully transfected to generate tetracycline-inducible RNAi cell lines. RNAi-mediated knockdown of the two candidate regulators did not significantly affect parasite growth or morphology.

Conclusions: The two genes analyzed thus far are unlikely to be essential for parasite viability. Gene expression analysis and VSG switching assays are in progress to determine the consequences of their knockdowns on VSG gene regulation.

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POSTER ON BOARD SHIFT 02
HOST IMMUNITY, MOLECULAR INTERACTIONS, AND MICROBIOME / MECHANISMS OF EVASION

SCHISTOSOMA MANSONI GRANULIN: A NOVEL LIGAND FOR CD177 WITH IMMUNOMODULATORY POTENTIAL

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Background and Aims: Schistosomiasis is caused by parasitic helminths that infect millions worldwide. In humans, schistosomes reside in the blood vessels of infected individuals, where they can evade the host's immune system. Identifying the specific modulators of immune cell function is an important step in the fight against this neglected tropical disease.

Methods: We used SAVEXIS, an ELISA-based method to identify new host:pathogen interactions. Cell surface and secreted proteins from *Schistosoma mansoni* somules and adults were used to interrogate an array of over 600 human leukocyte receptors. Selected interactions were confirmed by domain mapping, surface plasmon resonance, followed by flow cytometry and ELISA with human neutrophils.

Results: We identified a novel interaction between *S. mansoni* granulin (SmGRN) and CD177, a GPI-anchored glycoprotein predominantly expressed on neutrophils, which acts as a surface receptor for proteinase 3 (PR3). SmGRN specifically bound to CD177+ neutrophils and led to reduced CD66b expression on the cell surface, suggesting impaired degranulation. Interestingly, while increased PR3 was observed on the neutrophil surface in the presence of SmGRN, the PR3 enzymatic activity was reduced.

Conclusions: This study reveals a novel candidate playing a role in *S. mansoni* immune evasion. The interaction between SmGRN and CD177 suggests disruption of neutrophil-mediated inflammatory responses by interfering with PR3 signaling and degranulation. These findings provide new insights into the mechanisms of *S. mansoni* immunomodulation, particularly its manipulation of neutrophil function.

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POSTER ON BOARD SHIFT 02
HOST IMMUNITY, MOLECULAR INTERACTIONS, AND MICROBIOME / MECHANISMS OF EVASION

EVALUATION OF THE IMMUNOMODULATORY EFFECT OF BABESIA BOVIS TCTP PROTEIN ON THE BOVINE IMMUNE RESPONSE

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Background and Aims: *Babesia bovis* is a protozoan that causes bovine babesiosis and is proposed to secrete immunomodulatory proteins to enhance its survival. This study evaluated the role of its Translationally Controlled Tumor Protein (TCTP), a conserved protein with immunoregulatory functions, during infection.

Methods: The *tctp* gene was characterized across different isolates, confirming its conservation, transcription, and intraerythrocytic expression. Immunogenic peptides were designed using bioinformatic analysis and used to immunize cattle. Subsequently, the animals were challenged with a virulent strain of *B. bovis*, and clinical signs, parasitemia, and humoral response were assessed. Additionally, recombinant TCTP proteins from *B. bovis* (rBboTCTP) and *Bos taurus* (rBtTCTP) were expressed for use in lymphoproliferation assays with bovine PBMCs and B cells.

Results: The *tctp* gene was shown to be highly conserved among *B. bovis* isolates, and its transcription and expression in intraerythrocytic stages were confirmed. Immunization with TCTP peptides induced the production of specific antibodies with neutralizing capacity *in vitro* (32-24%). After challenge, immunized cattle exhibited less severe clinical signs and higher levels of total antibodies compared to non-immunized animals. In lymphoproliferation assays, bovine TCTP induced greater cellular proliferation compared to *B. bovis* TCTP, both in PBMCs ($p \leq 0.01$) and B cells ($p \leq 0.001$). When both proteins were incubated together, the observed proliferation was similar to that induced by *B. bovis* TCTP, both in PBMCs ($p \leq 0.0001$) and B cells ($p \leq 0.001$).

Conclusions: *B. bovis* TCTP acts as an immunomodulator, suppressing the proliferative response of host lymphocytes and revealing a novel immune evasion mechanism.

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SHIFT02-140

POSTER ON BOARD SHIFT 02
HOST IMMUNITY, MOLECULAR INTERACTIONS, AND MICROBIOME / MECHANISMS OF EVASION

LOSS OF COMPLEX-TYPE N-LINKED GLYCANS ATTENUATES MAXIMUM CELL DENSITY AND SUSCEPTIBILITY TO HUMAN SERUM OF TRYPANOSOMA BRUCEI BRUCEI

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Background and Aims: *Trypanosoma brucei* is a protozoan with a single flagellum that causes African trypanosomiasis. It is well known that the glycoprotein VSG, which covers its surface, functions to evade the host immune system. However, the role of the giant poly-*N*-acetyllactosamine chains (pNAL) of glycoproteins accumulating in the flagellar pocket of this organism remains unknown, with only a suggested involvement in endocytosis. The aim of this study is to reveal the function of these pNAL.

Methods: The genes encoding *N*-acetylglucosamine transferases TbGT11 and TbGT15, essential for pNAL formation, were doubly knocked out (DKO) in the bloodstream form *T. brucei* Lister strain 427, and the resulting phenotype was examined.

Results: The DKO exhibited a loss of reactivity with lectins recognizing pNAL and a reduction in glycoprotein molecular weight, confirming the absence of pNAL. Although the proliferation rate of the DKO was comparable to that of the wild-type, the maximum cell density was significantly lower than that of the wild-type. Furthermore, the function of the haptoglobin-hemoglobin receptor (HpHbR) present in the flagellar pocket was also impaired. The HpHbR also mediates uptake of trypanolytic factor (TLF) present in human serum; and the DKO exhibited resistance to lysis by human serum.

Conclusions: Although pNAL are not essential for *T. brucei* survival, they were thought to enhance the binding of extracellular molecules to receptors. The importance of the pNAL in the uptake of HpHb was particularly demonstrated. *T. brucei* appears to be less able to evade the action of TLF due to the pNAL.

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SHIFT02-141

POSTER ON BOARD SHIFT 02
HOST IMMUNITY, MOLECULAR INTERACTIONS, AND MICROBIOME / MECHANISMS OF EVASION

SINGLE-CELL TRANSCRIPTOME ANALYSIS OF GIARDIA INTESTINALIS REVEALS THE FIRST GRAPHICAL CELL CYCLE AND UNCOVERS NOVEL BIOLOGICAL INSIGHTS

Emma Stenlund[1], Ylva Veith[2], Katherine Mosquera[2], Johan Ankarklev[2], Staffan Svärd[1]
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Background and Aims: The non-invasive parasitic protozoan *Giardia intestinalis* infect humans by attaching tightly to intestinal epithelial cells of the small intestine. The motile, disease causing trophozoites differentiate into environmentally resistant cysts for transmission to new hosts via the fecal-oral route. A symptomatic infection causes acute symptoms like diarrhea and abdominal cramps but also long-term infections resulting in complications like digestive issues with nutrient malabsorption, weight loss and stunting in children as a possible outcome. Although the molecular mechanisms underlying giardiasis remain incompletely understood, the parasite is known to evade the adaptive host immune response through antigenic variation mediated by variant surface proteins (VSPs).

Methods: Using single-cell RNA sequencing, we present the first graphical representation of the *Giardia* cell cycle on single cell level.

Results: Cell cycle-specific transcriptional annotations were visualized in a UMAP format. We further investigate whether differentiation in *Giardia* is coordinated with cell cycle progression. Notably, we observe cell cycle-dependent regulation of VSP expression. These expression patterns will be compared with those observed in Argonaute and Dicer CRISPR knockout strains to assess the contribution of RNA silencing in cell cycle and VSP regulation.

Conclusions: This provides new insight into the coordination of cell cycle progression, differentiation, and immune evasion in *Giardia*.

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POSTER ON BOARD SHIFT 02
HOST IMMUNITY, MOLECULAR INTERACTIONS, AND MICROBIOME / CELL SIGNALING AND PARASITISM

UNLOCKING NEMATODE CULTURE: THE ROLE OF HOST LIPIDS IN THE SURVIVAL OF DIROFILARIA IMMITIS

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Institute of Parasitology, Sainte-Anne-de-Bellevue, Canada

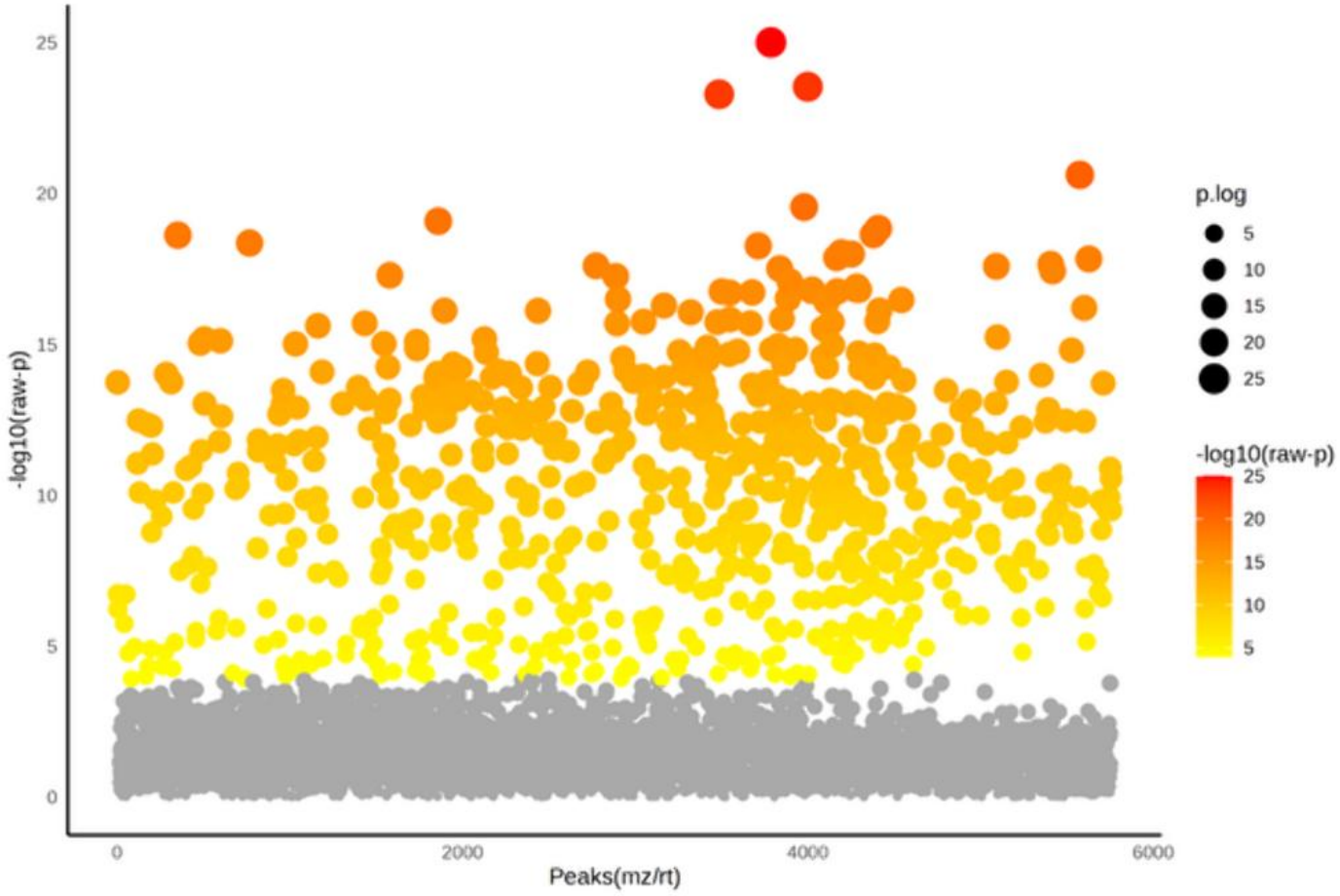
Background and Aims: *In vitro* maintenance of the canine heartworm, *Dirofilaria immitis*, remains challenging even in fetal bovine serum (FBS) supplemented media, limiting studies of parasite biology and drug discovery.Like other nematodes in ex vivo cultures, *D. immitis* rapidly reduces secretion of extracellular vesicles (EVs) and other soluble products within hours of removal from the host. We hypothesize that the lipid composition of conventional FBS-based culture media does not adequately recapitulate the host lipid environment required by the parasite. Because helminths depend on host derived lipids for metabolism, membrane formation and signaling, we therefore aimed to (1) identify lipid features present in dog serum and plasma that are reduced or absent in FBS, and (2) determine whether infection is associated with lipid remodeling that could inform the optimization of culture conditions for *D. immitis*.

Methods: Untargeted lipidomics analysis (liquid chromatography mass spectrometry, positive and negative ion modes) was performed on serum from *D. immitis*-infected and -uninfected dogs and on commercial FBS. Features were detected, aligned, and annotated in MS-DIAL, then analyzed and visualized in MetaboAnalyst using analysis of variance, principal component analysis (PCA), and fold change comparisons

Results: Across thousands of detected features, we observed widespread differences among groups (Fig.1). PCA separated FBS from dog serum, with PC1 (37.1%) and PC2 (33.3%) together explaining 70.4% of the total variance. Within dog serum, infected and uninfected samples showed broad shifts in lipid abundance , consistent with infection-associated differences in the serum

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lipidome.



Conclusions: The dog serum lipidome differs markedly from FBS and is further shaped by infection status, highlighting candidate host lipids not adequately represented in standard FBS supplementation. This findings will inform the refinement of lipid supplementation to improve *D. immitis in vitro* culture and better support downstream phenotypic studies.

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POSTER ON BOARD SHIFT 02
HOST IMMUNITY, MOLECULAR INTERACTIONS, AND MICROBIOME / CELL SIGNALING AND PARASITISM

CHARACTERIZATION OF THE ARGININE METHYLTRANSFERASE 5 (PRMT5) IN TROPHOZOITES OF ENTAMOEBA HISTOLYTICA SUBMITTED TO STRESS AND DURING PHAGOCYTOSIS

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Background and Aims: Post-translational modifications (PTMs) are key regulatory mechanisms that contribute to functional diversity of proteins by modulating their structure, activity, localization, stability, and interactions, thereby controlling essential cellular processes such as signaling, growth, and stress responses. Arginine methylation is a PTM catalyzed by protein arginine methyltransferases (PRMTs) that are classified into three main types, all of which can produce monomethyl arginine (MMA); additionally, type I and type II enzymes also catalyze asymmetric (ADMA) and symmetric (SDMA) dimethylation, respectively. Arginine methylation is involved in diverse cellular processes, including transcription regulation, DNA repair, apoptosis, and cellular stress adaptation. Several inhibitors targeting PRMTs have been developed, including EPZ015666, a selective inhibitor of PRMT5. The aim of this study was to characterize the PRMT5 enzyme of *Entamoeba histolytica* (EhPRMT5), which is currently the only known PRMT capable of catalyzing SDMA in this parasite.

Methods: To investigate the functional role of EhPRMT5, its activity was inhibited using EPZ015666 and the survival of trophozoites was analyzed under thermal, nutritional, and oxidative stress conditions.

Results: Under these stressful conditions, the treated amoebae showed increased cell death compared to untreated controls. Furthermore, we examined the rate of erythrophagocytosis in parasites treated with EPZ015666 and found that this virulence-related property was augmented in comparison to untreated controls.

Conclusions: Taken together, these results suggest that EhPRMT5 plays a regulatory role in the cellular stress response and negatively modulates the erythrophagocytosis of *E. histolytica*, probably through arginine methylation of proteins involved in DNA repair and apoptosis pathways, as well of those participating in virulence.

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HOST IMMUNITY, MOLECULAR INTERACTIONS, AND MICROBIOME / CELL SIGNALING AND PARASITISM

GENERATION AND EVALUATION OF KNOCKOUT CLONES OF SET20 AND SET23, HYPOTHETICAL METHYLTRANSFERASES IN TRYPANOSOMA CRUZI

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Background and Aims: *Trypanosoma cruzi*, the causative agent of Chagas disease, possesses many hypothetical proteins annotated or predicted in its genome that are yet to be studied. In particular, none of the SET lysine methyltransferases have been studied so far despite being an extended and ubiquitinous family, important for a myriad of cellular processes in different organisms, including *Trypanosoma brucei* as recently shown. In order to evaluate potential drug targets in *T. cruzi*, we aimed to study two unknown proteins that resemble well-characterized SET methyltransferases of model organisms and are homologous to nuclear proteins in *T. brucei*: TcSET20 and TcSET23.

Methods: To start unveiling their roles, we generated knockouts for each SET using CRISPR-Cas9 in epimastigotes, the replicative non-infective form of the parasite. Then, we isolated edited clones via limiting dilution.

Results: We confirmed the successful gene knockout of each protein by the extraction of genomic DNA, followed by long-read sequencing with Oxford Nanopore Technologies. Moreover, we corroborated the absence of off-target insertions of the selection marker. We observed that neither of these proteins is essential for cell viability and that TcSET23 knockout clones reach the stationary phase in LIT medium at a significantly lower concentration (around 15-20% less cells per milliliter) than the control strain.

Conclusions: We successfully obtained edited parasites for TcSET20 and TcSET23 and began evaluating differences with respect to the control strain. So far, we observed a significant growth difference in the TcSET23 knockout strain with respect to the control.

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SHIFT02-145

POSTER ON BOARD SHIFT 02
HOST IMMUNITY, MOLECULAR INTERACTIONS, AND MICROBIOME / CELL SIGNALING AND PARASITISM

EFFECT OF SILENCE OF THE HEAT SHOCK TRANSCRIPTION FACTOR 7 (EHHSF7) ON EHHSP20 Y EHHSP70 AND ITS LOCALIZATION IN ENTAMOEBA HISTOLYTICA DURING HEAT SHOCK STRESS

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Background and Aims: Heat shock transcription factors (HSFs) activate the transcription of stress response genes. *Entamoeba histolytica* possesses seven EhHSFs (EhHSF1-7), with EhHSF7 being the main factor involved in emetine stress response. In *E. histolytica*, 1967 genes containing Heat Shock Response Elements have been identified and could be recognized and regulated by EhHSF7. To evaluate the effect of EhHSF7 silencing and its subcellular localization during heat shock (HS) on the expression of stress response-related genes.

Methods: The *E. histolytica* HM1-IMSS clone A strain was used. Gene expression during HS and after EhHSF7 silencing using a specific DsiRNA was analyzed by Rt-qPCR. Subcellular localization was assessed by immunofluorescence microscopy using specific antibodies against EhHSF7 and EhHSP70.

Results: *E. histolytica* trophozoites exposed to HS showed decreased expression of six *Ehhsf* genes, with only *Ehhsf4* showing increased expression. The expression of the chaperone genes EhHSP20 and EhHSP70 increased in a time-dependent manner during HS. Silencing of EhHSF7 in trophozoites exposed to HS for 30 min resulted in decreased expression of EhHSP20 and EhHSP70. Immunolocalization analysis revealed that EhHSF7 relocalized during HS, being detected in both the

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nucleus and cytoplasm at 10 min. Additionally, protein levels varied during HS, with higher amounts of EhHSF7 observed at 20 min.

Conclusions: Heat shock induces a general decrease in EhHSF expression, while significantly increasing the expression of the chaperones EhHSP20 and EhHSP70. Reduced expression of these chaperones upon EhHSF7 silencing suggests that both genes are regulated by EhHSF7. Furthermore, HS induces dynamic changes in the subcellular localization of EhHSF7.

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SHIFT02-146

POSTER ON BOARD SHIFT 02
HOST IMMUNITY, MOLECULAR INTERACTIONS, AND MICROBIOME / CELL SIGNALING AND PARASITISM

DIFFERENTIAL ACTIVATION OF NLRP3 INFLAMMASOME PATHWAYS BY SECRETED PROTEINS OF LEISHMANIA MEXICANA

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Background and Aims: The inflammasome is a complex whose function is the maturation of the cytokines IL-1β and IL-18. Certain *Leishmania* species have evolved strategies to attenuate inflammasome activation in macrophages. Nevertheless, several studies have demonstrated that *Leishmania spp.* infection can activate the NLRP3 in macrophages, contributing to the restriction of parasite replication by the host. Our research has demonstrated that secreted proteins (SP) from parasites isolated from patients with lcutaneous leishmaniasis (LCL) induce IL-1β production in human macrophages. , the objective of the present study was to analyze the effect of SP from parasites isolated from patients with LCL on inflammasome activation.

Methods: Parasites were isolated from both clinical manifestations, and secreted proteins were obtained after 1 h of incubation for amastigotes and 7 h for promastigotes. These proteins were incubated for 24 h with murine macrophages. Subsequently, initial priming was performed with LPS, followed by a second stimulus with ATP. Finally, during the last part of the treatment, supernatants were collected and cells were lysed. The expression of NLRP3, IL-1β, caspase-1, and caspase-11 was analyzed by Western blot, while IL-1β release by macrophages was quantified by ELISA.

Results: The results showed that secreted proteins from both parasite stages and clinical manifestations differentially activate the canonical and non-canonical inflammasome pathways

Conclusions: These findings allow us to conclude that the differential activation of the NLRP3 inflammasome suggests an important mechanism used by the parasite to evade the innate immune response. This project was funded by CONAHCYT CBF2023–2024-734 and PAPIIT IN219025, awarded to Dr. María Magdalena Aguirre-García

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SHIFT02-147

POSTER ON BOARD SHIFT 02
HOST IMMUNITY, MOLECULAR INTERACTIONS, AND MICROBIOME / CELL SIGNALING AND PARASITISM

RBP7910 REGULATES MITOCHONDRIAL RNA PROCESSING AND DIFFERENTIATION IN TRYPANOSOMA BRUCEI

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Background and Aims: *Trypanosoma brucei* is a parasitic protozoan responsible for African trypanosomiasis. To complete its life cycle, it must transition between a mammalian bloodstream form (BF) and a tsetse fly procyclic form (PF). This differentiation requires a metabolic shift from glycolysis to oxidative phosphorylation. RNA-binding proteins (RBPs) contribute to this shift by processing mitochondrial mRNAs into functional transcripts required for energy production. RBP7910, a mitochondrial Z-RNA binding protein, may contribute to parasite survival and differentiation. However, previous studies used monomorphic cell lines, which bypass the critical transition through the ‘short stumpy’ stage and cannot complete the full *T. brucei* developmental cycle. Therefore, ongoing work uses pleomorphic cell lines to test whether RBP7910 regulates BF-to-PF differentiation, mitochondrial RNA editing, and mitochondrial activation during this transition.

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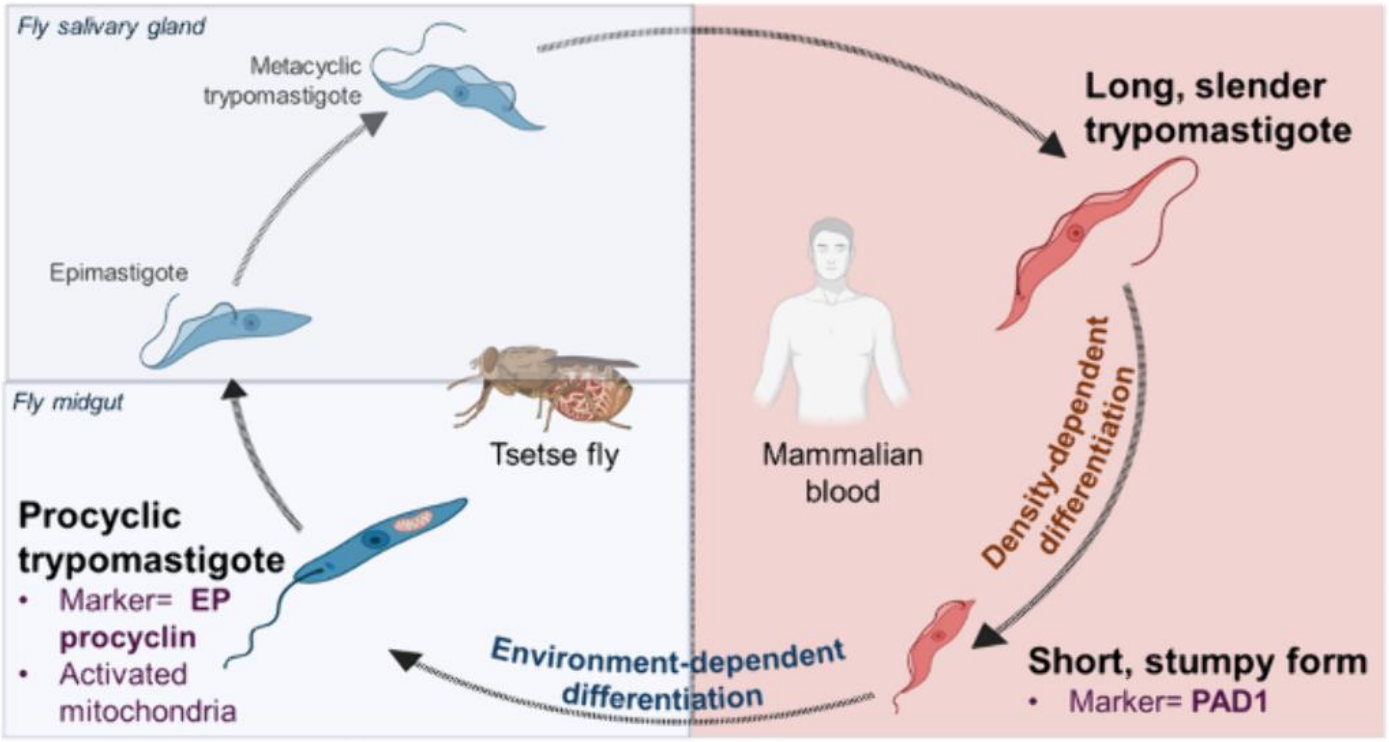


Figure 1. Life cycle of *T. brucei:* differentiation from the bloodstream forms to the insect form.

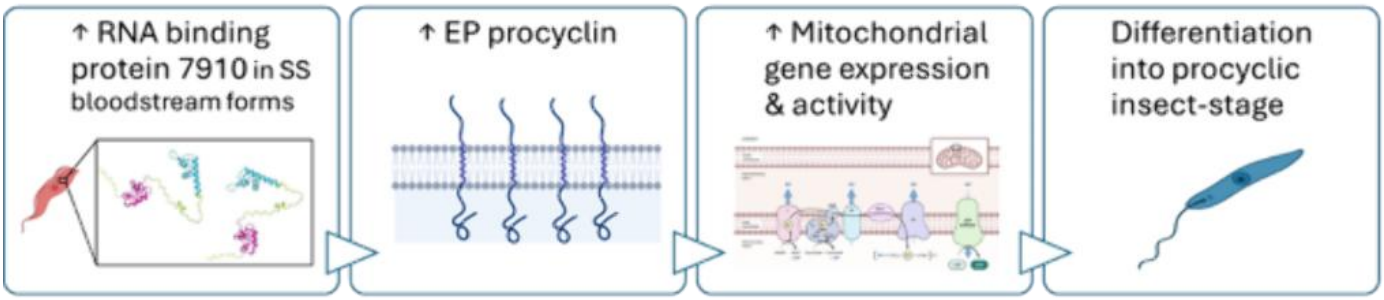


Figure 2. Schematic highlighting proposed role of RBP7910 in *T. brucei* differentiation [3].

Methods: Tetracycline-inducible RBP7910 overexpression and RNAi knockdown cell lines were used to assess mitochondrial RNA processing and differentiation. Completed monomorphic experiments examined EP procyclin expression and mitochondrial RNA editing after RBP7910 overexpression. Ongoing pleomorphic differentiation experiments use cold shock and *cis*-aconitate, with PAD1 and EP procyclin monitored by western blot, and edited/unedited cytochrome b (CYb) and ATPase 6 (A6) transcripts quantified by RT-qPCR and ddPCR.

Results: In monomorphic cell lines, RBP7910 overexpression induced EP procyclin expression, resembling differentiation toward the insect-stage despite the absence of environmental cues. RBP7910 overexpression also increased CYb editing, but not A6 editing.

Conclusions: RBP7910 may regulate selected electron transport chain transcripts, linking mitochondrial mRNA editing to mitochondrial remodeling during differentiation. Its overexpression promotes features of the BF-to-PF transition, suggesting a role in developmental regulation.

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SHIFT02-148

POSTER ON BOARD SHIFT 02
HOST IMMUNITY, MOLECULAR INTERACTIONS, AND MICROBIOME / CELL SIGNALING AND PARASITISM

THREE-DIMENSIONAL GENOME ARCHITECTURE AND DAC4-DEPENDENT CHROMATIN ORGANIZATION IN LEISHMANIA MEXICANA

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Background and Aims: *Leishmania* spp., the causative agents of leishmaniasis, display exceptional genome plasticity features enabling rapid adaptation to drug pressure. Genes are organized in long polycistronic units with limited transcriptional regulation, suggesting that epigenetic mechanisms and higher-order genome organization play critical roles in parasite biology. However, the three-dimensional (3D) genome architecture of *Leishmania* and its regulation remain unclear. This study aimed to improve the reference genome of *Leishmania mexicana* and to characterize its spatial organization, with a focus on the role of the histone deacetylase (DAC4).

Methods: An improved genome assembly of the *L. mexicana* M927 C9T7 strain was generated by integrating Oxford Nanopore long-read sequencing with Hi-C contact data (Illumina sequencing) using an innovative genome assembly pipeline. Repetitive elements were identified and gene annotations refined. High-resolution Hi-C contact matrices were constructed to analyze genome spatial folding. Comparison of Hi-C matrices between the DAC4 mutant and the wild type was performed to assess DAC4-dependent changes in chromatin spatial organization

Results: The improved assembly resolved 9 chromosomes from telomere to telomere. Hi-C maps of wild-type parasites revealed strong short-range chromatin interactions, limited inter-chromosomal contacts, and uniform intra-chromosomal organization with compartments, topologically associating domains and loops, indicating a compact and ordered 3D genome architecture. Large chromosomes appear to occupy specific territories while shorter chromosomes form interacting clusters. In contrast, DAC4 mutants exhibited alterations in chromatin folding, including changes in cis- and trans-interactions.

Conclusions: The data show improved genome assembly for *L. mexicana* and insights into its spatial organization. The impact of DAC4 on genome organization is in progress

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POSTER ON BOARD SHIFT 02
HOST IMMUNITY, MOLECULAR INTERACTIONS, AND MICROBIOME / CELL SIGNALING AND PARASITISM

FUNCTIONAL STUDY OF THE PRESENILIN-1 SIGNALING PATHWAY IN THE REGULATION OF BRAIN IMMUNE RESPONSE AND NEUROPROTECTION AFTER ANGIOSTRONGYLUS CANTONENSIS INFECTION

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Background and Aims: Presenilin-1 is a multifunctional protein involved in neuroinflammatory regulation through signaling pathways such as GSK-3β and Wnt/β-catenin; however, its role in infection-induced neuroinflammation remains poorly understood. *Angiostrongylus cantonensis* is a parasitic nematode that causes eosinophilic meningitis in non-permissive hosts, including humans and mice, with larvae developing into the fifth-stage (L_5) in the central nervous system. Despite albendazole being the primary clinical treatment, effective therapeutic strategies for cerebral angiostrongyliasis remain limited. This study aimed to investigate the role of Presenilin-1 in regulating neuroinflammatory responses and behavioral alterations induced by *A. cantonensis* infection.

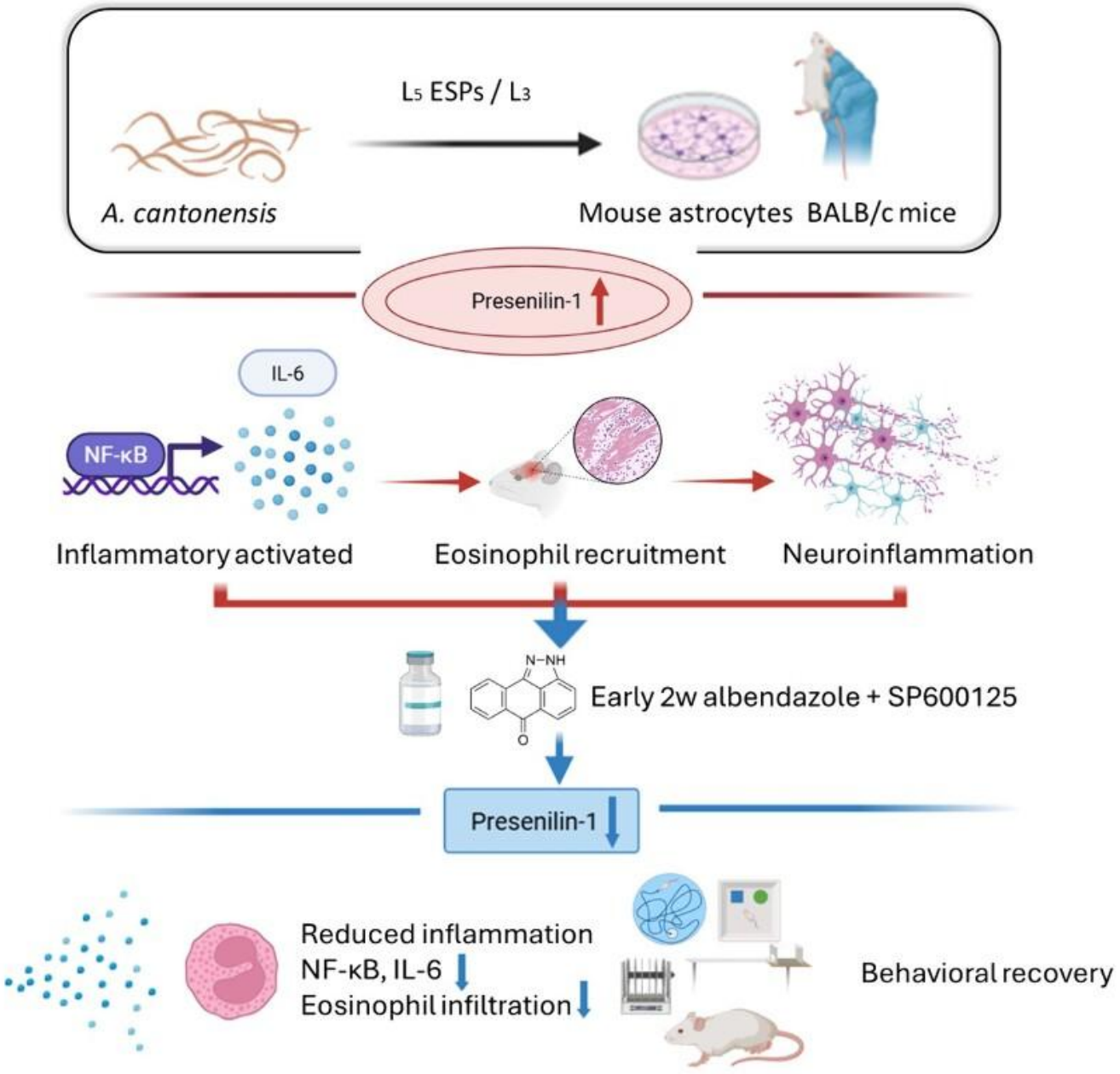
Methods: Mouse astrocytes were treated with L_5 excretory–secretory products (ESPs) to evaluate inflammatory signaling *in vitro*. BALB/c mice were infected with *A. cantonensis* and treated with albendazole alone or in combination with the JNK inhibitor SP600125. Expression of Presenilin-1, phosphorylated GSK-3β (Ser9), β-catenin, and inflammatory cytokines was assessed by Western blotting and immunohistochemistry. Serum cytokines were quantified by ELISA, while eosinophil infiltration and behavioral performance were evaluated using histopathological analysis and behavioral tests.

Results: ESP-treated astrocytes exhibited increased Presenilin-1 and GSK-3β expression, elevated NF-κB and IL-6 levels, and reduced β-catenin expression. *In vivo* infection induced Presenilin-1–associated neuroinflammation, characterized by increased NF-κB and IL-6 and decreased phosphorylated GSK-3β (Ser9), β-catenin, and IL-10 in multiple brain regions. Combined Early 2 week albendazole and SP600125 treatment attenuated neuroinflammation, reduced eosinophil infiltration and serum IL-12p40 levels, which improved infection-induced behavioral deficits.

Conclusions: These findings identify Presenilin-1 as a critical mediator of *A. cantonensis*–induced neuroinflammation and behavioral dysfunction, highlighting as a promising strategy for inflammation-

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related brain injury.



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POSTER ON BOARD SHIFT 02
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FUNCTIONAL ANALYSIS OF THE PLASMODIUM FALCIPARUM RETROMER-ASSOCIATED PROTEIN PFVPS29

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Background and Aims: Prior to egress, newly formed *Plasmodium falciparum* merozoites must generate apical organelles required for invasion of new host erythrocytes. Proteins destined for the apical complex traffic through the secretory pathway via the escort protein PfSortilin. In other eukaryotes, Sortilin is recycled back to the Golgi through the Retromer complex. However, the role of Retromer-mediated recycling in apical organelle biogenesis in *P. falciparum* remains unclear. We hypothesized that disruption of the Retromer component PfVPS29 would impair apical complex formation and interfere with the erythrocytic cycle.

Methods: Using the Selection-Linked Integration system, we generated a parasite line expressing PfVPS29 fused to a twin-FKBP and GFP tag (PfVPS29^Ks). PfVPS29 expression and localization were assessed throughout the erythrocytic cycle by fluorescence microscopy. Functional relevance was investigated using knock-sideways–mediated mislocalization and in vitro growth assays.

Results: PfVPS29 was expressed throughout the erythrocytic cycle and localized primarily to endosome-like structures, with partial localization to micronemes. Knock-sideways induction resulted in only partial mislocalization of PfVPS29 to the nucleus and caused a mild growth defect, indicating limited disruption of parasite development.

Conclusions: These results suggest that PfVPS29 contributes to intracellular trafficking in *P. falciparum* but is not strictly essential for in vitro erythrocytic growth. Residual properly localized PfVPS29 may be sufficient to support partial Retromer function and apical organelle biogenesis.

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POSTER ON BOARD SHIFT 02
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CRISPR-CAS9 REVEALS ISOFORM-SPECIFIC FUNCTIONS OF AMPK ALPHA SUBUNITS IN TRYPANOSOMA CRUZI

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Background and Aims: AMP-activated protein kinase (AMPK) of *Trypanosoma cruzi* is a heterotrimeric complex composed of α, β, and γ subunits. *T. cruzi* encodes two isoforms of the catalytic α subunit. AMPK functions as a central energy sensor, responding to changes in cellular energy status through AMP levels. Phylogenetic analyses indicate that duplication of the α subunit genes in kinetoplastids occurred independently of vertebrates, and that the α1 isoform accumulated more mutations, suggesting potential neofunctionalization. This study aimed to characterize the functional differences between the two α isoforms.

Methods: Knockout cell lines for each α isoform were generated using CRISPR-Cas9. Selected clonal populations were validated by Nanopore. Parasite proliferation was evaluated through growth curve analyses. The α-subunit phosphorylation patterns were assessed by western blot using an antibody recognizing the phosphorylated threonine residue. Potential alterations in cell-cycle progression were assessed by Flow cytometry. Parasites were cultured in media containing different carbon sources to evaluate growth, viability, and phosphorylation responses.

Results: Knockout of the α2 subunit resulted in a marked reduction in parasite growth during both exponential and stationary phases. Western blot analyses confirmed the absence of α1-associated bands in the corresponding knockout line, and increased phosphorylation was detected in α2 knockout lines compared to the control. Supplementation with 2% glucose significantly improved the growth of the α2 knockout parasites.

Conclusions: These results indicate that the α2 subunit plays a critical role in parasite growth under standard culture conditions, likely linked to glucose availability. Enhanced α1 phosphorylation in the absence of α2 suggests functional compensation between α isoforms.

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POSTER ON BOARD SHIFT 02
HOST IMMUNITY, MOLECULAR INTERACTIONS, AND MICROBIOME / CELL SIGNALING AND PARASITISM

CHARACTERIZATION OF A NOVEL APICOMPLEXAN-SPECIFIC CHIMERIC PROTEIN

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Background and Aims: *Toxoplasma gondii* is an intracellular pathogen with an exceptional ability to infect a wide range of host cells. The parasite infection is driven by a multitude of signaling cascades. Several proteins that regulate such signaling cascades have been reported, yet the complexity of the signaling network is poorly understood. We aimed to look at the signaling mechanisms that are governed by bi-functional proteins.

Methods: Subcellular expression and localization in *T. gondii* tachyzoites was performed by genomic tagging and immunofluorescence assays. Conditional mutant using tetracycline-regulatable system was used to examine the protein function and its physiological importance during the lytic cycle. Proteomics was performed to assess the pathways dysregulated upon knockdown in *T. gondii*.

Results: We report a novel chimeric protein that is conserved across apicomplexan parasites but absent in all other domains of life. This protein consists of an N-terminal KDAC domain and an IPK domain at the C-terminus. This fusion protein is essential for the lytic cycle of the parasite, and KDAC-IPK-depleted parasites display a severe growth defect due to defective replication. Proteomic analysis of the mutant suggests significant metabolic rewiring in the parasite, indicating its role in metabolic regulation.

Conclusions: This study characterizes a novel chimeric protein that is essential for the growth of the parasite. This shows a possible new player in the apicoplast metabolism of *T.gondii*. Our current research focuses on understanding the structural complexity of the protein to eventually place it as a strong candidate for the development of drugs against it, owing to the specificity of the protein to Apicomplexans.

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AUTOPHAGY-DRIVEN MODULATION OF METACYCLOGENESIS AND INFECTIVITY BY THE TCVPS34–TCVPS15 COMPLEX IN TRYPANOSOMA CRUZI

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Background and Aims: Autophagy and metacyclogenesis are key processes for the life cycle and infectivity of *Trypanosoma cruzi*, the causative agent of Chagas disease. TcVps34, a class III phosphatidylinositol 3-kinase, and its regulatory partner TcVps15 are core components of the autophagic machinery in this parasite. This study aimed to evaluate the role of TcVps34 and TcVps15 in metacyclogenesis and parasite infectivity.

Methods: *Trypanosoma cruzi* Y strain parasites overexpressing TcVps34 or TcVps15 were analyzed for autophagic activity using the *T. cruzi* autophagy marker TcAtg8 and monodansylcadaverine (MDC) staining. The activity of degradative compartments was evaluated using DQ™-Red BSA. *In vitro* host cell invasion assays were performed using Vero cells, and the expression of surface glycoproteins involved in infectivity was analyzed by flow cytometry. Parasite infectivity was assessed using murine infection models with adult male Balb/c mice.

Results: Overexpression of either TcVps34 or TcVps15 enhanced autophagic activity during the early stages of metacyclogenesis and increased the activity of degradative compartments. However, parasites overexpressing these proteins showed a significant reduction in host cell invasion. This decrease in infectivity was associated with reduced expression of the surface glycoprotein gp82, a key mediator of host cell entry, along with a moderate increase in gp90 levels, a negative regulator of parasite invasion. Interestingly, *in vivo*, mice infected with TcVps34-OE or TcVps15-OE parasites developed markedly lower parasitemia during the acute phase of infection compared to mice infected with wild-type parasites.

Conclusions: The TcVps34–TcVps15 complex is crucial for regulating autophagy and metacyclogenesis and also modulates *T. cruzi* infectivity and pathogenicity *in vivo*.

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SHIFT02-154

POSTER ON BOARD SHIFT 02
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CHROMATIN MODIFYING ENZYMES IN SCHISTOSOMA MANSONI MODULATE LNCRNA LEVELS AND ARE ESSENTIAL FOR PARASITE VIABILITY AND FERTILITY

Daisy Woellner-Santos[1], Adriana Da Silva Andrade Pereira[1], Gilbert De Oliveira Silveira[1], Helena Dos Santos Coelho[1], Lucas Ferreira Maciel[1], Vilaca Guimaraes De Oliveira[1], Ana Carolina Tahira[1], Sergio Verjovski-Almeida[1], Murilo Sena Amaral[2]
[1]Instituto Butantan, São Paulo, Brazil, [2]Instituto Butantan, Sao Paulo, Brazil

Background and Aims: Schistosomiasis remains a major public health challenge, with praziquantel as the sole therapeutic option despite its limited activity against juvenile worms and the looming threat of resistance. Epigenetic regulation and long non-coding RNAs (lncRNAs) have emerged as key components of Schistosoma mansoni biology, yet their interplay remains poorly understood.

Methods: Here, we investigated the roles of the chromatin-modifying enzymes EZH2 and LSD1 in S. mansoni adult worms using combined pharmacological inhibition and RNAi-mediated knockdown. We incubated S.mansoni adult worms with the EZH2 inhibitor GSK343 or the LSD1 inhibitor MC3935 and silenced EZH2 and LSD1, and analyzed parasite viability, motility, pairing, and reproductive organ integrity. We performed RNA-Seq re-analysis to elucidate transcriptional remodeling upon drug treatment. We also analyzed the single-cell and developmental stage expression profiles of five drug-responsive lincRNAs.

Results: Treatment with the EZH2 inhibitor GSK343 or the LSD1 inhibitor MC3935 disrupted parasite viability, motility, pairing, and reproductive organ integrity more profoundly than RNAi alone, indicating broader effects of small-molecule inhibition. RNA-Seq re-analysis revealed that both drugs trigger extensive, sex-biased transcriptional remodeling, including the differential expression of long intergenic non-coding RNAs (lincRNAs). Single-cell and developmental stage expression profiling of five drug-responsive lincRNAs uncovered distinct spatial and temporal enrichment patterns, while RT-qPCR validation confirmed that several lincRNAs, especially SmLINC101519 and SmLINC133371, are transcriptionally responsive to both EZH2 and LSD1 perturbations.

Conclusions: Together, our findings identify lincRNAs as critical downstream targets and potential effectors of chromatin regulation in S. mansoni, establishing a framework for exploring RNA-based interventions and epigenetic pathways as therapeutic targets in schistosomiasis

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SHIFT02-155

POSTER ON BOARD SHIFT 02
HOST IMMUNITY, MOLECULAR INTERACTIONS, AND MICROBIOME / CELL SIGNALING AND PARASITISM

LIPID PROFILING OF EXTRACELLULAR VESICLES SECRETED BY P. FALCIPARUM-INFECTED RED BLOOD CELLS

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Background and Aims: Plasmodium falciparum, the causative agent of malaria, was responsible for 570,000 deaths in 2024. During its intra-erythrocytic stage, the parasite depends on lipids for membrane biogenesis, haemoglobin digestion, and intercellular communication. Lipids are acquired through both de novo synthesis and scavenging from the host, making them key determinants of parasite growth and adaptation. Recent studies show that lipid composition undergoes major remodeling during gametocyte development. Extracellular vesicles (EVs), secreted by infected red blood cells, represent a major route for lipid trafficking and parasite communication. The biogenesis and lipid composition of EVs play critical roles in transmitting developmental and adaptive signals. This study aims to characterize the lipidome of EVs isolated from asexual stages to identify lipid signatures associated with sexual differentiation.

Methods: EVs were isolated using differential centrifugation from conditioned media of synchronized P. falciparum cultures. Lipid extracts are being analyzed by liquid chromatography-mass spectrometry (LC-MS). Data will be processed using an in-house bioinformatic pipeline to identify and quantify lipid species across parasite stages and to perform differential abundance analyses.

Results: Lipidomic profiling will identify distinct lipid species across stage-specific EVs. We expect differential lipid signatures between asexual stages, with enrichment of specific lipid classes in EVs from parasites undergoing sexual commitment. Stage-specific analyses are predicted to reveal lipid remodeling patterns that distinguish developmental trajectories, potentially identifying lipid mediators involved in gametocyte formation. Comparative analyses will highlight candidate lipids serving as signals in EV-mediated parasite communication during sexual differentiation.

Conclusions: This work will provide the first comprehensive lipidomic characterization of stage-specific P. falciparum extracellular vesicles and reveal lipid signatures associated with gametocyte development, highlighting potential targets for transmission-blocking interventions.

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SHIFT02-156

POSTER ON BOARD SHIFT 02
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EFFECTS OF EVS RELEASED FROM VIRULENT AND NON-VIRULENT TRYPANOSOMA CRUZI IN THE ACUTE PHASE TO EXPERIMENTAL MODEL IN CHAGAS DISEASE

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Background and Aims: The extracellular vesicles (EVs) shedding from *Trypanosoma cruzi* trypomastigotes modulate the parasite host interaction and release during the different stages of the life cycle. The aim of this study was to determine the immunomodulatory role of EVs released by trypomastigotes of the Y strain of *T. cruzi* in the experimental model of the Chagas disease (CD). In parallel, we evaluate the role of particles in the development of new therapies in CD and comparing the effects of trypomastigotes with different background of virulence of the *T. cruzi*.

Methods: BALB/c mice were pretreated with EVs isolated from *T. cruzi*trypomastigotes, using different particle concentrations and application routes. Virulent (P20) and non-virulent (P2) parasites of the Y strain were used. The parameters analyzed were parasitemia, survival, and histopathology from many organs of animals infected by *T. cruzi*.

Results: The animals were infected with trypomastigotes P20 and P2. The results showed an increase in parasitemia and tissue parasitism in animals infected with the P20 parasite. The pretreatment with EVs from P20 parasites and infection increased parasitemia and tissue parasitism in animals compared to groups of animals pretreated with P2 EVs and the control group.

Conclusions: The treatment of mice with the EVs isolated from parasite increases the amastigotes nests and inflammation in different organs of animals infected with *T. cruzi*. The immunization of animals with EVs and adjuvant decreases parasitemia. In contrast, animals previously treated with EVs from virulent parasite (P20) increase parasitemia and tissue parasitemia in contrast to treatment with EVs shedding from non-virulent parasite (P2).

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SHIFT02-157

POSTER ON BOARD SHIFT 02
HOST IMMUNITY, MOLECULAR INTERACTIONS, AND MICROBIOME / IMMUNOSUPPRESSION/HIV AND PARASITIC DISEASES (MALARIA, LEISHMANIASIS)

HIV AND MALARIA CO INFECTION IN INDONESIA EPIDEMIOLOGICAL TRENDS IMMUNOLOGICAL INTERACTIONS AND PUBLIC HEALTH IMPLICATIONS

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Background and Aims: Indonesia, a co-endemic setting in Southeast Asia, faces sustained malaria transmission alongside a growing human immunodeficiency virus (HIV) epidemic. Approximately 564,000 people are estimated to be living with HIV in 2025, while over 443,000 malaria cases occur annually, nearly 89 percent in Papua Province. Overlapping transmission increases the likelihood of co-infection and severe disease. This review synthesizes national epidemiological trends from 2020 to 2025 and examines key immunopathogenic mechanisms relevant to Indonesia.

Methods: An integrative narrative review was conducted using Indonesian national surveillance reports and literature identified through searches of PubMed/MEDLINE and Scopus. Search terms included HIV, malaria, co-infection, parasitemia, immune exhaustion, and severe malaria. Studies published between 2020 and 2025 were screened. National epidemiological data were incorporated to contextualize disease burden and species distribution.

Results: *Plasmodium falciparum* and *Plasmodium vivax* predominate in Indonesia. People living with HIV demonstrate higher parasite densities and increased risk of severe malaria, particularly in the setting of advanced immune suppression. HIV mediated depletion of CD4 positive T cells, immune exhaustion, and impaired germinal center responses reduce high-affinity opsonizing antibody formation and splenic clearance of infected erythrocytes, facilitating uncontrolled parasitemia. In falciparum infection, endothelial activation and adhesion molecule upregulation enhance cytoadherence and microvascular sequestration. Malaria induced inflammatory cytokines and nuclear factor kappa B signaling increase viral transcription and transient plasma viral load, reinforcing systemic immune activation.

Conclusions: HIV and malaria co-infection represents a biologically synergistic challenge in Indonesia. Integrated surveillance and coordinated clinical strategies are essential to reduce severe malaria risk and support elimination efforts.

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POSTER ON BOARD SHIFT 02
HOST IMMUNITY, MOLECULAR INTERACTIONS, AND MICROBIOME / IMMUNOSUPPRESSION/HIV AND PARASITIC DISEASES (MALARIA, LEISHMANIASIS)

TESTOSTERONE MODIFIES IMMUNE CELL POPULATIONS AND CIRCADIAN GENE EXPRESSION DURING PLASMODIUM BERGHEI ANKA INFECTION

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Background and Aims: Malaria is the most lethal parasitic disease, exhibits circadian rhythmicity, and shows sexual dimorphism. Male-biased severity has been linked to the immunosuppressive effects of testosterone (T), whose secretion follows a circadian pattern and modulates multiple immune functions. However, whether testosterone regulates the circadian genes *Bmal1* and *Clock* in immune cells remains unknown. This study examined the effects of testosterone on the expression of these genes during *Plasmodium berghei* ANKA infection.

Methods: Male CBA/Ca mice received testosterone plus letrozole (LT), a selective aromatase inhibitor that prevents testosterone conversion into oestrogens. Mice were infected with *P. berghei* ANKA, and testosterone levels, immune cell populations, and *Bmal1* and *Clock* expression in the hypothalamus and spleen were analysed at ZT3 (day) and ZT15 (night).

Results: At ZT3, LT administration did not alter testosterone levels but reduced CD3+, CD4+, and CD8+ cell counts. LT increased *Bmal1* expression in the hypothalamus and spleen, downregulated *Clock* in the hypothalamus, and enhanced it in the spleen; these changes were associated with increased parasitaemia. In contrast, at ZT15, LT increased free testosterone levels without affecting T-cell populations, suppressed *Bmal1* in both tissues, increased hypothalamic *Clock*, and downregulated splenic *Clock*. These nighttime changes were associated with lower parasitaemia compared with ZT3.

Conclusions: Testosterone and *Plasmodium* infection interact to differentially modulate immune cell populations and circadian gene expression depending on time of day. These findings reveal a chronobiological mechanism by which testosterone influences parasite clearance and infection outcome. **Funding:** PAPIIT IN223524, UNAM.

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HOST IMMUNITY, MOLECULAR INTERACTIONS, AND MICROBIOME / IMMUNOSUPPRESSION/HIV AND PARASITIC DISEASES (MALARIA, LEISHMANIASIS)

ADIPOCYTE FATTY ACID BINDING PROTEIN (A-FABP) AND MALARIA: EVALUATION OF INFLAMMATION AND LIPID PARAMETERS DURING PLASMODIUM SPP. INFECTION IN BRAZILIAN AMAZON

Cinthia Rodolphi[1], Lisa Oe[2], Isabela Soares[1], Ana Luiza Alencar[1], Barbara Baptista[1], Juliana Lemos[1], Carolina Pereira[1], Hugo Souza[3], Lucas De Queiroz[1], Rodrigo Martorano[4], Paula De Luca[1], Alvaro Bertho[1], Lilian Pratt-Riccio[1], Claudio Daniel-Ribeiro[1], Guy Caljon[2], Paulo Totino[1], Josué Lima-Junior[1]
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Background and Aims: Adipocyte Fatty Acid Binding Protein 4 (A-FABP) is predominantly expressed in adipocytes and macrophages, and elevated circulating levels have been associated with several inflammatory and metabolic diseases. Despite its potential relevance to malaria immunopathology, few studies have investigated A-FABP during *Plasmodium* infections. Therefore, this study aimed to explore associations between circulating A-FABP and other immunological and metabolic markers in individuals from Brazilian Amazon.

Methods: The individuals (n=130) were recruited from Brazilian endemic areas: 37 infected with *P. falciparum* (PF), 38 infected with *P. vivax* (PV), 39 uninfected cohabitants (CH), and 16 non-endemic controls (NEC). Plasmatic A-FABP levels were measured by ELISA; Th1, Th2, and Th17 cytokines were quantified using cytometric bead array (CBA); and cholesterol, triglycerides, and C-reactive protein (CRP) were assessed by colorimetric assays.

Results: A-FABP ranged from 9.7ng/mL to 23.8 ng/mL, however no significant differences in plasma concentrations were observed among the study groups. However, A-FABP levels were positively correlated with the pro-inflammatory cytokine IL-6 in the PV group and negatively correlated with the anti-inflammatory cytokine IL-4 in the CH group. As expected, CRP levels were increased in infected individuals. Regarding lipid parameters, the PF group exhibited significantly lower cholesterol levels compared with the PV group, while triglyceride levels did not differ significantly between infected groups.

Conclusions: Overall, these findings provide novel evidence linking A-FABP to IL-6 in uncomplicated *P. vivax* malaria and to IL-4 in individuals previously exposed to *Plasmodium*, thereby

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contributing to the understanding of malaria immunopathology. Further studies are warranted to confirm and expand these associations.

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HOST IMMUNITY, MOLECULAR INTERACTIONS, AND MICROBIOME / IMMUNOSUPPRESSION/HIV AND PARASITIC DISEASES (MALARIA, LEISHMANIASIS)

PD-1 EXPRESSION ON B-CELL SUBSETS DURING ACUTE MALARIA

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Background and Aims: Repeated malaria exposure shapes immune responses in endemic regions, but how immune regulatory pathways influence B-cell activation during acute infection remains unclear. Programmed cell death protein 1 (PD-1) is an inhibitory immune receptor extensively studied in T cells, yet its expression across human B-cell subsets during acute malaria, and its relationship to exposure history and parasite-binding B cells, is poorly defined. This study aimed to examine PD-1 expression across circulating B-cell subsets during acute malaria in individuals with different malaria exposure histories.

Methods: Peripheral blood mononuclear cells from 64 individuals were analyzed by flow cytometry, including malaria-exposed and malaria-naïve individuals with acute malaria, as well as malaria-exposed and malaria-naïve controls. PD-1 expression was assessed across major circulating B-cell subsets. *Plasmodium falciparum*–binding and non–binding B cells were compared within individuals. Associations with parasitemia and inflammatory markers, including osteopontin and interferon-γ, were explored.

Results: PD-1 expression patterns varied across B-cell subsets and according to malaria exposure history during acute infection. Differences were observed between memory B-cell compartments and between parasite-binding and non–parasite-binding B cells. Associations between PD-1 expression, parasite density, and inflammatory markers were subset-specific.

Conclusions: These findings indicate that PD-1 expression on B cells during acute malaria is shaped by both B-cell subset identity and malaria exposure history. The study provides insight into how regulatory pathways are engaged within the B-cell compartment during naturally acquired malaria and supports further investigation of humoral immune regulation in malaria-exposed populations.

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HOST IMMUNITY, MOLECULAR INTERACTIONS, AND MICROBIOME / IMMUNOSUPPRESSION/HIV AND PARASITIC DISEASES (MALARIA, LEISHMANIASIS)

EFFECT OF MALARIA INFECTION ON HIV-1 VIRAL LOAD AND THE IMPACT OF MALARIA TREATMENT ON MAINTAINING VIRAL SUPPRESSION AT LAGOS STATE UNIVERSITY TEACHING HOSPITAL, NIGERIA: FINDINGS AND LESSONS LEARNED

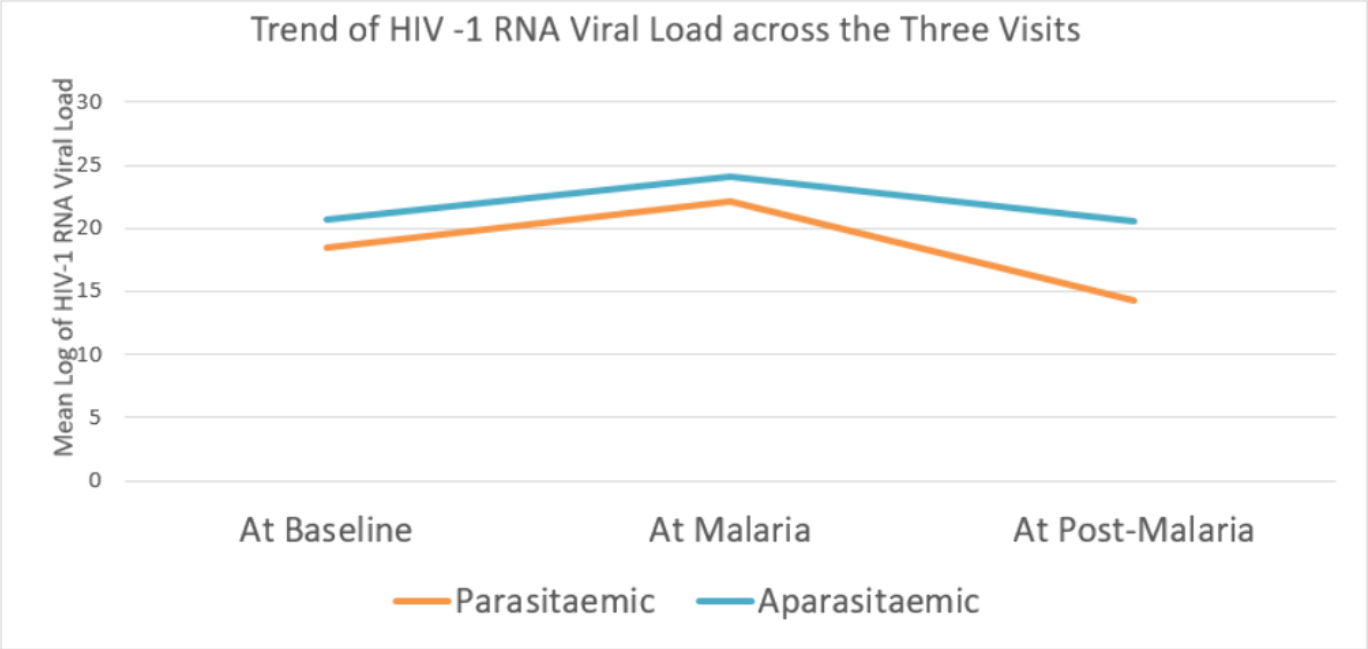
Jamiu Olabamiji[1], Isah Aliyu[2], Abdulhadi Kumurya[2], Abduljaleel Akinbami[3], Philip Ojo[4], Omosalewa Alao[3], Aderonke Agbaje[5]
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Background and Aims: Co-infection of Malaria and HIV, the two deadliest diseases of our era is a raising a serious concern in the management and control of these infectious diseases in Sub-Saharan Africa (SSA). The need to achieving and maintaining an undetectable viral load is key to HIV epidemic control (undetectable equals untransmitable “U = U”). This progressive cohort study reveals the effect of Malaria on HIV-1 replication among HIV infected individuals that has achieved viral suppression after six (6) months of treatment

Methods: At baseline blood samples were collected from 124 participants and were assayed for Malaria and HIV-1 viral load, 12 participants were excluded after baseline based on the exclusion criteria. During the first follow-up, 15 participants that were tested positive for malaria were place on Antimalarial treatment and the second follow-up samples were collected between six to eight weeks to assess the effect of effective malaria treatment on the HIV-1 Viral Load. 97 participants remain Aparasitaemic throughout the three visit, their result was analyzed and compared with the Parasitaemic group.

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Results:



The result reveal that the effect of Malaria infection on HIV-1 viral replication in the study group is not significant (p-value= .000). Hence, virally suppressed individual cannot transmit the infection during a malaria episode, also the increase in viral load during malaria visit reduce significantly after prompt and effective malaria treatment.

Conclusions: This study established that malaria infection does raise the HIV-1 RNA concentration but not to a level that will threaten viral suppression status and prompt malaria treatment is key to the sustenance.

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POSTER ON BOARD SHIFT 02
HOST IMMUNITY, MOLECULAR INTERACTIONS, AND MICROBIOME / IMMUNOSUPPRESSION/HIV AND PARASITIC DISEASES (MALARIA, LEISHMANIASIS)

PLASMODIUM PARASITES REGULATE THE INNATE IMMUNE SYSTEM VIA VESCILE-MEDIATED SECRETION OF DISTINCT MEMBERS OF THE PIR MULTIGENE FAMILY.

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Background and Aims: Malaria remains one of the most devastating parasitic diseases in humans, partly due to the parasite's capacity to modulate and circumvent host immune responses. A major contributor to this adaptability is the presence of large multigene families found across *Plasmodium* species. Among these, the Plasmodium Interspersed Repeat (PIR) which encodes for variant antigens which are thought to play key roles in shaping host–parasite interactions. While *P. falciparum* employs Variant Surface Antigens (VSAs) to regulate immune recognition, comparable mechanisms in species such as *P. vivax* and *P. yoelii* remain poorly defined.

Methods: In *P. yoelii*, the PIR (yir) repertoire comprises more than 800 members that are heterogeneously expressed within parasite populations, presenting challenges for functional analysis. To overcome this, we applied a selection-linked integration strategy to generate homogeneous parasite lines expressing defined *yir* genes.

Results: These transgenic lines revealed clear evidence of allelic exclusion, implicating heterochromatin-associated epigenetic regulation. In vivo infections with distinct YIR-expressing lines produced divergent infection outcomes, suggesting that individual YIRs exert distinct immunomodulatory effects. Functional assays with rodent macrophages demonstrated that specific YIRs suppress macrophage activation. Using transwell systems, we showed that this suppression is mediated by secreted factors rather than direct parasite–cell contact. Further characterization identified YIR proteins within extracellular vesicles (EVs).

Conclusions: Collectively, these findings reveal a previously uncharacterized role for YIR proteins in altering innate immune function through EV-associated delivery, providing new insight into the molecular strategies *P. yoelii* utilizes to persist within the host.

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HOST IMMUNITY, MOLECULAR INTERACTIONS, AND MICROBIOME / IMMUNOSUPPRESSION/HIV AND PARASITIC DISEASES (MALARIA, LEISHMANIASIS)

A NON-LETHAL MURINE MALARIA ALLEVIATES COLLAGEN-INDUCED ARTHRITIS VIA OVERLAPPING IMMUNOREGULATORY MECHNISMS BEYOND IFN-Γ AND IL-10

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Background and Aims: Parasitic infections can modulate host immunity and ameliorate autoimmune diseases. We previously reported that infection with the non-lethal murine malaria parasite *Plasmodium yoelii* 17XNL (Py) suppressed collagen-induced arthritis (CIA), a model of rheumatoid arthritis, and enhanced production of an anti-arthritic cytokine IL-10. Our subsequent study, however, demonstrated that Py still suppressed CIA in IL-10-knockout mice during parasitemia, suggesting the presence of IL-10-independent anti-arthritic mechanisms. As IFN-γ is another anti-arthritic cytokine and a key mediator of anti-malarial immunity, here we investigated the requirement of IFN-γ for the anti-arthritic effects of Py infection and sought to provide an integrated view of Py-induced immunoregulation in CIA.

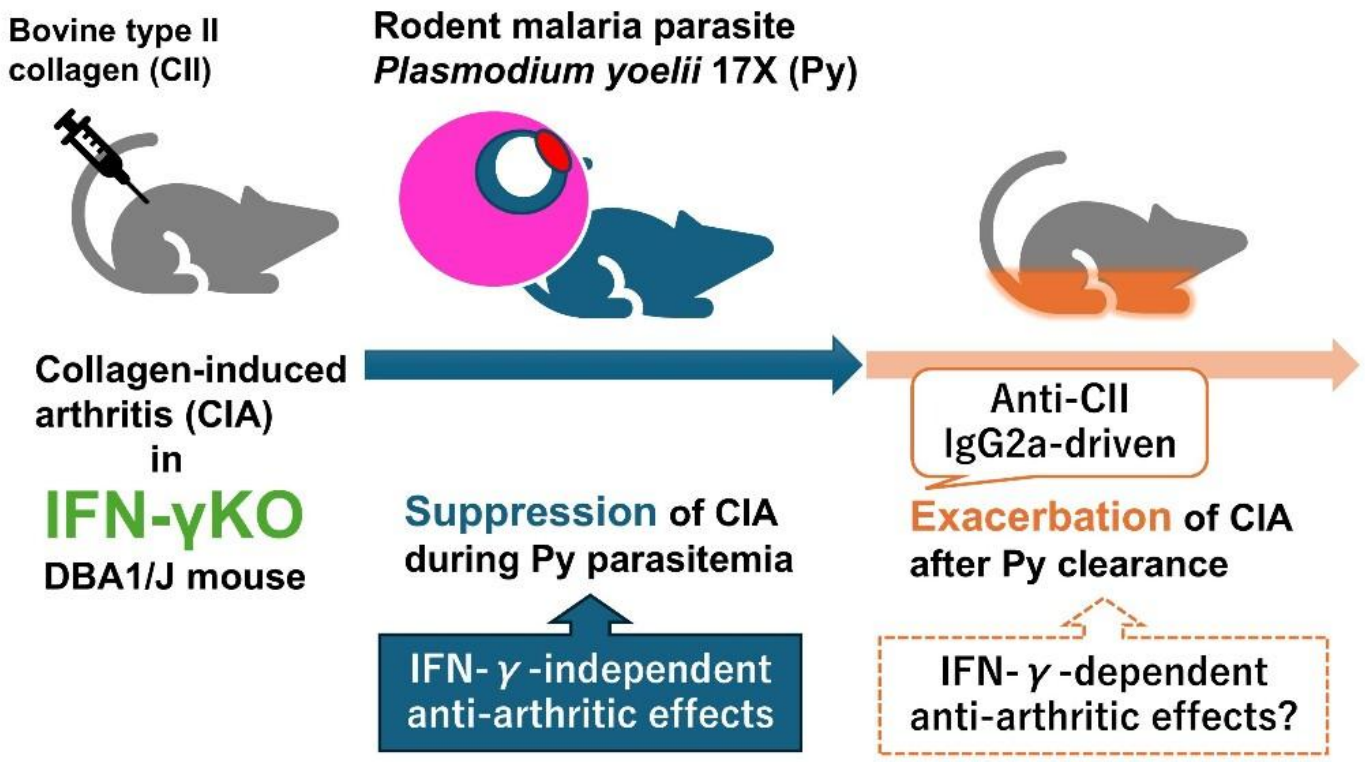
Methods: CIA was induced in IFN-γ knockout (IFN-γKO) and wild-type (WT) DBA/1J mice by immunization with bovine type II collagen (CII) emulsified in Freund's complete adjuvant. Two weeks after CII immunization, mice were inoculated with Py-infected erythrocytes. Arthritis severity was monitored over time, and serum anti-CII IgG levels and splenic cytokine production were measured.

Results: In IFN-γKO mice, Py infection suppressed CIA development and downregulated the pro-arthritic cytokines IL-17A and TNF-α during parasitemia. After parasite clearance, however, a marked rebound of arthritis was observed, accompanied by a surge in serum anti-CII IgG levels. Our findings indicate that host IFN-γ is not required for the suppression of CIA by Py infection during parasitemia, but may be important for restraining autoantibody production after parasite clearance.

Conclusions: Across WT, IL-10KO, and IFN-γKO mice, Py suppresses CIA during parasitemia, indicating that Py infection has anti-arthritic effects independent of IL-10 and IFN-

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HOST IMMUNITY, MOLECULAR INTERACTIONS, AND MICROBIOME / GUT IMMUNOLOGY AND ENTERIC PARASITES (GIARDIA, ENTAMOEBA, CRYPTOSPORIDIUM, ETC.)

UNVEILING PROTIST-BACTERIA INTERACTIONS THROUGH COMPUTATIONAL METABOLIC MODELING

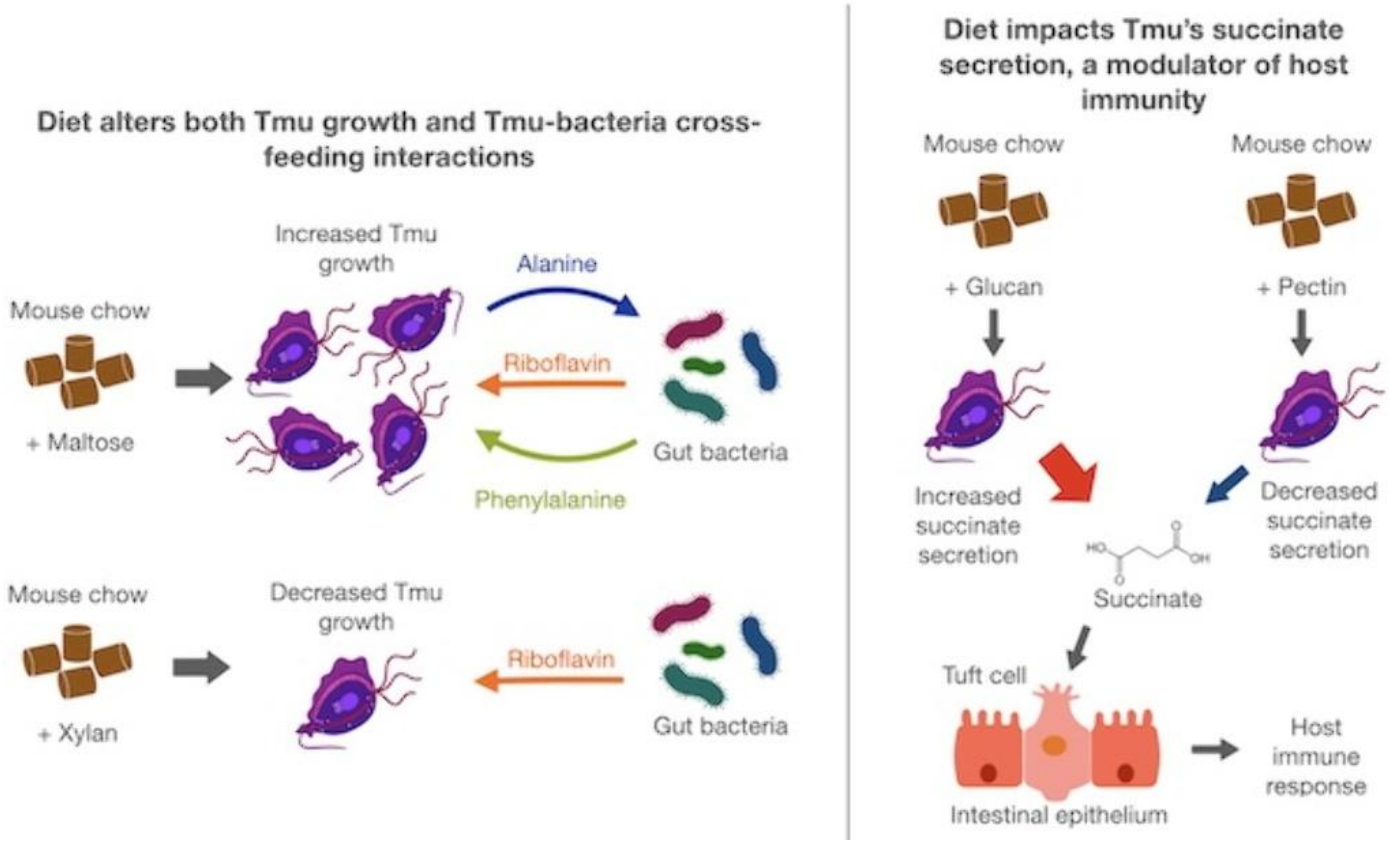
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Background and Aims: *Tritrichomonas musculus* (Tmu) is a common protist in the mouse gut. As many gut protists, Tmu is a pathobiont, capable of commensalism and pathogenesis. Previous studies suggest that gut bacteria could mediate the protists' switch to pathogens, at least in part, through metabolic interactions. To further explore the role of such interactions in Tmu pathogenesis we performed computational simulations of the mice gut.

Methods: We reconstructed genome scale metabolic models (GEMs) of Tmu and 266 mouse gut bacteria. We applied these GEMs to simulate the impact of 45 different diets on Tmu growth, and its metabolic interplay with gut bacteria, using the BacArena modeling platform. For the diets we considered regular mouse chow with 1 of 45 different saccharides as carbon source.

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Results:



Among the tested carbon sources, we found that glucose, maltose and maltose oligomers increased Tmu numbers, while xylan reduced them. The amount of secreted succinate, a key modulator of host immunity, depended on the diet as well, with glucan resulting in the highest succinate levels, and pectin in the lowest. Finally, we also identified cross-feeding interactions that depended on diet. Under maltose, Tmu feeds alanine to bacteria, and bacteria feed Tmu with phenylalanine and riboflavin. In contrast, when grown with xylan, bacteria feed Tmu with riboflavin but stop receiving alanine from it.

Conclusions: Our simulations revealed an important role of diet on Tmu growth, succinate secretion and Tmu-bacteria interactions. These diets will be validated *in vivo* to assess if we can promote commensalism in Tmu, and eventually other gut protists.

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HOST IMMUNITY, MOLECULAR INTERACTIONS, AND MICROBIOME / GUT IMMUNOLOGY AND ENTERIC PARASITES (GIARDIA, ENTAMOEBA, CRYPTOSPORIDIUM, ETC.)

TYPE 2 IMMUNITY EXACERBATES DIET-INDUCED EARLY LIFE STUNTING

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Background and Aims: Stunting occurs when insufficient nutrients are allocated to growth during early life, potentially resulting from nutrient-poor diets and energy demands from immune responses. However, the underlying mechanisms linking malnutrition, immune activation, and growth impairment remain unclear. We aimed to uncover these mechanisms by examining how type 2 immune responses impact nutrient allocation and growth trajectories during early life.

Methods: We developed a murine model in which weanling mice were placed on either a malnourished (low protein, low fat) or isocaloric control diet and subsequently infected with the small intestinal parasitic nematode *Heligomosomoides polygyrus bakeri* (*Hpb*). We measured weight gain, tail growth, and serum and hepatic levels of the growth stimulating hormone insulin-like growth factor 1 (IGF-1) and its inhibitor fibroblast growth factor 21 (FGF-21). To assess the role of type 2 immunity—the dominant response to *Hpb*—we used STAT6-deficient mice lacking IL-4 and IL-13 signaling.

Results: Malnourished mice exhibited reduced weight gain and tail growth compared to controls. Despite no effect of infection on control mice, infection of malnourished mice significantly exacerbated stunting. Stunted mice displayed reduced IGF-1 and elevated FGF-21, with this ratio greatly exaggerated in Hpb-infected malnourished mice. Genetic deletion of STAT6 completely restored the IGF-1/FGF-21 ratio to uninfected levels and eliminated Hpb-exacerbated stunting.

Conclusions: Type 2 cytokines limit growth to allocate nutrients for mounting robust immune responses. These findings reveal a mechanism by which IL-4/IL-13 signaling mediates the trade-off between growth and immunity during early life malnutrition and infection through an FGF-21-IGF-1 axis.

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HOST IMMUNITY, MOLECULAR INTERACTIONS, AND MICROBIOME / GUT IMMUNOLOGY AND ENTERIC PARASITES (GIARDIA, ENTAMOEBA, CRYPTOSPORIDIUM, ETC.)

IDENTIFYING THE REDOX PARTNERS OF GIARDIA CYTOCHROME B5: A COMPUTATIONAL AND PRACTICAL APPROACH

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Background and Aims: The metabolic pathway for heme biosynthesis is absent from *Giardia spp*, yet their genomes encode members of the cytochrome b_5 family; four such proteins occur in *Giardia intestinalis* (gCYTB5-I-IV). While well-studied in other organisms, the role of these redox proteins in Giardia is unclear, as it largely lacks known electron transfer partners such as cytochrome b_5 reductase (CYTB5R). However, the presence of cytochromes in a parasitic protist that does not produce heme suggests that they have important functional roles. As the small size of cytochromes b_5 and the transient nature of biological redox reactions have presented a challenge in identifying potential redox partners experimentally by *in-vivo* protein-protein interaction methods, an alternate strategy seems to be required to answer this question. While Giardia does not encode a CYTB5R, other flavoproteins may fill this donor role, and other redox-active proteins may be prospective electron acceptors.

Methods: Using the software AlphaFold 3, interactions between gCYTB5s and these potential partners can be modelled and evaluated to guide *in-vitro* enzyme assays experiments to test them.

Results: Such models suggest that gCYTB5s interact with a Giardia flavoprotein bearing 34% sequence identity to old yellow enzyme. Additionally, flavoproteins such as thioredoxin reductase, Giardia oxidoreductase 2, NADH oxidase, and Giardia flavohemoglobin also show possible interactions.

Conclusions: Recombinant prospective partners will be further tested for electron transfer with gCYTB5s spectroscopically by monitoring rates of NAD(P)H consumption and gCYTB5 reduction. Understanding the movement of electrons between these proteins will help us piece together the role that gCYTB5s play in Giardia.

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HOST IMMUNITY, MOLECULAR INTERACTIONS, AND MICROBIOME / GUT IMMUNOLOGY AND ENTERIC PARASITES (GIARDIA, ENTAMOEBA, CRYPTOSPORIDIUM, ETC.)

INFLUENCE OF IFN-GAMMA ON THE RESTRICTION OF CRYPTOSPORIDIUM

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Background and Aims: *Cryptosporidium* spp. parasites are an important cause of diarrheal disease that exclusively infect intestinal epithelial cells (IEC). Infection begins with oocyst ingestion and sporozoite invasion of IEC. Next, the parasite undergoes three rounds of asexual replication, followed by sexual differentiation, fertilization, and production of new oocysts. IFN-γ production is required to limit *Cryptosporidium*, and we recently demonstrated that IFN-γ must signal to IEC for optimal control. However, the mechanisms by which IFN-γ restricts *Cryptosporidium* replication remain poorly understood. Thus, this project aims to determine the stage of the *Cryptosporidium* life cycle impacted by IFN-γ.

Methods: Primary IEC will be grown as air-liquid interface (ALI) monolayers in transwells, allowing infection of the apical surface and addition of cytokines to the basolateral surface of IEC. Infected cells will be identified by qPCR and confocal microscopy. Importantly, confocal microscopy permits the identification of parasite's replication stage based on the number of nuclei observed.

Results: Our data demonstrate that pre-incubation of ALI organoids with IFN-γ limits parasite replication over time. Additionally, we have used confocal microscopy to analyze parasite growth in culture and identify infected IEC. Subsequent experiments will identify which replication stage is most impacted by IFN-γ.

Conclusions: Determining the stage impacted by IFN-γ will extend our knowledge of how IEC protect themselves and could lead to the identification of pathways involved in parasite control. This is pertinent, since understanding the IEC-intrinsic mechanisms of pathogen restriction could be applicable to other enteric pathogens.

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HOST IMMUNITY, MOLECULAR INTERACTIONS, AND MICROBIOME / GUT IMMUNOLOGY AND ENTERIC PARASITES (GIARDIA, ENTAMOEBA, CRYPTOSPORIDIUM, ETC.)

IT TAKES 4 TO TANGO: A SNARE QUARTET AT THE HEART OF PARASITE SURVIVAL

Mathi Funk, Corina Wirdnam, Carmen Faso
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Background and Aims: In *Giardia lamblia*, feeding organelles called peripheral endocytic compartments (PECs or PVs) mark the parasite-host interface. Four Soluble N-ethylmaleimide sensitive factor attachment protein receptor (SNARE) proteins were identified in an interactome study of PECs-associated proteins. SNAREs play a central role in membrane fusion events. We hypothesize that this SNARE quartet plays a pivotal role in both endo-and exocytosis by mediating fusion events between PECs and the plasma membrane (PM).

Methods: To test this hypothesis, we have three goals. 1) to determine the precise localization of each SNARE protein using lattice light sheet microscopy (LLSM), followed by a colocalization-analysis pipeline. 2) to understand the membrane fusion machinery in more detail using co-immunoprecipitation (Co-IP) and cross-linking mass spectrometry (XL-MS) to identify native interaction partners of the SNARE quartet and compute possible structural models for the fusion complex. 3) to demonstrate the role of this fusion machinery in endocytosis by expressing dominant-negative mutants of SNARE-associated proteins (Rab1a and NSF) in *Giardia* cells and applying an imaging-based uptake-phenotype analysis pipeline.

Results: Two novel markers, labelling exclusively the PM and PECs were introduced in LLSM experiments. Data from this approach are currently being fed into a bespoken colocalization-analysis pipeline. Furthermore, experimental data revealed interactions with canonical SNARE-associated proteins, including NSF, alpha-SNAP and Vacuolar Protein Sorting (VPS) proteins.

Conclusions: Taken together, our data so far point to the existence of a canonical SNARE complex. Preliminary imaging data discriminates between membrane compartments at the periphery of the cell, paving the way towards a high-resolution definition of each SNARE's location.

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HOST IMMUNITY, MOLECULAR INTERACTIONS, AND MICROBIOME / GUT IMMUNOLOGY AND ENTERIC PARASITES (GIARDIA, ENTAMOEBA, CRYPTOSPORIDIUM, ETC.)

FCGBP SUPPORTS MUC2 MUCUS STRUCTURAL INTEGRITY IN COLONIC BARRIER IMMUNITY AGAINST ENTAMOEBA HISTOLYTICA

Hayley Gorman, France Moreau, Sarah Wilson, Kris Chadee
University of Calgary, Microbiology, Immunology And Infectious Diseases, Calgary, Canada

Background and Aims: The first line of innate host defence in the gastrointestinal tract is the mucus layer. MUC2 mucin and FCGBP are major goblet cell proteins that form the mucus barrier to prevent pathogens from invading the underlying epithelial cells. However, the functional/cooperative role of FCGBP together with MUC2 is not well characterized. Here, we interrogated the function of FCGBP in response to the colonic parasite *Entamoeba histolytica* (*Eh*).

Methods: Native mucus was collected from LS174T goblet-like cells and organoid monolayers from healthy control patients grown in air-liquid interface (ALI), to investigate the effect of *Eh* secreted proteases (*Eh*SP) on MUC2 and FCGBP degradation quantified by immunoblotting. Villin Cre *Fcgbp*$^{fl/fl}$ and *Fcgbp*$^{fl-/fl-}$ littermates were used to interrogate innate host defences in response to *Eh* in the colon by quantifying *Eh*-induced pro-inflammatory cytokines by RT-PCR and a 32-plex cytokine panel.

Results: Time- and dose-dependent degradation of mucus derived from ALI showed rapid degradation of FCGBP with low concentrations of *Eh*SP prior to MUC2 degradation. *Fcgbp*$^{fl/fl}$ showed reduced pro-inflammatory cytokines in *Eh* colonic loops whereas *Fcgbp*$^{fl-/fl-}$ littermates elicited an increase in pro-inflammatory cytokines.

Conclusions: This study demonstrates that the cleavage of FCGBP is a prerequisite for degrading MUC2 in the mucus layer. Loss of Fcgbp dampened a proper innate immune response, suggesting a role for FCGBP in regulating inflammation in the gut to colonic pathogens. These data highlight the need for further research in understanding the cooperative role of MUC2-FCGBP interactions in conferring innate host defense in the gut.

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HOST IMMUNITY, MOLECULAR INTERACTIONS, AND MICROBIOME / GUT IMMUNOLOGY AND ENTERIC PARASITES (GIARDIA, ENTAMOEBA, CRYPTOSPORIDIUM, ETC.)

EFFECT OF CORABION ON OXIDATIVE STRESS IN EXPERIMENTAL AMEBIC LIVER ABSCESS

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Background and Aims: *Entamoeba histolytica* (*E. histolytica*) is the parasitic protozoan that causes amebiasis, an infection that occurs in humans. The parasite causes colitis and can disseminate from the intestinal wall to extraintestinal organs such as the liver, where it causes amebic liver abscess (ALA). ALA is characterized by a granulomatous inflammatory response with extensive areas of necrosis, with no correlation between the number of amebas and the extent of the lesion. Amebic damage is characterized by hepatic tissue destruction associated with a severe inflammatory and oxidative response. The present study aims to assess the effect of Corabion, a compound exhibits antioxidant activity, on evolution of damage and oxidative stress in ALA and its impact on disease progression.

Methods: *E. histolytica* trophozoites were cultured and used to induce ALA in hamsters by intrahepatic inoculation. Animals were divided in three groups, 1) control, 2) infected and 3) Corabion-treated. Corabion was administered orally before and after of amebas inoculation. At 12 h, 48 h, and 7 days, liver and blood samples were collected, and the tissue damage was quantified in each group, the oxidative stress was assessed through TBARS quantification.

Results: A decrease in the percentage of lesion was observed over time in Corabion-treated group compared to the infected group. In addition, a significant reduction in oxidative stress was observed in the hamsters treated with Corabion at 12 h of ALA evolution compared to infected group without treatment.

Conclusions: Corabion reduces the percentage of ALA and the oxidative stress during early stages of infection.

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HOST IMMUNITY, MOLECULAR INTERACTIONS, AND MICROBIOME / GUT IMMUNOLOGY AND ENTERIC PARASITES (GIARDIA, ENTAMOEBA, CRYPTOSPORIDIUM, ETC.)

NITRIC OXIDE DIOXYGENASE ACTIVITY OF GIARDIA INTESTINALIS FLAVOHEMOGLOBINS

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Background and Aims: The parasitic protist *Giardia intestinalis* possesses flavohemoglobin (gFlHb), an enzyme with nitric oxide dioxygenase (NOD) activity that oxidizes nitric oxide (NO) to nitrate (NO_3^-). Of the eight *G. intestinalis* assemblages (A-H), only those that infect humans (A, B, E) encode gFlHb. Research on gFlHb as a potential drug target has focused on gFlHb-A, despite sequence differences of 13-29% relative to gFlHb-B and E.

Methods: To determine if there were enzymatic differences among the gFlHb variants, all three were recombinantly expressed and their NOD activities were measured using a free radical analyzer equipped with a NO-sensitive electrode.

Results: Rates of NOD activity were comparable among variants (k_{cat}: 35-62 s^{-1}), as were their sensitivities towards the substrates NO (K_m: 0.62-0.89 μM) and NADH (K_m: 15-19 μM). Inhibition of NOD activity by miconazole (K_i: 5-13 μM) and cyanide (K_i: 1-3 μM) were comparable among all variants. However, nitrite (NO_2^-) a moderate inhibitor of gFlHb-A and E (K_i: 27 and 61 μM respectively) was a weak inhibitor for gFlHb-B (K_i could not be determined accurately); interestingly optical titrations show that NO_2^- binding affinity to ferric gFlHb was similar for all three variants (K_D: 275-343 μM). Structural comparisons revealed residue differences in substrate entry and product exit pathways connecting the active site.

Conclusions: The NOD rate parameters of gFlHbs are similar, but their different sensitivities towards NO_2^- signify differences in substrate access and product egress that need to be considered if this enzyme is to be examined as a drug target.

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DESPITE A HIGHLY DIVERGENT CLATHRIN LIGHT CHAIN, THE CLATHRIN SUBUNIT INTERFACE IS PRESERVED IN GIARDIA LAMBLIA

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Background and Aims: Clathrin-mediated endocytosis (CME) is conserved across eukaryotes, yet many eukaryotic lineages display differences in the ultrastructural organization and accessory proteins involved. *Giardia lamblia*, a unicellular enteroparasite, represents an extreme case of cellular streamlining, including the absence of canonical endosomes and lysosomes, which are replaced by peripheral endocytic compartments (PECs). These features make *Giardia* a powerful model to ask how far CME can be reduced while remaining functional, and which adaptations enable this streamlining. To address these questions, we investigate the role of clathrin assemblies at *Giardia* PECs by elucidating their structural configuration, with a focus on the divergent clathrin light chain analogue (ACLC).

Methods: *Giardia's* clathrin assemblies were analysed using immunoprecipitation–cross-linking mass spectrometry (XL-MS) to define protein interaction interfaces. The roles of functional domains of *Giardia* clathrin were investigated by analysing the subcellular localization of corresponding truncations using fluorescence microscopy and by assessing their native interactome using immunoprecipitation and mass spectrometry.

Results: XL-MS identified direct interactions between *Giardia* clathrin heavy chain (GlCHC) and ACLC at their C-terminal regions, corresponding to the canonical heavy–light chain interface. Truncation analyses showed that the clathrin trimerization domain enables the recruitment of GlCHC to clathrin assemblies at PECs, while mislocalizing truncations were being actively degraded.

Conclusions: Together, these data indicate that *Giardia* clathrin complexes contain a highly divergent clathrin light chain while retaining assembly principles commonly conserved across eukaryotes, including a conserved heavy–light chain interface and trimerization-dependent assembly. These findings highlight how deeply conserved molecular architectures can persist despite extreme sequence divergence and cellular streamlining.

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MODULATION OF VARIANT-SPECIFIC SURFACE PROTEIN 5G8 IN GIARDIA LAMBLIA DURING HOST INTERACTION

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Background and Aims: Variant-specific surface proteins (VSPs) are central to antigenic variation and immune evasion in *Giardia lamblia*. We previously identified VSP 5G8 as a highly immunogenic antigen; however, the mechanisms regulating its expression during host interaction remain unclear. This study aimed to evaluate the modulation of VSP 5G8 expression *in vivo* and *in vitro*, and to assess its ability to interact with the intestinal epithelial barrier.

Methods: Gerbils (*Meriones unguiculatus*) were infected with 2×10^6 *G. lamblia* trophozoites (GS-5G8(+) variant). Fourteen days post-infection, trophozoites were recovered from the small intestine. In parallel, trophozoites were co-cultured with human colon adenocarcinoma cells (Caco-2) and recovered after 12 h. VSP 5G8 expression was analyzed by flow cytometry and competitive ELISA. The capacity of 5G8 to cross the epithelial barrier was evaluated using a Transwell system.

Results: Trophozoites recovered *in vivo* exhibited a pronounced reduction in surface VSP 5G8 expression, decreasing from 35% to 5%. Similarly, *in vitro* co-culture with epithelial cells led to reduced surface expression (75% to 42%). Competitive ELISA confirmed a decrease in 5G8 production under both experimental conditions. Transwell assays demonstrated that *G. lamblia* induces the translocation of proteins, including VSP 5G8, across the epithelial barrier.

Conclusions: These findings demonstrate dynamic regulation of VSP 5G8 expression during host interaction and suggest parasite-driven mechanisms controlling antigen exposure. The *in vivo* and *in vitro* models presented here provide useful tools to further explore the role of VSPs in immune evasion and host–parasite interactions during giardiasis.

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ANTI-INFLAMMATORY EFFECTS OF BLASTOCYSTIS ST3 COLONIZATION AND HYALURONIC ACID IN EXPERIMENTAL COLITIS

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Background and Aims: Micro-eukaryotes represent a natural yet long-overlooked component of the gut microbiome and may contribute to immune balance and attenuation of intestinal inflammation. *Blastocystis* sp. is an intestinal protist with an unclear role in host health that is more frequently detected in asymptomatic individuals than in patients with IBD/IBS, suggesting a potentially benign or beneficial function. Hyaluronic acid (HA), an important component of the intestinal extracellular matrix, may exert anti-inflammatory effects on the intestinal mucosa and is considered a supportive agent. This study aimed to evaluate the anti-inflammatory effects of *Blastocystis* ST3 colonization and HA administration in experimental colitis.

Methods: Acute colitis was induced in outbred SPF Wistar rats using DNBS. In separate experiments, rats were either colonized with *Blastocystis* ST3 or treated orally with HA. Inflammation was assessed using clinical parameters, macroscopic intestinal evaluation, and cytokine gene expression; histopathology was additionally assessed in *Blastocystis*-colonized groups.

Results: *Blastocystis* ST3 colonization accelerated recovery from colitis, reduced TNFα and IL-1β expression, and improved clinical scores and histopathological findings. In rats without colitis, colonization was associated with increased expression of IL-4, IL-25, and IL-10. HA independently reduced TNFα expression in the same model.

Conclusions: *Blastocystis* ST3 colonization facilitates recovery from experimental colitis. Hyaluronic acid exerts an independent anti-inflammatory effect, particularly at the level of TNFα. Together, these findings suggest immunomodulatory effects of both agents and support future studies exploring their potential synergistic role in intestinal mucosal healing. **Acknowledgements** This work was funded by: OP JAK (MŠMT, Czech Republic), project No. CZ.02.01.01/00/23_020/0008499.

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THE IMPACT OF DIET ON TYPE 2 IMMUNITY AGAINST ENTERIC HELMINTH INFECTION THROUGH ALTERATION OF THE MICROBIOME AND METABOLOME

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Background and Aims: Helminth infections affect a quarter of the global population, causing malnutrition and increased morbidity. The establishment of protective immunity against helminths depends on a robust type-2 immunity. The precise regulatory mechanisms governing this response remain unresolved. Recent attention has turned towards the roles of microbiome and diet, both of which are recognised as modulators of inflammation. Evidence indicates that high-fat-diets facilitate *Trichuris muris* expulsion in mice, while diets rich in soluble plant fibres lead to chronic infection. Host- and microbiome-derived metabolites seemingly influence the host's type-1/type-2 immune balance. Nevertheless, the fundamental mechanisms driving these effects remain unexplored. Our objective is to elucidate how diets through the metabolome and microbiome influence shifts in immunity against *T. muris*. We seek to identify mechanisms responsible for the altered immunity arising from distinct diets and their associated changes in infection.

Methods: We conducted a mouse study involving dietary interventions with polyphenols before and during *T. muris* infection. Comprehensive metabolomic analysis and 16SrRNA sequencing were performed to discern differences in the effects of diets. The infection state and immune response were assessed through worm burden and serum antibody responses.

Results: Preliminary findings indicate substantial influence from diet and infection on the metabolome and microbiome. It appears that there is a clear shift in energy metabolism and a proliferation of facultative anaerobes once infected and on a diet consisting of 1% proanthocyanidins.

Conclusions: The identification of host- and microbiota-derived metabolites linked to type-2 immunity offers the possibility for interventions to promote immunity against helminth infection as well as therapeutics for type-2 driven inflammation.

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B- AND T-CELL EPITOPE IDENTIFICATION OF THE BIP PROTEIN FROM GIARDIA LAMBLIA

Karina Esparza[1], Thania Garzón[1], Lucila Rascón[1], David Ortega-Tirado[2], Carlos Velazquez[1]
[1]University of Sonora, Chemistry-biology, Hermosillo, Mexico, [2]Universidad de Sonora, Hermosillo, Mexico

Background and Aims: *Giardia lamblia* causes giardiasis, a major intestinal parasitic infection worldwide. There is currently no licensed vaccine for humans. Identifying parasitic immunogenic antigens is essential for developing new prophylactic strategies. The immunoglobulin binding protein (BIP) has been proposed as a immunogenic antigen. Its immunological characteristics remain poorly understood. This study aimed to immunologically characterize the BIP protein of *G. lamblia* and identify potential B- and T-cell epitopes.

Methods: Linear B-cell epitopes were predicted *in silico* using BepiPred 2.0, and conformational epitopes with ElliPro. To experimentally validate these predictions, polyclonal antibodies were generated by immunizing BALB/c mice with recombinant BIP. Monoclonal antibodies anti-BIP protein were produced. These antibodies were used in Western blot assays to identify immunoreactive fragments, which were analyzed by mass spectrometry to determine their amino acid sequences. T-cell epitopes (I-A^k haplotype) were predicted using NetMHCII 3.2.

Results: Immunoinformatics analysis identified multiple linear and conformational B-cell epitopes located in exposed regions of the protein structure. Western blot assays showed that both polyclonal and monoclonal antibodies recognized several protein fragments, including an approximately 7 kDa fragment detected by both antibody types. Predicted T-cell epitopes showed strong binding affinity to mouse MHC class II I-A^k, with some residues overlapping predicted B-cell epitopes.

Conclusions: The BIP protein contains immunoreactive B- and T-cell epitopes supported by *in silico* predictions and experimental validation. Our data highlight BIP as an antigen of interest and laying the groundwork for future multipeptide vaccine strategies against *G. lamblia*

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HOST IMMUNITY, MOLECULAR INTERACTIONS, AND MICROBIOME / GUT IMMUNOLOGY AND ENTERIC PARASITES (GIARDIA, ENTAMOEBA, CRYPTOSPORIDIUM, ETC.)

A SELF-LIMITING TYPE I IMMUNE-STROMAL CELL NETWORK PROTECTS INTESTINAL BARRIER INTEGRITY DURING INFECTION

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Background and Aims: Intestinal host defence depends on the interplay between immune mediators, structural elements, and microbial cells. Helminths are large tissue-invasive parasites that are well-tolerated suggesting an intercellular dialogue exists maintaining host fitness. In intestinal helminth infection, we found a type 1 immune signature protecting host mortality without altering parasite load defining a new mechanism of disease tolerance. This defense strategy was driven by IFNg-producing CD8 T cells in an antigen-independent, gut microbiota-dependent manner. Our aim was to define the intercellular network necessary for the canonically cytotoxic IFNg to mediate host defence without creating excessive collateral damage.

Methods: Mice were infected with the enteric murine roundworm *Heligmosomoides polygyrus bakeri* (*Hpb*) while intestinal tissue architecture and immune profile were determined during the early tissue-invasive phase of infection. Using adoptive cell transfer, transgenic reporter mice and bone marrow chimeric approaches, we explored an intercellular network driving disease tolerance.

Results: Eliminating IFNg receptor signaling provoked host morbidity to high *Hpb* doses including increased granuloma size, localized tissue bleeding, myofibroblast remodelling and loss of protective neutrophil recruitment to the infection site. Probing IFNg signaling in discreet cell compartments revealed an immune-smooth muscle cell interplay critical for promoting host fitness and barrier integrity. Further studies suggest that IFNg-dependent, stromal-derived prostaglandins quench IFNγ signaling, limiting collateral damage to type 1 immune-mediated host defense.

Conclusions: Our studies describe an unexpected role for microbiota-dependent IFNg-producing CD8 T cells in limiting tissue damage to intestinal helminth infection and reveal a self-limiting immune-stromal cell network that could be targeted for protection against tissue injury.

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HOST IMMUNITY, MOLECULAR INTERACTIONS, AND MICROBIOME / GUT IMMUNOLOGY AND ENTERIC PARASITES (GIARDIA, ENTAMOEBA, CRYPTOSPORIDIUM, ETC.)

ENTERIC EUKARYOTIC COLONISATION DURING PREGNANCY AND INFANCY IN A MALNOURISHED POPULATION: IMPLICATIONS FOR MATERNAL AND CHILD HEALTH

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Background and Aims: Enteric parasites and other gut eukaryotes are highly prevalent in low-resource settings and may influence nutrition, immune function, and early development, yet remain understudied during pregnancy and infancy. This study aimed to characterize patterns of enteric eukaryotic colonisation in mothers and infants in a malnourished population, and how these eukaryotic communities, along with bacterial communities and host factors influence maternal and child health.

Methods: We conducted a longitudinal cohort study of 400 pregnant women aged 17-24 years in Matiari District, Pakistan. Stool samples were collected from mothers during pregnancy and postpartum, and from infants at 3 and 12 months of age. Enteric eukaryotic communities were profiled using 18S rDNA sequencing, alongside 16S rDNA sequencing of bacterial communities. Maternal anthropometry, gestational weight gain, birth outcomes, infant growth (WHO z-scores), clinical data, diet, medication use, and environmental exposures were recorded.

Results: Enteric eukaryotes were common but showed marked age-specific colonisation patterns. *Blastocystis*, often co-occurring with *Entamoeba* and *Endolimax*, was prevalent in maternal samples during pregnancy and postpartum. In contrast, infants showed higher colonisation by fungi and *Pentatrichomonas,* with some overlap with maternal protist profiles. G*iardia* were also detected in a subset of infants. Ongoing analyses are examining co-occurrence patterns among protists and fungi, and their associations with bacterial community structure and host nutritional and health outcomes.

Conclusions: Enteric eukaryotes display distinct, age-dependent colonisation patterns and may compete for ecological niches in the gut. Integrating eukaryotic gut microbiome communities into maternal-child health studies is essential for understanding nutrition and health during pregnancy in high-burden settings.

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HOST IMMUNITY, MOLECULAR INTERACTIONS, AND MICROBIOME / HOST MICROBIOME AND PARASITIC INFECTION

PROTIST–BACTERIOME ASSOCIATIONS ACROSS THE HUMAN–DOG INTERFACE IN A WESTERNIZATION GRADIENT

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Background and Aims: Intestinal protists are common components of the gut ecosystem, yet their ecological relationships with bacterial communities across human–animal interfaces remain poorly understood. This study aimed to examine how Westernization shapes intestinal protist communities and their associations with the gut bacteriome in humans and their companion dogs.

Methods: We applied a paired dog–owner design across rural and urban populations in Romania representing traditional and urban lifestyles and integrated these data with harmonized cohorts from the Czech Republic representing a Westernized population context. Fecal samples from 256 humans and 192 dogs were screened for *Blastocystis* sp., *Giardia intestinalis*, *Dientamoeba fragilis*, and *Entamoeba* spp. using quantitative molecular detection and genetic subtyping. Gut bacterial communities were profiled by 16S rRNA gene sequencing using the Illumina MiSeq platform.

Results: Protist prevalence and genetic diversity were highest in rural settings and declined toward urban environments in both humans and dogs. Along this Westernization gradient, bacterial alpha diversity decreased and inter-individual microbiome variability increased in both hosts. In humans, colonization by *Blastocystis* sp. and *Giardia intestinalis* was consistently associated with higher bacterial diversity and shifts in community structure. In dogs, *Giardia intestinalis* was associated with increased bacterial diversity, whereas *Blastocystis-associated effects* were weak or absent. No consistent microbiome associations were observed for *Dientamoeba fragilis*.

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Conclusions: These data demonstrate that protist–bacteriome associations are host-specific and shift along a Westernization gradient, even between shared human-dog environments. **Acknowledgment:** This work was supported by a grant from the Czech Science Foundation (22-04837S) to KJ.

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HOST IMMUNITY, MOLECULAR INTERACTIONS, AND MICROBIOME / HOST MICROBIOME AND PARASITIC INFECTION

THE IMPACT OF ELEVATED TEMPERATURE ON THE MICROBIOME OF A TREMATODE AND ITS CRUSTACEAN HOST

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Background and Aims: An animal's microbiome plays crucial roles in all aspects of its life, and therefore quantifying the effect of temperature on animal microbiomes becomes increasingly important in the context of global climate change. This study investigated whether experimental exposure to higher temperature as well as the duration of the exposure period modifies the microbial diversity and species composition of the trematode, *Maritrema poulini* and its freshwater isopod host, *Austridotea annectens*.

Methods: Naturally infected isopods were assigned to three laboratory treatments consisting of exposing them to ambient (17 °C), warm (22 °C), and warmer (24 °C) temperatures for either 14 days or 28 days, creating a prolonged exposure period. The bacterial component of the host and parasite microbiomes were genetically characterised and compared among all three temperature treatments and between two temporal periods.

Results: We found a decrease in diversity for: 1) the isopod microbiome as temperature increased, at both phylum and genus level; 2) the *M. poulini* microbiome as the exposure time increased, at all taxonomic levels tested. In terms of species composition, we found both temperature and exposure time modified the host and parasite microbiomes at several taxonomic levels.

Conclusions: The study shows that both temperature and exposure time can influence both the diversity and species composition of host and parasite microbiomes, with greater impact on species composition than on diversity. The findings suggest that global warming will modify the microbial communities of hosts and parasites, with unforeseen consequences.

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HOST IMMUNITY, MOLECULAR INTERACTIONS, AND MICROBIOME / HOST MICROBIOME AND PARASITIC INFECTION

THE ROLE OF FERMENTABLE FIBRES IN TRICHURIS MURIS INFECTION: HOST SUSCEPTIBILITY AND MICROBIAL INTERACTIONS

Anne Majgaard Jensen[1], Amalie Vedsted-Jakobsen[1], Eirikur Andri Þormar[2], Louise Von Gersdorff Jørgensen[1], Morten Tønsberg Limborg[2], Andrew Richard Williams[1]
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Background and Aims: Parasitic helminths infect over a billion people worldwide and contribute to malnutrition and morbidity. These infections involve complex interactions with the host immune system and gut microbiota (GM), while the contribution of the worm-associated microbiome remains poorly understood. Dietary components, such as fermentable fibres, can modulate GM and may influence host susceptibility to infection. Here, we investigated how dietary fermentable fibres and antibiotic-mediated disruption of the GM affect susceptibility to *Trichuris muris* infection and the stability of the worm microbiome.

Methods: Mice were fed diets containing fermentable fibres or non-fermentable fibres for 14 days before *T. muris* infection. Worm burdens were quantified to assess host susceptibility. Host gut microbiome composition was analysed using 16S rRNA gene sequencing. In a separate experiment, mice received broad-spectrum antibiotics to deplete the GM, and worm infectivity and microbiome composition were assessed in antibiotic-treated and untreated mice.

Results: Mice fed fermentable fibres exhibited significantly higher worm burdens, indicating that fermentable fibres increase susceptibility to *T. muris* infection. Host GM composition differed markedly between dietary groups; however, the worm-associated microbiome remained stable across diets. Antibiotic treatment significantly altered host GM composition but did not affect worm burden or worm microbiome diversity. Worm-associated microbial communities were highly similar between antibiotic-treated and untreated mice.

Conclusions: These findings demonstrate that dietary fermentable fibres enhance susceptibility to *T. muris* infection, but this effect does not appear to be mediated by changes in the host GM. Moreover, the stability of the worm-associated microbiome, even following antibiotic-induced perturbation, highlights its resilience.

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HOST IMMUNITY, MOLECULAR INTERACTIONS, AND MICROBIOME / HOST MICROBIOME AND PARASITIC INFECTION

MONEPANTEL-BASED ANTHELMINTIC TREATMENT INDUCES SHIFTS IN GASTROINTESTINAL MICROBIOTA IN SHEEP

Carlos Lanusse, Maria Mate, Luis Alvarez, Juan Liron
CIVETAN, Tandil, Argentina

Background and Aims: Gastrointestinal nematodes remain one of the most significant biological constraints to sustainable livestock production worldwide, with well-recognized economic and productivity losses. Beyond their direct effects on the host, there is growing recognition that nematode colonization actively shapes the gastrointestinal (GI) environment, altering the ecological niche where parasites and microbial communities coexist.

Methods: In this context, understanding bacteria–nematode interactions has become increasingly relevant for the development of innovative and integrative parasite control strategies. The goal of the current study was to assess how a monepantel-based anthelmintic treatment influences GI microbial communities in sheep.

Results: Seventeen (17) days after treatment, only a low number of *Oesophagostomum radiatum* and *Trichuris ovis* were recovered from the large intestine of treated animals, indicating effective nematode reduction. Concomitantly, marked shifts in the abomasal microbiota were observed following treatment, including a significant decrease in the bacterial family *Mycoplasmataceae* family (Log2FC= -4, Padj= 0.001) and a pronounced increase in *Methanobacteriaceae* (Log2FC= 2.9, Padj= 0.018). These changes suggest that monepantel-induced nematode removal is associated with rapid restructuring of abomasal microbial community.

Conclusions: Although the mechanistic basis of interactions among mycoplasmas, methanogenic archaea and nematode populations requires further investigation, our results support the concept that parasite control interventions can have broader ecological consequences within the GI ecosystem, highlighting the potential of parasite–bacterial community interactions as a novel framework for the development of innovative and sustainable parasite control strategies.

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POSTER ON BOARD SHIFT 02
HOST IMMUNITY, MOLECULAR INTERACTIONS, AND MICROBIOME / HOST MICROBIOME AND PARASITIC INFECTION

SUPPLEMENTATION WITH ZINC OXIDE NANOPARTICLES REDUCES GREENHOUSE GAS EMISSIONS AND CONTROL ENDOPARASITE INFECTION IN LAMBS

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Background and Aims: Methane (CH_4) is a byproduct of ruminal fermentation, a process where microbes in the rumen break down feed. Increased methane production leads to higher greenhouse gas emissions and lower feed efficiency. We investigated the effect of ZnO nanoparticles (NPs) supplementation on CH_4 emissions, growth parameters, and parasitological status in lambs infected with the gastrointestinal nematode *Haemonchus contortus*.

Methods: 24 lambs were divided into four groups: control (Con), Con supplemented with ZnO-NPs (Zn), infected group (Inf), and Inf with ZnO-NP supplement (Inf+Zn). ZnO-NP supplementation lasted for 40 days, and four open-circuit respiratory chambers were used to measure CH_4 over the last nine days. Average daily gain (ADG) and dry matter digestibility (DMD) were evaluated. Quantification of eggs per gram of faeces (EPG) was performed using the modified McMaster method.

Results: Significant differences in DMD and ADG were found between the Inf and Inf+Zn lamb groups. CH_4 emission in the Inf group was higher (by 21%) than in the Con group. ZnO-NPs significantly reduced CH_4 emissions (20%) in the Inf+Zn group. In the Inf group, high EPG was maintained until the end of the experiment. In the Inf+-Zn group, the EPG reduction began on day 33 and continued until day 38.

Conclusions: Supplementation with ZnO nanoparticles slowed parasite infection dynamics, increased body weight by improving dry matter digestibility, and reduced methane emissions in lambs. *Acknowledgment:* The study was supported by funds from the Ministry of Education of the Slovak Republic and the Slovak Academy of Sciences (VEGA 2/0007/25).

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CHARACTERIZING INTERACTIONS BETWEEN TRICHOMONAS VAGINALIS AND VAGINAL MICROBIOTA LACTOBACILLUS GASSERI AND GARDNERELLA VAGINALIS

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Background and Aims: *Trichomonas vaginalis* is a flagellated protozoan parasite whose infection is associated with an imbalance in the cervicovaginal microbiome. A cervicovaginal microbial community dominated by *Lactobacillus gasseri* is found in some women and is categorized as community state type II (CST-II). CST-II is associated with resilience to infections in the female reproductive tract and may protect against bacterial vaginosis. Whereas, Gardnerella vaginalis is found in women with bacterial vaginosis and a cervicovaginal microbial community characterized by reduced amounts of Lactobacillus species (CST-IV).

Methods: We hypothesize that cervicovaginal bacteria may display differential susceptibility to *T. vaginalis* antibacterial activity. To test this, we performed total killing assays in a synthetic media that mimics vaginal secretions, called Serrador's Lactobacillus-adapted Iscove's Media-vaginally adapted (SLIM-V).

Results: In 3hr co-incubation assays, we found that *T. vaginalis* does not affect the viability of *L. gasseri*, and similarly the presence of *L. gasseri* did not affect the viability of *T. vaginalis*. Whereas, *T. vaginalis* displayed antibacterial activity against G. vaginalis, reducing the colony formation by 54% compared to the *G. vaginalis* only control. Growth of *T. vaginalis* was not affected by exposure to *G. vaginalis*.

Conclusions: Our studies employing comparable experimental conditions in SLIM-V media reveal a potential difference in antibacterial activity exerted by *T. vaginalis*, which we are investigating further. Further mechanistic study of the interactions between *T. vaginalis* and the cervicovaginal microbiome could lead to improved methods of treatment for those infected with *T. vaginalis* or to try to counteract the effects vaginal dysbiosis.

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OPTIMIZING NESTED PCR FOR SENSITIVE DETECTION OF PLASMODIUM MALARIAE IN LABORATORY SETTINGS IN SOUTHERN VIETNAM

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Background and Aims: Nested polymerase chain reaction (Nested PCR) is a highly sensitive technique for detecting infectious diseases, particularly in cases with low parasitemia such as malaria. This study aimed to optimize a Nested PCR protocol for the specific detection of *Plasmodium malariae*, focusing on refining cycling conditions, annealing temperatures, and procedural parameters to ensure reliable performance in laboratory settings in Southern Vietnam.

Methods: The assay targeted the *P. malariae* 18S rRNA gene using the primer pairs PLU5/PLU6 and MAL1/MAL2. Cycle numbers were optimized at 25, 30, and 35 cycles for the first round, and 25 and 30 cycles for the second round. The annealing temperature for the MAL1/MAL2 primers was evaluated across a range of 54–60°C. PCR products were visualized using the ChemiDoc-It2 imaging system.

Results: The MAL1/MAL2 primer pair demonstrated high specificity for *P. malariae*, with no cross-amplification observed for other *Plasmodium* species, including *P. falciparum*, *P. vivax*, *P. ovale*, and *P. knowlesi*, or for common microbial contaminants. Optimal conditions were identified as 35 cycles in the first PCR round and 25 cycles in the second round, with an annealing temperature of 54°C for the MAL1/MAL2 primers. Under these conditions, the assay achieved a limit of detection of 0.5 parasites/µL.

Conclusions: The optimized protocol is well-suited for laboratories in Southern Vietnam.

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MICROBIOTA-BASED ORAL EXPOSURE TO ALPHA-GAL-EXPRESSING BACTERIA REDUCES AVIAN MALARIA SUSCEPTIBILITY THROUGH NATURAL ANTIBODY-MEDIATED IMMUNITY

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Background and Aims: Natural antibodies against galactose-α-1,3-galactose (α-Gal) have been implicated in protection against several pathogens, including *Plasmodium* spp. However, experimental evidence linking microbiota-driven α-Gal immunity to malaria resistance in birds remains limited. We conducted a controlled experimental infection study in domestic canaries, *Serinus canaria*, to assess whether oral exposure to α-Gal expressing bacteria modulates anti-α-Gal antibody responses and susceptibility to avian malaria.

Methods: Canaries were orally administered with either live *Escherichia coli* O86:B7, live *E. coli* Nissle 1917, heat-killed *E. coli* Nissle 1917, or phosphate-buffered saline as a control. Following bacterial exposure, birds were subjected to bites of *Culex pipiens* mosquitoes infected with *Plasmodium relictum*. Anti-α-Gal IgY and IgM levels were quantified by ELISA, and infection outcomes were evaluated.

Results: Oral exposure to live *E. coli* strains reduced the proportion of infected birds compared with control and heat-killed treatments, whereas non-viable bacteria did not confer protection. Exposure to live bacteria was associated with distinct anti-α-Gal antibody responses, including increased IgM levels following infection and strain-dependent induction of IgY responses. Although peak parasitemia did not differ significantly among groups, birds exposed to live bacteria showed reduced variability and lower parasitemia.

Conclusions: Our results provide experimental evidence that microbiota-based oral exposure to α-Gal-expressing bacteria can shape anti-α-Gal immunity and reduce susceptibility to avian malaria. The study highlights the ecological relevance of host-microbiota-parasite interactions and supports α-

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Gal-targeted, microbiota-based strategies for controlling vector-borne infections in birds. This research was funded by a grant (No. S-MIP-25-42) from the Research Council of Lithuania.

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POSTER ON BOARD SHIFT 02
HOST IMMUNITY, MOLECULAR INTERACTIONS, AND MICROBIOME / HOST MICROBIOME AND PARASITIC INFECTION

CHICORY (CICHORIUM INTYBUS) MODIFIES THE RUMINAL ENVIRONMENT OF PARASITE-BURDENED LAMBS

Daniel Petrič[1], Matej Leško[1], Dominika Batťányi[1], Adam Cieślak[2], Sylwester Ślusarczyk[3], Marián Várady[4], Zora Váradyová[1]
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Background and Aims: Chicory is a perennial herb that is considered a high-quality feed containing phytochemicals and trace elements that can improve the growth of grazing lambs burdened with endoparasites. We investigated whether meadow pasture enriched with experimentally sown chicory could favorably affect growth parameters and the ruminal environment of lambs with endoparasites.

Methods: Two fenced plots were established: one with meadow grassland and another with 25% chicory reclamation. Sixteen lambs infected with *Haemonchus contortus* were divided equally between the two plots and grazed for 144 days. The phytochemical composition of both pastures was analyzed using ultra-high-resolution mass spectrometry. Growth parameters, ruminal microbial fermentation, and parasitological status of lambs were analyzed.

Results: The levels of phytochemicals on both pastures were higher in May and July compared to September. The number of eggs per gram of faeces decreased significantly in both groups of lambs from day (D) 48 onwards, exceeding 95% after D103. From D89 to D144, lambs grazing on chicory pasture gained more body weight ($p<0.001$), had higher ammonia nitrogen content in their rumen ($p<0.05$), and lower propionate concentrations ($p<0.05$). The total bacterial population and abundance of Streptococcus bovis were also higher ($p<0.05$).

Conclusions: Pasture enriched with chicory improved the utilisation of nutrients, microbial fermentation in the rumen, and lamb growth performance, while slowing down the dynamics of endoparasite infection. This study was supported by the Scientific Grant Agency of the Ministry of Education of the Slovak Republic and the Slovak Academy of Sciences (VEGA 2/0007/25).

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HOST IMMUNITY, MOLECULAR INTERACTIONS, AND MICROBIOME / HOST MICROBIOME AND PARASITIC INFECTION

'OMICS INSIGHTS INTO THE ROLE OF PROTIST-MICROBIOME INTERACTIONS IN PREGNANCY OUTCOMES OF IRON-DEFICIENT MICE

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Background and Aims: The gut microbiome plays a pivotal role in modulating host susceptibility to pathogenic protists, particularly under nutritional stress and during pregnancy. Iron deficiency, prevalent in many populations, exacerbates parasitic infections and adversely affects maternal and fetal health. As many enteric protists can exhibit both commensal and pathogenic behaviour, this study explores how model pathobiont *Tritrichomonas musculis* (Tmu) interacts with gut microbiota under iron-deficient conditions, with a focus on pregnancy and birth outcomes.

Methods: Female C57BL/6 mice received iron-replete (45 ppm) or deficient (5 ppm) diets for 2 weeks prior to oral gavage with 1×10^6 Tmu protists or saline. Caecal DNA and RNA were collected at baseline (pre-mating), third trimester, and 4-8 weeks postpartum from dams and available pups. 16S rRNA data were analyzed with QIIME2, and metatranscriptomes using Metapro. Differential abundance and gene expression were evaluated with phyloseq and MaAsLin3; functional changes were explored through GO term and KEGG pathway enrichment.

Results: Iron deficiency markedly reduced litter size and caused pup mortality within two days. Tmu colonization was unaffected by diet and did not alter pregnancy outcomes. However, preliminary 16S rRNA and metatranscriptomic analyses revealed substantial microbiome alterations—shifts in diversity, composition, and gene expression—driven by both iron deficiency and Tmu colonization. Notably, maternally transmitted Tmu accelerated microbiome diversity maturation in pups. Functional pathway enrichments induced by Tmu and micronutrient status are under investigation.

Conclusions: Our findings demonstrate synergistic effects of diet, microbiome, and protist colonization, with implications for maternal and neonatal health in settings with high levels of nutritional deficiencies and enteric protists.

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PROBIOTIC MODULATION OF BEHAVIORAL ALTERATIONS IN A RAT MODEL OF CHRONIC TOXOPLASMA GONDII INFECTION

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Background and Aims: Toxoplasma gondii is linked to neuroinflammation and behavioral alterations. Elucidating its immunomodulatory strategies targeting the gut–brain axis may facilitate the development of interventions to mitigate these effects. This study evaluated whether probiotic administration could restore healthy behavior in experimental chronic toxoplasmosis and identified the immunoregulatory pathways involved.

Methods: Seventy-five male Rattus norvegicus were randomized into five groups: G1 (T. gondii), G2 (T. gondii followed by Lactobacillus rhamnosus administration), G3 (L. rhamnosus followed by T. gondii), G4 (L. rhamnosus), and a control group. Cerebral IL-10 and TNF-α concentrations were measured by enzyme-linked immunosorbent assay at 15 days post-infection (dpi) to establish baseline values for subsequent analysis. Behavioral assessments were conducted at 60 dpi using the elevated plus maze and open field tests. Data were analyzed using the Kruskal–Wallis test or analysis of variance (α=0.05).

Results: Chronic infection in G1 was associated with behavioral alterations suggestive of anxiety-like behavior. The probiotic administration prior to infection showed modulatory effects in G3, as rats exhibit behavior similar to healthy controls. There were no significant differences in cerebral IL-10 and TNF- α levels between groups at 30dpi, which suggests that immunomodulation happens at a later stage and can play a role in the behavioral normalization observed.

Conclusions: *L. rhamnosus* demonstrated potential modulatory effects on behavioral outcomes in chronic toxoplasmosis, possibly through immune-regulatory mechanisms involving IL-10 and TNF- α. Research on both parameters at 90 days post-infection is underway. These findings support further investigation of gut–brain interventions in parasitic neuroinflammation.

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FACTORS DETERMINING SPECIES-SPECIFIC MICROBIAL SIGNATURES IN PARASITIC HELMINTHS SHARING AN INSECTIVOROUS BAT HOST

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Background and Aims: There is increasing evidence that parasitic metazoans maintain close associations with microbial communities. However, for most helminths, particularly those that parasitize wildlife, the origin of these interactions and their role in the evolution of parasitism remain poorly understood. This study aimed to evaluate the factors that determine the composition and structure of the microbiome associated with helminths coexisting in the same host, an insectivorous bat.

Methods: We used the insectivorous bat *Peropteryx kappleri* and its associated helminths as a model system. The microbiome of 41 helminths belonging to four trematode species and one nematode species was characterized in different intestinal and biliary microhabitats, along with host-associated samples. Bacterial communities were analyzed using the V4 region of the 16S rRNA gene. Microbiome structure was assessed by relative abundance analysis, alpha and beta diversity, and multivariate comparisons. Differentially abundant taxa were identified using DESeq2, and finally, the phylosymbiosis signal among trematode species was assessed by comparing helminth genetic distances with microbial distances.

Results: The helminth microbiome composition was partially influenced by the host microhabitat; however, each helminth species exhibited a distinctive microbial signature. No phylogenetic signals were detected among trematode species. Compared to the bat host, helminths showed a greater relative abundance of microbial taxa with symbiotic potential, including lineages commonly associated with arthropods, which could represent intermediate hosts.

Conclusions: Overall, our results indicate that helminth microbiomes are primarily determined by species identity, rather than by the host environment, and highlight their relevance for the establishment, development, and transmission of parasites.

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TUFT CELLS PLAY A CRITICAL ROLE IN HELMINTH-INDUCED ATTENUATION OF INTESTINAL AND NEUROINFLAMMATION DURING DIET-INDUCED OBESITY.

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Background and Aims: Progressive neuroinflammation is increasingly linked to obesity and dietary imbalance and is thought to be influenced by signals originating from the gastrointestinal tract through the gut–brain axis. While this axis provides a conceptual framework for understanding how gut-derived signals affect gut-brain function, the cellular mechanisms that initiate and transmit these signals remain poorly defined. Intestinal tuft cells are rare epithelial chemosensory cells best known for sensing parasitic infections and initiating type 2 immune responses. Whether tuft cells also participate in gut–brain communication and metabolic regulation remains unclear.

Methods: To investigate the role of tuft cells in gut–brain axis signaling, we used a mouse model of diet-induced obesity combined with genetic tuft cell deficiency. Mice were infected with the intestinal helminth *Heligmosomoides polygyrus* to activate tuft cell–dependent pathways. Metabolic outcomes, including body weight, were monitored following infection. Transcriptomic profiling was performed in gut and brain tissues, and flow cytometry was used to evaluate changes in intestinal immune responses.

Results: Helminth infection robustly activated tuft cells and was associated with significant weight loss in tuft cell–competent mice, indicating restoration of metabolic regulation. In contrast, tuft cell–deficient mice failed to exhibit comparable weight loss, demonstrating the requirement of tuft cells for these effects. Transcriptomic analyses of gut and brain tissues revealed marked reversals of diet-induced inflammation following *H polygyrus* infection in wild-type mice but not tuft cell-deficient mice.

Conclusions: Helminth infection reverses intestinal and neuroinflammation induced by diet-induced obesity of activating tuft cell-dependent type 2 immune circuits.

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HOST-PARASITE-MICROBIOME INTERACTIONS OF PROCYON LOTOR AND BAYLISASCARIS PROCYONIS

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Background and Aims: The intestinal helminth *Baylisascaris procyonis* was introduced to Germany at the beginning of the 20th century together with its main host, the North American raccoon (*Procyon lotor*), from where both spread throughout Europe. New research findings imply that the microbial composition of raccoons plays a significant role in the invasion process. This study aims to examine the interdependent relationships between invasive raccoons, the intestinal microbiome, and the parasite to investigate the effects of *B. procyonis* infection on the holobiont.

Methods: The gut and fecal microbiome of raccoons from two study areas was examined using Oxford Nanopore sequencing and analyzed for population- and infection-modulated effects. Additionally, the gut microbiome of *Baylisascaris procyonis* was characterized and correlated with that of the host.

Results: The results show a high microbial diversity in raccoon feces. Furthermore, the results suggest that the microbial composition of the helminths intestinal microbiome is visibly different from that of raccoons, but likely to be acquired from the host microbiome. So far, no significant effects of helminth infection on the bacterial composition of the fecal microbiome have been observed.

Conclusions: The first results indicate a high level of microbial diversity in the raccoon's fecal microbiome, which reflects its generalist lifestyle. The close co-evolutionary link between *Baylisascaris procyonis* and raccoons is becoming apparent. More in-depth investigations of the raccoon's gut microbiome at the site of infection are now being carried out to provide more detailed information about infection-modulated effects.

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HOST IMMUNITY, MOLECULAR INTERACTIONS, AND MICROBIOME / HOST MICROBIOME AND PARASITIC INFECTION

WHO CARRIES BLASTOCYSTIS? INSIGHTS FROM A POPULATION-BASED STUDY OF GUT EUKARYOTES AND HUMAN HEALTH

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Background and Aims: *Blastocystis* is a common intestinal microeukaryote estimated to colonize one billion people worldwide. Despite this ubiquity, its impact on the gut microbiome and host health remains unresolved. Most carriers are asymptomatic and recent work links its presence to healthier cardiometabolic outcomes. To better understand the characteristics of *Blastocystis* carriage, we analyzed samples from a large, population-based cohort recruited by random invitation, with extensive questionnaire-based and clinical phenotyping from the Copenhagen metropolitan area.

Methods: Fecal samples from 2,460 adult participants from The Danish Study of Functional Disorders were analyzed using amplicon-based next-generation sequencing of the 16S/18S rRNA genes for species-level taxonomic assignment. Binary outcomes were analyzed using logistic regression and continuous outcomes using linear regression. Categorical predictors were modelled using reference-level contrasts. All models were adjusted for age and sex, with Benjamin-Hochberg false discovery rate correction (BH-FDR) applied across tested predictors.

Results: Preliminary results from the first 1,579 analyzed samples showed *Blastocystis* carriage in 28.5% of participants (**Table 1**), with ST3 (31.1%) and ST4 (28.4%) as the predominant subtypes. Bacterial communities in carriers showed higher alpha diversity, reflected in Chao1 ($p = 2.12e-24$) and Shannon diversity ($p = 4e-04$) indices. Regression models summarized age- and sex-adjusted associations between participant characteristics and *Blastocystis* carriage following BH-FDR, including differences in bacterial diversity metrics, gastrointestinal symptom intensity, and body

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composition measures (**Figure 1**).

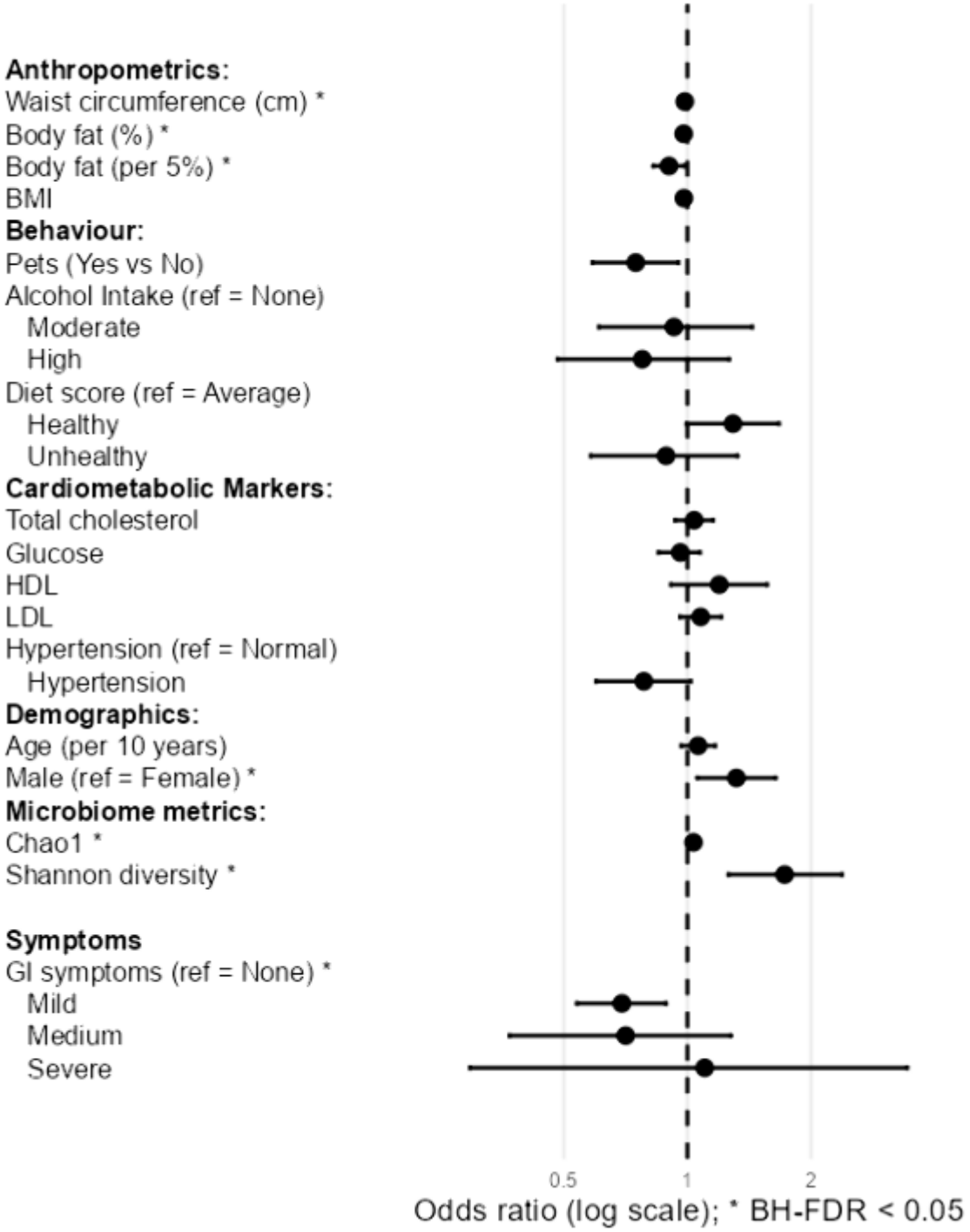
Table 1

Characteristic	Blastocystis Negative N = 1,129[1]	Blastocystis Positive N = 450[1]
Age	55 (47, 64)	57 (47, 65)
Gender		
F	613 (54%)	212 (47%)
M	516 (46%)	238 (53%)
BMI	25.6 (23.1, 28.3)	25.4 (23.1, 28.1)
Body fat (%)	29 (23, 36)	28 (22, 34)
Waist Circumference (cm)	89 (80, 98)	89 (78, 98)
GI Symptom Intensity		
none	685 (61%)	309 (70%)
mild	376 (34%)	114 (26%)
medium	45 (4.0%)	14 (3.2%)
severe	9 (0.8%)	4 (0.9%)
Chao1	74 (63, 85)	82 (74, 92)
Shannon Diversity	2.81 (2.56, 3.03)	2.88 (2.63, 3.08)

[1] Median (Q1, Q3); n (%)

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Figure 1. Adjusted odds ratios for Blastocystis carriage



Conclusions: Together, these findings reinforce the view of *Blastocystis* as a marker of gut microbial diversity and highlight consistent differences in host body composition and gastrointestinal symotoms among carriers in a large, population-based cohort.

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BLASTOCYSTIS IS NOT A PATHOGEN YOU CATCH: IT IS A CORE GUT EUKARYOTE YOU LOSE

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Background and Aims: *Blastocystis* is still framed as a pathogen you catch, despite being among the most frequently detected gut eukaryotes. We tested a deliberately contrarian model: in many hosts, *Blastocystis* behaves as a cyclic resident whose detectability tracks ecosystem state, and the health-versus-disease debate is often a problem of context, perturbation, and subtype biology rather than a simple yes/no infection.

Methods: We combined longitudinal sampling with a European cohort study. *Blastocystis* detection and subtyping used qPCR and amplicon sequencing; bacterial community structure with metagenomics; functional shifts were assessed by faecal ^{1}H NMR metabolomics. To move beyond correlations, we also performed in vitro experiments using multiple *Blastocystis* subtypes, testing responses to bacterial material (whole community fractions and cell-free components) to assess subtype-specific interactions with bacterial-derived cues.

Results: Longitudinally, antibiotic-associated loss of bacterial diversity coincided with a transient loss of *Blastocystis* signal followed by re-detection as the microbiome recovered, arguing against straightforward clearance and highlighting time-dependent false negatives. In the cohort, *Blastocystis* positivity was common and associated with higher bacterial alpha diversity. In vitro, subtypes showed distinct, reproducible responses to bacterial components, supporting the idea that *Blastocystis* biology is modulated by bacterial context and that subtype heterogeneity can generate apparently contradictory clinical associations.

Conclusions: These findings challenge the reflex that a positive result equals causation. Blastocystis may often be a marker and potentially a mediator of ecosystem state, while cyclic abundance and subtype-specific bacterial responsiveness help explain persistent inconsistencies across studies. Progress now requires longitudinal, subtype-aware designs that explicitly model perturbation before assigning pathogenicity.

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SHIFT02-195

POSTER ON BOARD SHIFT 02
HOST IMMUNITY, MOLECULAR INTERACTIONS, AND MICROBIOME / HOST MICROBIOME AND PARASITIC INFECTION

ZINC OXIDE NANOPARTICLE SUPPLEMENTATION MODULATES PARASITIC INFECTION AND RUMEN ENVIRONMENT IN GRAZING LAMBS

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Background and Aims: Research is needed to better understand the anthelmintic effects of ZnO nanoparticles (ZnO-NPs) in animals. We examined the impact of ZnO-NPs on the parasitological status and rumen environment of lambs infected with *Haemonchus contortus*.

Methods: Experimentally infected lambs were randomly divided into two groups of ten and grazed on pasture while receiving either a basal concentrate - control group or a concentrate supplemented with ZnO-NPs – experimental group. Pasture vegetation was characterized using phytosociological relevés, emphasizing medicinal properties and nutritional value. Fecal egg counts were monitored from day 14 to day 107 post-infection. Ruminal fermentation parameters, microbiota, microbial enzymatic activities, and ruminal histopathological changes were evaluated.

Results: Pastures grazed by the control group were dominated by plant species with medicinal properties, while the experimental group grazed on vegetation that was richer in plants with nutritional value. A significant reduction in fecal egg output was observed earlier in the experimental group (from day 42 onward) than in the control group. The experimental group exhibited higher ruminal concentrations of ammonia nitrogen, n-butyrate, and n-valerate; increased protozoal abundance; and elevated α-amylase and xylanase activities. Histological analysis revealed mild alterations in rumen papillae uniformity and inflammatory changes in the lamina propria.

Conclusions: Gastrointestinal nematode infection was markedly reduced, likely due to the combined medicinal and nutritional properties of the pasture vegetation. ZnO-NP supplementation further enhanced this effect due to its strong anthelmintic potential. This study was supported by funds from the Slovak Research and Development Agency (APVV-21-0301 and APVV-SK-PL-23-0004).

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SHIFT02-196

POSTER ON BOARD SHIFT 02
HOST IMMUNITY, MOLECULAR INTERACTIONS, AND MICROBIOME / HOST MICROBIOME AND PARASITIC INFECTION

ACUTE TRYPANOSOMA CRUZI INFECTION REMODELS THE GUT MICROBIOME AND ITS PREDICTED FUNCTIONAL LANDSCAPE IN A MURINE MODEL

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Background and Aims: Chagas disease, caused by *Trypanosoma cruzi*, produces multisystemic alterations that may involve the gut–microbiota axis. This study evaluated the impact of acute *T. cruzi* infection on the composition, diversity, and predicted function of the intestinal microbiome in a murine model.

Methods: BALB/c mice were infected with *T. cruzi* and analyzed 50 days post-infection. Parasite burden in heart and intestine was quantified by qPCR. Intestinal microbiome composition was assessed by 16S rRNA gene sequencing. Alpha and beta diversity metrics were calculated, and taxonomic differences were determined. Correlations between parasite burden and bacterial families were evaluated. Functional inference analysis was performed using PICRUSt.

Results: Parasite burden in heart and intestine was significantly higher in infected groups than in non-infected controls ($p < 0.05$). Infection increased alpha diversity (Shannon, Simpson, observed families). Beta diversity analysis (Bray–Curtis PCoA) revealed distinct microbial profiles by infection status (PERMANOVA $R^2 = 0.72$, $p = 0.001$). Infected mice showed higher relative abundance of *Muribaculaceae, Lachnospiraceae, Clostridiaceae,* and *Bacteroidaceae*, whereas *Lactobacillaceae* predominated in controls. Intestinal parasite burden correlated negatively with Bacteroidaceae and positively with *Akkermansiaceae* and Eubacterium. Functional inference indicated enrichment of pathways related to immune, digestive, circulatory, metabolic, and cellular signaling processes.

Conclusions: Acute *T. cruzi* infection significantly alters the diversity, composition, and predicted functions of the gut microbiome in BALB/c mice. These changes are associated with intestinal parasite burden and suggest infection-driven dysbiosis that may contribute to disease pathophysiology. Microbiota-targeted interventions could represent novel adjunct strategies for Chagas disease management.

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POSTER ON BOARD SHIFT 02
HOST IMMUNITY, MOLECULAR INTERACTIONS, AND MICROBIOME / HOST MICROBIOME AND PARASITIC INFECTION

TFEB REGULATION ALLOW AUTOPHAGY BALANCE, STEMNESS MAINTENANCE AND METABOLIC STRESS CONTROL IN LARVAL STAGE BY ECHINOCOCCUS LARVAL STAGE

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Background and Aims: Lysosome-mediated signaling and transcription programs sense the metabolism status and regulate autophagy. We have demonstrated that cestodes modulate the AMPK-TORC1 axis to ensure their development under nutrient deprivation and hypoxia. Here, we analyzed Transcription Factor Ephrussi Box(TFEB) molecular regulation in *Echinococcus*.

Methods: We obtained *in vitro* cultured metacestodes from infected CF-1 mice, and microcysts from protoscoleces cultured with growth factors. Electron microscopy, *in toto* confocal inmunolocalization (using phospho-AMPK-αThr172-CS#2535, phospho-TOR-Ser2448-CS#5536, MAP-LC3β-SC-28266, and TFEB#PA5-96632 antibodies) and qPCR assays were performed from control and treated parasites with AMPK-TOR-TFEB agonists/antagonists.

Results: We demonstrated that lysosomes act as scaffolds of Eg-AMPK and Eg-TOR activated-state in protoscoleces and metacestodes, respectively. Eg-TFEB showed conserved structure, with Rag-GTPases binding domain to recruit it at lysosomal surface and phosphorylable serines by the Eg-TORC1 involved in its cytoplasmic retention. In differentiated cells, insulin activates Eg-TORC1, which phosphorylates and sequesters Eg-TFEB in the cytoplasm. In metformin and rapamycin presence, Eg-TORC1 inhibition allowed nuclear translocation of Eg-TFEB, promoting the expression of autophagy, lysosomal, and metabolic genes, even its own transcription, by binding to CLEAR motifs identified in corresponding promoters. Eg-TFEB dephosphorylation at Eg-TORC1-targeted sites was demonstrated modulating protein phosphastases (thapsigargin, cyclosporine A and okadaic acid). Likewise, lysosomotropic drugs as trehalose and chloroquine induced nuclear translocation of Eg-TFEB. In microcyst, regardless of Eg-TOR activation, Eg-TFEB showed nuclear localization inducing autophagy activation. Finally, anti-metacestodial effects was showed by eltrombopag disrupting the TFEB-DNA interaction.

Conclusions: Parasite forms showed TORC1-dependent-Eg-TFEB nuclear translocation, but undifferentiated microcyst cells induce TFEB activity through Eg-TOR-independent manner.

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SHIFT02-198

POSTER ON BOARD SHIFT 02
HOST IMMUNITY, MOLECULAR INTERACTIONS, AND MICROBIOME / VECTOR MICROBIOME AND TRANSMISSION

BACTERIAL COMPOSITION OF POLYGENIS (POLYGENIS) BOHLSI BOHLSI (WAGNER, 1901) (SIPHONAPTERA: RHOPALOPSYLLIDAE) ASSOCIATED WITH THRICHOMYS FOSTERI (RODENTIA: ECHIMYIDAE) IN THE BRAZILIAN PANTANAL WETLAND

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Background and Aims: Fleas (Insecta: Siphonaptera) are important vectors of zoonotic bacterial pathogens. However, the bacterial communities hosted by fleas remain poorly understood, especially regarding the interactions between non-pathogenic bacteria and pathogens, as well as the factors shaping these bacterial communities. This study aimed to explore the bacterial composition and diversity between female and male *Polygenis* (*Polygenis*) *bohlsi bohlsi* fleas collected from free-living *Thrichomys fosteri* (Rodentia: Echimyidae) in the Nhecolândia region, Pantanal, Mato Grosso do Sul state, central-western Brazil.

Methods: Herein, bacterial profiling of fleas was performed using a Next-Generation Sequencing approach targeting the V3–V4 hypervariable region of the 16S rRNA gene to assess community structure.

Results: Bacterial diversity in *Polygenis* fleas varied by sex, and significant differences were observed in alpha and beta diversity analyses. Pseudomonadota was the predominant phylum (80.9 ±27.9%), and two bacteria exhibited a sex-biased pattern of infection: *Bartonella* spp. was detected only in males (36.9% ±44.3; prevalence = 3/4) and *Wolbachia* spp. dominated in females (82.3% ±23.8; prevalence=12/12) and was less abundant in males (2.9% ±3.0, prevalence=3/4). Phylogenetic analysis positioned *Bartonella gltA* sequences detected in two fleas within the same clade as *Bartonella harrusi*. While the *Wolbachia wsp* and 16S rRNA sequences detected in *P. (P.) bohlsi*

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bohlsi grouped within the supergroups S (associated with pseudoscorpions), *gatB* sequences grouped with V (cat flea–related).

Conclusions: These findings suggest sex-based microbiome differences and raise questions about the role of *Wolbachia* spp. in the vector competence of *Polygenis* fleas for *Bartonella* spp.

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SHIFT02-199

POSTER ON BOARD SHIFT 02
HOST IMMUNITY, MOLECULAR INTERACTIONS, AND MICROBIOME / VECTOR MICROBIOME AND TRANSMISSION

BACTERIAL MICROBIOME ACROSS THE LIFE STAGES OF THE HUMAN-BITING BLACK FLY SIMULIUM ASAKOAE SPECIES-GROUP (DIPTERA: SIMULIIDAE) IN THAILAND

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Background and Aims: Black flies (Diptera: Simuliidae) are vectors of medical and veterinary importance, transmitting various pathogens to humans and animals, including the causative agents of onchocerciasis. Although their microbiomes have increasingly been studied recently, these studies have focused solely on specific life stages using short-read sequencing technology. In Thailand, the *Simulium asakoae* species-group is one of the most abundant groups of black flies, of which certain members are vectors of filarial worms and blood protozoa, making them ideal models for microbiome research. Since understanding microbiome dynamics across the life stages of vectors is crucial for the development of novel vector control strategies, bacterial communities of the *S. asakoae* species-group were characterized.

Methods: Larvae, pupae, and newly emerged adults of the *S. asakoae* species-group collected from Chiang Mai province, northern Thailand were profiled by Nanopore full length 16S rRNA gene sequencing.

Results: Bacterial community analysis across life stages identified four dominant phyla (Firmicutes, Actinobacteria, Proteobacteria, and Bacteroidetes) with stage-specific shifts, including *Bacillus circulans* and *Listeria ivanovii* dominance in larvae and newly emerged adults, while *Aquabacterium olei* and *B. circulans* predominated in pupae. Among the 1,163 bacterial species identified, only 59 species (3.5%) were shared across all life stages, with 153 taxa showing differential abundance.

Conclusions: By utilizing long-read sequencing in this study, it offers greater taxonomic resolution, especially at the species level, and provides more accurate estimation of species richness than short-read sequencing. Overall, the results contribute to our understanding of insect–microbe interactions

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during development and provide a foundation for microbiome-based approaches to black fly management and vector control.

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SHIFT02-200

POSTER ON BOARD SHIFT 02
HOST IMMUNITY, MOLECULAR INTERACTIONS, AND MICROBIOME / VECTOR MICROBIOME AND TRANSMISSION

MICROBIOME ANALYSIS OF RHIPICEPHALUS SANGUINEUS COMPLEX TICKS FROM A ROCKY MOUNTAIN SPOTTED FEVER HOTSPOT IN CHIHUAHUA, MEXICO

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Background and Aims: Chihuahua in Northern Mexico ranks among the top three states with the most cases of Rocky Mountain Spotted Fever (RMSF). Tick surveillance has focussed primarly in pathogen screening and control programs. We present a pilot screening of the microbiome of *Rhipicephalus sanguineus* complex ticks collected from an endemic area of RMSF transmission.

Methods: Genomic DNA was extracted from ticks collected from a canine host (*Canis lupus familiaris*). Microbiome characterization was performed through full-length 16S rRNA gene long-read sequencing (27f/1492r primers) on the PacBio Sequel IIe platform. Bioinformatic processing was carried out within the QIIME 2 framework, utilizing the DADA2 (denoise-ccs) algorithm to generate amplicon sequence variants (ASVs) with a length filter between 1000 and 1600 bp. Taxonomic identification was conducted using a VSEARCH-based consensus classifier, referencing the Greengenes2 database (v. 2024.09) with a 97% identity threshold. Finally, the taxonomic composition of the samples was determined through analysis of relative abundance profiles.

Results: Sequence analysis revealed a diverse microbiota in ticks, characterized by a high relative abundance of environmental genera, including *Bradyrhizobium, Fictibacillus*, and *Microvirga*. Additionally, typical commensals of the canine host's skin surface include *Staphylococcus* and *Kocuria*. Moreover, the detection of taxa with high functional versatility, notably *Comamonas, Janibacter*, and *Chryseobacterium*, highlights the complexity of the internal microbiome.

Conclusions: Identified bacteria suggest: 1) close interaction between the tick and soil or vegetation during its free-living periods; 2) active microbial exchange at the parasite-canine host interface during

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hematophagy, and 3) maintaining physiological homeostasis and nutrient processing within the arthropod's digestive tract.

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SHIFT02-201

POSTER ON BOARD SHIFT 02
HOST IMMUNITY, MOLECULAR INTERACTIONS, AND MICROBIOME / VECTOR MICROBIOME AND TRANSMISSION

IS MALATHION ONCOGENIC FOR MOSQUITOES? A TRANSCRIPTOMIC AND HISTOLOGICAL STUDY OF ADULTS DERIVED FROM MALATHION EXPOSED LARVAE

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Background and Aims: Malathion is a widely used organophosphate insecticide in agriculture and public health, particularly for mosquito control. Here, we investigated whether larval exposure to environmentally realistic concentrations of malathion affects tissue integrity and gene expression in adult *Aedes aegypti*, the primary dengue vector worldwide.

Methods: Larvae were exposed under conditions simulating moderate to severe environmental contamination. RNA from exposed and unexposed mosquitoes (both sexes) was used to prepare stranded cDNA libraries following rRNA depletion, and sequencing was performed on an Illumina NovaSeq platform (100 bp paired-end). Reads were mapped to the AaegL5.0 reference genome using STAR, and gene counts were generated with HTSeq after filtering low-abundance genes. Differential expression analysis was conducted with DESeq2, followed by redundancy analysis and functional enrichment (GO and KEGG). In parallel, histological analyses were performed on serial sections to assess tissue integrity and detect potential lesions.

Results: No neoplastic or pre-neoplastic lesions were observed in adults exposed to malathion during larval development. However, transcriptomic analyses revealed significant changes in genes involved in mitochondrial function, energy metabolism, and detoxification pathways, indicating persistent physiological effects. These responses were more pronounced in females, with upregulation of detoxification genes (e.g., cytochrome P450s), olfactory-related genes, and stress-response pathways, suggesting potential implications for metabolic resistance.

Conclusions: Overall, early-life exposure to malathion induces lasting molecular responses in adult mosquitoes without detectable tissue damage. These findings highlight the capacity of mosquitoes to mount adaptive transcriptomic responses to environmental pollutants, with potential implications for the evolution of insecticide resistance and vector control strategies.

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SHIFT02-202

POSTER ON BOARD SHIFT 02
HOST IMMUNITY, MOLECULAR INTERACTIONS, AND MICROBIOME / VECTOR MICROBIOME AND TRANSMISSION

METATRANSCRIPTOMIC RNA SEQUENCING REVEALS TRANSCRIPTIONALLY ACTIVE MICROORGANISMS AT THE RHIPICEPHALUS MICROPLUS–HOST INTERFACE

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Background and Aims: Passive surveillance underpins animal disease detection, while active surveillance is typically limited to known pathogens, increasing the risk that emerging agents remain undetected. Tick-borne diseases pose major threats to animal production, biosecurity, and One Health. Although *Rhipicephalus microplus* is a recognised vector of several important pathogens, its broader microbial community remains poorly characterised in South Africa. This study aimed to characterise the transcriptionally active viral and bacterial communities under field conditions to inform One Health surveillance.

Methods: RNA metatranscriptomics was used to detect actively replicating viruses and bacteria, complemented by targeted bacterial metagenomics (16S rRNA) to assess bacterial diversity in some 500 *R. microplus* ticks collected across South Africa. Several bioinformatics pipelines were used for mapping and annotation. Alpha diversity (Shannon entropy, Pielou's evenness) and beta diversity (Bray–Curtis, Jaccard, UniFrac) metrics were calculated, and UpSet plots were used to identify shared and unique bacterial taxa.

Results: High-priority tick-associated viruses, including Jingmen tick virus, Wuhan tick virus 2, and Lihan tick virus, were detected in a high proportion of samples, with RNA-seq reads confirming active replication and enabling the development of PCR-RFLP detection assays. Bacterial profiling identified 564 genera across 38 phyla, including genera with high zoonotic relevance, e.g. *Coxiella burnetii*, *Clostridium sensu stricto*, *Salmonella*, and *Borrelia*. Beta-diversity analyses revealed significant geographic structuring of tick microbiomes.

Conclusions: This first comprehensive metatranscriptomic study establishes a foundation for One Health–aligned surveillance, rapid diagnostics and identifies a core tick microbiome with potential targets for integrated control strategies.

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SHIFT02-203

POSTER ON BOARD SHIFT 02
HOST IMMUNITY, MOLECULAR INTERACTIONS, AND MICROBIOME / VECTOR MICROBIOME AND TRANSMISSION

UNRAVELLING THE MICROBIOME OF THE AUSTRALIAN CATTLE TICK (RHIPICEPHALUS AUSTRALIS): STAGE-SPECIFIC MICROBIAL DIVERSITY AND SYMBIOTIC ASSOCIATIONS

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Background and Aims: Ticks are among the most important vectors of pathogens in animals. *Rhipicephalus australis* (the Australian cattle tick) transmits several economically important pathogens, including *Anaplasma* and *Babesia* spp. Increasing evidence suggests that the tick microbiome can influence pathogen colonisation, persistence and transmission, thereby shaping vectorial capacity; however, the microbiome of *R. australis* remains poorly characterised. This study characterised bacterial communities in larval and adult *R. australis* collected from cattle farms in Queensland during 2024.

Methods: Ticks were surface-decontaminated, preserved in 70% ethanol, and subjected to DNA extraction, followed by PCR amplification of the 16S rRNA gene (V4 region). Sequencing was performed on the Illumina NextSeq 1000 platform and downstream analyses were conducted in QIIME 2 and R, incorporating rigorous contaminant filtering.

Results: A diverse assemblage of bacterial taxa was identified, including *Arsenophonus, Acinetobacter*, *Brevibacterium*, *Brachybacterium*, *Coxiella*, *Corynebacterium*, *Serratia*, *Stenotrophomonas*, *Lactococcus*, *Escherichia-Shigella, Salmonella, Staphylococcus* and *Providencia*. Several taxa were consistently detected across both life stages, suggesting conserved or potentially symbiotic associations, while others were stage-specific, indicating dynamic shifts in microbial composition during tick development. Microbial network analyses further revealed distinct interaction structures between larval and adult stages.

Conclusions: These findings provide the first detailed insights into life stage–associated microbiome variation in the Australian cattle tick and highlight the potential role of microbial communities in tick biology and pathogen transmission. This work establishes a foundation for exploring microbiome-

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based strategies to improve tick control and enhance livestock health and productivity in Australia and beyond.

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SHIFT02-204

POSTER ON BOARD SHIFT 02
HOST IMMUNITY, MOLECULAR INTERACTIONS, AND MICROBIOME / VECTOR MICROBIOME AND TRANSMISSION

MICROSPORIDIA MB PRESENCE IS ASSOCIATED WITH PLASMODIUM FALCIPARUM ABSENCE IN WILD ANOPHELES GAMBIAE MOSQUITOES IN OGUN STATE, NIGERIA

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Background and Aims: The experimental blocking of *Plasmodium falciparum* transmission by *Microsporidia MB* in *Anopheles* mosquitoes highlights a biological control potential against malaria. However, transmission efficiency in the host and the parasite transmission-blocking effect are yet fully understood in field investigations. In this study, we assessed the association between *Microsporidia MB* infection and *Plasmodium* prevalence in wild-caught *Anopheles* mosquitoes in Ogun State, Nigeria where the compatibility of the local *Anopheles* population with microsporidia is not well-known.

Methods: Wild-caught adult *Anopheles* mosquitoes and third-to fourth-instar larvae (L3–L4) were collected from 44 locations across 13 local government areas (LGAs) in Ogun State. Individual genomic DNA was extracted using partially destructive Lysis C buffer. Species identification of *Anopheles* mosquitoes and detection of *Plasmodium* infection were performed using a multi-locus amplicon sequencing panel targeting 62 loci across mosquito and parasite genomes. Microsporidia were detected using PCR and sequencing.

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Results: *Microsporidia MB* was widespread (47.9%) in dominant and secondary malaria vector species, with higher prevalence in the adults (58.4%) than larvae (38.7%). Although *P. falciparum* infection was detected in *Microsporidia MB*-positive *An. coluzzii* and *An. funestus* group, *Microsporidia MB* presence was significantly associated with *P. falciparum* absence in *Anopheles. gambiae s.s.* (p = 0.0098), compared to a 12.3% prevalence of *P. falciparum* in *Microsporidia MB*-negative *An. gambiae s.s.*

Conclusions: The *Microsporidia MB*–associated absence of *P. falciparum* in *An. gambiae* s.s. under non-experimental conditions, affirms its potential as malaria elimination tool, with possible variation in transmission-blocking effect across vector species.

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SHIFT02-205

POSTER ON BOARD SHIFT 02
HOST IMMUNITY, MOLECULAR INTERACTIONS, AND MICROBIOME / VECTOR MICROBIOME AND TRANSMISSION

MICROBIOME OF HYALOMMA DROMEDARII (IXODIDA: IXODIDAE) TICKS: VARIATION IN COMMUNITY STRUCTURE WITH REGARD TO SEX AND HOST HABITAT

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Background and Aims: *Hyalomma dromedarii* ticks are the main hematophagous ectoparasites of camels, harboring a variety of microbes that can affect tick vector competence and pathogen transmission. To better understand the tick microbiome influenced by sex and host habitat, we analyzed the bacterial community of *H. dromedarii* male and female ticks collected from camel farms, livestock markets, and slaughterhouses, representing the range of major habitats in the UAE.

Methods: Tick samples were collected during 2022 and 2023. A total of 40 ticks (male (15), female (15), and nymph (10)) were selected from tick samples collected from camels and processed for genomic DNA and next-generation sequencing using the Illumina MiSeq platform.

Results: We obtained 151,168 read counts, and these formed 237 operational taxonomic units representing 11 phyla, 22 classes, 77 families, and 164 genera. The phyla Actinomycetota, Bacillota, Bacteroidota, Pseudomonadota, and Fusobacteriota were the most abundant. The bacterial genus *Corynebacterium* dominated the microbiomes of farm-collected female *H. dromedarii* ticks, while *Proteus* dominated the microbiomes of farm-collected male *H. dromedarii* ticks. In comparison, the microbiomes of *H. dromedarii* ticks collected from slaughterhouse samples were dominated by genus *Francisella* in both males and females.

Conclusions: Our results confirm that the bacterial microbiomes of *H. dromedarii* ticks vary by sex and habitat settings. Furthermore, recent findings could deliver insight into the differences in the ability of camel ticks to acquire, maintain, and transmit pathogens in various habitats that may impact the tick vector competence of medically and agriculturally important species in the Middle East and North Africa (MENA) region and Asia.

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SHIFT02-206

POSTER ON BOARD SHIFT 02
HOST IMMUNITY, MOLECULAR INTERACTIONS, AND MICROBIOME / VECTOR MICROBIOME AND TRANSMISSION

GENOMIC TECHNOLOGIES FOR CONTROL OF CATTLE TICKS

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Background and Aims: Ticks and the pathogens they carry are a significant burden on global health and agriculture. The tick microbiome has been identified as a target for control strategies, but few studies have investigated the microbiome of discrete tissues or utilised an un-biased metagenome approach. *Rhipicephalus australis* (Australian cattle tick) belongs to a cryptic species group, including the invasive *R. microplus*. This study aimed to characterise the salivary gland and gut tissues of *R. australis* to identify possible control targets.

Methods: Gut and salivary gland (SG) tissues were dissected from 60 semi-engorged adult female ticks with 3 replicates for each tissue. DNA was sequenced twice using Oxford Nanopore Technology long read sequencing with adaptive sampling to deplete the tick genome followed by enrichment for bacterial species of interest. Assembled reads were classified using Kraken2, with a stringent confidence threshold of 0.01.

Results: There was no statistical difference in alpha diversity between gut and SG tissues ($p = 0.4$), but ordination analysis revealed differences in species composition between the tissues. A Coxiella-like endosymbiont was identified confined to the SG and after full genome assembly has been termed *CLE-Ra*. *CLE-Ra* has gene pathways consistent with Vitamin B and Folic Acid synthesis but is lacking virulence genes found in its relative, the pathogenic *Coxiella burnetii*.

Conclusions: This study has identified a potential bacterial target for tick control applicable across the *R. microplus* clade and has revealed recent evolutionary changes in tick endosymbionts during divergence of an invasive tick species.

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SHIFT02-207

POSTER ON BOARD SHIFT 02
DIAGNOSTICS, HOST-PARASITE INTERACTION AND EMERGING TECHNOLOGIES / BIOMARKER DISCOVERY AND DIAGNOSTIC INNOVATION

IDENTIFICATION AND VALIDATION OF ONCHOCERCA VOLVULUS ANTIGEN IN HUMAN URINE FOR A COMPANION DIAGNOSTIC

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Background and Aims: Despite several decades of mass drug administration and elimination-related activities, human onchocerciasis still represents a major parasitic threat of blindness and skin disease. A major challenge encountered by the elimination program is the lack of a suitable accurate and non-invasive diagnostic tool as currently used methods are either invasive or not suitable for monitoring activities. Herein, we describe the identification and characterization of *Onchocerca volvulus* heat shock protein 70 (*Ov*HSP70) as a novel diagnostic biomarker in urine, and its ability to monitor ivemectin treatment.

Methods: This nematode-specific antigen was identified through LC-MS after differential SDS-PAGE using urine-derived protein extracts from *O. volvulus*-infected patients in Cameroon. Polyclonal antibodies were generated in rabbits after cloning and expression of *Ov*HSP70 in *Escherichia coli,* and its ability to differentiate between urine samples from infected patients and healthy controls in a hypoendemic area of onchocerciasis. Correlation analysis between biomarker intensity and ivermectin treatment times was done on R.

Results: This proof-of-concept diagnostic showed a sensitivity of 87% and specificity between 37% and 100%. This antigen detection method indicated its ability to determine treatment endpoints with ivermectin, as the antigen intensity negatively correlated with number of treatment times, useful in treatment surveillance and monitoring disease recrudescence.

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Conclusions: These results provide an excellent basis for further studies to develop a non-invasive diagnostic assay for human onchocerciasis using urine samples. Such a urine-based diagnostic assay will be of major importance for the elimination program of human onchcerciasis in endemic countries.

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SHIFT02-208

POSTER ON BOARD SHIFT 02
DIAGNOSTICS, HOST-PARASITE INTERACTION AND EMERGING TECHNOLOGIES / BIOMARKER DISCOVERY AND DIAGNOSTIC INNOVATION

SELF-ACTUATED MICROFLUIDIC CHIPLET FOR MULTIPLEX NUCLEIC ACID AMPLIFICATION ASSAY

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Background and Aims: Effective diagnosis of comorbidities and infectious diseases that present similar symptoms requires point-of-need assays capable of co-detecting and differentiating among multiple co-endemic pathogens to enable timely, precision medicine and effective control measures. We previously developed a two-stage isothermal amplification assay dubbed Penn-RAMP to address this need. Penn-RAMP's first stage comprises a recombinase polymerase amplification (RPA), which amplifies all targets of interest in a single reaction chamber for a short duration. The RPA amplicons are then aliquoted into multiple loop-mediated isothermal amplification (LAMP) reaction chambers, each customized with pre-dried primers to amplify a single target or a group of targets.

Methods: To enable Penn-RAMP at the point of need, we describe here a self-actuated Penn-RAMP chiplet that accommodates the Penn-RAMP assay. Our chiplet employs temperature-controlled phase change valves and capillary valves to self-aliquot first-stage amplicons into multiple (five) second-stage reaction chambers and to seal these chambers. The functionality of our device is demonstrated by co-detecting target pathogens.

Results: The analytical performance of our chiplet is comparable to that of the benchtop Penn-RAMP assay and surpasses that of standalone LAMP assays by at least 10-fold.

Conclusions: The self-actuated chiplet can be operated standalone with purified nucleic acids or as the downstream amplification module of a sample preparation cassette. Our detection platfrom numerous other applications that could benefit from rapid detection, such as animal and human pathogens and food contaminants.

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SHIFT02-209

POSTER ON BOARD SHIFT 02
DIAGNOSTICS, HOST-PARASITE INTERACTION AND EMERGING TECHNOLOGIES / BIOMARKER DISCOVERY AND DIAGNOSTIC INNOVATION

QUANTITATIVE MORPHOMETRIC ANALYSIS OF ENTAMOEBA HISTOLYTICA TROPHOZOITES TREATED WITH ARTEMISININ

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Background and Aims: Artemisinin demonstrates significant amebicidal activity against *Entamoeba histolytica* (*E. histolytica*) (IC_{50} = 600 µM) with Gal/GalNAc lectin expression alterations. However, quantitative morphological characterization correlating molecular alterations with structural modification remains unexplored. This study develops an automated image analysis system to quantify morphometric changes in *E. histolytica* trophozoites treated with artemisinin.

Methods: Optical microscopy images of *E. histolytica* trophozoites (HM1:IMSS strain) treated with artemisinin (600 µM, 40× magnification) were processed using automated algorithms for: (1) adaptive segmentation, (2) morphometric extraction (area, circularity, aspect ratio), (3) GLCM texture analysis, (4) integrated optical density (IOD), and (5) vacuole quantification. Statistical comparisons used ANOVA between artemisinin and control groups

Results: Artemisinin-treated trophozoites showed: (1) 42.3% area reduction ($p<0.001$); (2) decreased circularity (0.78±0.06 to 0.54±0.12, $p<0.001$), indicating membrane irregularity; (3) 2.8-fold increased cytoplasmic heterogeneity (GLCM entropy: 3.2±0.4 vs 1.1±0.2, $p<0.001$); (4) elevated IOD (1847±312 vs 982±156, $p<0.01$); and (5) increased vacuolization (8.4±2.1 vs 3.2±0.8 vacuoles/cell, $p<0.001$)

Conclusions: Automated morphometric analysis successfully quantifies structural modifications in artemisinin-treated *E. histolytica*, establishing quantitative correlations between morphological changes consistent with viability loss and molecular alterations that correlate with Gal/GalNAc lectin modification previously demonstrated. This multi-scale approach validates artemisinin's amebicidal mechanism and provides a computational framework for anti-amebic drug screening based on quantifiable microscopic parameters

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SHIFT02-210

POSTER ON BOARD SHIFT 02
DIAGNOSTICS, HOST-PARASITE INTERACTION AND EMERGING TECHNOLOGIES / BIOMARKER DISCOVERY AND DIAGNOSTIC INNOVATION

PREDICTIVE AND PROGNOSTIC ROLES OF MATRIX METALLOPROTEINASES AND THEIR INHIBITORS IN CARDIAC REMODELING IN CHAGAS DISEASE

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Background and Aims: Chronic Chagas disease has a heterogeneous clinical course, ranging from an indeterminate asymptomatic phase to severe cardiac involvement. Predicting progression to cardiomyopathy and fibrosis remains a major challenge. Extracellular matrix remodeling is central to pathogenesis, with matrix metalloproteinases (MMP-2 and MMP-9) contributing through degradation of structural components and modulation of inflammatory pathways. Their activity is regulated by tissue inhibitors of metalloproteinases(TIMPs), and the MMP/TIMP balance may reflect disease progression. The aim of this study was to evaluate plasma levels of MMP-2, MMP-9, and TIMPs in chronic Chagas disease patients and compare them with other cardiomyopathies, assessing their biomarker potential.

Methods: Plasma samples were analyzed by multiplex immunoassay from individuals in the indeterminate form (IND, n=25) and patients with Chagas cardiomyopathy (CARD-B1, n=24; B2, n=24; C, n=32). Patients with idiopathic heart disease (IHD, n=15), rheumatic fever (RF, n=16), and non-infected controls (NI, n=25) were included.

Results: MMP-2 emerged as a potential early biomarker of replacement fibrosis during initial cardiac remodeling in patients in the indeterminate form, suggesting predictive value for disease progression. In contrast, MMP-9 was associated with advanced fibrosis and more severe remodeling in established cardiomyopathy. Tissue inhibitors of metalloproteinases (TIMPs) regulate MMP activity and help maintain extracellular matrix homeostasis. Disruption of the MMP/TIMP balance may contribute to pathological remodeling, supporting the relevance of TIMPs as biomarkers for disease monitoring and prognostic assessment in cardiac disease.

Conclusions: These results highlight MMPs and TIMPs as potential biomarkers of Chagas cardiomyopathy progression; however, further studies are needed to validate their clinical utility and therapeutic potential.

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POSTER ON BOARD SHIFT 02
DIAGNOSTICS, HOST-PARASITE INTERACTION AND EMERGING TECHNOLOGIES / BIOMARKER DISCOVERY AND DIAGNOSTIC INNOVATION

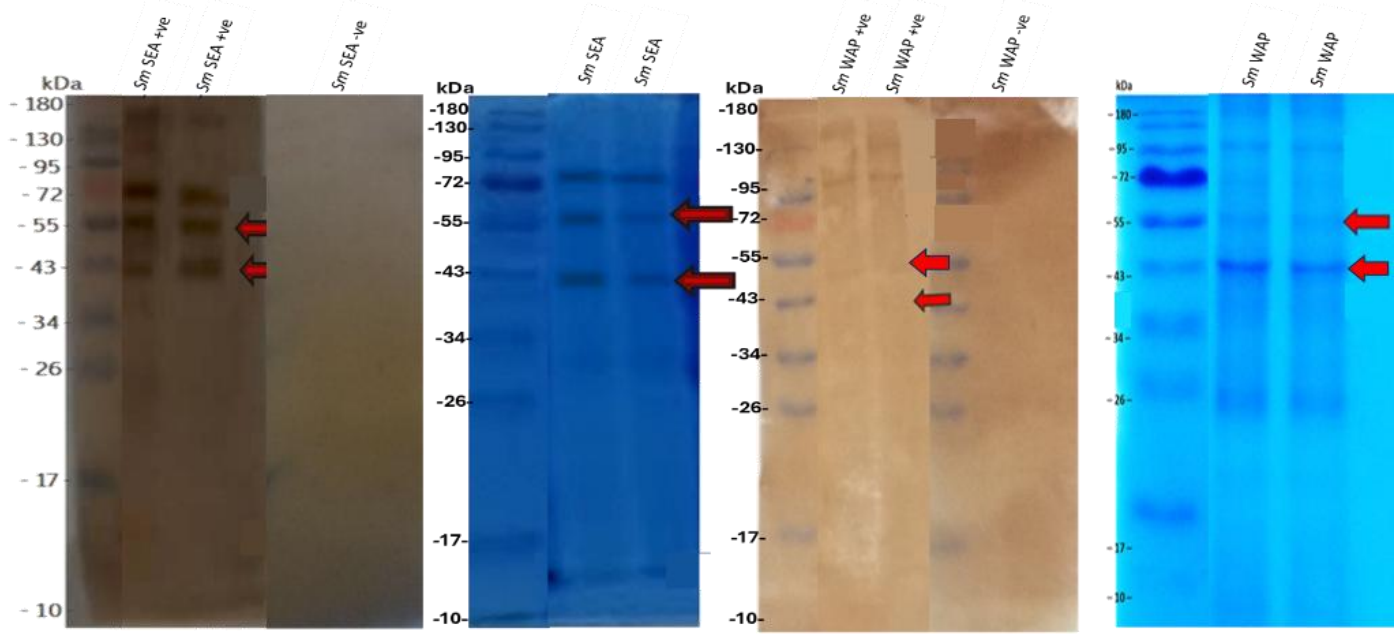
PROTEOMIC DISCOVERY OF IMMUNOGENIC SCHISTOSOMA PROTEINS FOR NEXT-GENERATION DIAGNOSTICS

Blessing Babalola[1], Kabirat Sulaiman[2], Tajudeen Oriade[1], Oyetunde Oyeyemi[1]
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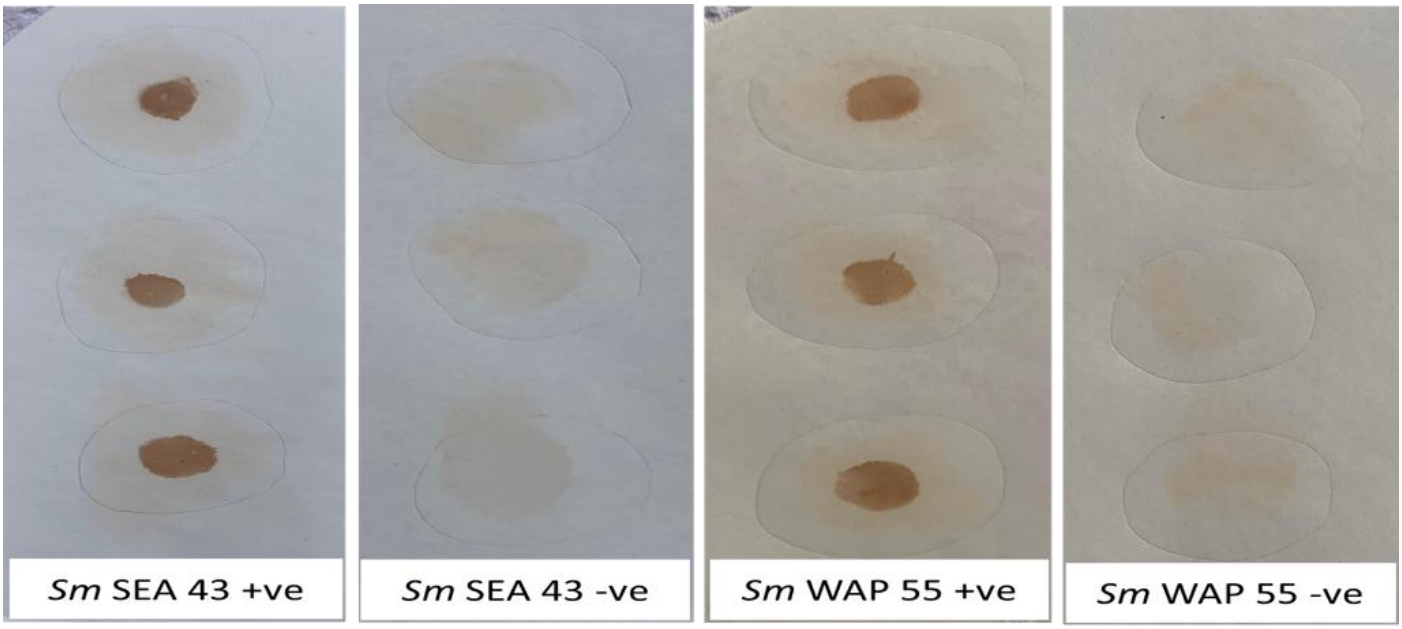
Background and Aims: Schistosomiasis remains a major parasitic disease in Africa, with low-intensity infections often undetected by conventional diagnostics using crude antigens. Defined immunogenic proteins offer a pathway toward more accurate and reliable diagnostic tools.This study assessed two immunoreactive proteins eluted from *Schistosoma mansoni* soluble egg antigen (SEA) and worm antigen protein (WAP) as potential field-ready diagnostic biomarkers.

Methods: Immunoreactive proteins from *S. mansoni* SEA and WAP were identified from crude samples by SDS-PAGE, with corresponding Western blot performed using patient sera to confirm reactivity. Bands were then excised, eluted, and quantified by BCA assay, evaluated via ELISA on 40 urine samples (20+ / 20−), and further assessed by dot blot using pooled urine. Diagnostic indices, including AUC, sensitivity, and specificity, were analysed with GraphPad Prism 8.0.1 and MedCalc.

Results:



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Two proteins were successfully eluted: 43 kDa (SEA) and 55 kDa (WAP), both showed excellent diagnostic performance with 95% sensitivity and specificity, and high AUC (0.95; 95% CI: 0.87-1.00). ELISA separated positive (0.38±0.23) and negative (0.07±0.03) samples (P <0.0001). Dot blot assays showed that *Sm*-eluted proteins recognised *S. haematobium* samples, demonstrating cross-species diagnostic potential.

Conclusions: The 43 kDa (SEA) and 55 kDa (WAP) proteins demonstrate strong diagnostic performance and cross-species recognition, highlighting their potential as next-generation laboratory biomarkers to enhance schistosomiasis surveillance and inform future diagnostic development.

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POSTER ON BOARD SHIFT 02
DIAGNOSTICS, HOST-PARASITE INTERACTION AND EMERGING TECHNOLOGIES / BIOMARKER DISCOVERY AND DIAGNOSTIC INNOVATION

INVOLVEMENT OF THE PP2C PROTEIN PHOSPHATASE IN STRUCTURAL AND MORPHOLOGICAL CHANGES OF LEISHMANIA MEXICANA PROMASTIGOTES

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Background and Aims: Leishmaniasis is a disease caused by parasites of the genus *Leishmania* spp., whose main cellular hosts are neutrophils and macrophages. The parasite flagellum is essential for the establishment of infection, as it enables motility through chemotaxis. In previous laboratory studies, the protein phosphatase PP2C was localized in the flagellar pocket and the flagellum of *Leishmania mexicana* (*L. mexicana*) promastigotes. Furthermore, this protein belongs to the serine-threonine phosphatase family and therefore may participate in flagellar morphology and development. The main focus of this study was to analyze the role of PP2C in knock-out (KO) promastigotes by evaluating its impact on growth, cell viability, expression and enzymatic activity, as well as the morphological changes during culture.

Methods: KO promastigotes were generated by homologous recombination targeting the gene encoding the PP2C phosphatase.

Results: Wild-type (WT) and KO promastigotes did not show significant differences in their growth curves. However, KO parasites exhibited irregular morphologies compared with WT parasites. Finally, Western blot analysis did not detect the PP2C protein at any time point during culture in KO parasites.

Conclusions: The results suggest that PP2C may play a relevant role in parasite morphology, indicating a possible structural function in *L. mexicana*, with implications for motility and pathogenicity. Nevertheless, further studies are required to confirm the specific involvement of PP2C in the parasite. This project was funded by CONAHCYT CBF2023-2024-734 and UNAM-DGAPA-PAPIIT IN212422 awarded to Dr. María Magdalena Aguirre García.

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SHIFT02-213

POSTER ON BOARD SHIFT 02
DIAGNOSTICS, HOST-PARASITE INTERACTION AND EMERGING TECHNOLOGIES / BIOMARKER DISCOVERY AND DIAGNOSTIC INNOVATION

ANTI-TRICHURIS TRICHIURA IGG4 AS AN AUXILIARY MARKER FOR STOOL-BASED DIAGNOSIS IN ENDEMIC SETTINGS

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Background and Aims: Stool-based diagnosis remains central for *Trichuris trichiura* detection but may miss low-intensity infections, particularly in endemic settings under repeated anthelmintic interventions. We aimed to evaluate whether anti-*T. trichiura* IgG1 and IgG4 could serve as auxiliary serological markers to complement stool-based diagnosis under realistic endemic conditions.

Methods: Serum samples from endemic areas in northeastern Brazil and non-endemic controls from Uberlândia, Brazil, were tested by ELISA for anti-*T. trichiura* IgG1 and IgG4 using adult crude extract. Performance was assessed by ROC analysis comparing Trichuris-monoinfected or all Trichuris-positive individuals with endemic copro-negative controls. Same-area robustness and analytical specificity were also evaluated.

Results: In the monoinfected scenario, IgG4 outperformed IgG1, with AUC 0.771, sensitivity 77.8%, and specificity 85.2%, whereas IgG1 showed AUC 0.619, sensitivity 50.0%, and specificity 77.8%. When all *Trichuris*-positive individuals were included, IgG4 maintained higher specificity than IgG1 (85.2% versus 66.7%) with AUCs of 0.709 and 0.673, respectively. In same-area analysis restricted to Pacatuba, IgG4 retained better performance among monoinfected individuals. Across comparator parasitological groups, IgG4 showed lower apparent cross-reactivity than IgG1, particularly against *Ascaris*-positive, *Schistosoma*-positive, and non-helminth parasite-positive groups.

Conclusions: Anti-*T. trichiura* IgG4 showed the best balance between discrimination and analytical specificity, supporting its use as an auxiliary marker to complement stool-based diagnosis. This approach may be particularly useful for surveillance and monitoring in endemic settings where stool-based methods may underestimate low-burden infections.

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POSTER ON BOARD SHIFT 02
DIAGNOSTICS, HOST-PARASITE INTERACTION AND EMERGING TECHNOLOGIES / BIOMARKER DISCOVERY AND DIAGNOSTIC INNOVATION

CHARACTERIZATION OF DNA APTAMERS FOR THE DETECTION OF E. HISTOLYTICA TROPHOZOITES

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Background and Aims: Intestinal amebiasis caused by the protozoan parasite *Entamoeba histolytic*a, affects thousands of people worldwide each year. Microscopic techniques are the first choice for detecting the parasite; however, its similarity to non-pathogenic species complicates diagnosis. Therefore, new methodologies such as ssDNA or ssRNA aptamers obtained through the Bio-SELEX methodology are atractive tools that can contribute to the development of novel diagnostic methods. For this reason, the aim of the present study was to characterize two ssDNA aptamers that recognize membrane proteins (MPs) of *E. histolytica* trophozoites.

Methods: Two ssDNA aptamers (40 nucleotides) labeled with biotin at the 5' end and obtained through 13 rounds of positive selection using the Bio-SELEX strategy were evaluated in Dot-Blot and ELASA assays to assess the recognition of *E. histolytica* MPs. Pull-down experiments were also performed for protein target identification.

Results: The two most enriched 40-nucleotide aptamers (APM_1 and APM_2) obtained from the Bio-SELEX strategy recognized *E. histolytica* MPs with greater affinity up to a concentration of 100 ng/µL. However, only APM_2 specifically recognized *E. histolytica* MPs without showing a detectable signal for proteins from other microorganisms. Finally, the protein recognized by both aptamers has a molecular weight between 15 and 25 kDa.

Conclusions: The implemented Bio-SELEX strategy allowed the obtaining of two ssDNA aptamers that recognize MPs of *E. histolytica*.

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POSTER ON BOARD SHIFT 02
DIAGNOSTICS, HOST-PARASITE INTERACTION AND EMERGING TECHNOLOGIES / BIOMARKER DISCOVERY AND DIAGNOSTIC INNOVATION

IDENTIFICATION OF SPECIFIC EPITOPES FROM RECOMBINANT LEISHMANIA INFANTUM PROTEINS FOR IMPROVED DIAGNOSIS OF VISCERAL LEISHMANIASIS

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Background and Aims: Visceral leishmaniasis (VL) diagnosis is limited by cross-reactivity and variable accuracy of current serological assays. Previous studies reported cross-reactivity among recombinant *Leishmania infantum* antigens and other trypanosomatids. This study aimed to identify species-specific linear epitopes and develop a chimeric antigen to improve diagnostic performance in dogs and humans.

Methods: Sequence similarity of six *L. infantum* proteins (rK39, rK28, rLci1, rLci2, rLci5, and rLci12) was analyzed using BLASTp and Clustal Omega. Linear IgG epitopes were mapped by peptide microarrays using canine and human sera from *L. infantum*, *Trypanosoma cruzi*, and control groups. Immunoreactive and discriminatory peptides were selected for chimeric protein design. The construct was expressed in *Escherichia coli*, purified, and evaluated by enzyme-linked immunosorbent assay. Diagnostic accuracy was assessed by receiver operating characteristic analysis.

Results: In silico analysis revealed high similarity between rLci1 and rLci12 and *T. cruzi* proteins, whereas rLci2 and rLci5 showed greater specificity for *Leishmania* spp. Microarrays identified 15 canine and 29 human immunoreactive epitopes, with 11 highly specific sequences. The chimeric antigen demonstrated strong discriminatory capacity. In dogs, receiver operating characteristic analysis showed an area under the curve of 1.000, with 100% sensitivity and specificity. In humans, the area under the curve was 0.9597, with 96% sensitivity and 91% specificity. Optimal reactivity was observed at 100 ng per well.

Conclusions: Epitope-guided design enabled the development of a highly accurate chimeric antigen for VL diagnosis. This approach represents a promising strategy to reduce cross-reactivity and improve serological testing in canine and human populations.

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POSTER ON BOARD SHIFT 02
DIAGNOSTICS, HOST-PARASITE INTERACTION AND EMERGING TECHNOLOGIES / BIOMARKER DISCOVERY AND DIAGNOSTIC INNOVATION

CORRELATION OF PARASITE LOAD AND CIRCULATING IMMUNE COMPLEXES AS DIAGNOSTIC MARKERS IN CANINE LEISHMANIOSIS.

Clara Gómez-Velasco[1], Ana Montoya[1], Nuria Parody[2], Jerónimo Carnés[2], Jaime Heredia-Caldeiro[1], Juan Barrera[1], Efrén Estévez-Sánchez[1], Juliana Sarquis[1], Daniela García-Rupérez[1], Pablo Moraleda[1], Guadalupe Miró[1]
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Background and Aims: Canine leishmaniosis (CanL), caused by *Leishmania infantum*, is a chronic disease with variable clinical manifestations resulting from complex parasite-host's immune response interactions, which complicates diagnosis and monitoring. High parasite burdens and sustained antibody production promote the formation of circulating immune complexes (CIC), which may deposit in tissues, causing severe clinical outcomes. While real-time PCR (qPCR) reliably quantifies parasite load, serum CIC levels have been previously associated with disease severity. This study assessed the relationship between tissue parasite load and CIC levels in naturally infected dogs as complementary diagnostic markers in CanL.

Methods: Forty dogs naturally infected were clinically classified according to LeishVet guidelines, along with twenty non-infected controls. Parasite load was quantified by qPCR in lymph node aspirates and oral swabs, while CIC levels were measured in serum using a validated *Leishmania*-specific PEG-ELISA.

Results: Dogs with higher lymph node parasite loads presented increased CIC levels, reaching values up to 2 OD (cut-off > 1.536 OD), whereas dogs with low or undetectable parasite loads generally showed low CIC concentrations (negative under 0.3 OD). Oral swab samples yielded lower parasite loads; however, *Leishmania* DNA was mainly detected in sick dogs with higher CIC levels.

Conclusions: Lymph node sampling remains the gold standard for molecular detection of *L. infantum*, while oral swabs constitute a reliable non-invasive alternative when lymph node sampling is not feasible, particularly in advanced disease stages. CIC levels and parasite load followed similar patterns consistent with disease severity, supporting a multimodal diagnostic approach to improve clinical decision-making and long-term monitoring of CanL.

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POSTER ON BOARD SHIFT 02
DIAGNOSTICS, HOST-PARASITE INTERACTION AND EMERGING TECHNOLOGIES / BIOMARKER DISCOVERY AND DIAGNOSTIC INNOVATION

DETECTION OF NAEGLERIA FOWLERI IN PRIMARY AMEBIC MENINGOENCEPHALITIS ANIMAL MODELS USING FOWLERPAIN-2 ANTIBODY

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Background and Aims: *Naegleria fowleri* is a free-living amoeba that causes fatal primary amoebic meningoencephalitis (PAM) in humans. PAM progresses rapidly and severely, leading to an almost universally fatal outcome if not diagnosed and treated promptly. This study aimed to generate *Naegleria*-specific antibodies and evaluate their specificity to facilitate the rapid and accurate diagnosis of PAM caused by *N. fowleri*.

Methods: A polyclonal peptide antibody targeting the fowlerpain-2 protein of *N. fowleri* was generated. Antibody reactivity was assessed using ELISA, and western blot analysis was conducted to evaluate cross-reactivity with other amoebae that causing amoebic encephalitis. Antibody specificity was further examined in a co-culture system consisting of *N. fowleri* and the human brain cell, followed by immunocytochemistry (ICC). Additionally, PAM murine models were established through infection with *N. fowleri* trophozoites, and brain tissues were collected for histopathological examination using hematoxylin and eosin (H&E) staining and immunohistochemistry (IHC).

Results: ELISA and western blot analyses demonstrated that the fowlerpain-2 antibody specifically reacted with *N. fowleri*. ICC results from the co-culture system confirmed that the antibody did not react with human cells but selectively recognized *N. fowleri*. In the PAM mouse model, IHC analysis verified that the fowlerpain-2 antibody specifically detected *N. fowleri* in infected mouse brain tissues.

Conclusions: These findings demonstrate that the fowlerpain-2 antibody specifically detects *N. fowleri* in both in vitro and in vivo models. This study provides important experimental evidence supporting the development of an antibody-based diagnostic platform for the rapid and accurate diagnosis of PAM.

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DIAGNOSTICS, HOST-PARASITE INTERACTION AND EMERGING TECHNOLOGIES / BIOMARKER DISCOVERY AND DIAGNOSTIC INNOVATION

A CAMELID SINGLE-DOMAIN ANTIBODY-BASED PAN-TRYPANOSOMA BRUCEI RECOGNITION PLATFORM TO ENABLE ONE HEALTH DIAGNOSTICS

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Background and Aims: *Trypanosoma brucei* causes African Trypanosomosis in humans and animals, posing interconnected health and economic burdens across endemic regions. Current diagnostics largely rely on antibody detection and cannot discriminate active from past infections, limiting surveillance, treatment monitoring, and integrated One Health control strategies. This study aimed to develop a camelid single-domain antibody (sdAb)–based antigen recognition platform for pan-specific detection of active *T. brucei* infections across host species.

Methods: A panel of camelid sdAbs targeting *T. brucei* enolase (*Tbr*ENO), a highly conserved antigen across all *T. brucei* subspecies, was generated and evaluated in sandwich ELISA. Optimal sdAb pairs were selected based on sensitivity, specificity, and performance in complex biological matrices. The platform was validated using recombinant proteins, plasma from experimentally infected mice, and *T. b. rhodesiense*- and *T. b. gambiense*-infected human samples, and its ability to monitor infection dynamics and treatment outcome was assessed in a mouse model.

Results: The sdAbsR3-77/sdAbR2-103 sandwich ELISA selectively detected *Tbr*ENO with high analytical sensitivity and no cross-reactivity with related protozoan enolases. It outperformed microscopy in mice, enabling real-time monitoring of antigenemia. The *Tbr*ENO signal rapidly disappeared after treatment, confirming its test-of-cure potential. In humans, it detected early and late *T. b. rhodesiense* and advanced *T. b. gambiense* infections, while remaining negative in uninfected controls.

Conclusions: This camelid sdAb-based pan-*T. brucei* recognition platform enables accurate detection of active infections across host and subspecies, addressing critical diagnostic. By

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supporting infection-specific diagnosis and treatment monitoring within a conserved-antigen framework, it provides a robust foundation for One Health diagnostics and integrated disease surveillance.

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LARGE SCALE PROPAGATION OF SCHISTOSOMA JAPONICUM FOR THE GENERATION OF RESEARCH REAGENTS

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Background and Aims: Schistosomiasis is a significant disease burden for over 300 million people across the tropics, with 3 major species causing the most morbidity and mortality: *Schistosoma haematobium, S. japonicum* and *S. mansoni*. *S. japonicum* control is complicated by its zoonotic promiscuity: over 40 mammals have been identified as reservoir hosts including bovines, water buffalo, pigs, dogs, cats, and rats. Understanding transmission dynamics, developing new anti-helminthics, and testing vaccine candidates are all research objectives that will require access to abundant life cycle stages of *S. japonicum* and its invertebrate host, *Oncomelania hupensis*.

Methods: The Schistosomiasis Resource Center at BRI sought to achieve large scale propagation of both Chinese and Philippine strains of *S. japonicum*. Breeder aquaria holding ≥ 500 mixed sex adult *O. hupensis* allow sexual reproduction and high yield egg collection. Water, temperature, lighting, and food sources have been optimized for expanded breeding. Juvenile *O. hupensis* are exposed to miracidia en masse to develop a stable patent population, and cercariae are recovered via shed or by crushing the snail to collect this larval form. Outbred mice are exposed to 40-50 cercariae and euthanized 6-8wk post-infection to perfuse adult schistosomes and isolate parasite eggs lodged in the enlarged liver.

Results: In the laboratory setting, large scale propagation of *S. japonicum* allows access to critical research material including cercariae-positive snail hosts, viable or frozen schistosomes and eggs, and infected rodents.

Conclusions: These reagents have advanced our understanding of pulmonary hypertension, aid in the identification of praziquantel alternatives, and help elucidate the mechanisms of anti-helminthics.

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DETECTING CIRCULATING MICRORNAS IN A MOUSE MODEL OF CANINE DIROFILARIASIS

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Background and Aims: *Dirofilaria immitis* is a filarial nematode that causes canine heartworm disease. Recent studies have shown that immunodeficient NSG mice can sustain *D. immitis* infection for ≥15 weeks, making them potential surrogate hosts for heartworm clinical research. This could help to overcome the logistical and challenges around using domestic dogs for experimental infections. Previous papers have reported heartworm micro (mi)RNAs in infected dog sera, suggesting these as potential diagnostic biomarkers that could improve heartworm diagnosis. The goal of this study was to determine if *D. immitis* microRNAs (miRNAs) could be detected in the plasma of infected mice, and to compare these profiles with those reported for canine *D. immitis* infections.

Methods: Plasma samples were collected from NGS mice on days 5, 28 and 70 post infection with *D. immitis*, and from time matched sham infected control mice. RNA was extracted from plasma, sequenced for small RNAs, and analysed using the mirdeep2 algorithm.

Results: Thirty-one *D. immitis*-derived miRNAs were detected in infected samples at day 70, and absent from sham samples. Only six of these were detected in single day 5 or day 28 replicates. This data appears qualitatively comparable to miRNA datasets from infected canines at infection timepoints consistent with presence of adult parasites.

Conclusions: This work supports the use of NSG mice as a surrogate host for *D. immitis* infection and could accelerate biomarker and clinical discovery research for canine heartworm. Ongoing work is analysing the impact of *D. immitis* RNA secretion on host cell transcription.

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DYNAMICS OF SPECIFIC ANTI TRYPANOSOMA CRUZI ANTIBODIES IN PATIENTS UNDERGOING CHEMOTHERAPEUTIC TREATMENTS

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Background and Aims: Assessing therapeutic efficacy in chronic Chagas disease is a major challenge; conventional serological reversion can take 10 to 20 years, and PCR is limited by intermittent parasitemia. We recently developed the Atlas of Antigens and Epitopes of Chagas Disease. In this work, we aimed to identify specific anti-*Trypanosoma cruzi* antibody profiles that serve as early biomarkers of treatment response.

Methods: We analyzed serum samples from 36 chronic patients enrolled in the E1224 clinical trial (Benznidazole, E1224, and Placebo arms) at recruitment, end-of-treatment (Day 65), and 12-month follow-up. We utilized high-density peptide microarrays (CHAGASTOPE-v2) containing 392,299 peptides to monitor longitudinal dynamics of antibody repertoires across patients.

Results: Longitudinal analysis revealed that patients exhibit three distinct profiles of antibody response over time: seronegativization, seropositivization, and no change. Notably, a considerable number of patients in the placebo group exhibit changes in their antibody profiles over time, suggesting natural oscillatory dynamics. We determined that the antibody repertoire is structured into stable and dynamic compartments, with the latter showing fluctuations. Using these data we prioritized *Trypanosoma cruzi* antigenic markers, based on signal magnitude, change and seroprevalence. Prioritized markers reveal treatment response at day 65 and 12 months.

Conclusions: Longitudinal profiling of antibody responses in chronic Chagas disease patients using peptide arrays revealed heterogeneous dynamics, including natural oscillations independent of treatment, and correlations with treatment in some patients. Selected peptide markers detect immunological changes earlier than conventional serology, supporting their evaluation as candidate biomarkers of treatment response. Prioritized peptides were synthesized for development of validation in ELISA-based assays (underway).

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SIMPLIFYING MOLECULAR DIAGNOSTICS IN RESOURCE-LIMITED SETTINGS: DNA EXTRACTION UTILIZING A COTTON-SYRINGE

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Background and Aims: Field-applicable molecular diagnostics are crucial tools in the control and elimination of neglected tropical diseases (NTDs). To achieve these would require simplifying laboratory-based sample processing of complex samples such as stools, which we sought to develop.

Methods: Using the NTD parasite *Schistosoma japonicum* Philippine strain (*SjP*) as the model organism, we employed a cotton-based DNA extraction method using a cotton-syringe device on spiked and endemic stool samples. DNA extraction efficiency was evaluated by measuring the difference in qPCR cycle threshold (ΔCt) between the extracted and inputted parasite DNA. Diagnostic performance was evaluated using Kato-Katz microscopy (KK) and kit DNA extraction with qPCR as reference tests.

Results: The optimized cotton-syringe DNA extraction method was estimated to have a 25.96% efficiency, achieving a comparable performance compared to commercial kits, with a qPCR limit of detection of 0.52 *SjP* target DNA copies/µl of sample. Combined with a handheld, battery-operated homogenization device, the method enables qPCR detection of seeded stool samples with at least 1 *SjP* egg per gram of stool. In the validation using endemic human stool samples (n=100) from the Philippines, the cotton-syringe with qPCR was able to detect 30 out of 31 positive individuals based on KK resulting in a sensitivity of 96.77%. Using KK and qPCR of kit DNA extracts as a composite reference, the cotton-syringe has a sensitivity of 87.768%, a specificity of 92.16%, and a kappa value of 0.80, indicating a strong agreement.

Conclusions: Cotton-syringe presents a simplified, pipette-free DNA extraction that can facilitate field-based molecular testing in resource-limited, NTD-endemic communities.

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FROM BIOMARKER DISCOVERY TO CLINICAL USE: CLIA VALIDATED SEROLOGICAL DIAGNOSTICS FOR HUMAN LIVER FLUKE INFECTIONS

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Background and Aims: Human liver fluke infections caused by *Clonorchis sinensis* (Cs), *Opisthorchis viverrini* (Ov), and *Fasciola hepatica* (Fh) remain underdiagnosed due to limited access to sensitive and scalable diagnostics. Chronic Cs and Ov infections are associated with increased risk of cholangiocarcinoma, underscoring the need for reliable serological tools for screening and surveillance in both endemic and non-endemic settings.

Methods: A biomarker-driven approach, integrating literature review, *in silico* analysis, and high-density peptide microarray identified candidate antigens for Cs and Ov. ELISA and lateral flow (LF) assays were evaluated using sera from individuals with stool confirmed infection status. Sensitivity (Sn), specificity (Sp) and Matthews Correlation Coefficient (MCC) were determined by ROC analysis. A commercially available *F. hepatica* IgG ELISA was also validated using immunoblot-confirmed positive sera.

Results: The Cs ELISA showed 96% sensitivity (n=31), 97% specificity (n=207), AUC 0.97. The Cs LF showed 81% sensitivity (n=21) 100% specificity (n=50), AUC 0.94. The Ov ELISA showed 58% sensitivity (n=12), 97% specificity (n=222), AUC 0.76, and MCC of 0.47, whereas Ov LF showed 91% sensitivity (n = 21), 80% specificity (n = 305), AUC 0.92. The *F. hepatica* IgG ELISA showed 95% sensitivity (n=20), 100% specificity (n=20), acceptable intra- and inter-assay precision, and a well-defined reference and reportable range, supporting CLIA implementation.

Conclusions: These assays represent the first CLIA-based serologic test menu for Cs, Ov, and Fh available outside endemic regions. Serology provides a scalable alternative to stool microscopy, supporting screening and surveillance in both endemic and non-endemic populations.

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DIENTAMOEBA FRAGILIS REVIEW OF EPIDEMIOLOGY (DARE) IN AUSTRALIA – ANALYSIS OF INFECTIONS DIAGNOSED BETWEEN 2015 AND 2024 IN A PRIVATE PATHOLOGY SETTING.

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Background and Aims: *Dientamoeba fragilis* (*D.fragilis*), a commonly detected protozoan, remains understudied.

Methods: A 10 year (2015 – 2024) retrospective review of 980965 faecal samples tested at Douglass Hanly Moir Pathology, Sydney, Australia.

Results: 441036 (45%) of samples underwent both PCR and microscopy with only 0.6% received in sodium acetate-acetic acid-formalin (SAF). 59732 (13.5%) were positive by PCR (11.5% by Seegene 2023-24 (2nd after Blastocystis at 13%), 14.8% by Roche 2015-2022) with 1789 (3%) of these also microscopy-positive, with trophozoites demonstrated only in SAF in 29.8%. Median PCR cycle threshold (Ct) values for SAF microscopy +/++/+++ samples were 30.3/28.8/26.4 while for non-SAF samples they were 24.5/23.2/22. 1077/1789 (60%) of the PCR and microscopy-positive samples had only *D. fragilis* detected. Females comprised 57.9% of cases, and median age was 30 yrs with 39.7% being children. In a subset analysis of isolated *D.fragilis* in 144 patients with ≥ 2 samples collected during 2023-2024, two age peaks were noted: 0-9 yrs (35%) and 40-59 yrs (29%). The most common symptoms were diarrhoea (55%) and abdominal pain/bloating (41%) and median/average duration of symptoms was 9/19 weeks. A minority of samples (12/144, 8%) had concurrent eosinophil counts and faecal calprotectin co-requested with no eosinophilia evident and borderline/high calprotectin uncommon (3/12, 25%).

Conclusions: In New South Wales, Australia, the detection of *D,fragilis* was common and SAF collections (while infrequently requested) were more sensitive for microscopic detection based on PCR Ct value. Infections with only *D.fragilis* detected were seen most frequently in children, were usually symptomatic and not usually associated with eosinophilia or elevated calprotectin.

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DIAGNOSTICS, HOST-PARASITE INTERACTION AND EMERGING TECHNOLOGIES / NEW DEVELOPMENT IN DIAGNOSTICS OF PARASITIC INFECTION

THE SPENT SUBSTRATE OF EDIBLE MUSHROOM PLEUROTUS OSTREATUS HAS ACTIVITY AGAINST THE PLANT-PARASITIC AND FREE-LIVING NEMATODES NACOBBUS ABERRANS

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Background and Aims: *Nacobbus aberrans* is an economically important plant-parasitic nematode responsible for significant crop losses. Due to the negative effects of chemical nematicides on human health, the environment, and non-target organisms. This study evaluated a sustainable alternative by assessing the nematicidal activity of a crude hexane extract obtained from the spent substrate of *Pleurotus ostreatus*. The extract was tested against the free-living nematodes *Caenorhabditis elegans* and *Panagrellus redivivus*, as well as against *N. aberrans*.

Methods: *In vitro* bioassays were performed by exposing second-stage juveniles (J2) to four extract concentrations (10–1.25 mg/mL) for species-specific incubation periods. Ivermectin and Tween 80 served as positive and negative controls, respectively. Mortality was determined microscopically based on larval responsiveness. Data were analyzed using one-way ANOVA followed by Tukey's test ($p < 0.05$).

Results: The highest mortality occurred at 10 mg/mL, reaching 72.2% ± 3.6 in *C. elegans*, 57.0% ± 11.5 in *P. redivivus* after 17 h, and 28.5% ± 2.9 in *N. aberrans*. Reduced efficacy was observed at lower concentrations, indicating a concentration-dependent effect. Exposed nematodes showed behavioral and morphological alterations consistent with physiological stress.

Conclusions: The crude hexane extract from *P. ostreatus* spent substrate demonstrated nematicidal activity against *N. aberrans* and free-living nematodes, with greater susceptibility in non-parasitic species. These findings support *P. ostreatus* as a source of bioactive metabolites for sustainable nematode management and highlight the usefulness of *C. elegans* and *P. redivivus* as model

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organisms for evaluating environmental implications in integrated pest management and soil ecosystem conservation.

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EVALUATION OF PLASMODIUM FALCIPARUM HISTIDINE RICH PROTEIN (PFHRP2/3) GENE DELETIONS IN PARASITE ISOLATES AND IMPLICATIONS FOR MALARIA CONTROL

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Background and Aims: Malaria rapid diagnostic tests (mRDTs) are the second most commonly used diagnostic test for malaria in Nigeria. Unfortunately, there are reports of deletions in Pfhrp2/3, leading to false mRDT-negative results and under-treatment of malaria. This study was designed to evaluate the prevalence of *Pf*hrp2/3 gene deletions in parasite isolates in Ibadan, Nigeria.

Methods: Two hundred and fifty-nine (259) patients presenting with clinical signs and symptoms of malaria were enrolled following written informed consent. Blood samples were screened microscopically for positive malaria parasites. *Pf*hrp2-based-mRDT was performed following the manufacturer's instructions. Genomic DNA of parasites was extracted from 1mL of blood sample using a modified Chelex DNA extraction protocol. *Pf*hrp2 and *Pf*hrp3 genes were amplified using specific primers in conventional polymerase chain reaction (PCR) with *Pf*3D7 genomic DNA as a positive control. PCR amplicons were resolved on an agarose gel, with band sizes matched to the control. The results were correlated with parasite densities and mRDTs to assess diagnostic accuracy and gene deletions.

Results: 57/259 had microscopically confirmed *Pf* infection with a mean parasite density of 2,151 (SEM: 1,676) parasites/μL of blood, giving the prevalence of malaria by microscopy to be 22%, and 51/259 were positive for *Pf*-mRDTs, giving a prevalence of 19% by *Pf*-mRDTs. PCR analysis was carried out, and one false negative was confirmed due to the hrp2 deletion; this was also confirmed with the hrp3 deletion

Conclusions: This study is a pilot study and a proof of principle that *Pf*hrp2/3 deletion is present in the selected study area.

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MOLECULAR CHARACTERIZATION AND RECOMBINANT EXPRESSION OF BABESIA OVIS SBP4 AS A POTENTIAL SERODIAGNOSTIC ANTIGEN

Sezayi Ozubek[1], Sercan Keskin[2], Mehmet Can Ulucesme[1], Arda Eyvaz[1], Mehmet Alatas[1], Mehmet Ziya Doymaz[3], Munir Aktas[1]
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Background and Aims: Ovine babesiosis caused by *Babesia ovis* is a tick-borne disease affecting sheep in tropical and subtropical regions, resulting in economic losses. Advanced diagnostic methods and identification of vaccine candidate antigens are crucial for effective ovine babesiosis control. Despite its clinical relevance, only a few useful serological markers are available for accurate diagnosis.The SBP4 protein, initially identified in *B. bovis*, has demonstrated excellent performance as a serological antigen in ELISA for bovine babesiosis. In this study, *B. ovis* spherical body protein 4 (SBP4) was evaluated its activity with sera from infected sheep, suggesting its potential for future serodiagnostic application.

Methods: Using the *B. bovis* SBP4 as a query, BLAST searches were conducted on an uncurated *B. ovis* genome assembly, followed by PCR amplification and sequencing of a newly identified Bo-SBP4. Characterization of this novel gene and protein was carried out using bioinformatics analysis, and ELISA.The serological response to Bo-SBP4 protein during acute and post-infection periods was determined in sheep experimentally infected with the *B. ovis*-Alacakaya strain. Five sheep were experimentally infected, and blood and serum samples were collected daily for the first 30 days post-infection and biweekly for one year after the acute phase.

Results: The presence of specific antibodies against rBoSbp4 was tested in serum samples, and antibodies in some experimental animals remained positive for 400 days post-infection.

Conclusions: The BoSBP4 protien shows potatial as a candidate for developing a novel serological diagnostic test to detect *B. ovis*. This work was financially supported by Scientific and Technological Council of Türkiye (TUBITAK)(project number: 222O123).

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EXPLAINABLE AI-BASED CEREBRAL MALARIA DIAGNOSIS: BRIDGING ACCURACY WITH HUMAN-UNDERSTANDABLE DECISIONS

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Background and Aims: Cerebral malaria (CM), a severe neurological condition caused by Plasmodium falciparum infection, is characterized by high mortality rates, primarily in children under the age of five in Sub-Saharan Africa. It is made difficult to diagnose due to overlapping symptoms with other mosquito-borne diseases, leading to frequent misdiagnosis. This study aims to develop an interpretable and accurate machine learning model for CM diagnosis.

Methods: Four classifiers—Support Vector Machine (SVM), Logistic Regression, Naïve Bayes, and XGBoost—were trained on a severe malaria medical database. The best-performing model, XGBoost, was interpreted using Explainable AI (XAI) techniques: SHAP (SHapley Additive exPlanations) for global interpretability and LIME (Local Interpretable Model-agnostic Explanations) for local, case-by-case explanations.

Results: XGBoost-based platform showed good predictive performance and was able to differentiate cerebral malaria from uncomplicated malaria and other vector-borne illnesses with high sensitivity. SHAP and LIME provided clinically useful data on feature contributions and local predictions.

Conclusions: Combining high-performance predictive models with post-hoc explanation techniques enables the development of diagnostically accurate and interpretable devices. This could enable clinical decision-making, build trust, and improve timely diagnosis of cerebral malaria in resource-limited settings.

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PRELIMINARY DEVELOPMENT OF AN IMMUNOCHROMATOGRAPHIC TEST FOR EARLY DETECTION OF CANINE VISCERAL LEISHMANIASIS

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Background and Aims: Background: Canine visceral leishmaniasis (CVL), caused by Leishmania infantum, is a serious parasitic disease. Domestic dogs in endemic regions act as primary reservoirs for the parasite. Early diagnosis, control, and regular screening of dogs are essential for effective disease management. This study aims to develop a low-cost immunochromatographic test (ICT) for detecting anti-Leishmania antibodies in dogs.

Methods: Methods: Overall, 93 canine serum samples were collected from endemic and non endemic areas of CVL in Iran. Rabbit anti-canine antibodies were conjugated with gold nanoparticles, and strips were coated with Leishmania antigens. A drop of serum was added to each strip, and a positive result was indicated by two red lines. The validity of ICT for the detection of CVL in the field was determined with comparing to direct agglutination test (DAT) as gold serological test on 40 sera with anti-Leishmania antibodies at titers ≥1:320 considered as positive control as well as 53 sera with no anti-Leishmania antibodies including 10 collected from healthy dogs and 43 from other infectious diseases considered as negative control sera.

Results: Results: A sensitivity of 82.5% (CI 95%.78.1-86.9) and specificity of 90.5% (CI 95%. 87.7-92.5) were found at a 1:320 cut off titer when DAT confirmed cases were compared with negative control. The agreement (0. 871) was found between ICT and DAT using kappa analysis.

Conclusions: Conclusion: ICT and DAT showed good agreement. Further studies on test validation in larger populations from endemic and non-endemic areas are recommended.

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POSTER ON BOARD SHIFT 02
DIAGNOSTICS, HOST-PARASITE INTERACTION AND EMERGING TECHNOLOGIES / NEW DEVELOPMENT IN DIAGNOSTICS OF PARASITIC INFECTION

A RAPID TEST FOR THE DETECTION OF CIRCULATING ANODIC ANTIGENS IN URINE FOR THE DIAGNOSIS OF SCHISTOSOMA HAEMATOBIUM AND SCHISTOSOMA MANSONI INFECTION

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Background and Aims: The World Health Organization states the need for improved, sensitive, specific, rapid, diagnostics for schistosomiasis surveillance and control. Laboratory-based assays targeting circulating anodic antigens (CAAs) are well-proven diagnostics for all *Schistosoma* spp., but point-of-care (POC) tests are lacking. We have developed a urine-based CAA lateral flow assay (CAA-LFA) and tested the relative diagnostic performance across different transmission and species contexts.

Methods: We used three sample-sets from distinct transmission settings: *Schistosoma mansoni* (Uganda), *Schistosoma haematobium* (The Gambia), and a mixed species, low intensity dataset from returning travellers and migrants (Italy) to test our CAA-LFA. The datasets include established diagnostics: Kato-Katz thick smear egg counts, urine microscopy egg counts, a commercially available POC circulating cathodic antigen (POC-CCA), haemoglobin urinalysis strips, and our CAA-LFA prototype. In the absence of a perfect reference, we modelled the relationship between the established diagnostics and the CAA-LFA. We fitted the model using a Bayesian framework to infer the true, unobservable, infection status of an individual, and estimated the sensitivity and specificity of our CAA-LFA.

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Results: We estimated sensitivities and specificities of each diagnostic, examining variations in performance across transmission landscapes. Additionally, we investigated the performance of our CAA-LFA in relation to model-inferred infection intensities. We will present our findings, which provide evidence on the performance of our CAA-LFA in *S. mansoni* endemic, *S. haematobium* endemic, and co-endemic settings.

Conclusions: Overall, these findings expand our understanding of which diagnostic tools are most effective in given transmission contexts and help support decision-making in schistosomiasis surveillance, control, and elimination programs.

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DIAGNOSTICS, HOST-PARASITE INTERACTION AND EMERGING TECHNOLOGIES / NEW DEVELOPMENT IN DIAGNOSTICS OF PARASITIC INFECTION

RETHINKING ECTOPARASITE DIAGNOSTICS: SKIN-SURFACE DNA AS A PLATFORM FOR DETECTION AND SURVEILLANCE OF CATTLE LICE

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Background and Aims: Ectoparasite detection requires careful visual observation, which is difficult to standardize, insensitive and not scalable. We are developing a novel diagnostic approach for detecting ectoparasite DNA on the host's skin surface. We have focused on cattle lice because improved diagnostics are urgently needed to enable more targeted and sustainable parasiticide use.

Methods: We have developed 18S rDNA metabarcoding to detect lice DNA from simple skin swabs and determine the relative abundance of different species. We have also developed and optimized species-specific real-time fluorescence LAMP (Rt-LAMP) assays (18S and COX-1) for the two major lice species *Linognathus vituli* and *Bovicola bovis* for use as rapid diagnostic tests. We applied these assays to a longitudinal beef calf study in Alberta to investigate the localization and dynamics of lice DNA on the bovine skin surface over time.

Results: The longitudinal study showed that the detection of bovine skin surface lice DNA matches known louse predilection sites, *B. bovis* was most predominant around the tail head, and *L. vituli was* most predominant on the withers and neck. The detection of lice DNA also matched the expected dynamics of infection, peaking in Feb/Mar before declining, but with the relative proportion of *B. bovis* increasing over the winter.

Conclusions: We have shown that louse DNA can be detected on the bovine skin surface and used to monitor lice infestations. This powerful tool could also be applied to routine diagnostics and surveillance for many other ectoparasites of domestic animals, wildlife and humans.

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DIAGNOSTICS, HOST-PARASITE INTERACTION AND EMERGING TECHNOLOGIES / NEW DEVELOPMENT IN DIAGNOSTICS OF PARASITIC INFECTION

COMPARISON BETWEEN ITS1 AND HSP70 TARGETS IN THE DIAGNOSIS OF AMERICAN CUTANEOUS LEISHMANIASIS IN PATIENTS FROM AN EXTRA-AMAZONIAN REGION: IDENTIFICATION OF L. (L.) AMAZONENSIS

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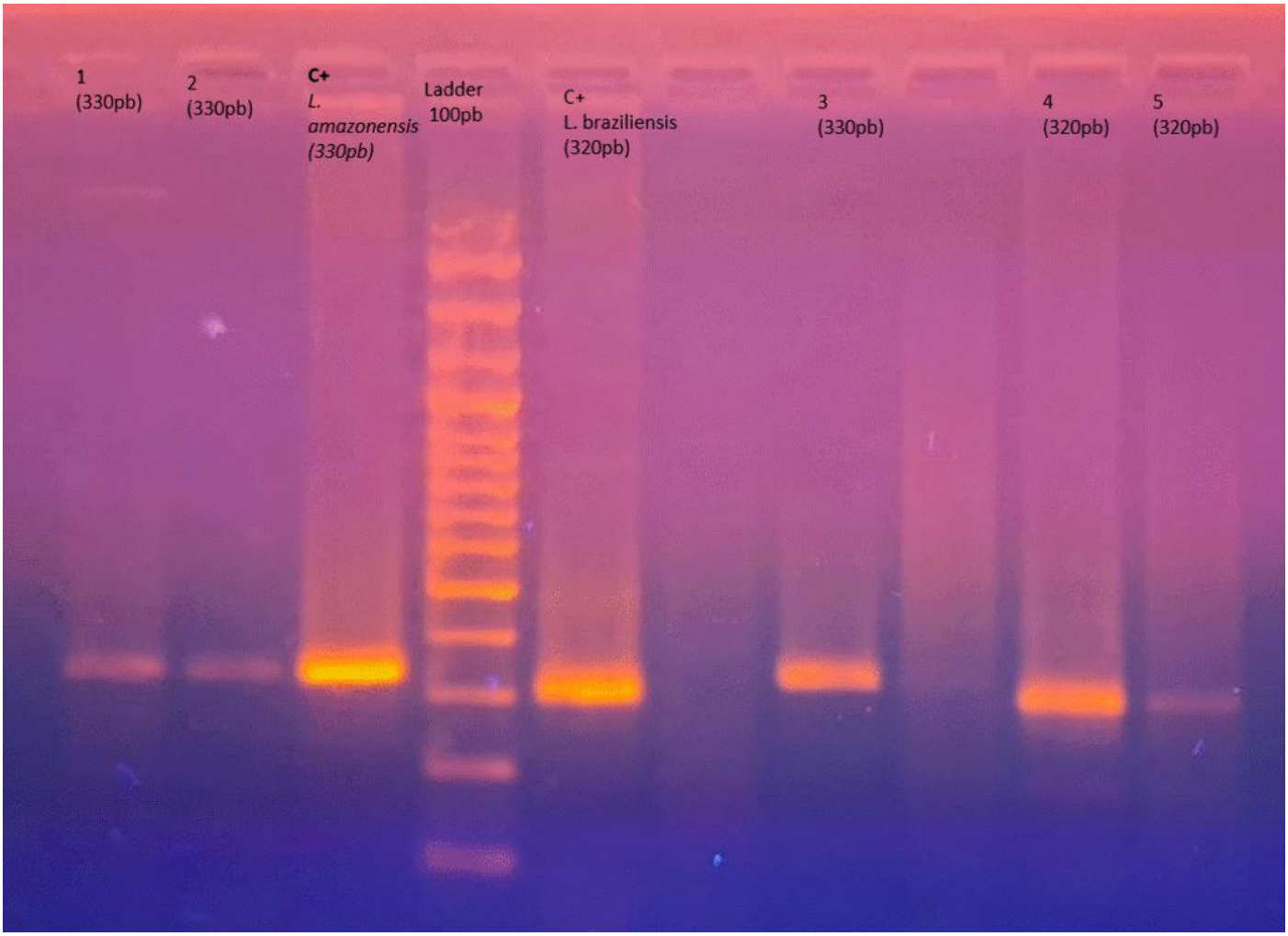
Background and Aims: The diagnosis of American Cutaneous Leishmaniasis (ACL) is based on clinical and epidemiological criteria and confirmed by parasitological methods.There is still no established consensus on the best PCR-target to be used. It is also crucial to have rapid molecular tests capable of identifying the *Leishmania* species. Our objectives are to evaluate the ITS1-PCR and nested hsp70-PCR, to analyze their respective sensitivities and specificities in cases of suspected ACL, and to assess their capacity for rapid species identification.

Methods: A total of 112 samples were obtained from patients at the Instituto de Infectologia Emílio Ribas (IIER) in São Paulo, Brazil. The samples underwent DNA extraction followed by conventional PCR-ITS1 and nested-hsp70 PCR; results were visualized on a 2% agarose gel. Five amplicons were also subjected to nested-hsp70 PCR, followed by sequencing and RFLP analysis.

Results: ITS1-PCR and nested-hsp70-PCR showed sensitivities of 66.67% and 60.38%, and specificities of 82.46% and 96.61%, respectively. ITS1-PCR enabled the identification of *L. (L.) amazonensis* (330 bp) directly from the amplicons obtained after electrophoresis(Fig.1). Confirmation that the species was indeed *L. amazonensis* was achieved through sequencing and RFLP of the nested-hsp70 amplicons. Figure 1-Agarose gel electrophoresis of PCR-ITS1 amplicons 1,2,3,4 and 5. Lanes 1,2 and 3:330 bp amplicons; Lanes 4 and 5:320 bp amplicons.Positive control (+):*L.*

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amazonensis (330 bp).Positive control (+): *L. braziliensis* (320 bp).100 bp DNA ladder.



Conclusions: ITS1-PCR showed superior sensitivity compared to nested-hsp70-PCR and identified L. amazonensis without the need for sequencing or RFLP procedures, which are labor-intensive and increase the costs of species identification.**Acknowledgements:**FAPESP2023/16087-0 and FMUSP

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DIAGNOSTICS, HOST-PARASITE INTERACTION AND EMERGING TECHNOLOGIES / NEW DEVELOPMENT IN DIAGNOSTICS OF PARASITIC INFECTION

GENETIC ANALYSIS OF LEISHMANIA (L.) INFANTUM POPULATIONS FROM DIFFERENT EPIDEMIOLOGICAL TRANSMISSION SCENARIOS IN THE STATE OF SÃO PAULO, BRASIL

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Background and Aims: *Leishmania (Leishmania) infantum* is the species responsible for visceral leishmaniasis(VL) in Brazil.Genotypic differences of the parasite have been associated with disease severity and potentially different epidemiological scenarios.In the state of São Paulo,Brasil, two distinct transmission scenarios of VL have been described: one with occurrence of both canine and human visceral leishmaniasis(scenario 1) and another only canine visceral leishmaniasis(scenario 2). These diferences in transmission scenarios may be related to different genotypes of *L.(L.)infantum,* and our hypothesis is that the populations circulating in these scenarios are genetically distinct. Our objectives are to investigate this by assessing the possible existence of diverse parasite genotypes in these scenarios through whole-genome sequencing of *L. infantum*.

Methods: A total of 40 promastigote isolates from dogs in different endemic areas were selected.DNA was extracted from promastigotes, and libraries were prepared using the Illumina DNA Prep protocol.Thirty-four samples were sequenced, yielding 21 files that were analyzed in Galaxy

Results: After cleaning and quality assessment, 19 isolates were mapping against different reference genomes using the program Geneious Prime. Mapping metrics indicated an average identity greater than 96%, highlighting polysomy in chromosome 31.Heatmap analysis of the variants revealed two major clusters:one with a higher number of SNPs and another with a more balanced profile.An intersection plot of variants suggested genomic proximity.Our results demonstrated that there is no correlation between a particular genotype and the occurrence of canine or human disease.

Conclusions: There is no correlation between a particular genotype and the occurrence of canine or human disease, as initially hypothesized.

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DIAGNOSTICS, HOST-PARASITE INTERACTION AND EMERGING TECHNOLOGIES / NEW DEVELOPMENT IN DIAGNOSTICS OF PARASITIC INFECTION

A NEWER FASTER ALTERNATIVE TO LARVAL CULTURES

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Background and Aims: Management of gastrointestinal nematodes (GIN) poses a major risk to livestock production and animal welfare globally. Monitoring GIN species is essential for effective parasite management, but current larval culture methods for identification are slow, limiting timely decisions. A rapid alternative is now commercially available to identify *Haemonchus contortus*, *Teladorsagia circumcincta*, Ostertagia ostertagi, Trichostrongylus axei, *Trichostrongylus colubriformis*, *Trichostrongylus vitrinus*, *Nematodirus spathiger*, and Cooperia spp. This assay provides species-level information within three days of sample arrival, enabling on-farm management.

Methods: Multiplex qPCR panels were developed to identify the major sheep and cattle GIN in New Zealand from faeces. Analytical sensitivity and specificity for each of the targets were determined. Storage conditions of the samples were assessed to define the optimal transport conditions for farmers to send to the laboratory for testing. Diagnostic sensitivity and specificity are continuing to be evaluated using field samples.

Results: Analytic specificity was 100% for all assays apart from *Trichostrongylus colubriformis* which also detected the closely related *T. vitrinus*. Work is ongoing to improve this specificity. Analytical sensitivity of the assay was between 2-10 copy numbers for all targets.

Conclusions: This assay enables producers to make parasite management decisions based on GIN species-level information. It is highly sensitive and specific, representing a practical tool for improving GIN control. Acknowledgements: this test was developed by scientists at Awanui Veterinary Laboratories and funded by Beef + Lamb New Zealand. We thank the producers and veterinarians for the input they have provided to the development of this test.

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DEVELOPMENT AND APPLICATION OF SPECIES-SPECIFIC LOOP-MEDIATED ISOTHERMAL AMPLIFICATION (LAMP) ASSAYS FOR THE RAPID DETECTION OF BLOOD FLUKES OF BLUEFIN TUNAS

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Background and Aims: Blood flukes of the genus *Cardicola* (Digenea: Aporocotylidae) are well-documented health concerns infecting farmed and ranched bluefin tuna species, where infections can lead to economic losses. While recombinase polymerase assays (RPA) have been developed for two *Cardicola* species, the high degree of sequence conservation across all three described species requires an alternative method capable of supporting confident species-level discrimination. Therefore, the aims of this study were to design, optimise and validate LAMP for the detection of *C. forsteri, C. orientalis* and *C. opisthorchis* and apply the assays to Pacific bluefin tuna cultured in Japan.

Methods: This research developed and validated loop-mediated isothermal amplification (LAMP) assays for three blood fluke species described to infect bluefin tunas, *C. forsteri*, *C. orientalis* and *C. opisthorchis* targeting the ITS-2 region of ribosomal DNA. In addition, these assays were applied to DNA extracted from three sample types targeting sites of significant pathology, the heart, gill necropsy and biopsy from 15 Pacific bluefin tuna farmed in Japan. Results were then compared to outputs of quantitative polymerase chain reactions (qPCR), a method considered the gold standard for blood fluke detection in Australia.

Results: Species-specific LAMP assays were successfully optimised and validated for *C. forsteri*, *C. orientalis* and *C. opisthorchis* detection. Application and comparison of LAMP to qPCR showed LAMP assays to be more sensitive.

Conclusions: This constitutes the first development and application of LAMP diagnostics to all three described *Cardicola* species infecting bluefin tunas, providing a rapid, cost-effective and user-friendly alternative to standard molecular diagnostics, informing management and treatment decisions.

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DIAGNOSTICS, HOST-PARASITE INTERACTION AND EMERGING TECHNOLOGIES / NEW DEVELOPMENT IN DIAGNOSTICS OF PARASITIC INFECTION

LABORATORY DIAGNOSTIC CAPACITY FOR FOODBORNE ZOONOTIC TREMATODES IN NORTHERN VIETNAM: A SURVEY OF HEALTH FACILITIES AND EVALUATION OF A PILOT TRAINING COURSE

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Background and Aims: Foodborne zoonotic trematodes (FBT) represent a significant public health issue in tropical regions, including Vietnam. Diagnostic accessibility is often limited to the provincial level, creating challenges for timely treatment in remote areas. This study aimed to assess the FBT diagnostic capacity of district and provincial health centers in two Northern Vietnamese provinces and evaluate the efficacy of a pilot laboratory training course.

Methods: In June 2022, a survey was conducted at 14 health centers in Yen Bai and Thanh Hoa provinces using questionnaires for 28 staff and 14 managers, alongside a 9-topic quality management checklist. Subsequently, a 3-day training course was organized for 30 participants. The curriculum covered theoretical knowledge and four diagnostic techniques: Kato-Katz, Formalin-ether concentration technique (FECT), Flukefinder, and Enzyme-linked immunosorbent assay (ELISA).

Results: Baseline assessments revealed weak laboratory quality systems and limited diagnostic capacity. While all 14 laboratories performed direct fecal smears, only 64.3% utilized the Kato-Katz technique, and none had experience with FECT or Flukefinder. Existing ELISA systems were non-functional due to lack of samples or project expiration. Post-training evaluations showed significant improvement: mean knowledge scores increased from 11.27±3.03 to 17.80±1.35 ($p < 0.001$). Furthermore, the proportion of participants able to correctly identify eight types of helminth eggs rose from 10% to 100%.

Conclusions: Significant gaps exist in resources and technical expertise for FBT diagnosis at the local level. Strengthening laboratory infrastructure and implementing standardized quality management through continuous training are essential for improving patient care and FBT control in endemic regions.

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COMPARATIVE STUDY OF MULTIPLEX PCR ASSAYS FOR DETECTING TWENTY HUMAN PATHOGENIC CRYPTOSPORIDIUM SPECIES

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Background and Aims: *Cryptosporidium hominis* and *Cryptosporidium parvum* account for most human cryptosporidiosis cases, and commercial multiplex PCR assays are mainly designed to detect these two species. However, more than twenty additional *Cryptosporidium* species or genotypes are known to infect humans, including emerging and outbreak-associated species. The ability of routine molecular assays to detect these rare species remains insufficiently documented, potentially leading to an underestimation of their epidemiological impact. This study was conducted by the French National Reference Center for Cryptosporidiosis, whose missions include reducing the underestimation of this long-neglected parasite.

Methods: We assessed the performance of 12 PCR assays commonly used in France for the detection of 20 *Cryptosporidium* species pathogenic to humans. Species detection range and cycle threshold (Ct) values from quantitative PCR assays were collected and descriptively compared across kits. Hypotheses explaining inter-assay discrepancies were explored

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Results: Detection performance varied markedly according to species. All assays reliably detected *C. hominis* and *C. parvum*, but substantial discrepancies were observed for less common species. Several assays failed to detect multiple rare or emerging species, whereas others showed broader detection spectra. Ct values varied for identical samples, reflecting differences in analytical sensitivity and target design.

Conclusions: Such variability may contribute to underdiagnosis and underestimation of the epidemiological burden of *Cryptosporidium* spp. Improved assay design and greater transparency regarding species detection spectra are essential for accurate diagnosis and surveillance.

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DIAGNOSTICS, HOST-PARASITE INTERACTION AND EMERGING TECHNOLOGIES / NEW DEVELOPMENT IN DIAGNOSTICS OF PARASITIC INFECTION

TENTATIVE ANALYSIS OF BIOMARKERS ASSOCIATED WITH FILARIASIS IN MOUNGO DIVISION, LITTORAL, CAMEROON

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Background and Aims: Filariasis is a significant cause of morbidity and a public health concern in most tropical countries. These diseases are usually contracted in childhood and most often diagnosed in adulthood. This study aimed to identify biochemical markers associated with filariasis and the endobacteria present in microfilariae. This was a cross-sectional analytical study within a hospital setting. Persons aged from six years and above were included in the study. They were then clustered into asymptomatic and symptomatic.

Methods: Data was collected from February, 2023 to June, 2023. Blood, urine and skin samples were collected and analysed by microscopy to identify microfilariae. The plasmatic and urinary markers were evaluated by spectrophotometry and protein fraction was monitored by electrophoresis.

Results: The data was analysed using software-SPSS 25 with the significant threshold set at 0.005. A total of 55 persons were included in the study, representing 74.32% of the total population questioned. The sex ratio (F/M) was 2.66. The infection rate was 18.2% Onchocerca volvulus, 54.54% Mansonella perstans, 22.72% Loa loa and 4.54% Wuchereria bancrofti. Protein levels were 35% higher in the asymptomatic with no parasite, 80% in the asymptomatic with detected parasite and 91.67% in the symptomatic. The decrease in albumin fraction and increase in gamma fractions seem to be correlated with the presence of parasites in the blood.

Conclusions: These results suggest that protidemia and serum protein are significant indicators in the diagnosis of filariasis. Keywords: Microfilariae; Biomarkers; Moungo; Cameroon

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DIAGNOSTICS, HOST-PARASITE INTERACTION AND EMERGING TECHNOLOGIES / NEW DEVELOPMENT IN DIAGNOSTICS OF PARASITIC INFECTION

FIRST INSIGHT INTO DRUG RESISTANCE GENETIC MARKERS, GLUCOSE-6-PHOSPHATE DEHYDROGENASE AND PHYLOGENETIC PATTERNS OF MISDIAGNOSED PLASMODIUM VIVAX MALARIA IN FAR NORTH REGION, CAMEROON

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Background and Aims: There are growing reports on the prevalence of non-falciparum species and submicroscopic infections in sub-Saharan African countries but little information is available from Cameroon.

Methods: A hospital-based cross-sectional study was carried out in four towns (Douala, Maroua, Mayo-Oulo, and Pette) from three malaria epidemiological strata (Forest, Sahelian, and Soudanian) of Cameroon. Malaria parasites were detected by Giemsa light microscopy and polymerase chain reaction (PCR) assay. Non-falciparum isolates were characterized and their 18S gene sequences were BLASTed for confrmatory diagnosis.

Results: PCR assay detected malaria parasites in 82.4% (98/119) patients, among them 12.2% (12/98) were asymptomatic cases. Three Plasmodium species viz. P. falciparum, P. ovale curtisi and P. vivax, and two co-infection types (P. falciparum+P. vivax and P. falciparum+P. ovale curtisi) were found. The remaining infections were mono–infections with either P. falciparum or P. ovale curtisi. All non–falciparum infections were symptomatic and microscopic. The overall proportion of submicroscopic infections was 11.8% (14/119). Most asymptomatic and submicroscopic infection cases were self-medicated with antimalarial drugs and/or medicinal plants. On analysis, P. ovale curtisi sequences were found to be phylogenetically closer to sequences from India while P. vivax isolates appeared closer to those from Nigeria, India, and Cameroon. No G6PD-d case was found among non-falciparum infections

Conclusions: This study confrms our previous work on circulation of P. vivax and P. ovale curtisi and the absence of P. knowlesi in Cameroon. More studies are needed to address non-falciparum malaria along with submicroscopic infections for efective malaria management and control in Cameroon.

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DNA EXTRACTION COMPARISON OF MOSQUITOES AND BREAKING THE COLD CHAIN IN QPCR XENOMONITORING OF WUCHERERIA BANCROFTI

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Background and Aims: Vector monitoring is becoming an increasingly important tool for mass drug administration stopping decisions. However, extraction of DNA from vectors and maintaining cold chain storage of reagents needed for detection can cause financial challenges and limit utility in many settings.

Methods: Here, we tested three different DNA extraction methods (Qiagen DNeasy Blood and Tissue Kit, NaOH-based crude extraction method, TE-based crude extraction method) in *Anopheles sp.* infected with *Wuchereria bancrofti*. Mosquito pools of different sizes were macerated, and the mosquito slurry was divided into three separate pools. Each pool underwent DNA extraction using one of the three methods. Next, the samples were tested via qPCR for the presence of *W. bancrofti* DNA and results were compared across extraction methods. All samples were then retested , except the polymerase (TaqPath ProAmp) was heated to 40°C for 4 weeks and then refrozen at -20°C before use.

Results: Detection of *W. bancrofti* DNA was nearly as robust in the NaOH extracted samples as the Qiagen DNeasy extracted samples. Furthermore, heating the polymerase did not significantly impact the sensitivity of the qPCR.

Conclusions: Together, this data demonstrates the potential use of an economical extraction method for mosquito DNA and the robustness of TaqPath ProAmp in qPCR detection.

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EVALUATION OF THE PERFORMANCE OF GLYCINE IN THE RECOVERY OF TOXOPLASMA GONDII OOCYSTS IN SANDY SOIL SAMPLES FOR ENVIRONMENTAL SURVEILLANCE.

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Background and Aims: Soil is an important environmental reservoir of *Toxoplasma gondii* oocysts and plays a relevant role in the epidemiology of toxoplasmosis. The standardization of methodologies for the recovery of oocysts from environmental matrices is essential to support environmental surveillance studies. The aim of this study was to evaluate the sensitivity of oocyst recovery at different known concentrations using 1M glycine as an eluent for sandy soil samples, combined with detection by real-time PCR (qPCR).

Methods: Ten-gram samples of sandy soil were artificially contaminated with three known concentrations of oocysts (15.000; 1.000; and 500), in triplicate, placed in 50mL tubes. The oocysts were kept in contact with the soil for 1 hour at room temperature, after which 30mL of 1M glycine was added. The samples were homogenized using a shaker for 30 minutes to promote oocyst recovery. They were then subjected to sedimentation for 5 minutes and the supernatant was transferred to new tubes and centrifuged at 2.100×g for 15 minutes. The pellets were used for DNA extraction with the DNeasy PowerSoil Kit® and the amplification was performed by qPCR using the QuantStudio5®.

Results: All samples showed amplification at the three tested concentrations. The mean cycle threshold values were 31,06 for 15.000 oocysts 33,41 for 1.000 oocysts, and 34,82 for 500 oocysts.

Conclusions: The use of 1M glycine proved to be effective for the recovery of *Toxoplasma* oocysts from sandy soil at different concentrations, representing a suitable choice for environmental studies. However, additional experiments are still needed to determine the detection limit of the method, especially at lower oocyst concentrations.

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DIAGNOSTICS, HOST-PARASITE INTERACTION AND EMERGING TECHNOLOGIES / NEW DEVELOPMENT IN DIAGNOSTICS OF PARASITIC INFECTION

EVALUATING THE DIAGNOSTIC GAP: A STRATIFIED COMPARISON OF NGS METABARCODING AND MICROSCOPY FOR INTESTINAL PARASITE IDENTIFICATION IN A PEDIATRIC COHORT.

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Background and Aims: Conventional microscopy is limited by low parasitic loads and inability to differentiate morphologically identical species. Next-Generation Sequencing (NGS) offers superior resolution. This study evaluates species-specific agreement to identify diagnostic gaps in intestinal protozoa and nematodes.

Methods: A comparative study was conducted on 65 fecal samples from children under 5 years old. Molecular identification involved NGS metabarcoding of the 18S rDNA V4 region, while morphological analysis was performed via concentrated microscopy. Statistical metrics included Cohen's kappa (κ kappa) and Positive Agreement.

Results: Agreement was highly parasite-dependent. Nematodes: Almost perfect agreement for *Ascaris sp.* (κ / kappa=0.893) and Hookworms (κ / kappa=0.846). Taxonomic Resolution: NGS resolved 100% of the *Entamoeba* complex cases as the commensal *Entamoeba dispar*. Diagnostic Gaps: NGS detected a prevalence of *Blastocystis spp.* nearly tenfold higher than microscopy. Interestingly, *Cystoisospora belli* was identified only via NGS. Null Concordance (κ / kappa=0.000): A systematic molecular detection failure was observed for *Endolimax nana*, *Iodamoeba bütschlii*, *Giardia lamblia*, *Chilomastix mesnili*, and *Cyclospora cayetanensis*, which were detected only by microscopy. Polyparasitism: NGS detected 4.2 species/child vs. 1.8 by microscopy.

Conclusions: While microscopy is robust for nematodes and certain protozoan cysts, it is insufficient for the "protozoome" and taxonomic differentiation. The systematic failure of NGS to detect specific protozoa highlights the need for optimized lysis and primer inclusivity. However, the superior detection of *Blastocystis* and *Cystoisospora*, alongside the resolution of *E. dispar*, proves that 18S V4 NGS is vital to bridge diagnostic gaps and avoid overtreatment in public health.

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EVALUATION OF CONVENTIONAL AND FOUR REAL-TIME PCR METHODS FOR THE DETECTION OF LEISHMANIA ON FIELD-COLLECTED SAMPLES IN ETHIOPIA

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Background and Aims: Microscopic examination is the gold standard for parasite detection. Various molecular approaches targeting different gene fragments have been developed, showing high sensitivity and specificity. This study compared different diagnostic methods for the detection of *Leishmania* in Ethiopia (most probably caused by *L. aethiopica*) on different sample types collected from a suspected CL lesion.

Methods: 111 patients suspected of CL, from 37 rural villages from Southern Regional State in Ethiopia between May and July 2018. Giemsa-stained smears were examined microscopically for amastigote detection, while the first sample was subjected to five molecular tests. DNA extracts were tested by a conventional PCR targeting ITS-1, and three probe-based real-time PCRs targeting 18S rDNA for universal detection of *Leishmania* and CL and VL kDNA, designed to respectively detect *L. donovani* and *L. aethopica*, and RNA extracts with a SYBR Green-based RT-PCR assay targeting spliced leader (SL) RNA.

Results: Regarding DNA molecular approaches of skin scrap samples, the kDNA PCRs showed the best result, followed by the 18S rDNA PCR assay, while the conventional ITS-1 PCR resulted in a rather low sensitivity. Detection of *Leishmania* RNA with the SL RNA RT-PCR assay correlated well with the PCR results seen on DNA extracts of skin scraping. Skin scrapings resulted in a higher positivity rate and lower Ct-values than blood filter paper spots for the real-time PCR assays.

Conclusions: The recently developed real-time PCR assay targeting kDNA of *L. aethiopica* and SL-RNA provides promising results forthe diagnosis of CL in Ethiopia, with the best results obtained on skin scraping samples.

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FIVE WAYS TO FIND A PARASITE: COMPARISON OF FIVE MOLECULAR ASSAYS FOR THE DETECTION OF STRONGYLOIDES SPP.

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Background and Aims: Strongyloidiasis, caused by nematodes of the genus *Strongyloides*, is a neglected tropical disease with significant public health implications. Accurate diagnostics are essential for disease surveillance and control, yet traditional stool-based and serological methods have limitations in sensitivity and specificity. Molecular assays offer an alternative; however, their diagnostic performance for *Strongyloides* remains uncertain.

Methods: In this study, we compared four probe-based real-time PCR (qPCR) assays and one loop-mediated isothermal amplification (LAMP) assay using human faecal DNA samples from Gabon and Papua New Guinea with both *Strongyloides stercoralis* and *Strongyloides fuelleborni* infections.

Results: Our results revealed discordance between assays, with no single primer set identifying all positive samples across all assays. One assay was identified as specific for *S. stercoralis* only, which raises issues about the specificity of molecular diagnostics used in some *Strongyloides* epidemiological surveys, particularly with increasing identifications of *S. fuelleborni* infections in humans from Asia. Of the assays examined, four were probe based qPCR assays and a LAMP assay. On sequencing positive amplicons, enteric bacterial sequences were identified for two assays. However, subsequent testing and BLAST results identified that the specificity of the probe meant that this was not an issue when running the assay as designed.

Conclusions: These findings underscore the need for improved molecular tools and the necessity for thorough and comprehensive assay validation prior to employment for strongyloidiasis diagnosis in endemic settings.

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RELEVANCE OF THE ALBENDAZOLE TREATMENT PROTOCOL COMMONLY USED IN SHEEP IN FRANCE TO CONTROL DICROCOELIUM DENDRITICUM INFECTION

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Background and Aims: Hepatobiliary trematodosis caused by *Dicrocoelium dendriticum* leads economic losses in ruminant production. With increasing reports of helminth resistance to benzimidazoles, monitoring the efficacy of albendazole against dicrocoeliosis is essential. This trial aims to evaluate the effectiveness and relevance of the treatment protocol traditionally used in sheep farming, based on albendazole for controlling *D. dendriticum* infection.

Methods: Sixty ewes naturally infected with *D. dendriticum* were randomized equally according to similar *Dicrocoelium* egg counts : one group treated with albendazole at the entrance to the sheepfold (Day 0) at an oral dose of 15 mg/kg body weight and one untreated control group. Coproscopic analysis were performed on Days 0, 14, and 28 post-treatment to assess faecal egg count reduction. On Day 28, six ewes from each group were slaughtered to count the flukes present in their livers.

Results: On day 0, the average number of fluke eggs per gram of feces was 205.14 ± 181.40 in the control group and 233 ± 233 in the treated group. Coprological therapeutic albendazole efficacy reached 3.11 % on Day 14 and 81.04 % on Day 28 ($P < 0.05$). Liver necropsy revealed a significant reduction of 59.79% in fluke load for the treated group ($P = 0.02$) with an average of 568 ± 430 flukes counted; it was 1413 ± 562 for the control group .

Conclusions: This results claimed insufficient effect of oral albendazole formulation, despite a decrease in egg excretion and a decrease in the number of adults in the liver.

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FIELD PERFORMANCE OF THE BAERMANN TECHNIQUE: EXPERIMENTAL AND NATIONAL DATA ON CATTLE LUNGWORM (DICTYOCAULUS VIVIPARUS)

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Background and Aims: *Dictyocaulus viviparus,* is a major cause of parasitic bronchitis in grazing cattle in temperate regions. Diagnosis relies on Baermann method for detecting L1 larvae or serology (ELISA). Although ELISA has high analytical sensitivity, processing time delay clinical decision-making. The Baermann method can detect an ongoing infection, but is perceived to have lower sensitivity under field conditions. This study aimed to investigate if reduced field sensitivity reflect methodical limitations or pre-analytical factors, focusing on fecal sample mass and handling.

Methods: Following a literature review to identify methodological variables influencing larval recovery, fecal samples (n=77) from six cattle farms were analyzed using the Baermann method. Experimental larval recovery were conducted using diluted naturally infected feces (10-200g) and spiked negative samples with known larval counts (10-100g). Eight year (2014-2024) of national diagnostic data from Swedish Veterinary Agency were analyzed for seasonal trends.

Results: Twenty of the 77 samples were positive (26%). Experimental trails demonstrated an inverse relationship between fecal mass and larval recovery, decreasing from 38% (10g) to 6% (100g) in spiked samples. Literature and experimental data indicated that storage time and temperature have big effects on larval recovery. National data showed seasonality in percentage of positive samples, with highest diagnostic positivity between August and November.

Conclusions: The Baermann method appears sensitive under optimal conditions, but increased fecal mass and delayed processing may reduce recovery efficiency. Low field sensitivity can therefore reflect pre-analytical constraints rather than methodological limitations. Standardizing sampling and protocols could improve routine lungworm diagnosis.

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EVALUATION OF A RAPID ISOTHERMAL LAMP ASSAY FOR MALARIA DETECTION AND PLASMODIUM FALCIPARUM IDENTIFICATION

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Background and Aims: Rapid detection of malaria, particularly *Plasmodium falciparum,* is essential due to the risk of severe disease. Many molecular assays detect Plasmodium without species differentiation. This study evaluated the performance of the Biosynex Ampliflash Malaria real-time isothermal LAMP assay for detection of *Plasmodium* species and identification of *P. falciparum* in EDTA blood.

Methods: Intermediary precision was evaluated over four days by four operators using 16 spiked *P. falciparum* samples (low or medium concentration) and 3 negative samples. Diagnostic performance evaluated by comparison with the Alethia® Malaria DNA amplification assay using 13 clinical samples representing four human Plasmodium spp. (*P. falciparum, P. vivax, P. ovale*, and *P. malariae)*, and the two zoonotic *P. knowlesi* and P. *cynomolgi*. Analytical sensitivity was assessed using dilution series of known parasitaemia of *P. falciparum* and *P. knowlesi*.

Results: Precision met expected result in all replicates with no false positives. High overall agreement was observed between methods. One *P. falciparum sample* with very low parasite load (*laboratory developed* PCR Cp value= 40) was negative in the *P. falciparum* reaction but positive in the *Plasmodium* spp. reaction of the Biosynex kit. Two highly diluted *P. knowlesi* samples were detected by Alethia® only, consistent with results close to the limit of detection.

Conclusions: The Biosynex Ampliflash Malaria LAMP assay demonstrated high intermediary precision, good analytical sensitivity, and diagnostic performance comparable to Alethia®, with the added advantage of *P. falciparum* identification. The assay is robust and suitable for routine clinical use for rapid malaria diagnosis.

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EVALUATION OF THREE PCR PROTOCOLS FOR DETECTION OF TOXOPLASMA GONDII DNA IN CARNIVORE SAMPLES

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Background and Aims: *Toxoplasma gondii* is a widespread zoonotic parasite infecting a broad range of vertebrate hosts, including humans. It threatens wildlife health, and it is an important foodborne pathogen. Detection of *T. gondii* in tissue is challenging because cysts are unevenly distributed and present in low concentrations. PCR-based methods offer alternatives, although sensitivity may vary with sample size. These challenges are particularly relevant for wildlife surveillance. This study evaluated a lysate-based approach using up to 100 grams of tissue, and compared three DNA extraction and PCR protocols for detecting *T. gondii* in wildlife carnivores.

Methods: Heart, brain, and muscle samples from six carnivore species (river otters, Arctic and red foxes, martens, fishers and coyotes) were homogenized to produce lysates. Three protocols were evaluated: (1) magnetic capture DNA extraction using 25-50 mL of lysate followed by qPCR; (2) commercial DNA extraction from 200 mL of lysate followed by PCR targeting the 188 bp within the 529 bp repeat element; and (3) the same extraction as protocol 2 followed by a nested PCR targeting the ITS-1 region.

Results: Estimated *T. gondii* prevalence was 66.7%, 31.3% and 41.5% for protocols 1, 2 and 3, respectively. Agreement was fair to moderate between protocols (k = 0.33-0.40). Protocol 1 showed higher sensitivity likely due to the larger sample volumes analyzed.

Conclusions: Magnetic capture combined with qPCR showed improved detection of *T. gondii* in wildlife tissues. More sensitive and validated detection methods are needed for wildlife research and food safety, particularly for Indigenous communities that rely on country foods for food security.

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PULSED-POWER INDUCED INACTIVATION OF TREMATODES IN FRESHWATER FISH

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Background and Aims: Opisthorchiasis, a food-borne parasitic infection caused by consuming raw or undercooked freshwater fish infected with *Opisthorchis viverrini*, represents a significant public health challenge. *O. viverrini* is recognized as a risk factor for cholangiocarcinoma, leading the WHO to target its elimination. Building on previous success in inactivating *Anisakis* in marine fish, this study investigates the efficacy of pulsed-power technology as a non-thermal method to inactivate trematode metacercariae in freshwater fish while preserving fish meat quality.

Methods: Freshwater fish, *Tanakia lanceolata* infected with *Metagonimus katsuradai* were subjected to pulsed-power treatment using a lab-built circuit. Fish were placed in a reactor with conductivity-adjusted water and exposed to 50 shots at charged voltages ranging from 10 to 30 kV. Following treatment, metacercariae were collected via artificial gastric digestion. The metacercariae activity level was determined by calculating the excysted rate after incubation in trypsin solution at 37°C. Trematode morphology was also examined under a microscope to assess structural damage.

Results: The results demonstrated a significant reduction in parasite activity. While untreated metacercariae showed a 62% excysted rate, the 30 kV treatment group reduced the rate to only 0.3%. Microscopic observations confirmed tissue degradation and obscured trematode outlines in treated samples, indicating parasite death rather than temporary paralysis. Furthermore, the temperature increase during treatment was kept within 10°C, maintaining the raw state of the fish.

Conclusions: This study confirms that pulsed-power is an effective, non-thermal intervention for trematode inactivation. Ongoing research focuses on optimizing pulse intensity and clarifying the specific mechanisms of inactivation.

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NANOPORE LONG-READ METABARCODING FOR COMPREHENSIVE CHARACTERISATION OF PARASITIC NEMATODES IN HUMANS AND ANIMALS: A DEVELOPMENT AND DIAGNOSTIC VALIDATION STUDY

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Background and Aims: Parasitic nematodes impose a substantial burden on human and animal health; however, advanced diagnostic tools for their accurate characterisation are scarce. One nascent approach is metabarcoding, which enables the simultaneous characterisation of all parasitic nematodes in a sample, termed the nemabiome. However, earlier iterations of this approach could not detect socioeconomically important genera such as *Strongyloides* and *Trichuris*.

Methods: We used a comparative molecular target approach to identify optimal barcoding genes and create a novel nemabiome assay capable of detecting all vertebrate-infecting parasitic nematodes. We leveraged the long-read sequencing capabilities of the Oxford Nanopore Technologies platform to ensure high taxonomic resolution. The diagnostic performance of the assay was benchmarked against that of quantitative PCR (qPCR), using kappa (κ) statistics and diagnostic sensitivity and specificity assessment.

Results: As shown through our recent publication in *The Lancet Microbe* we identified optimal barcoding genes for nematodes in all parasitic clades with validation using positive controls confirming the assay's ability to detect parasites from 24 genera. Diagnostic specificity was 99.6% (95% CI 98.8–99.9), diagnostic sensitivity was 86.0% (81.4–89.9), and diagnostic agreement was substantial (κ≥0.69) or high (κ≥0.81) between the nemabiome assay and qPCR. Moreover, our assay revealed a greater diversity of nematodes (n=11) than conventional molecular techniques.

Conclusions: This nemabiome assay, unlike target-specific molecular methods (qPCR), facilitates the detection of previously unidentified parasites, including zoonotic and cryptic species, and elucidates interhost transmission pathways and animal reservoirs. Greater employment of this method will strengthen parasite control programmes, including the management of emerging and re-emerging parasitic threats.

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PARASITE SMOOTHIES: ASSESSING WHOLE-HOST HOMOGENISATION AS A NEW TOOL FOR PARASITE DETECTION AND IDENTIFICATION

Kamil Hupalo[1,2,3], Celine Sassor[1], Mia Fuhren[1], Dominik Buchner[3], Daniel Grabner[1,2], Bernd Sures[1,4,5,6]
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Background and Aims: Despite their importance in ecosystem functioning, parasites remain the most neglected components of biodiversity monitoring. This is partly due to the methodological challenges associated with their detection and identification combined with ongoing taxonomic impediment. Current traditional morphological and molecular approaches are time-consuming, labour-intensive, and require specialized expertise. Here, we explore a novel approach to studying parasites through whole-host homogenisation followed by DNA-based identification of parasites. Our goal was to validate whether this novel approach is reliable and to evaluate if it could become a complementary method for studying parasites, providing a time-efficient solution for a parasite scan with limited taxonomic expertise.

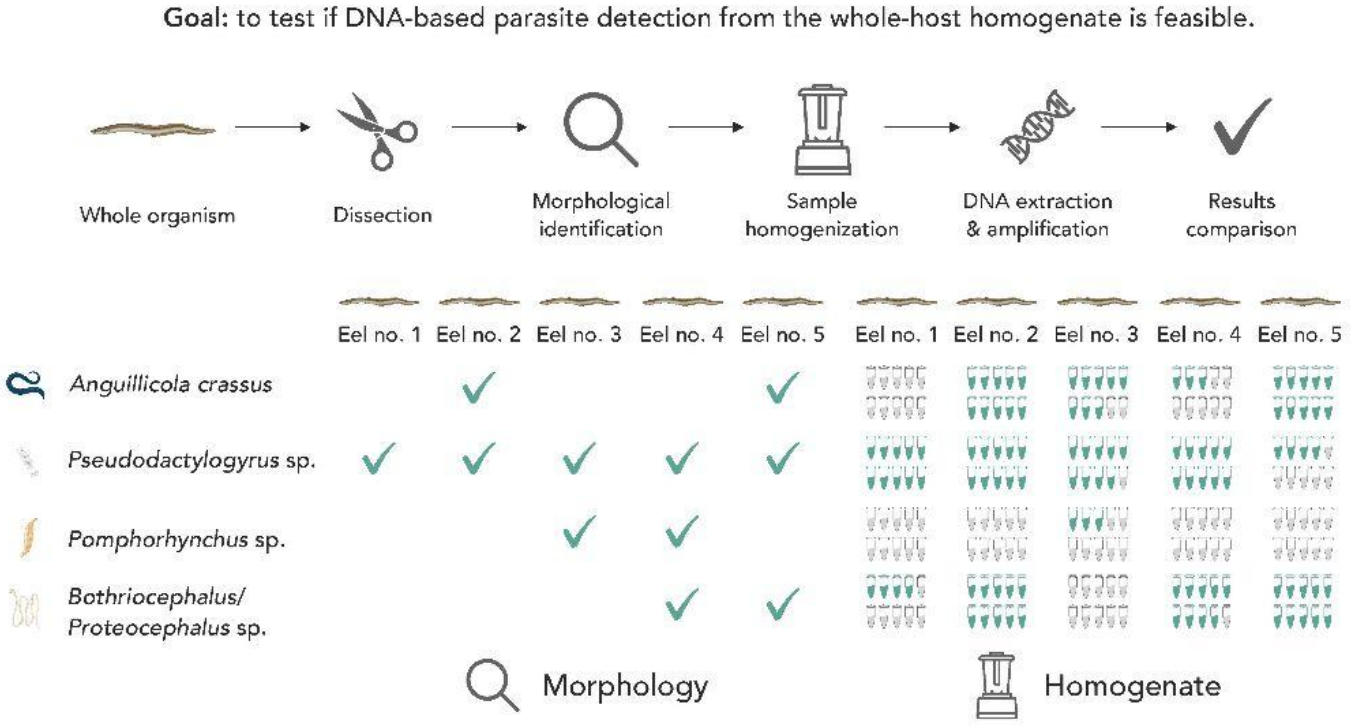
Methods: To test the method's efficiency, we analysed selected specimens of European eel as model hosts. Their parasites were first identified morphologically and afterwards, all the parasites with entire host tissue were homogenised together using a commercial blender. Molecular identification of the parasites was conducted using DNA barcoding with a set of parasite-specific primers.

Results: After homogenisation and DNA-based identification, we successfully detected all parasite taxa identified during morphological analyses, even including instances where they had not been detected morphologically. A notable exception were acanthocephalans, which showed low levels of molecular detection.

Conclusions: Despite certain limitations, the detection of parasites directly from the whole-host homogenate shows high potential for efficient and accurate parasite detection, in some cases even surpassing morphological identification. The further development of the method, particularly through exploration of DNA metabarcoding, could improve the reliability of parasite assessments and broaden

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the scope of parasite detection, aiding in proper parasite recognition.



Outcome: DNA-based parasite detection from the whole-host homogenate is feasible and comparable with morphology. Upon optimisation and further development, it can serve as a powerful tool for studying parasites.

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HAVING IMPACT ON MONITORING PARASITE DIVERSITY – TESTING AND OPTIMIZING A NOVEL EDNA-BASED APPROACH FOR A HOLISTIC PARASITE SCAN.

Kamil Hupalo[1,2,3], Celine Sassor[1], Mia Fuhren[1], Daniel Grabner[1,2], Dominik Buchner[3], Milen Nachev[1], Sebastian Prati[1,2,4], Florian Leese[2,3], Isabel Blasco Costa[5], Eloïse Rochat[5], Chris Selbach[6], Rachel Paterson[7], Bernd Sures[1,8,9,10]
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Background and Aims: Although parasites are essential components of a healthy ecosystem, they remain the most neglected components of biodiversity monitoring. Monitoring of parasite biodiversity is time-consuming, labor-intensive, requires specialised expertise and remains ethically problematic, as it involves sacrificing many host individuals. Thus, it is necessary to develop less invasive and non-lethal monitoring approaches. Environmental DNA (eDNA) offers a potential solution, particularly for studying aquatic parasites, by directly detecting them from the environment without sampling the hosts. There is growing evidence that eDNA has proven useful for detecting single parasite species. However, there are very few studies simultaneously targeting multiple parasite species within and across defined parasite groups. Moreover, there is no consensus on the optimal sampling conditions for detecting certain parasite groups via eDNA. Therefore, our goal was to evaluate a novel eDNA-based approach for a holistic parasite scan through multiplexed DNA metabarcoding. We also aimed to find optimal sampling conditions for detecting aquatic parasites by testing different sample types, filter pore sizes and water volumes.

Methods: For that, we analysed parasite diversity focusing on helminths as our target parasite group and European eels as our host model from both experimental and field samples, collected monthly from riverine and lake ecosystems.

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Results: Here, we will present the first results showcasing the feasibility and performance of multiplexed eDNA metabarcoding for the simultaneous detection of multiple parasite groups using eDNA.

Conclusions: Our results showcase a novel eDNA-based approach for studying parasites, offering an efficient pathway for integrating parasite diversity assessments into existing monitoring programs.

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BABESIA CANIS AND BABESIA VOGELI AS CAUSATIVE AGENTS OF CLINICAL BABESIOSIS IN DOGS, IN SOUTH-EASTERN ROMANIA: A MOLECULAR APPROACH

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Background and Aims: Canine babesiosis (CB) is a tick-borne disease with a significant impact on the health of dogs worldwide. Currently, CB is increasingly reported in many European countries, including as an emerging and rapidly expanding disease. Therefore, its continuous monitoring in both endemic and non-endemic areas, including by molecular tools, are of high relevance and research interest. The aim of this study was to investigate the genetic diversity of *Babesia* spp. as causative agents of clinical babesiosis in a tick-endemic area (Dobrogea) in South-eastern Romania.

Methods: Twenty-three naturally infected dogs diagnosed with various clinical forms of babesiosis were investigated by means of clinical history, physical examination and hematological tests. Dog blood samples were further subjected to molecular investigation using PCR, sequencing analyses, and genetic characterization.

Results: Subsequently, *Babesia canis* (95.7%) and *Babesia vogeli* (4.3%) were molecularly confirmed as causative agents of babesiosis in the investigated dogs. Among the *B. canis* isolates, two genotypes were distinguished based on two nucleotide substitutions (GA→AG) observed in 18S rRNA gene sequences, with the AG genotype predominating (54.5% of samples), while the GA variant was identified in 9.1% of samples. In the remaining isolates (36.4%) both variants were identified. Additionally, mixed infection with *B. vogeli* and *Ehrlichia canis* resulted in severe disease.

Conclusions: This is the first report for the presence of genetically heterogenic *B. canis* strains in dogs with clinical babesiosis in Romania. However, further studies are needed to clarify the potential relationship between the genetic structure of the causative agents and the clinical course of disease

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REVEALING THE SILENT INVADER: RAPID ANTIBODY RECOGNITION TOOL OF A STAGE-SPECIFIC S. MANSONI BIOMARKER

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Background and Aims: Schistosomiasis is a major neglected tropical disease caused by *Schistosoma* blood flukes, with *Schistosoma mansoni* widely prevalent in sub-Saharan Africa, the Middle East, and South America. In line with the WHO 2030 roadmap, early and accurate diagnosis before mass drug administration is a key priority. However, existing diagnostic tools remain limited in sensitivity and field applicability. This study aims to develop a portable diagnostic based on antibody recognition of the life-cycle–dependent expression of *S. mansoni* Venom Allergen-Like (SmVAL) proteins, enabling early detection of *S. mansoni* infection.

Methods: Two SmVAL isoforms were heterologously expressed in *E. coli* and structurally characterized using NMR, SAXS, and circular dichroism. Polyclonal antibodies were generated, affinity-matured, and characterized by surface plasmon resonance, SAXS, and fluorescence spectroscopy. To improve robustness, reproducibility and field suitability, camelid-derived nanobodies and molecularly imprinted polymers were also developed against the same SmVAL targets.

Results: Both SmVAL isoforms were identified as stable proteins with well-defined structural features, suitable for diagnostic targeting and structural characterization. Antibodies showed high specificity and strong binding affinities, while nanobodies and molecularly imprinted polymers provided enhanced stability without compromising sensitivity. Integration of these recognition elements into a portable diagnostic prototype enabled sensitive detection of circulating SmVALs at sub-picogram per milliliter concentrations, prior to egg patency.

Conclusions: The resulting early diagnostic tool could offer enhanced reproducibility, sensitivity, and stability through SmVAL recognition, while improving cost-effectiveness compared to current serological or molecular methods. This diagnostic device may contribute to transmission interruption in endemic settings and pave the way to more precise drug administration.

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EVALUATION OF A COMMERCIAL ELISA TEST FOR THE DIAGNOSIS OF TRICHINELLOSIS

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Background and Aims: A quick and reliable diagnosis of trichinellosis is the basis for timely therapy administration and prevention of severe form of the disease. We analyse the success of a commercial enzyme-linked immunosorbent assay (ELISA) for serological screening of trichinellosis.

Methods: A retrospective study included 121 patients with clinical suspicion of trichinellosis diagnosed at the Parasitological Laboratory, the University Clinical Center of Serbia (UCCS) in Belgrade from September 2022 to May 2023. Patients' sera were initially and secondary tested with a qualitative ELISA test for the detection of specific anti-Trichinella IgG antibodies (Bioactiva Diagnostica GmbH, Bad Homburg, Germany). A confirmatory Western Blot (WB) (LDBIO Diagnostics, Lyon, France) was conducted in 33 patients. The ELISA test and WB was performed and interpreted according to the manufacturer's instructions.

Results: The study group included 88 patients from UCCS and 33 from other medical institutions. Fifty-six men and 65 women, were tested. Cross-reactivity was analyzed in 62 patients. The control group included 11 healthy patients, 2 with trichinellosis, and one each with strongyloidiasis and echinococcosis. Trichinellosis was diagnosed in 21 patients. The patients with a negative ELISA, tested before the third week following exposure. After repeated testing, conducted on five patients, seroconversion was recorded in 2 patients. WB was positive in 12 patients. In the control group, two patients had trichinellosis.

Conclusions: Despite the lack of sensitivity in the early stages of infection, there is a significant absence of cross-reactivity with Toxocara. Further evaluation of this test is required.

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DETECTING RESIDUAL TRANSMISSION USING MALARIA-LAMP: HIDDEN LOW-DENSITY PARASITE CARRIERS AND RISK FACTORS IN PALAWAN, THE PHILIPPINES

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Background and Aims: Despite the intensive malaria control supported by the Global Fund, malaria incidence in Palawan, the Philippines, has plateaued, suggesting the persistence of residual malaria foci sustained by asymptomatic and low-density parasite carriers undetectable by conventional diagnostics. This study aimed to identify hidden parasite reservoirs using highly sensitive nucleic-acid amplification methods and to characterize associated epidemiological and behavioral risk factors to inform targeted elimination strategies.

Methods: Reactive case detection was conducted in three municipalities in southern Palawan. Index cases were identified from the surveillance database, and household members and neighboring residents within a 1-km radius were screened by rapid diagnostic test (RDT). Dried blood samples were analyzed by loop-mediated isothermal amplification (LAMP) at the University of the Philippines Manila and by PCR at JIHS. A structured questionnaire assessed demographic, behavioral, and household risk factors associated with malaria exposure.

Results: Among 1,428 participants, RDT detected asymptomatic low-density infections in 2.9%, whereas LAMP/PCR identified malaria parasite DNA in 12.2%, revealing a substantial hidden reservoir of infection. *Plasmodium falciparum* and *P. vivax* predominated, with occasional mixed and minor species infections. Questionnaire analysis indicated significant associations between infection risk and inconsistent mosquito-net use, agricultural or forest-related occupations, and nighttime outdoor activities, suggesting ongoing exposure to vector habitats.

Conclusions: A considerable proportion of malaria infections in Palawan remain undetected by routine surveillance. Integration of high-sensitivity diagnostics such as LAMP into reactive surveillance, combined with targeted interventions addressing behavioral risk factors, is essential for interrupting residual transmission and achieving malaria elimination in the Philippines.

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IMPLEMENTATION OF A THREE-TEST ALGORITHM FOR CLINICAL SEROLOGICAL TESTING FOR CHAGAS DISEASE

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Background and Aims: Chagas Disease (CD), caused by *Trypanosoma cruzi*, affects millions worldwide but remains significantly under-diagnosed. While early diagnosis is vital for treatment efficacy, the low sensitivity of direct detection methods and variable serological test performance present challenges. Public health guidelines recommend performing two independent serological tests in parallel, with a third to resolve discrepancies. However, most clinical laboratories offer only single-assay screening or serial testing. We aimed to validate and implement a combination of FDA-cleared and laboratory-developed tests (LDTs) to provide the recommended diagnostic pathway.

Methods: We validated three serological assays in our CLIA laboratory: an FDA-cleared ELISA, an LDT multiplex immunoassay, and an LDT TESA immunoblot. To our knowledge, this represents the first commercial parallel-testing-plus-tiebreaker protocol for CD. We implemented an algorithm utilizing the ELISA and TESA immunoblot in parallel, with reflex to the multiplex immunoassay as a tiebreaker for discordant results.

Results: Validation of the ELISA and multiplex immunoassay demonstrated 100% positive percent agreement (PPA) and 100% negative percent agreement (NPA). The TESA immunoblot was validated with a different specimen set and showed 100% PPA and 96.7% NPA. To date, 22.4% of specimens tested positive, with approximately 10% of all specimens requiring tiebreaker resolution due to discordant results.

Conclusions: Prospective clinical studies with adequate statistical power are needed to identify the optimal assays for United States populations at risk for CD, but the availability of the recommended 3-test algorithm within a single commercial clinical laboratory significantly advances the accessibility and accuracy of CD serological testing in the U.S.

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NEAR-INFRARED SPECTROSCOPY DETECTS DENGUE VIRUS IN AEDES AEGYPTI BUT DOES NOT DIFFERENTIATE BETWEEN SEROTYPES 1 AND 4

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Background and Aims: Dengue remains one of the most important arboviral diseases worldwide. Near infrared spectroscopy (NIRS) has emerged as a rapid, non destructive, and high throughput alternative to detect arboviral infections in *Aedes aegypti* mosquitoes, with >90% accuracy for detecting dengue, Zika and chikungunya. This study evaluated whether NIRS can detect DENV infection in *Aedes aegypti* and differentiate between serotypes 1 and 4.

Methods: Laboratory reared female *Aedes aegypti* were orally infected with DENV-1 or DENV-4 through artificial blood meals, while control groups received non infected blood. Mosquitoes were analyzed at 10 and 14-days post infection. NIR spectra were collected from head/thorax and mosquito abdomen regions. Infection status was confirmed by RT qPCR, and only PCR-positive samples were included in model development. Artificial neural network models were trained, validated, and tested to classify infection status and serotype based on spectral data.

Results: NIRS reliably distinguished DENV infected from uninfected mosquitoes, achieving approximately 88% accuracy in test datasets. Spectra from the abdomen yielded the highest predictive performance, with accuracy exceeding 98% in some analyses. Model was consistent across both post infection timepoints. However, the performance in independent test sets declined markedly for serotype differentiation. Sensitivity for detecting DENV 4 remained high, whereas discrimination of DENV 1 was limited, and direct differentiation between the two serotypes was not reliable.

Conclusions: NIRS represents a promising rapid screening tool for detecting DENV-infection in *Aedes aegypti* mosquitoes. While it cannot currently discriminate DENV serotypes, its speed, scalability, and non destructive nature support its integration into entomovirological surveillance programs as a complementary early warning approach.

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ESTIMATION OF THE VALIDITY OF NEUROIMAGING IN THE DIAGNOSIS OF NEUROCYSTICERCOSIS: A SYSTEMATIC REVIEW AND BAYESIAN LATENT CLASS META-ANALYSIS (PRELIMINARY RESULTS)

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Background and Aims: Neurocysticercosis is caused by the larvae of the neglected zoonotic parasite *Taenia solium* infecting the brain and remains a major cause of neurological morbidity in endemic regions. Neuroimaging (cranial CT or MRI) is the reference test for neurocysticercosis diagnosis but remains imperfect, as its accuracy depends on image quality and reader expertise. This review aims to summarize the evidence on the performance of neuroimaging as an imperfect diagnostic test for neurocysticercosis.

Methods: We searched MEDLINE, EMBASE, Web of Science, Global Health, Global Index Medicus, and SCIELO for observational studies evaluating human neurocysticercosis using at least two diagnostic tests, one of which was neuroimaging, and with sufficient data to reconstruct contingency tables. Each study was evaluated according to the eligibility criteria by two independent reviewers, with disagreements resolved by consensus. A Directed Acyclic Graph was developed to inform the analytical strategy. Planned analyses include meta-analysis using hierarchical Bayesian latent class models to estimate misclassification-adjusted sensitivity and specificity of neuroimaging.

Results: Fifty-three studies were eligible for inclusion. Data extraction and risk-of-bias assessment are ongoing. Included studies cover multiple endemic settings, imaging modalities (CT and MRI), and study designs.

Conclusions: These preliminary results highlight that substantial evidence exists to assess the diagnostic validity of neuroimaging for neurocysticercosis while accounting for test imperfection. Final analyses will provide misclassification-adjusted accuracy estimates, which could be used to adjust for misclassification error when estimating the prevalence of neurocysticercosis in various

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populations and the association between risk factors and neurocysticercosis. This data will also inform future diagnostic research.

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USING ENVIRONMENTAL DNA TO SURVEIL GASTROINTESTINAL PARASITES IN SHEEP FARMS IN NORTHERN IRELAND

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Background and Aims: Environmental DNA (eDNA) is a powerful biodiversity monitoring tool, offering a non-invasive and highly sensitive approach. In our previous study (McFarland et al. 2024), we reported for the first time the use of eDNA from herbage samples to identify trematode parasites and their intermediate hosts. Expanding on these techniques, we report now the detection of nematode parasite DNA from herbage samples.

Methods: A longitudinal study was conducted across four farms, with eight sampling timepoints, between 4/2023 and 9/2024. At each visit, environmental and faecal samples were collected. eDNA was extracted from samples and then analysed using droplet-digital PCR (ddPCR) with primer-probes assays. Results were correlated with faecal egg counts (FECs).

Results: FECs were strongly and positively correlated with ddPCR droplet counts for *Teladorsagia circumcincta* detected in herbage samples (Pearson's r=0.876, p=0.006). In contrast, *Calicophoron daubneyi* showed a weak negative correlation (r=-0.33, p=0.126). Environmental conditions influenced eDNA recovery, with rainfall exerting the greatest effect across all four farms. Among strongyle species, *T. circumcincta* was the most abundant, as determined by ddPCR and Illumina next-generation sequencing.

Conclusions: Our findings represent an alternative strategy for supporting helminth infection monitoring. Grazed pasture grass shows strong potential as a practical eDNA sampling matrix source for detecting gastrointestinal parasite populations. Integrating this novel technique with weather data could support farmers with targeted treatment decisions. Future applications may aid in the monitoring of potential anthelmintic resistance, further improving parasite management strategies.
MCFARLAND, C., et. al., 2024. Environmental DNA, 6, e70027.

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SEQUENCING CLINICAL SPECIMENS IDENTIFIES CRYPTOSPORIDIUM FROM UNEXPECTED SOURCES

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Background and Aims: Cryptosporidiosis became a nationally notifiable disease in the United States in 1994. It is predominantly considered a waterborne illness that occurs after becoming infected with *Cryptosporidium parvum* or *Cryptosporidium hominis*, the two species that typically infect humans. However, identifying other *Cryptosporidium* species that infect humans could suggest contact with the environment, domestic animals, livestock or wild animals are also contributing factors to cryptosporidiosis infections. Our aims were to identify species, other than *C. parvum* and *C. hominis,* that are causing infections and determine their distribution within New York State (NYS).

Methods: Real-time PCR, Sanger sequencing, and targeted next generation sequencing (tNGS) were employed to identify *Cryptosporidium* species in stool specimens.

Results: From 2022 through 2025, over 200 clinical human specimens were identified as containing a *Cryptosporidium* species other than *C. parvum* or *C. hominis*. Frequently observed species include *C. mortiferum*, *C. felis*, and *C. ubiquitum*. *C. mortiferum* was found ubiquitously throughout NYS primarily between June and October during the surveillance period with *C. felis* identified most often in New York City. Additional species were also observed, although at lower frequencies.

Conclusions: This study highlights three important findings, 1) the public health importance of *Cryptosporidium* species other than *C. parvum* and *C. hominis* 2) once identified, prevention measures regarding specific species can be relayed to the public to aid in decreasing infections and 3) molecular epidemiology is an effective tool for assisting with public health safety by identifying potential transmission routes for cryptosporidiosis.

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THE PROFICIENCY TESTING SCHEMES IN PARASITOLOGY – AN IMPORTANT TOOL TO INCREASE THE ACCURACY OF THE PARASITOLOGICAL LABORATORY DIAGNOSIS

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Background and Aims: Medical laboratories from Romania participate in external quality control programs (EQC) offered by the CALILAB Association Bucharest, Romania-based .The coproparasitological exam is included in a specific scheme for laboratory specialties. In our country, medical laboratories must participate in EQC programs as part of the accreditation process according to EN ISO 15189.

Methods: Between 2020-2025, besides the CALILAB team, we examined the results reported by participants in EQC schemes. During this period, 37 rounds of EQC were organized, with a total of 15701 results reported, ranging from 156 to 690 participants per round/participation. In each round, participants in EQC schemes received digital images, representing the object of the proficiency test (PT) adminitered by the CALILAB Association to the medical laboratories in Romania. The purpose of these images was to either identify parasites in PT or to confirm absence of parasitic elements.

Results: During the analyzed period, participants reported 15701 results to the EQC scheme "parasitological examination"; the percentage of "acceptable" results obtained by the participants in the EQC scheme varied between 82.99% and 100% over the 37 rounds of 2020–2025.

Conclusions: After a proper analyse of the results, we noticed as follow: the samples without parasitic elements, are always a challenge due to the presence of a multitude of non-specific forms that can generate a false positive/negative responses; a good performance of the medical laboratories participants in the EQC scheme contributes to increasing the accuracy of laboratory parasitological diagnosis.

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PERFORMANCE OF A NOVEL MULTI-ANTIBODY SCHISTOSOMA HAEMATOBIUM SEROLOGY PANEL IN SIX COUNTRIES

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Background and Aims: Female genital schistosomiasis (FGS) is a chronic condition caused by infection with the S. haematobium parasite. The diagnostic landscape for schistosomiasis is limited, and the majority of techniques rely on the detection of eggs in urine, indicating an active infection rather than a history of infection. In this study, we incorporated both urine filtration microscopy in the field and a novel enzyme-linked immunoassay (ELISA) to detect eggs and IgG4/IgG1 antibodies in participants.

Methods: Dried blood spot samples and urine aliquots were collected from 9,540 participants (ages 15-60) in 2 archetypes each across 6 countries (13 total districts). The urine samples were filtered and assessed by trained microscopists directly in the field, while DBS were assessed in the laboratory on a panel of two ELISAs: Sh. SAP1 (IgG1) and Sh. Quad (IgG4).

Results: The prevalence of antibodies against Sh. Quad correlated most strongly with the presence of egg in urine, while antibodies against Sh. SAP1 were high in all regions, but highest in high-prevalence districts. Variation in SAP1 and Quad prevalences were observed between countries, likely caused by differences in schistosomiasis prevalence and MDA coverage in the region, while within countries, Sh. Quad showed more variation than SAP1.

Conclusions: The Sh. ELISAs have proven to be a valuable tool in determining a history of infection for S. haematobium. For some women with confirmed FGS, the Sh. ELISAs proved to be a potentially critical screening tool when combined with a tailored symptom screen, particularly in areas where diagnosis via colposcopy is not possible.

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EVALUATION OF A NOVEL PINWORM COLLECTION DEVICE

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Background and Aims: Accurate laboratory detection of *Enterobius vermicularis* relies on effective perianal specimen collection, stability, and safe handling. The traditional gold-standard collection method uses adhesive tape, which can present handling challenges for patients, caregivers, and laboratory staff. This study evaluated the performance, specimen stability, and clinical comparability of a newly available ParaClick® Pinworm Paddle relative to established collection methods, with the goal of validating an easier-to-collect and safer alternative for routine use.

Methods: Comparative validation was performed using three perianal collection types: ParaClick® paddles, Swube™ paddles, and clear adhesive tape. Blinded negative samples and positive egg- and adult worm-mimic samples were evaluated. Overall agreement, precision, and accuracy were assessed through microscopic examination by multiple technologists. Specimen stability was evaluated at room temperature at intervals through 10 days post-collection. Stability, transport conditions, repeatability, reproducibility, and analytical performance characteristics were analyzed in accordance with laboratory validation standards.

Results: Across 450 blinded examinations, the ParaClick® device demonstrated 99.8% overall percent agreement, with 100% positive percent agreement and 99.3% negative percent agreement compared with gold-standard methods. Intra-assay and inter-assay precision were 100%. All three collection methods produced satisfactory results for 10 days post-collection when stored and transported at room temperature, establishing validated specimen stability times. The ParaClick® device enabled secure closure after collection and examination through a sealed viewing window, reducing handling risks.

Conclusions: The new ParaClick® Pinworm Paddle is diagnostically equivalent to the established gold-standard collection method and other commercially available pinworm collection devices while offering improved safety, ease of collection, and validated specimen stability for up to 10 days.

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COMPARISON OF THREE STOOL CONCENTRATION FILTER DEVICES TO SUPPORT DIGITAL OVA AND PARASITE EXAMINATION AND OPERATIONAL RESILIENCE IN THE CLINICAL PARASITOLOGY LAB

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Background and Aims: Effective stool concentration is essential to optimize organism recovery and improve diagnostic sensitivity for ova and parasite examination (O&P), particularly in digital and AI-assisted slide review where debris reduction and organism clarity can significantly impact detection. Increasing supply chain disruptions further necessitate validated, equivalent backup concentration devices. This study evaluated the ParaDevice (Reingenuity), ParaPak SpinCon (Meridian), and Mini Parasep SF (Apacor) stool concentration systems for diagnostic equivalence and workflow compatibility.

Methods: Forty preserved stool specimens (20 parasite-positive and 20 parasite-negative), including both 10% formalin/Zinc-PVA and single-vial alcohol-based preservatives, were processed using all three concentration filters. For each filter, specimens were examined by three methods: manual wet mount microscopy, manual microscopy of permanently stained slides, and AI-assisted review of digitally scanned permanent-stain slides using the Hamamatsu NanoZoomer360 and Techcyte software. Results were compared for organism detection and identification, with discrepant findings resolved through repeat review and reprocessing. Workflow efficiency and ease of handling were also evaluated.

Results: Across 120 total evaluations, initial concordance ranged from 88–98% among the three filters. After discrepant analysis, concordance reached 100% for manual microscopy evaluations and 98–100% for digital review. One slide (0.8%) demonstrated a persistent discrepancy, yielding 99% overall agreement.

Conclusions: All three stool concentration filters demonstrated acceptable sensitivity and specimen quality for manual and digital O&P examination. The ParaDevice and Mini Parasep SF devices supported efficient routine workflows, while the SpinCon filter's increased handling complexity and spill risk limit its practicality, supporting its role mainly as a backup option during supply chain disruptions

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CONSEQUENCES OF POLYPARASITISM ON ANAEMIA AMONG PRIMARY SCHOOL CHILDREN IN HARARE, ZIMBABWE

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Background and Aims: Anaemia is a big issue for school kids in Sub-Saharan Africa, often worsened by parasitic infections like schistosomes, malaria, and worms. Co-infections can tank haemoglobin levels and iron status. This study looked at how single and multiple parasitic infections impact anaemia among primary school kids in Zimbabwe,Harare.Aim was to investigate the impact of single versus parasitic infections on anaemia and iron deficiency anaemia in Zimbabwean school kids.

Methods: The study involved 609 primary school kids in Zimbabwe,Harare.They tested for P. falciparum (blood), S. haematobium (urine), S. mansoni, hookworm, Trichuris, Ascaris (stool). Checked haemoglobin and ferritin levels to assess anaemia and iron deficiency anaemia.

Results: Infections were common: S. haematobium (52.3%), P. falciparum (27.9%), S. mansoni (22.7%), hookworm (23.7%). Overall, 48.4% of kids had anaemia; 38.1% had iron deficiency anaemia. Kids with P. falciparum, S. haematobium, and hookworm had lower haemoglobin levels. Those with multiple infections had almost 2x more anaemia and iron deficiency anaemia vs kids with single infections. Co-infections with malaria + schistosomes + worms had the highest anaemia rates

Conclusions: Co-infections with malaria and worms significantly increase anaemia risk in school kids. Findings highlight the need for combined deworming and malaria control in schools to tackle anaemia in Zimbabwe's endemic areas. Integrated interventions can help reduce the burden.

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NON-THERMAL INACTIVATION OF ANISAKIS LARVAE IN RAW FISH USING PULSED POWER TECHNOLOGY

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Background and Aims: *Anisakis* larvae pose a significant food safety risk in raw seafood, and current countermeasures such as freezing and heating compromise product quality. This study aimed to evaluate pulsed power technology as a non-thermal method for inactivating *Anisakis* larvae in raw fish while maintaining sensory and physicochemical quality.

Methods: *Anisakis* larvae in fish muscle were treated using a pulsed power system with parallel-plate electrodes. Electrical parameters, including applied voltage, electrode gap, conductivity, and pulse energy density, were varied systematically. Larval inactivation was assessed by motility loss, and fish quality was evaluated by sensory and physicochemical analyses.

Results: Pulsed power treatment successfully inactivated *Anisakis* larvae under uniform electric field conditions. Inactivation depended on pulse-specific electrical parameters rather than total energy input alone. Quality evaluations showed minimal changes in sensory attributes and freshness indicators compared with untreated samples, whereas freezing caused noticeable quality deterioration.

Conclusions: Pulsed power technology enables effective non-thermal inactivation of *Anisakis* larvae while preserving raw fish quality. The results highlight the importance of pulse design under uniform electric fields and demonstrate the potential of pulsed power as a practical food safety intervention for raw seafood products.

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DEVELOPMENT AND ASSESSMENT OF AN OPTIMIZED MICROSCOPY PROTOCOL FOR THE DIAGNOSIS OF MALE GENITAL SCHISTOSOMIASIS BY DETECTION OF SCHISTOSOMA EGGS IN SEMEN

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Background and Aims: Male genital schistosomiasis (MGS) results when eggs deposited by the parasitic trematode *Schistosoma haematobium* become trapped within the male reproductive organs. Microscopy of semen samples for the detection of *Schistosoma* eggs is typically utilized for diagnosis, however, there is currently no standardized protocol. The aim of this study was to develop a standardized protocol for the detection of *Schistosoma* eggs in semen and compare the performance of filtration and centrifugation concentration techniques. Additionally, we aimed to optimize the protocol for usage in resource-constrained settings through the exploration of alternative, low-cost filters.

Methods: Aliquots of pooled human semen were spiked with known quantities of *Schistosoma haematobium* eggs. Samples were assigned to either centrifugation or filtration with five replicates per experimental condition. Slides were examined under a microscope and egg counts recorded. The limit of detection for each method was calculated using Probit analysis. Several commercially available coffee filters were investigated as potential alternatives to polycarbonate filters.

Results: The limit of detection for semen microscopy with filtration was 2 eggs, while the limit of detection for centrifugation was 5 eggs. Coffee filters were successfully used to trap and visualize *Schistosoma* eggs (Figure 1), with AeroPress filters demonstrating good visualization and

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sensitivity.

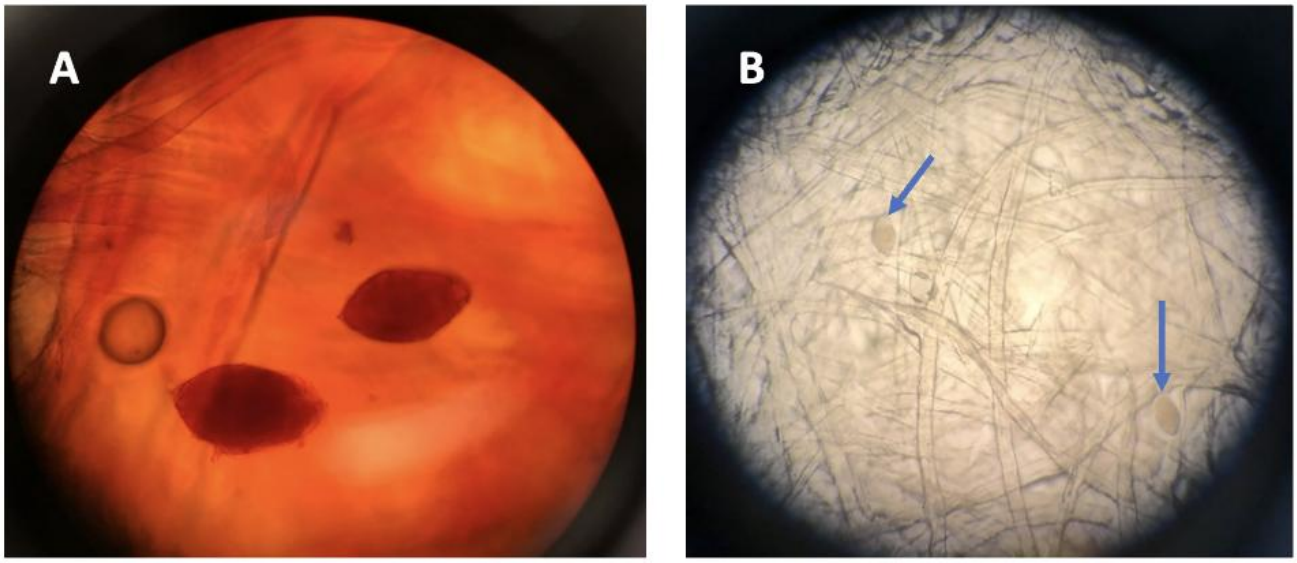


Figure 1. *Schistosoma haematobium* eggs trapped on a commercially available (AeroPress) coffee filter. **A.** Filter stained with iodine showing two *S. haematobium* eggs at 40X magnification. **B.** Unstained filter showing visualization of two *S. haematobium* eggs at 10X magnification.

Conclusions: This study presents the first evaluation of semen microscopy processing methods and identifies an optimized, standardized protocol for the detection of *Schistosoma* eggs from semen. We show in a proof-of-concept that easy to use coffee filters can be used in place of standard filters without substantial reduction in ease of visualization or sensitivity.

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DIAGNOSTICS, HOST-PARASITE INTERACTION AND EMERGING TECHNOLOGIES / NEW DEVELOPMENT IN DIAGNOSTICS OF PARASITIC INFECTION

A NOVEL PORTABLE SEQUENCING PLATFORM RESOLVES THE GLOBAL DIVERSITY OF LEISHMANIA SPECIES

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Background and Aims: Leishmaniases are targeted for elimination as a public health problem by 2030 under the WHO Neglected Tropical Diseases (NTDs) road map 2021-2030. Achieving these goals requires innovative diagnostic tools to strengthen global surveillance, particularly under rapidly shifting epidemiological conditions. We developed and validated a novel long-read metabarcoding sequencing assay to accurately capture *Leishmania* species diversity.

Methods: A metabarcoding assay targeting HSP70 gene was established using Oxford Nanopore Technologies' portable MinION™ platform integrated with a curated bioinformatic pipeline and reference database for species-level identification. Optimisation used 14 cultured *Leishmania* species and 26 contrived samples simulating sympatric and co-infection scenarios. Diagnostic performance was benchmarked against a qPCR using sandflies, human skin biopsies, saliva, and lesion scarification slides and dog blood, from both the Old and New Worlds. Additionally, HSP70 metabarcoding was applied to assess the diagnostic specificity of a widely employed kDNA-targeting qPCR assay in *Leishmania infantum* diagnostics.

Results: The assay accurately identified all 14 species in cultured and contrived samples with 99.65–100% identity against reference database. Field validation showed weak to perfect agreement with HSP70-qPCR (Cohen's Kappa) and detected more positives in most clinical samples. Importantly, previously unreported *Leishmania* species were identified in dogs and sandflies from Europe, and

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multi-species co-infections were detected in humans and dogs from Brazil. kDNA qPCR testing revealed amplification of seven non-*L. infantum* species, highlighting potential misdiagnosis without confirmatory typing.

Conclusions: This metabarcoding assay enables robust species-level identification and co-infection detection across diverse sample types, outperforming conventional assays and supporting global surveillance and WHO NTD control targets.

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COMPARING QUANTITATIVE AND DIGITAL PCR DETECTION OF CRYPTOSPORIDIUM PARVUM IN LEAFY GREENS USING GBLOCK AS A COPY-NUMBER BENCHMARK

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Background and Aims: *Cryptosporidium* is an important protozoan parasite that can be transmitted through food and water. Detection of *Cryptosporidium* relies primarily on immunomagnetic separation (IMS) and immunofluorescence microscopy (IFM), which is sensitive but costly and time-consuming. Molecular methods may be more easily implemented in routine monitoring, yet limitations remain in quantification and sensitivity at low oocyst occurrence. Digital PCR (dPCR) is an advanced version of quantitative PCR (qPCR), which provides absolute endpoint quantification and theoretically higher precision than qPCR. This study aims to compare the performance of dPCR and qPCR using synthetically constructed DNA sequences (Gblock), followed by artificial contamination of leafy greens.

Methods: This study consists of 3 phases. Phase 1 involves optimization of the dPCR and qPCR assays using Gblock as copy-number standard. In phase 2, performance of dPCR and qPCR are determined using purified oocysts, with its DNA extracted by DNeasy Power Soil kit. Finally, leafy greens are artificially contaminated and efficacy and sensitivity of dPCR and qPCR are compared. IMS-IFM is also employed to compare with molecular quantification for phase 2 and 3.

Results: The study is ongoing and the results include performance data on dPCR and qPCR assays for both quantitative and qualitative methods, including but not limited to Limit of Detection (LOD), sensitivity, Limit of Quantification (LOQ) and percentage oocyst recovery.

Conclusions: The study will generate essential data on the efficiency and performance of dPCR and qPCR methods, which help enhance the design and interpretation of both epidemiological investigations and experimental research of protozoa in food matrices.

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COMPARATIVE EVALUATION OF CONVENTIONAL PCR ASSAYS TARGETING EMA GENES FOR DETECTION OF THEILERIA HANEYI

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Background and Aims: *Theileria haneyi* is an emerging equine piroplasm that escapes detection by several assays designed for *Theileria equi*. Although a USDA-developed nested PCR is widely used, it has practical limitations, and the analytical sensitivity of previously reported conventional PCR assays remains poorly defined. This study evaluated the analytical sensitivity of newly designed *T. haneyi*–specific conventional PCR assays and compared them with established methods.

Methods: Six primer sets targeting equi merozoite antigen genes (*EMA10*, *EMA11*, and *EMA12*), which are present in *T. haneyi* but absent in *T. equi*, were developed. Two-fold serial dilutions of parasite-positive blood samples were tested, with parasite concentrations expressed as infected red blood cells per microliter (iRBC/μL). Five dilution levels were analyzed with ten replicates each, and Probit regression was used to estimate the 95% limit of detection (LoD95).

Results: The USDA nested PCR assay showed an LoD95 of 1.84 iRBC/μL, whereas the assay reported by Bhoora *et al*. yielded an LoD95 of 5.41 iRBC/μL. Among the newly designed assays, Set A (*EMA10*) and Set D (*EMA12*) demonstrated the highest sensitivity, with LoD95 values of 1.29 and 1.02 iRBC/μL, respectively. In experimentally infected horses, the USDA nested PCR assay and Set D became positive at 21 dpi, whereas Set A yielded positive results at 20 dpi.

Conclusions: These results demonstrate that conventional PCR assays targeting *T. haneyi*–specific EMA genes can achieve analytical sensitivity comparable to nested PCR while offering practical advantages for routine diagnostic and research applications.

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EFFECT OF ARTEMISININ COMBINATION THERAPY AND SERUM PROTEIN PROFILE ON PLASMODIUM FALCIPARUM INFECTION AMONG IN-PATIENTS IN KADUNA STATE, NIGERIA.

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Background and Aims: Background and Aims Malaria remains a major tropical disease caused by Plasmodium parasites and transmitted by infected female Anopheles mosquitoes (Zareen *et al.*, 2016). The principal species responsible for severe morbidity and mortality are Plasmodium falciparum and Plasmodium vivax (WHO, 2022). The World Health Organization recommends Artemisinin-based Combination Therapies (ACTs) as first-line treatment for uncomplicated P. falciparum malaria due to their high efficacy and reduced risk of resistance (Smithus *et al.*, 2010; Zou *et al.*, 2021). This study evaluated the therapeutic response to ACTs and their impact on serum protein profiles among malaria-infected patients in selected hospitals in Kaduna State, Nigeria.

Methods: Methods Thirty-five confirmed malaria patients were recruited from hospitals across three senatorial zones of Kaduna State, Nigeria. Diagnosis was established by microscopy. Blood samples were collected before and after treatment, and serum protein levels were determined using the Biuret method. Data were analyzed using descriptive statistics and Student's t-test at $p \leq 0.05$.

Results: Results Most patients (88.6%) received artemether–lumefantrine-based ACTs, while 11.4% received other combinations. In one hospital, 8 of 9 patients treated with a specific ACT brand experienced relapse, whereas no relapses were recorded with other brands. Mean serum protein values remained within normal reference ranges before and after treatment, with no statistically significant difference ($p >$
0.05).

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Table 1. The therapeutic results of some commonly used ACT malaria drugs from four selected LGA's Hospitals in Kaduna State.

Nos.	LGA Hospitals	LGA	No. of ACTs used	Type of ACTs (Brand Names)	ACT Active ingredients	No.pre-treatment malaria patients	No of relapsed post malaria treated patients
1.	Jowako specialist Hospital.	K/N	09	Lonart-Ds	A/L	09	08
			01	P-Alaxin	D/P	01	Nil
2.	Gwanna Awan general Hospital.	K/S	05	Artemef	A/L	05	Nil
			03	Lumartem	A/L	03	Nil
3.	Kagarko General Hospital.	Kagc	06	Artemether &	A/L	06	Nil
			05	Lumefantrine Lonart-Ds	A/L	05	Nil
4.	Hajia Sawaba general Hospital.	Zaria	03	Co-artem	A/L	03	Nil
			01	Duo-Cotecxin	A/P	01	Nil
			01	Artemether & fansider	A/SP	01	Nil
			01	Artesunate & Fansider	AT/SP	01	Nil
Total	**4 major Hospitals used**	**4 LGAs**		**9 brand types of ACTs used**	**31AL,1DP, 1AP,1ASP, 1ATSP**	**35**	**08**

A/L= Artemether&Lumefantrine, D/P= Dihydroartemisinin &Piperaquine, A/P= Artemether & Piperaquine, A/SP=Artemether&Sulfadoxine-pyrimethamine, AT/SP= Artesunate&Sulfadoxine-pyrimethamine, ACT= Artemisinin Combination Therapy, LGAs= Local Government Areas, K/S=Kaduna-south, K/N=Kaduna-north, Kagc=Kagarko

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Table 2. The values of the different protein serum profile of malaria patients in Kaduna state

Serum Protein Profile	Normal /Standard Range (g/L) n=35	Mean values of patients ±SD (g/L)		
		Pre-treatment	Post-treatment	P-values
Total protein	60-82	73.5 ± 4	73.0 ± 3	0.46
Albumin	30-52	41.5 ± 3	40.8 ± 2	0.63
Globulin	23-35	32.1 ± 5	31.8 ± 4	0.25

Conclusions: Conclusions The findings indicate adherence to national ACT policy and suggest that ACT treatment did not significantly alter serum protein profiles in the study population. Also, all the serum protein profiles among the patients investigated were found within the standard reference ranges.

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RECONSTRUCTING MEDIEVAL PARASITIC INFECTIONS USING TARGETED ANCIENT DNA FROM ARCHAEOLOGICAL SEDIMENTS

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Background and Aims: Ancient DNA (aDNA) approaches provide valuable insights into the history of human health and disease. Recent methodological advances enable recovery of aDNA from environmental samples such as latrines, archaeological sediments, and other anthropogenic deposits, opening new possibilities for reconstructing past parasite diversity. The aim of this study was to develop and evaluate advanced ancient metagenomic tools for detecting parasitic helminths from environmental samples associated with past human activities.

Methods: We conducted a pilot study using environmental samples from the Czech Republic archaeologically dated to the 11th–13th centuries. DNA was extracted from sediments and analysed using a targeted capture probe panel designed to enrich complete mitochondrial genomes of parasitic helminths. Results were compared with data obtained from classical microscopic examination and basic molecular diagnostic approaches previously applied to the same samples.

Results: Microscopic and basic molecular analyses provided a baseline for evaluating the targeted capture approach. Targeted enrichment enabled species-level identification of helminths and detection of parasite DNA in samples where specific parasite eggs had not been detected, although *Trichuris* and *Ascaris* remained the most prevalent parasites. Comparisons of pre- and post-capture datasets demonstrated efficient enrichment, increased detection of parasite diversity, and the potential for partial reconstruction of ancient helminth mitochondrial genomes.

Conclusions: Targeted capture of parasite aDNA represents a robust and sensitive approach for detecting ancient parasites from environmental samples. Integrating metagenomic parasite screening into archaeological research enhances reconstructions of past parasitic infections, health, evolutionary processes, and human–animal interactions.

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HEAT-TRANSFER-BASED BIOSENSORS AS A NOVEL LABEL-FREE APPROACH FOR THE DETECTION OF NEGLECTED DISEASES

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Background and Aims: Neglected tropical diseases such as malaria and leprosy remain major public health challenges due to the lack of field-deployable diagnostic tools. To address this gap, we worked on a label-free biosensing platform based on the Heat Transfer Method (HTM), which detects variations in interfacial thermal resistance resulting from specific biomolecular interactions at functionalized metal surfaces.

Methods: For malaria, *Plasmodium falciparum* schizont-stage parasites were cultured in human erythrocytes, and used to generate surface-imprinted PDMS–graphene oxide receptor layers on aluminum substrates. These layers enabled label-free detection by monitoring changes in thermal resistance upon binding of infected red blood cells at the sensor interface. For leprosy, aluminum chips were sequentially hydroxylated, silanized with APTES, activated with glutaraldehyde, and functionalized with a recombinant multiepitope protein specific to *Mycobacterium leprae*. The biofunctionalized chips were integrated into the HTM setup, where diluted serum samples from patients, healthy controls, tuberculosis cases, and contacts flowed over the sensor surface at 37°C, allowing real-time monitoring of binding-induced variations in interfacial thermal resistance and label-free detection of anti-*M. leprae* antibodies.

Results: Our studies demonstrate that HTM enables sensitive detection of *P. falciparum*-infected erythrocytes using MIP receptor layers, with detection limits below 0.5% parasitemia. In addition, the biofunctionalized HTM sensors successfully identified anti-*M. leprae* antibodies in diluted serum (1:200) from individuals exposed to *M. leprae*.

Conclusions: This physical transduction principle eliminates the need for optical or enzymatic labeling, offering a robust and cost-effective diagnostic strategy. Overall, the HTM approach provides a portable, easy-to-fabricate, and low-reagent-consumption platform aligned with the development of sustainable point-of-care diagnostics for resource-limited settings.

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STANDARDIZATION, OPTIMIZATION AND VALIDATION OF A QPCR ASSAY FOR THE DIAGNOSIS OF BOVINE CYSTICERCOSIS

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Background and Aims: Bovine cysticercosis is a zoonosis that causes significant economic losses in the beef industry. Samples collected during *post-mortem* inspection at slaughterhouses are sent to the Canadian Food Inspection Agency for confirmation by microscopy and, if necessary, histopathology. However, infected carcasses may contain degenerated or calcified cysticerci, making confirmation challenging. In addition to low sensitivity, the current diagnostic method is time-consuming and require specialized expertise. This study aimed to further evaluate and validate a real-time PCR with melting curve analysis assay for routine diagnosis of bovine cysticercosis.

Methods: A previously developed qPCR-MCA assay targeting *Taenia saginata* was evaluated using 162 experimentally generated cysticerci archived for over 10 years. Results were compared with previous histopathology and immunohistochemistry findings. Assay specificity was assessed using field samples with lesions of alternative etiologies. Additionally, 50 freshly collected field samples imported from Brazil were tested, including viable and calcified cysts collected from carcasses condemned for cysticercosis.

Results: The qPCR-MCA assay correctly identified 153/162 (94%) degenerated cysticerci as *T. saginata*, compared with 149/162 (92%) by IHC and 61/162 (38%) by histopathology. Among the 50 Brazilian field samples, 43/50 tested positive, including all 20/20 viable cysts and 23/30 calcified lesions.

Conclusions: The qPCR-MCA assay demonstrated high sensitivity and superior performance compared with histopathology for detecting degenerated bovine cysticerci. The assay also showed promising performance for the detection of calcified field lesions, highlighting its potential as a rapid and reliable confirmatory diagnostic tool for routine use at CFIA laboratories to support food safety and trade.

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DETECTION OF SPARGANUM PROLIFERUM IN FECES FROM A HEALTHY CHEETAH FROM A ZOO IN QUEBEC: EXAMPLE OF THE USE OF PRECISION MOLECULAR DIAGNOSTIC TOOL

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Background and Aims: *Sparganum proliferum* is a parasitic cestode belonging to the genus *Spirometra* in the Diphyllobothriiidae family. It is notable for its unusual and aggressive proliferative behavior during its larval stage in human and animal hosts, which distinguishes it from other members of the *Spirometra* genus. The parasite typically involves multiple hosts in its life cycle: Copepods act as the first intermediate host, followed by amphibians, reptiles, or small mammals as second intermediate hosts. The definitive host are carnivorous mammals. Unlike typical *Spirometra*, *Sparganum proliferum* exhibits extensive asexual proliferation within the host tissues leading to widespread dissemination and formation of multiple larval masses that can cause severe tissue damage, chronic inflammation, and fatal if left untreated.

Methods: Feces from two healthy cheetahs born in a Quebec Zoo were submitted for routine testing. After zinc sulfate double centrifugation, eggs with morphology of *Spirometra* were observed under the microscope. In order to confirm the species, nucleic acid was extracted from both fecal samples and amplification with pan-cestode 12s rRNA genes primers were attempted. Amplicons were submitted for Sanger sequencing.

Results: After blast analysis, results from both animals showed high 97% identity with a *Sparganum proliferum* isolates.

Conclusions: Cases of proliferative sparganosis in wild animals are extremely rare. To our knowledge this is the first detection of the *Sparganum proliferum* parasite in North America. This finding underscores the importance of molecular tools for the accurate diagnosis of parasites, in order to improve surveillance of emerging parasitic threats in wildlife.

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DETECTION OF PARANOPLOCEPHALA SPP. CESTODES IN FECES OF A DOMESTIC RABBIT FROM QUEBEC: UTILITY OF THE PRECISION OF MOLECULAR DIAGNOSTIC TOOLS

Christian Savard[1], Jeffrey Tereski[2], Valerie Proulx[1], Joan Pena[1], Martine Germain[3], Pablo Jimenez Castro[2], Christian M. Leutenegger[2]
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Background and Aims: *Paranoplocephala* spp. is a genus of cestodes belonging to the Anoplocephalidae family. These parasites are primarily known to infect rodents, particularly voles and mice. In most cases, there are no clinical signs, but a high parasite burden can lead to digestive disorders such as episodes of diarrhea and chronic or intermittent abdominal pain. As for rabbits, there is no solid scientific evidence indicating that *Paranoplocephala* can directly infect them.

Methods: Cestodes were observed on feces from a domestic rabbit housed in the province of Quebec, Canada. In order to confirm the species of the observed worms, nucleic acid was extracted and amplification with pan-cestodes 12s rRNA genes primers was attempted. Amplicons were submitted for Sanger sequencing.

Results: After blast analysis of the obtained sequence, results showed the highest identity (82%) with an isolate of *Paranoplocephala* spp. found in *Neodon irene*, commonly known as Irene's vole or Chinese bush vole. The moderate identity with this isolate could indicate a different species from the same genus. However, more sequencing results will be needed.

Conclusions: The *Paranoplocephala* genus parasites primarily infect wild rodents or other hosts such as equids. To date there is no clear evidence that it infects domestic or wild rabbits. To our knowledge this is the first evidence of *Paranoplocephala* spp. cestodes developing to the adult stage in a domestic rabbit from North America. This shows the importance of molecular tools for the accurate identification of cestode infections and for detecting unexpected host-parasite associations.

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SCHOOL-LEVEL SCHISTOSOMIASIS SURVEILLANCE WITH SMARTPHONE-BASED POC-CCA INTERPRETATION: FIELD PERFORMANCE AND QUANTITATIVE SIGNAL METRICS IN WESTERN KENYA

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Background and Aims: Schistosomiasis remains endemic across Western Kenya, where school-aged children carry the highest infection burden. MEDSCAN, a smartphone-based POC-CCA interpretation platform, was deployed for at-scale field surveillance. We characterized its diagnostic performance and quantitative signal output across a four-county study.

Methods: Between 2025 and 2026, 13,165 POC-CCA tests were performed at 148 schools across four Western Kenyan counties (Migori, Homa Bay, Kisumu, Siaya). Each analyzed test recorded a visual read, an algorithm-derived result, and quantitative line and background signal metrics. We assessed algorithm–visual agreement and benchmarked four signal-based positivity metrics against the visual reference.

Results: MEDSCAN delivered sub-school-level prevalence estimates across all four counties—finer-grained resolution than typical district-aggregated surveillance—with cross-sectional positivity of 48.7% (5,516/11,326) by visual read. The algorithm agreed with visual reads in 73% of tests; 32% of algorithm-positive reads were visually negative, possibly indicating algorithmic detection of antigen below the human band-detection threshold and identifying a population for future validation. Among signal-based positivity metrics, background-corrected ratios performed strongest in the field: the test-to-interpeak (T:I) ratio achieved κ=0.51 (visual-inclusive) and κ=0.72 (visual-strict), exceeding control-normalized alternatives.

Conclusions: MEDSCAN supports population-scale schistosomiasis surveillance with sub-school-level resolution. Its quantitative signal output extends detection beyond visual band reading and enables field-calibrated positivity metrics, even allowing simple determination of intensity of infection. Together these features position smartphone POC-CCA interpretation as a scalable and cost-efficient option for disease mapping.

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ADDED VALUE OF MULTIPLEX PCR IN THE DIAGNOSIS OF INTESTINAL HELMINTH INFECTIONS OF LOW-ENDEMICITY AREA IN SENEGAL

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Background and Aims: Intestinal helminthiases remain a major public health concern, particularly in low-resource countries. Their diagnosis traditionally relies on optical microscopy, which is operator-dependent and presents important limitations in low-transmission contexts. The objective of this study was to evaluate the contribution of multiplex PCR in confirming positive cases detected by microscopy among preschool and school-aged children.

Methods: This study was conducted as part of a project evaluating the impact of mass drug administration on intestinal parasitic infections. A total of 191 children aged 4 to 15 years, with a positive stool concentration result using the simplified Ritchie method, were included. Stool samples were analyzed using the Kato–Katz technique and multiplex PCR. Statistical analyses were performed using R software (version 2025.05.00), with the significance level set at 5%.

Results: The overall prevalence of intestinal helminth infections was 7.3% using the Kato–Katz method and 38.2% using multiplex PCR. The Kato–Katz detected only single species infections, whereas multiplex PCR identified both single infections (27.7%) and multiple infections (10.5%).

Conclusions: These findings demonstrate that multiplex PCR significantly improves the detection of single infections and co-infections compared with conventional microscopy. In low-transmission settings, multiplex PCR represents a valuable complementary tool for the epidemiological surveillance of intestinal helminthiases.

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DIAGNOSTICS, HOST-PARASITE INTERACTION AND EMERGING TECHNOLOGIES / NEW DEVELOPMENT IN DIAGNOSTICS OF PARASITIC INFECTION

OPTIMISING SAMPLE STORAGE AND PREPARATION FOR THE LOW-RESOURCE DNA-BASED DIAGNOSIS OF ACTIVE FEMALE GENITAL SCHISTOSOMIASIS

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Background and Aims: Female genital schistosomiasis (FGS) is caused by *Schistosoma haematobium* eggs becoming trapped in the reproductive organs. DNA-based diagnostics for FGS remain limited in low-resource settings partially because storage and extraction methods can demand higher-resources. This study investigated optimal sample storage from swabs and DNA extraction methods for FGS molecular diagnosis.

Methods: Nylon flocked swabs were spiked with single *S. haematobium* eggs. Swabs were stored in four different storage media at four different temperatures over multiple time periods (up to 28 days), before being processed with a standard DNA extraction method. Additionally, swabs were stored at 22°C for 24 hours, in five storage conditions, before being processed using 11 DNA extraction methods. All DNA extracts were analysed using the *Schistosoma* ITS-2-qPCR to measure DNA yield (CT value). All data were analysed using StataNow.

Results: Each storage variable (temperature, time point and storage media) had a statistically significant impact on DNA yield ($F(3, 235) = 7.24$, media: $p=0.0001$, temperature: $p=0.0319$, timepoint: $p<0.0001$). DNA was detected from swabs exposed to all storage variables. When analysis was performed to see if combinations of conditions had compound effects on DNA yield, the combination of temperature and timepoint had a significant impact ($F(3, 219) = 2.45$ $p=0.0319$). There was a decline in DNA yield with an increase in temperature and time.

Conclusions: Analytical data show that DNA can be obtained from *S. haematobium* eggs on swabs preserved and stored under low-resource condition and describe conditions that permit accessibility of molecular diagnostics for FGS in low-resource settings.

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QUALITATIVE EVALUATION OF TOXOPLASMA GONDII OOCYST RECOVERY IN SANDY SOIL SAMPLES USING 0.1% NONYLPHENOL ETHOXYLATE.

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Background and Aims: Soil is an important environmental reservoir of *Toxoplasma gondii* oocysts and plays a relevant role in the epidemiology of toxoplasmosis. The standardization of methodologies for oocyst recovery from environmental matrices are essential to support environmental surveillance studies. The aim of this is study is qualitatively evaluate the sensitivity of the Nonylphenol Ethoxylate (0.1%) (IGEPAL CO-630) solution for the recovery of *Toxoplasma* oocysts from sandy soil samples using microscopy, aiming at its application in environmental studies.

Methods: *Toxoplasma* oocysts were stained with DAPI to improve visualization under fluorescence microscopy. Different concentrations (1000, 500, 250, 100, 50, and 10 oocysts/mL) were prepared and inoculated into 5 g of sandy soil in triplicate. Samples were maintained at room temperature for 1 hour, followed by the addition of 10mL of Nonylphenol Ethoxylate (0.1%) solution and homogenization using vortex. After centrifugation at 2000×g for 20 minutes, the supernatant was discarded and sucrose solution was added. Samples were centrifuged at 400×g for 5 minutes, and additional sucrose was added until a positive meniscus was formed. A coverslip was placed on the tube and removed after 40 minutes for microscopic examination using a fluorescence microscope.

Results: *Toxoplasma* oocysts were detected in all tested concentrations, demonstrating that the Nonylphenol Ethoxylate (0.1%) solution was capable of qualitatively recovering oocysts from sandy soil samples.

Conclusions: The Nonylphenol Ethoxylate (0.1%) solution is a viable alternative for the qualitative recovery of *Toxoplasma gondii* oocysts from sandy soil using microscopy. However, due to its low sensitivity, the method should be combined with molecular techniques to enhance detection reliability.

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COMPARISON OF THE ABBOTT SD BIOLINE OV16 AND NOVEL ONCHOCERCIASIS TEST PLUS RAPID DIAGNOSTIC TESTS FOR ONCHOCERCIASIS SEROPREVALENCE ASSESSMENT IN ANGOLA

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Background and Aims: Onchocerciasis is a neglected tropical disease associated with severe skin and ocular complications. Mass drug administration (MDA) decisions rely on serological diagnostic tools, but more specific and sensitive tests are needed. We compared two rapid diagnostic tests (RDTs): commercially available Abbott SD Bioline Ov16 RDT, which detects anti-Ov16 IgG4, and Onchocerciasis Test Plus (OT+) RDT, which detects IgG4 against Ov16 and a combination of Ov33 and OvOC3261.

Methods: During the baseline survey of EKAIO, a cluster-randomised trial comparing moxidectin vs ivermectin MDA against onchocerciasis in Angola, approximately 2600 dried blood spots (DBS) were collected from participants aged ≥ 1 year. DBS were stored at room temperature, then frozen until eluted for testing using both RDTs. Abbott tests were read at 24h while OT+ tests at 30min. For participants aged ≥ 5 years, 1728 skin snips were examined for microfilarial positivity and density through microscopy.

Results: Seropositivity was 35.7% by Abbott RDT and 25.9% by OT+ RDT. Based on overall positive and negative agreement, and Cohen's kappa coefficient, preliminary analysis suggests a moderate-to-substantial concordance between the RDTs. Test results were also compared to skin-snip microfilarial status and load, showing a sensitivity of 70.11% for Abbott and 65.28% for OT+.

Conclusions: The high seropositivity confirms substantial onchocerciasis exposure and a high-transmission setting. This study is one of the first comparisons between the widely used Abbott RDT and the novel OT+ RDT in an onchocerciasis-endemic setting. These findings will help clarify whether this novel RDT can improve onchocerciasis diagnostics and support mapping, surveillance and MDA decisions.

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TRAVELLERS RETURNING TO DENMARK WITH HYPEREOSINOPHILIA AND ANCYLOSTOMA INFECTIONS ONLY DETECTED THROUGH THE USE OF BROAD-SPECIFICITY AMPLICON-BASED NGS

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Background and Aims: In non-endemic countries such as Denmark, hookworm infections in travellers may be rare and difficult to detect due to low-grade shedding of eggsand lack of suspicion and relevant test capacity. We present two cases of hookworm infections observed in 2025 and only detected through amplicon-based next-generation sequencing (NGS).

Methods: Both hookworm cases were detected by the in-house amplicon-based next-generation (NGS) assay (Statens Serum Institut, Copenhagen, Denmark), which makes use of three primer pairs targeting eukaryotic nuclear ribosomal genes. In both cases, primary diagnostic work-up had failed to provide the diagnosis.

Results: Both infections were acquired during 2-week holidays in Asia and were characterised by hypereosinophlia. Case 1 got infected with *Ancylostoma ceylanicum* during holiday in the Philippines, whereas Case 2 contracted infection with *Ancylostoma duodenale/caninum* in Thailand. The prepatent period of hookworm infections has been reported to 5–9 weeks. Notably, in Case 1, the NGS assay accurately detected hookworm DNA in what could be considered the prepatent period (Day 45 after travel start), where no eggs were observed in stool. For Case 2, faecal samples were obtained later in the infection, resulting in the demonstration of a couple of eggs in stool (one egg per slide), but only after careful re-examination. Case 1 and 2 were subsequently treated with good effect with Ivermectin (single-dose) and Albendazole (3-day course), respectively.

Conclusions: Our cases demonstrate that amplicon-based NGS is a simple and useful diagnostic add-on in situations where routine screening for eosinophilia and abdominal pain after exposure in the tropics fails to provide the diagnosis.

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RADIOFREQUENCY-BASED MACHINE LEARNING DIAGNOSTICS FOR DETECTING PARASITISM-ASSOCIATED ANEMIA IN GOATS

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Background and Aims: BACKGROUND AND AIMS Anemia caused by gastrointestinal parasitism is a major constraint to small ruminant productivity. Sustainable livestock production requires rapid, non-invasive, and scalable diagnostic tools that reduce animal handling stress. This study evaluated an artificial intelligence–driven diagnostic framework integrating radiofrequency-based non-destructive testing (RF-NDT) features with machine learning to detect parasitism-associated anemia in goats.

Methods: METHODS Radiofrequency spectral data were collected from goats spanning FAMACHA categories 1 to 3. unsupervised spectral clustering was applied to characterize dielectric patterns associated with anemia severity. Supervised machine learning models were developed for prediction, including regression models (Linear Regression, Random Forest, Support Vector Regression) and classification models (Multilayer Perceptron, Support Vector Machine, K-Nearest Neighbors). Model performance was assessed using R^2, root mean square error (RMSE), accuracy, F1 score, and area under the receiver operating characteristic curve (ROC-AUC).

Results: RESULTS AI-driven analysis revealed distinct dielectric signatures across anemia states. FAMACHA 1 animals showed a dominant cluster at 8.43 GHz explaining 93.7% of variance, while FAMACHA 2 shifted toward 9.33 GHz with reduced uniformity (88.7%). FAMACHA 3 exhibited dual clusters at 9.89 and 8.23 GHz explaining 91.0% of variance. Linear Regression demonstrated near-perfect fit ($R^2 \approx 1.00$; RMSE = 0.07), while Random Forest achieved robust performance ($R^2 = 0.79$). The Multilayer Perceptron delivered highest classification performance (accuracy = 0.84; F1 = 0.83; ROC-AUC = 0.94).

Conclusions: CONCLUSIONS Artificial intelligence integrated with RF sensing enables automated, non-invasive detection of parasitism-driven anemia through physiologically meaningful dielectric signatures. This framework supports scalable, field-deployable decision-making for herd health management.

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OBTAINING VIABLE ASCARIS SUUM EGGS BY IN VITRO CULTURE OF ADULT FEMALES AND FECES FROM SLAUGHTERHOUSE PIGS

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Background and Aims: *Ascaris* spp. are globally prevalent soil-transmitted helminths, with *Ascaris lumbricoides* infecting humans and *Ascaris suum* infecting pigs. These species are morphologically and genetically similar and capable of cross-infection, underscoring their zoonotic relevance. Owing to their exceptional environmental resistance, *Ascaris* eggs are widely used in sanitation and parasite viability studies; however, reproducible methods to obtain large quantities of standardized viable eggs remain limited. This study evaluated two complementary egg procurement approaches from naturally infected pigs: fecal egg recovery and in vitro culture of adult female worms.

Methods: Adult *A. suum* worms and fecal samples were collected at slaughter from naturally infected pigs. Adult females were cultured in vitro, either alone or co-cultured with males, in a PBS-based liquid medium at 37°C for seven days, and egg output was quantified. In parallel, eggs were recovered from feces using an adapted flotation–sieving–sedimentation protocol, concentrated to a defined final volume, and quantified microscopically.

Results: Preliminary results indicate that both approaches successfully yielded viable *A. suum* eggs suitable for downstream experimental applications. No significant differences in total egg output were observed between females cultured alone and females co-cultured with males after seven days (n=12 per group; $p>0.05$), with mean recoveries of 147573 and 156182 eggs per female, respectively. The fecal recovery protocol produced concentrated egg suspensions with variable yields depending on host infection intensity (mean: 30136 eggs per gram).

Conclusions: In conclusion, both methods provide complementary and scalable strategies for generating high-quality *Ascaris suum* egg batches, supporting their use in experimental parasitology and environmental health research.

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MENINGOENCEPHALITIS CAUSED BY BALAMUTHIA MANDRILLARIS IN A YOUNG ORANGUTAN (PONGO PYGMAEUS PYGMAEUS)

Thomas Charpentier[1], Thomas Rivière[1], Karim Lemberger[2], Hélène Yera[3], Thierry Petit[1]
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Background and Aims: *Balamuthia mandrillaris* has been responsible for approximately 200 human cases since its description in 1986. Several cases have been reported in great apes, and it is an important emerging pathogen.

Methods: A two-and-a-half-year-old female Bornean orangutan (*Pongo pygmaeus pygmaeus*) was anesthetized due to lethargy and an inability to cling to her mother, which began the day before. The clinical examination reveals no major abnormalities, while blood tests show moderate lymphocytosis and a slightly elevated C-reactive protein level. Despite the supportive treatment administered, her condition deteriorates rapidly and she dies the following day.

Results: At autopsy, several whitish, necrotic lesions associated with petechiae were found in the occipital and temporal regions of both cerebral hemispheres. Histological examination revealed severe meningoencephalitis with numerous intralesional amoeba trophozoites. PCR testing for the DNA of several free-living amoebae was positive for *B. mandrillaris* in the kidney and brain. Sequencing of 16S mitochondrial rDNA confirmed the presence of *B. mandrillaris* DNA in the tissues. Initial serological tests (IIFA) revealed the presence of anti-*Balamuthia* antibodies in the young female, three weeks before death. Cultures taken from environmental samples reveal the presence of *Acanthamoeba* and *Balamuthia* in the soil.

Conclusions: Free-living amoebae are emerging pathogens that must be considered in the differential diagnosis of neurological disorders. This is the first case of balamuthiasis in mainland France.

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FIELD-READY DETECTION OF TOXOPLASMA GONDII IN MEAT SAMPLES BY REAL-TIME PCR

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Background and Aims: Human infection with *Toxoplasma gondii* mainly occurs through the consumption of undercooked meat containing tissue cysts. However, because infected animals rarely show clinical signs, detection during slaughterhouse inspection is limited, and no standardized method is available for detecting the parasite in meat. In this study, we aimed to develop a real-time PCR method for detecting *Toxoplasma* DNA to identify contamination in meat.

Methods: Primers and probes targeting the B1 gene were used (Fangwei *et al.,* 2019). Genomic DNA (gDNA) extracted from tachyzoites obtained by *in vitro* culture, as well as intact tachyzoites, were used to evaluate detection limits using a conventional real-time PCR system (QuantStudio 5, ABI) and a portable PCR device (PicoGene® PCR1100, GoFoton). To simulate the detection of *Toxoplasma* contamination in meat, parasite DNA detection was also performed using mixtures containing meat juice prepared from commercially available chicken meat on both devices.

Results: Using the conventional system, 10 fg of gDNA and a single tachyzoite were detectable. When mixed with meat juice, the detection limit for gDNA increased 100-fold to 1 pg, whereas a single tachyzoite remained detectable. The portable device showed comparable sensitivity for gDNA detection to that of the conventional system; however, detection of a single tachyzoite mixed with meat juice required heat pretreatment of the mixture.

Conclusions: In conclusion, this real-time PCR method allows detection of a single *Toxoplasma* organism in meat juice without nucleic acid extraction, indicating its potential applicability for meat inspection. Furthermore, the portable device may facilitate highly sensitive *Toxoplasma* testing outside conventional laboratory settings.

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TISSUE-SPECIFIC TRANSCRIPTOMIC INSIGHTS INTO MYXOZOAN INFECTIONS: IMMUNE AND STRESS RESPONSES IN THE KIDNEY AND HEAD CARTILAGE OF RAINBOW TROUT

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Background and Aims: By damaging target tissues and compromising the host immune system, *Myxobolus cerebralis* and *Tetracapsuloides bryosalmonae* remain persistent threats to salmonids. RNA sequencing was used to assess transcriptomic responses in the kidney and head cartilage (HC) of rainbow trout during single and co-infections with these two myxozoans.

Methods: Fish were exposed to single infections (Mc and Tb), and 30 days later half of the fish from each group were subjected to sequential co-infections (Mc^+ and Tb^+) to assess the combined effects of both pathogens. Transcriptomic analysis was conducted using kidney and HC tissues collected 60 days post co-infection.

Results: In the kidney, Tb fish had pronounced transcriptional alterations linked to chronic inflammation, immune complex clearance, and *IL-8* mediated responses. Tb^+ fish exhibited broad immune activation in the kidney leading to dysregulated inflammatory responses, most likely induced by the subsequent infection with *M. cerebralis*. HC responses in Tb+ fish shifted toward *IL-23*/Th17 driven response alongside reduced developmental and extracellular matrix processes. In HC, Mc fish had activated Th17, *IL-23*, phagolysosome and *IL-6* cytokine pathways. In contrast, Mc^+ fish showed enhanced pathogen processing and elevated metabolic responses accompanied by suppression of cytokine and tissue repair processes. In the kidney of Mc^+ fish, the immune response shifted towards adaptive immune engagement.

Conclusions: This study gives novel insights into how sequential myxozoan co-infection reshapes host immunity in a tissue specific pattern.

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EXPLORING THE SINGLE CELL ATLAS OF JUVENILE F. HEPATICA: ADVANCING LIVER FLUKE BIOLOGY

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Background and Aims: *Fasciola spp.* liver fluke place a significant disease burden on ruminant livestock worldwide and cause fasciolosis/fascioliasis, a neglected tropical zoonosis. While bile duct-dwelling adult fluke result in chronic disease, it is the migrating juveniles that cause acute fasciolosis, often resulting in death. A single drug, triclabendazole (TCBZ), is efficacious in the treatment of acute fasciolosis and increasing reports of TCBZ resistance underscores the need for novel drug target identification/validation. At present, this is undermined by our inability to fully unravel both the complexity of the parasite's fundamental biology and the mechanisms of TCBZ resistance.

Methods: We used a combination of single cell RNAseq and fluorescence *in situ* hybridisation (FISH) to identify and validate 33 transcriptionally distinct cell clusters within both TCBZ-susceptible and TCBZ-resistant juvenile *F. hepatica*. Results of differential expression analyses were subsequently exploited to functionally interrogate a novel drug target candidate using RNA interference (RNAi).

Results: Single cell clusters identified include stem cells and those of the gut, tegument, nervous system and musculature. Cell clustering and FISH-mediated transcript localisation revealed unexpected heterogeneity within the parasite's tegument, most notably between the transcriptional profiles of TCBZ-susceptible and TCBZ-resistant tegument clusters. RNAi-mediated knockdown of a proteasome endopeptidase subunit upregulated in the TCBZ-resistant juvenile tegument dysregulated stem cell proliferation, reduced growth and ultimately, proved lethal.

Conclusions: Taken together, these data provide a unique insight into the cellular complement of both TCBZ-susceptible and TCBZ-resistant juvenile *F. hepatica* and serve as a molecular blueprint for therapeutic target identification in this pathogenic life stage.

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METAGENOMIC AND AMPLICON ANALYSIS OF SANDFLY FEEDING HABITS AND LEISHMANIA PRESENCE IN EAST AFRICA

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Background and Aims: Determining sandfly feeding patterns is essential for understanding its environmental behavior and developing effective strategies to control *Leishmania* transmission.

Methods: Sandfly gut DNA was first analyzed via shotgun WGS as an initial broad survey of 14 samples across Kenya, Sudan, and Ethiopia, identifying feeding habits and pathogen presence to guide future research. Secondly, amplicon metabarcoding utilized multiple markers: ribulose-bisphosphate carboxylase (rbcL) for plant feeding analysis, cytochrome C oxidase subunit I (COI) for vertebrate blood meal identification, heat shock protein 70 for high-resolution *Leishmania* species differentiation, and 28S rDNA as a secondary marker for blood meals and plant matter.

Results: Data was classified using tools such as Kraken2 and Taxometer, and compared against RT-qPCR results for pathogen confirmation. WGS analysis identified *Leishmania donovani* DNA in high parasite load samples - confirmed by RT-qPCR - establishing a threshold for metagenomic detection. WGS also showed a broad vertebrate feeding profile. Next, rbcL amplicon sequencing revealed a consistent signature; *Moringa*, a nutritious and drought-resistant subsistence food in arid regions, was the most abundant plant detected; *Aristolochia*, a genus known in traditional medicine for its anti-parasitic properties, was second. Furthermore, the presence of *Vitis* DNA suggests vineyards may serve as important sugar feeding grounds. COI sequence analysis is ongoing to determine blood meal sources.

Conclusions: Identifying these predictable plant and mammal feeding habits offers a strategic advantage for understanding the environmental behavior of sandflies and potential new avenues for vector control. Additionally, vertebrate blood meal profiles identify animal hosts to screen for infection, mapping reservoirs in endemic regions.

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MOLECULAR CHARACTERIZATION OF MALARIA PARASITES BY NEXT-GENERATION SEQUENCING OF DNA EXTRACTS FROM GLASS SLIDES

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Background and Aims: Malaria remains a critical global health burden. Microscopy is the diagnostic gold standard, so blood smeared on glass slides is a widely available specimen type with a long shelf life, when stored appropriately. However, the utility of blood smears as substrates for next-generation sequencing (NGS) remains largely unexplored. This study aimed to evaluate a workflow optimized for DNA recovery from blood smears for downstream *Plasmodium* NGS.

Methods: A protocol was optimized to recover DNA from blood smears. Extracted DNA was evaluated using a published quantitative (q)PCR and three NGS methodologies: selective whole genome amplification (sWGA) for *P. falciparum* (Pf), Illumina AmpliSeq for *P. vivax* (Pv), and an amplicon-based NGS assay for *Plasmodium* spp. mitochondrial (Mt) sequences.

Results: Of 16 extracts prepared from Pf positive slides, one yielded sWGA data amenable to bioinformatic analysis. Among 31 Pv-positive extracts tested, 19 yielded sufficient AmpliSeq data for analysis. These 19 extracts were among 22 prepared from smears made ≤ 3 years before extraction. The Mt NGS method was applied to 11 extracts and 6 yielded partial *Plasmodium* Mt genomes of sufficient completeness for species identification. For all NGS methods, DNA extracts producing a sequencing result obtained a qPCR Ct value below 37.

Conclusions: Blood smears are a viable sample type for *Plasmodium* NGS, though performance is contingent on qPCR Ct, smear age (<3 years), and sequencing methodology. While sWGA was largely unsuccessful, AmpliSeq yielded data for hundreds of loci across multiple samples. The Mt assay generated multiple near-complete organellar genomes.

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COHESIN AND THE 3D GENOME ARCHITECTURE OF TRYPANOSOMA BRUCEI

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Background and Aims: *Trypanosoma brucei* is the causative agent of African Trypanosomiasis. *T. brucei* can evade host antibodies by switching its variant surface glycoprotein (VSG) coat. *T. brucei* has ~2,500 VSG genes, primarily located in silent subtelomeric regions. The active VSG gene is transcribed from a telomeric expression site (ES), while the others remain transcriptionally repressed. We showed that *T. brucei* chromosomes are organized into compartments A, which harbour core transcribed genes, and B, which corresponds to subtelomeric VSG-rich silent regions. The *T. brucei* genome contains other hallmarks of eukaryotic 3D genome organization, TADs and loops. The mechanisms underlying the formation of TADs and loops appear independent of the loop extrusion model. ChIP-seq showed that cohesin is enriched in telomeric ESs, some compartment boundaries and subtelomeric regions, indicating functions such as insulation of silent vs transcribed regions. Our aim is to generate conditional null mutants of cohesin subunits in *T. brucei* to determine how the lack of cohesin may affect genome spatial organization and transcription.

Methods: We are generating conditional null mutants of cohesin subunits in *T. brucei* and will use hydroxyurea-induced cell cycle synchronisation, short- and long-read sequencing methods, Hi-C and ChIP-sequencing to evaluate cohesin's role in genome organization and how it may change over the cell cycle.

Results: Hi-C and ChIP-seq analyses will enable comparison of genome organization and cohesin localization across cell-cycle stages in wild-type and cohesin depleted *T. brucei*.

Conclusions: We will test the role of cohesin in silent subtelomeric regions throughout the cell cycle and its contribution to VSG gene regulation.

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DIFFERENTIAL REGULATION AND SUBCELLULAR LOCALIZATION OF VIRULENCE FACTORS DISTINGUISH NAEGLERIA FOWLERI FROM RELATED NON-PATHOGENIC SPECIES

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Background and Aims: Primary Amoebic Meningoencephalitis (PAM), caused by *Naegleria fowleri*, is a fatal infection with limited treatment options. Since non-pathogenic species like *N. lovaniensis* may share virulence genes, we hypothesized that pathogenicity depends on differential regulation or post-translational modifications. Elucidating these mechanisms is critical for identifying specific therapeutic targets and vaccine candidates. This study evaluated the expression and localization of four virulence factors; Nfa1, Naegleriapore A (NPA), Cathepsin B (CatB), and Mp2CL5, across some *Naegleria* species with distinct pathogenic potentials.

Methods: Comparative analyses were performed on high- and low-virulence *N. fowleri*, *N. australiensis*, *N. lovaniensis*, and *N. gruberi*. Semi-quantitative RT-PCR was used to assess mRNA levels, while SDS-PAGE and confocal microscopy were employed to evaluate protein profiles and subcellular localization.

Results: Transcriptional analysis showed high-virulence *N. fowleri* and *N. australiensis* exhibit relative superior mRNA expression for NPA and Mp2CL5 compared to low-virulence strains. While CatB mRNA levels were qualitatively similar across species, immunocytochemistry revealed critical post-translational divergences: pathogenic strains concentrated CatB in pseudopods and extracellular vesicles—structures essential for invasion, whereas non-pathogens displayed diffuse cytoplasmic distribution. Additionally, Nfa1 expression was significantly reduced in *N. gruberi*.

Conclusions: *N. fowleri* pathogenicity is driven by a sophisticated regulatory network governing the abundance and trafficking of effector proteins, rather than simple gene presence. Uncovering these specific regulatory divergences highlights precise molecular markers, offering promising avenues for the development of novel therapeutic strategies and vaccines against PAM.
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POSTER ON BOARD SHIFT 02
DIAGNOSTICS, HOST-PARASITE INTERACTION AND EMERGING TECHNOLOGIES / GENOMICS, TRANSCRIPTOMICS, AND PROTEOMICS IN PARASITE RESEARCH

RESOLVING PARASITE IMMUNOMODULATORS TO A SINGLE-CELL TRANSCRIPTOMIC LEVEL

David Cruces-Gonzalez[1], Edouard Delanoe[2], Shashini Perera[1], Aralia Leon-Coria[1], Holly Liu[1], Erich Schwarz[3], Henry Mcsorley[4], James Wasmuth[2], Constance Finney[1]
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Background and Aims: Helminths can expertly manipulate the immune response of their hosts trough the release of a vast array of immunomodulators. These carefully timed and targeted proteins are very effective. They enable some helminths to remain in their hosts for decades and limit the hosts' ability to become immune to re-infection. Applying omics, we propose to create a systematic pipeline to discover helminth immunomodulators.

Methods: The Finney and Wasmuth labs produced the first and only single cell and multi-stage bulk transcriptomes for the murine parasite *Heligmosomoides bakeri*. Using the bulk transcriptomes, we confirmed the expression pattern of known immunomodulators across infection. Based on the single cell adult (10 days post infection-dpi) transcriptomic atlas, we have identified a small cluster of cells which express most described immunomodulators. To confirm the function of this cell cluster as a pharyngeal secretory cluster, we performed RNAscope on fresh frozen sections.

Results: According to bulk transcriptomics, HpARI-2 is expressed early in infection, HpBARI-2 is expressed throughout infection and HpTGM-6 is expressed later in infection. We successfully located these proteins to the pharynx at various time points including 2, 3, 5, 7 and 10 dpi. We have also confirmed the presence of other highly expressed genes in the cluster to be restricted to the pharynx and possess a characteristic immunomodulator biochemical signature. We are now using the pharyngeal secretory cluster as a way of identifying potential new immunomodulators.

Conclusions: Testing of candidate proteins is currently ongoing guided by computational predictions. Immunomodulators can be applied as novel antiparasitic and anti-inflammatory therapeutics.

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CHARACTERIZING VARIANT SURFACE GLYCOPROTEIN GENE EXPRESSION IN TRYPANOSOMA BRUCEI GAMBIENSE AFTER TSETSE FLY TRANSMISSION

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Background and Aims: *Trypanosoma brucei gambiense* is a tsetse fly-transmitted parasite causing human African trypanosomiasis. In the human host, the parasite evades the immune system through antigenic variation of its variant surface glycoprotein (VSG) coat. In *T.b.brucei*, early VSG expression follows a semi-predictable pattern that dissipates as infection progresses. Whether this also applies to the human-pathogenic *T.b.gambiense* remains unclear as experimental data are currently lacking. This study aims to characterize the early VSG expression landscape of *T.b.gambiense* in the bloodstream following natural tsetse fly transmission.

Methods: *Glossina morsitans morsitans* tsetse flies were experimentally infected with wild-type *T.b.gambiense* isolates and allowed to bite mice, simulating natural transmission. Bloodstream-stage parasites were collected at defined time points, and total RNA was extracted. VSG transcript repertoires were analyzed using targeted VSG sequencing to assess expression diversity and dynamics.

Results: VSG sequencing revealed a restricted repertoire of *T.b.gambiense* VSGs during the early bloodstream stage, dominated by a small number of dominant VSGs. These early VSG expression patterns were partially reproducible across infections in the experimental animals, either initiated by different tsetse flies or among multiple mice that were infected by the same fly. However, differences in the relative contribution of individual VSGs were observed between mice and across infections mediated by different flies, highlighting variability in VSG dominance.

Conclusions: These findings provide the first insights into early VSG expression dynamics of *T.b.gambiense* following natural tsetse fly transmission, suggesting a semi-predictable pattern. This work contributes to a better understanding of VSG dynamics during the earliest infection stages of this important human parasite.

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DIAGNOSTICS, HOST-PARASITE INTERACTION AND EMERGING TECHNOLOGIES / GENOMICS, TRANSCRIPTOMICS, AND PROTEOMICS IN PARASITE RESEARCH

ASSESSING CRYPTIC DIVERSITY OF PROSTHENORCHIS ELEGANS IN COSTA RICA

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Background and Aims: *Prosthenorchis elegans* is an acanthocephalan parasite of Latin American mammals that attaches to the gastrointestinal lining. Necropsies of wildlife reveal infections across diverse host species, with severe and occasionally fatal pathology. Despite its prevalence, little is known about the parasite, including whether a single species infects multiple hosts or if cryptic speciation is present. Here, we examine the genetic diversity of *P. elegans* from mammalian host species and integrate genetic measures of host specificity with clinical data to better understand host–parasite interactions.

Methods: We extracted DNA and sequenced the ITS-2 rDNA and COX-1 mtDNA regions from 21 *Prosthenorchis* worms collected from four native mammal species and one species rescued from the pet trade during opportunistic necropsies. We also performed long-read DNA sequencing and generated the first *Prosthenorchis* whole-genome sequence. We conducted phylogenetic analyses to assess genetic relationships among parasites from different hosts, and assessed clinical records from infected animals to evaluate host-specific variation in infection severity.

Results: *Prosthenorchis* infections were found in raccoons, kinkajous, squirrel monkeys, capuchin monkeys and a tamarin rescued from the pet trade. COX-1 variation was minimal but ITS-2 variation mirrored host phylogeny, with parasites collected from the same species clustering closely, suggesting cryptic speciation. 20 infections were recorded, including 18 monkeys with 6 fatalities and severe pathology in the remainder.

Conclusions: Our results reveal strong phylogenetic signals and high host specificity in *Prosthenorchis*. Because infections in primates are often severe or lethal, these findings could motivate future research on risk factors, and on mitigation strategies for disease management.

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FIRST GENOMIC DATA FOR THE CAPUCHIN MONKEY LUNGWORM FILARIOPSIS BARRETOI REVEALS PANMIXIA ACROSS HOST SOCIAL GROUPS

Megan Henriquez[1], Hadjira Hamou[2], Suheidy Morales[3], Dennise Ortiz[4], Uxia Rico[4], Valleria Villero[4], Paul Gordon[5], Brooklyn Christopher[5], Mario Baldi[6], Alicia Rojas[7], Fernando Campos[8], Katharine Jack[9], Amanda Melin[2]
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Background and Aims: Foundational hypotheses in parasite ecology suggest that animal social groups serve as “biological islands”, hindering parasite transmission between groups. Here, we use molecular approaches to analyze genetic data derived from the highly prevalent platyrrhine lungworm *Filariopsis barretoi*. Our goal was to explore its genetic diversity with respect to white faced capuchin (*Cebus imitator*) social organization to determine whether host social proximity facilitates parasite gene flow with observable effects on genetic population structure.

Methods: Using adult worms collected from necropsies of adult capuchins, we generated a draft Filariopsis genome and created custom primers targeting the ITS-2 rDNA genetic marker. Then, following a modified ‘Nemabiome’ protocol, we sequenced Filariopsis DNA extracted from fecal samples collected from 56 wild capuchins representing 6 social groups in the Sector Santa Rosa population in Guanacaste, Costa Rica. We calculated UniFrac genetic distances to determine if parasite genetic similarity was structured by host sex, group, or matriline.

Results: We found that genetically, parasites did not cluster by sex ($p = 0.356$), group ($p = 0.087$), nor matriline ($p = 0.363$) and that Filariopsis is panmictic across the broader capuchin population.

Conclusions: This suggests that parasite gene flow is not limited by host sociality and is shaped by a combination of factors such as host ecology, parasite life history, and habitat that maintain broader

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ecological connectivity. These findings highlight the utility of molecular parasite characterization in the non-invasive study of epidemiology in wild populations and lay the groundwork for future studies of disease transmission in wild primates.

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DIAGNOSTICS, HOST-PARASITE INTERACTION AND EMERGING TECHNOLOGIES / GENOMICS, TRANSCRIPTOMICS, AND PROTEOMICS IN PARASITE RESEARCH

REMODELLING OF LIVE SCHISTOSOME GLYCOSYLATION USING MANNOSIDASE INHIBITORS

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Background and Aims: Schistosomiasis is a neglected tropical disease caused by blood flukes, affecting over 250 million people worldwide. Schistosomes modulate the host's immune response to facilitate chronic infection. Immunomodulatory mechanisms are mediated in part by parasite-derived glycans. Additionally, cellular and molecular processes such as protein folding and intercellular communication are dependent on glycosylation, suggesting that glycans play pivotal roles in schistosome development. Our research aims to create live *Schistosoma mansoni* adult worms and schistosomula with remodelled glycosylation to unravel the glycobiology of schistosome development and host-parasite interaction.

Methods: We applied kifunensine and swainsonine, inhibitors of α-mannosidases involved in the protein N-glycosylation pathway, to *ex vivo* and *in vitro* cultured adult worms and schistosomula and analysed their effect on schistosome glycosylation and phenotype by glycomics and imaging approaches.

Results: Exposure to the inhibitors during culture changed the N-glycosylation profile from complex into hybrid and oligomannosidic N-glycan types. In adult worms this resulted in a significantly decreased expression of the immunogenic GalNAcbeta1-4GlcNAc motif, in particular in the tegument. The treatments did not affect adult worm morphology or motility. In contrast, schistosomula developed malformation and swelling of the gut after four weeks of culture in the presence of α-mannosidase inhibitors, accompanied by a rapid and strong change in the overall N-glycan profile that is reversible upon removal of the inhibitors after one week.

Conclusions: Schistosomes are amenable to live N-glycan-remodelling. This opens new avenues for studying the role of schistosome glycosylation during parasite development and in parasite-host interaction.

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COMPARATIVE GENOMICS OF THE MAJOR HUMAN HOOKWORM NECATOR AMERICANUS

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Background and Aims: From 170 to 400 million people are infected by hookworms. Of these hookworms, ~80% are *Necator americanus*, whose natural host is humans or great apes. The previous reference genome for *N. americanus* was assembled in the early 2010s from a strain (Anhui) genetically adapted to hamster hosts. Our goal is to assemble chromosomally-scaffolded genomes from both wild and laboratory *Necator* strains and species, compare them to Anhui, and identify genetic changes associated with host-specificity.

Methods: We used Nanopore, Illumina, and Omni-C sequencing with RAVEN, RACON, POLCA, and Ragtag to assemble genomes, and BUSCO to determine their completeness. We used RNA-seq with BRAKER2 or BRAKER3 to predict protein-coding genes. We used OrthoFinder and CAFE to identify changes in gene family size.

Results: We assembled a chromosomal reference genome for a laboratory strain of *N. americanus* not fully adapted to hamsters (Aroian). We also assembled chromosomal genomes for

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two wild isolates of *N. americanus* from sub-Saharan Africa and one wild isolate from east Asia, with two non-*americanus* Necator species as outgroups (*N. gorillae* and *N.* sp. type III), and a chromosomal reassembly of Anhui. We predicted 22,260-27,564 genes in these genomes, with completeness scores of 90.7-96.6%. Gene family size differences of Anhui identify proteins implicated in host-parasite interactions. Analyses of individual gene changes through SNP mapping and dN/dS statistics are ongoing.

Conclusions: Further characterization of these genomes will provide our first view of native hookworm genetics, and allow identification of genes that vary with host-specificity in *Necator*.

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DIAGNOSTICS, HOST-PARASITE INTERACTION AND EMERGING TECHNOLOGIES / GENOMICS, TRANSCRIPTOMICS, AND PROTEOMICS IN PARASITE RESEARCH

SPATIAL HETEROGENEITY IN ANOPHELES VECTOR DIVERSITY, PLASMODIUM FALCIPARUM INFECTION RATES, AND SYMBIONT PREVALENCE IN EIGHT CAMEROONIAN VILLAGES: INSIGHTS FOR TARGETED MALARIA CONTROL.

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Background and Aims: Malaria transmission in Cameroon shows spatial variability due to diverse Anopheles vectors. This study aimed to characterize species composition, Plasmodium falciparum infection rates, and symbiont presence across eight sites to inform targeted control.

Methods: Mosquitoes (8627) were collected from eight sites using Human Landing Catch (HLC) and CDC light traps (Anopheles, Culex, Mansonia). Species identification used morphology, Wilkins PCR-ID (n=316), and MinION sequencing (n=266, HotShot DNA extraction). P. falciparum was detected by CSP ELISA (n=5131) and qPCR (n=266). Wolbachia sp. and microsporidia were screened by PCR-gel electrophoresis (n=266).

Results: Anopheles gambiae s.l. dominated (5009, 83%), followed by A. pharoensis (709), A. ziemanni (117), and minor species. Wilkins PCR-ID revealed A. gambiae s.s. predominant, with A. coluzzii (76), A. arabiensis (11), and A. funestus funestus (30). MinION sequencing (n=266) explicitly confirmed: A. gambiae s.s. (5, Ngong), A. arabiensis (1, Ngong), A. coluzzii (7: 3 Ngong, 4 Tibati), A. funestus (47: 17 Esse, 13 Kekem, 17 Tibati), A. pharoensis (20: 17 Yangha, 3 Tibati), plus rare A. coustani (1), A. nili (1, Esse), A. rufipes (1, Esse). CSP ELISA detected 128 P. falciparum positives (2.5%): 80 gambiae (Esse), 37 (Belabo: 36 gambiae, 1 funestus), 5 gambiae (Maka), 2 (Tibati: 1 funestus, 1 gambiae), 2 (Yangha: 1 gambiae, 1 pharoensis). qPCR confirmed 19 positives (7.1%), mainly A. funestus in Esse/Kekem. Wolbachia infected 23/266 (8.6%), microsporidia 45/266 (16.9%).

Conclusions: A. gambiae s.l., A. funestus and A. pharoensis drives transmission. MinION revealed finer diversity. Moderate infection rates and symbiont prevalence suggest potential for innovative, site-specific interventions (e.g., Wolbachia-based) in Cameroon.

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GENOME SKIMMING REVEALS A MULTIPARTITE MITOCHONDRIAL GENOME IN THE PARASITIC NEMATODE DIOCTOPHYME RENALE

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Background and Aims: *Dioctophyme renale*, the giant kidney worm, is a parasitic nematode of medical and biological relevance that infects a wide range of mammals, including humans. Despite its importance, genomic information for this species is limited, with only partial mitochondrial and ribosomal sequences available, placing it within Clade I nematodes. Because mitochondrial genomes are valuable for phylogenetic and evolutionary studies, this work aimed to characterize the complete mitochondrial genome of *D. renale* and to assess its structure and phylogenetic relationships within Clade I.

Methods: An adult male *D. renale* specimen was recovered from a domestic dog in Argentina. High-molecular-weight genomic DNA was extracted and sequenced using Oxford Nanopore long-read technology. Mitochondrial reads were identified by similarity searches, assembled de novo, iteratively polished, and annotated using automated tools with manual curation. Comparative genomic and phylogenetic analyses were performed using mitochondrial protein-coding genes.

Results: Long-read sequencing revealed that the mitochondrial genome of *D. renale* is multipartite, consisting of eleven circular minichromosomes, each containing a single protein-coding gene and an associated non-coding region. This architecture was consistently supported by independent library preparation strategies. Phylogenetic analyses clustered *D. renale* with other vertebrate-parasitic Clade I nematodes, while the fragmented mitogenome structure appears to represent an independent evolutionary innovation.

Conclusions: This study provides the first complete characterization of a multipartite mitochondrial genome in *D. renale*, expanding knowledge of mitogenome diversity in nematodes. The long-read genome-skimming approach presented here offers a robust strategy for generating mitochondrial genomic resources in non-model parasitic species.

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DEVELOPING MALDI-TOF MASS SPECTROMETRY FOR LIVESTOCK PARASITE AND ARTHROPOD DISEASE-VECTOR RAPID DIAGNOSTICS

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Background and Aims: Arthropods such as ticks and lice are harmful viral and bacterial disease vectors, with invasive species establishing themselves in western Canada due to climate change, ecosystem/landscape change, and animal movement. Traditionally, arthropod identification has relied on morphology or molecular DNA-based methods. Morphological identification requires high expertise and is time-consuming. Misidentification is not uncommon because of outdated identification keys or when distinguishing closely related species. Molecular identification methods involve complex workflows and are challenging to scale for rapid, high-throughput diagnostics. Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) is an established technology widely used in diagnostic laboratories for routine bacterial identification. However, its application to parasites remains underexplored. We are investigating the use of MALDI-TOF MS as a rapid, scalable, and reliable method for parasite identification.

Methods: Arthropods were collected from a wide range of domestic and wild animal host species, undergoing a detailed morphological examination prior to dissection. Part of each arthropod was dissected for MALDI-TOF MS, while the rest was used for molecular identification. Spectra from each individual were added to a reference library and analyzed using spectrum analysis programs to validate identification specificity.

Results: Protein spectra were generated from lab-bred and field ticks. We have built a spectra library using 3-6 ticks of five species and challenged the library. Test ticks have generally been successfully identified to their respective species. We are currently expanding the library to include more species of arthropods.

Conclusions: MALDI-TOF MS is a promising technology that has the potential to be adapted for use in parasitology diagnostic labs for parasite identification.

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GENOME-WIDE ANALYSIS OF TELADORSAGIA CIRCUMCINCTA UNDER CONTRASTING TREATMENT REGIMES REVEALS STRONGER SELECTION FOR IVERMECTIN RESISTANCE FOLLOWING INTENSIVE DRENCHING

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Background and Aims: *Teladorsagia circumcincta* is the predominant and highly-resistant gastrointestinal nematode of sheep in the UK, causing major production losses and welfare impacts. Control relies on anthelmintics like ivermectin, but intensive use has led to widespread resistance. The genetic mechanism underlying ivermectin resistance and the influence of management practices on its evolution remain poorly understood. This study aimed to investigate the genetic basis of ivermectin resistance and estimate the strength of selection acting under contrasting treatment regimes.

Methods: Samples were collected during a 6-year field trial that was comparing two ivermectin drenching strategies: neo-suppressive treatment (NST), in which the entire flock was treated every four weeks, and targeted selective treatment (TST), in which individual lambs were treated based on growth performance. Faecal samples were collected bi-weekly, strongyle eggs then pooled by treatment and time point and hatched to first-stage larvae (L_1). DNA was extracted from pools of 200 L_1s followed by whole-genome sequencing. Genome-wide differentiation and allele frequency analyses were performed to detect signatures and strength of selection.

Results: Genome-wide F_{ST} scans identified a single QTL associated with ivermectin resistance spanning 32–42 Mb on Chromosome 5. This QTL habours several genes, however, most previously proposed ivermectin resistance candidate genes are located outside this region. Cochran–Mantel–Haenszel tests identified about 471 high-confidence variants while time-series modelling revealed significantly stronger selection in NST compared to TST populations.

Conclusions: Ivermectin resistance in *T. circumcincta* is strongly associated with a single QTL on Chromosome 5 and animal management strategies strongly influence the genomic evolution of the resistance.

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NGS APPROACH IN MITOCHONDRIAL GENOTYPING OF ECHINOCOCCUS GRANULOSUS SURGICAL STRAINS FROM THE FRENCH NATIONAL REFERENCE CENTER FOR ECHINOCOCCOSES

Perrine Duquet[1], Coralie Barrera[1], Carine Gnide[2], Louis Bohard[3], Laurence Millon[1], Jenny Knapp[1]
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Background and Aims: The *Echinococcus granulosus*, the infectious agent of cystic echinococcosis (CE), exhibits considerable genetic diversity, partly due to livestock trade. The French Echinococcosis Reference Center (NRC-E) receives surgical specimens of CE lesions from a wide geographical area and with different clinical forms, for expert analysis and species identification. The objective of this study was to develop a bioinformatics pipeline to process mitogenome data for the genotyping of *E. granulosus* strains, facilitate haplotype classification and help to trace back parasite circulation.

Methods: Recruitment was carried out at the NRC-E over the period 2021-2025 for a total of 35 patients with cysts and hydatid fluid specimens received for routine genotyping. Seven PCRs targeting contiguous mitochondrial loci were performed to sequence the full mitogenome (17641 pb). Bioinformatic processing and variant calling were performed in Shell language, from Illumina sequencing of endpoint PCR products. Genetic diversity was represented using a phylogenetic tree and a haplotype network.

Results: An average of 20 mutations were observed between the samples studied and the reference sequence. Phylogenetic analysis allowed for strict distinction of the *E. granulosus* s.l. species. Within the *E. granulosus* s.s. samples, high genetic diversity was observed, with no clear geographical trend.

Conclusions: The bioinformatics pipeline developed here for the study of the genetic diversity of operational strains of *E. granulosus* s.l. allows complex NGS data to be processed for epidemiological and clinical purposes. It will be used to try to better understand the circulation of this cosmopolitan parasite.

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DIAGNOSTICS, HOST-PARASITE INTERACTION AND EMERGING TECHNOLOGIES / GENOMICS, TRANSCRIPTOMICS, AND PROTEOMICS IN PARASITE RESEARCH

HIGH THROUGHPUT TARGETED LONG READ MITOGENOMICS REVEALS GLOBAL GENETIC DIVERSITY OF DIROFILARIA SPECIES

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Background and Aims: *Dirofilaria* species are widespread zoonotic parasites, and detailed genetic characterization is crucial for understanding their diversity and epidemiology. This study aimed to establish a high-throughput, robust, and cost-effective method for generating complete mitochondrial genomes to support global assessments of *Dirofilaria* genetic diversity.

Methods: High quality DNA was amplified using two long overlapping fragments (8.9–10 kb) via long range PCR, whereas lower quality samples were processed using six shorter overlapping fragments (~2.5 kb). Amplicons were sequenced with Oxford Nanopore Technologies, and reads were analysed through a dedicated pipeline incorporating Porechop, Nanoplot, and Chopper for trimming; Mitgard and Qualimap for de novo assembly and mapping; AGORA and GeSeq for annotation; and RAxML and iTOL for maximum likelihood phylogenetic reconstruction.

Results: Eighty-nine samples (adult worms, microfilariae, and extracted DNA) were collected from nine countries. Sequencing succeeded for 74 samples, yielding 61 complete and 13 partial mt genomes. Complete genomes included *D. immitis* (n = 30), *D. repens* (n = 27), and *D. asiatica* (n = 4). Coverage depth was consistently high (107.4×–905.6×). Phylogenetic analyses showed clear species-specific clustering of all newly generated sequences together with published *Dirofilaria* and other nematode mitogenomes.

Conclusions: This study demonstrates the efficiency of targeted full mitogenome sequencing using overlapping long-read amplicons. The workflow provides a scalable tool for generating high-quality mitogenomes and enables comprehensive evaluation of *Dirofilaria* genetic diversity. The approach enhances taxonomic resolution and supports epidemiological surveillance and control of filarial infections. **Funding**: FWF Grant DOI: 10.55776/PIN2522823; National Science Centre, Poland, OPUS LAP, grant number 2023/51/I/NZ6/00988.

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GENOMIC INSIGHTS INTO THE BEECH LEAF DISEASE NEMATODE LITYLENCHUS CRENATAE AND ITS NOVEL WOLBACHIA ENDOSYMBIONT WLCRE

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Background and Aims: The beech leaf disease that severely affects American beech trees (*Fagus grandifolia*) has spread to 14 US states since its first observation in Ohio in 2012. We aim to sequence and analyze the genome of the causative plant parasitic nematode (PPN) *Litylenchus crenatae mccannii*.

Methods: Nematodes were purified from beech-tree leaves collected on New England Biolabs campus in Ipswich, Massachusetts (USA). Metagenomic assembly, taxonomic binning, and gene annotation was performed using reads from Oxford Nanopore and Illumina Novaseq instruments.

Results: We obtained a 103.4 Mb *L. crenatae mccannii* genome distributed over 158 scaffolds with the largest scaffold being 10.695 Mb, N50 scaffold size of 4.2 Mb and encodes ~9,000 proteins. Phylogenomic analysis across 14 plant-parasitic nematodes (PPNs) and 3 free-living nematodes confirms the placement of *L. crenate* in the infra-order Tylenchomorpha. In the nematode genome, we identified 2 functional copies of the bacterial *panB* gene, which encodes the first committed step in the pantothenate (vitamin B5) biosynthesis pathway. We identify the endosymbiotic *Wolbachia w*Lcre and assembled its complete circular genome at 1,323,581 bp, with 1,300 protein coding genes, including all components of Riboflavin pathway, and almost complete heme biosynthesis pathway. A phylogenomic analysis placed *w*Lcre close to the supergroup L. Additional bacterial and fungal genomes associated with the diseased leaves were also obtained.

Conclusions: Together, these genome sequences would serve as important resources to understand the biology of this parasitic nematode and potential identification of treatment or management strategies.

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GENETIC DIVERSITY AND POPULATION STRUCTURE OF HAEMONCHUS CONTORTUS REVEALED BY WHOLE GENOME SEQUENCING

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Background and Aims: *Haemonchus contortus* is a major gastrointestinal parasite of small ruminants, and its extensive genetic variation promotes adaptation and anthelmintic resistance. Resolving genome-wide patterns of genetic variation is essential for interpreting resistance markers, and informing parasite control strategies. While reduced-representation approaches have provided important insights into population structure, whole genome sequencing (WGS) is needed to fully characterise population differentiation. This study aims to provide new insights into the genetic variation of *H. contortus* populations in Sweden and lay the groundwork for understanding adaptation and drug resistance.

Methods: This study performed WGS of pooled individuals from *H. contortus* isolates collected from fourteen Swedish sheep farms. Nucleotide diversity and pairwise F_{ST} were calculated, and highly differentiated SNPs were prioritised and assigned to their corresponding genes, and analysed by Gene Ontology (GO) enrichment.

Results: Within-population nucleotide diversity across *H. contortus* populations was high and comparable, while genome-wide differentiation between most population pairs was low. Three population pairs showed stronger differentiation, with the highest F_{ST} values (~0.14-0.16) and 15,315, 15,993, and 16,307 significantly differentiated SNPs, corresponding to 99, 96, and 103 candidate genes. GO enrichment revealed overrepresentation of terms including molecular function regulator activity, GTPase regulator activity, and nervous system development.

Conclusions: Swedish *H. contortus* isolates showed high genome-wide diversity and low differentiation, consistent with high gene flow, while moderate divergence in a small number of population pairs indicates geographical structuring. Candidate genes with enrichment of regulatory and nervous system–related functions, suggesting non-random divergence potentially driven by selection. These findings provide a genomic baseline for future studies.

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SENSITIVE AND RELIABLE METHOD FOR LRV2 DETECTION IN LEISHMANIA MAJOR

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Background and Aims: *Leishmania* is known to host multiple species of viruses of the family *Pseudototivirudae*, such as leishmaniaviruses (LRV1 and LRV2). The LRV2 genome comprises dsRNA and its presence has been linked to the increased chances of developing more severe mucocutaneous leishmaniasis over the cutaneous form, spread of the parasites, and elevated resistance to treatments.

Methods: A traditional screening for RNA viruses relies on gel-based identification of dsRNA bands, yet it was not clear how sensitive this approach is. In this work, we addressed confirm sensitivity of virus identification by direct comparison of gel-based visualization and RNA-sequencing followed by Transcriptomic analyses, which represents the gold-standard unbiased approach currently used to ascertain viral presence.

Results: Our dataset included 40 *L. major* isolates from Central Asia, 8 of which were shown to contain dsRNA viruses by wet-lab screening. Whole transcriptome analysis also revealed LRV2 presence in the same subset of isolates.

Conclusions: We concluded that gel-based identification is sensitive enough, at least in the case of *L. major* and LRV2.

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SMALL RNAS IN MALARIA EVS: UNCOVERING TRIGGERS OF GAMETOCYTOGENESIS

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Background and Aims: Malaria, primarily caused by *Plasmodium falciparum*, remains a major global health threat. Transmission depends on gametocytes the only stage capable of infecting mosquitoes. While EV protein cargo has been studied, their RNA content, particularly small non-coding RNAs, remains largely unexplored.
Objective 1: *Isolate and characterize stage-specific EVs*. Objective 2: *Profile EV small RNA cargo of stage-specific EVs via sequencing*.

Methods: Extracellular vesicles were isolated from conditioned media using differential centrifugation and size exclusion chromatography. EVs were characterized by TEM, NTA and western blot. RNA was extracted using TRIzol and Direct-zol kits for small RNA sequencing.

Results: Both DC and size exclusion chromatography (SEC) were evaluated for the isolation of extracellular vesicles from the three synchronized stages *P. falciparum* cultures. Comparative analyses showed that while SEC improved EV purity by reducing soluble protein contamination, it was not efficient as a standalone isolation method and required prior concentration by ultracentrifugation. Small RNA sequencing revealed a diverse repertoire of EV-associated RNAs. Preliminary analyses show distinct stage-specific small RNA signatures, with differential abundance of specific RNA species between ring-, trophozoite-, and schizont-derived EVs. Bioinformatic analyses are ongoing to identify candidate small RNAs potentially involved in regulating gene expression pathways.

Conclusions: This work provides the first comprehensive characterization of the small RNA content of stage-specific *P. falciparum* extracellular vesicles. Identifying small RNAs that are differentially packaged into EVs points to possible signals involved in EV-driven gametocytogenesis. These findings establish the basis for future functional studies and may help identify new targets to block malaria transmission.

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TRANSCRIPTOMIC ANALYSIS OF T-CELL EXHAUSTION GENES IN CHRONIC CHAGAS DISEASE PATIENTS UNDER NIFURTIMOX TREATMENT

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Background and Aims: This study aims to characterize the transcriptomic impact of nifurtimox on PBMCs on patients with chronic chagas disease, caused by Trypanosoma cruzi, leading to CD8+ T-cell exhaustion and inflammatory imbalance. We assessed the impact of Nifurtimox on proinflammatory chemokines, glycolysis persistence via HIF1A, lipid metabolism shifts, and mitochondrial biogenesis, supporting the transition toward anti-inflammatory profiles and the reversion of CD8+ T-cell exhaustion through transcriptomics.

Methods: Bulk RNA-Seq analyzed PBMCs from 14 individuals: untreated chronic Chagas (n=6), nifurtimox-treated (n=4), and healthy controls (n=4). Bioinformatics via Galaxy included Trimmomatic, Hisat2 (GRCh38), and htseq-count. Data processing in IDEP 2.4.3 used EdgeR transformation and filtering, yielding 16,822 quality-passed genes. Differential expression was assessed via Kruskal-Wallis and Dunn's post-hoc tests. PCA and boxplots were generated in R to evaluate transcriptomic variability between groups and compare the expression levels of specific genes associated with inflammation, metabolism and the reversion of CD8+ T-cells.

Results: Nifurtimox treatment induced a distinct transcriptomic signature, promoting a memory and regulatory phenotype. Downregulation of ACKR3, VSIG2, IL10, and proinflammatory chemokines (CXCL9, CXCL10) suggests a shift toward immune quiescence. Conversely, IL7R and CTLA4 were upregulated, indicating T-cell exhaustion reversal. While MGLL decreased, suggesting lipid-driven anti-inflammatory effects, IFNG (Gene) and glycolytic genes remained stable due to nifurtimox-induced oxidative stress via HIF1A. Finally, increased PPARGC1B expression across patient groups supports the generation of CD8+ memory T-cells.

Conclusions: Nifurtimox acts as a molecular switch, reversing T-cell exhaustion and restoring immune homeostasis. By modulating lipid metabolism and memory-associated genes like PPARGC1B, treatment promotes functional recovery and metabolic reprogramming.

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WHEN VIRUS MEETS PARASITE: MOLECULAR RESPONSE OF GIARDIA TO GIARDIAVIRUS

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Background and Aims: *Giardiavirus* (GLV) is a small dsRNA virus infecting *Giardia duodenalis*. Two closely related viral subtypes, GLVHP and GLVCAT, display different infection phenotypes. This study aimed to investigate the molecular basis of these divergent outcomes through an integrated transcriptomic and proteomic analysis of *Giardia* WBC6 experimentally infected with GLVHP (WBiHP) or GLVCAT (WBiCAT).

Methods: Total RNA and proteins were extracted from trophozoites at different time points during a 72h time course for RNA-seq and label-free proteomics analyses. RNA-seq reads were aligned to WBC6 reference genome and gene expression was quantified. Proteins were analysed by Data-Independent Acquisition mass spectrometry and quantified using DIA-NN. Differential expression analyses were performed using edgeR (transcriptomics) and limma (proteomics). Integrated analyses assessed mRNA–protein concordance and functional enrichment.

Results: Transcriptomic alterations were prominent at early time points in WBiHP and more limited and delayed in WBiCAT. Differentially expressed genes showed minimal overlap between infections, except for large gene families such as VSPs. Stress-related genes were upregulated only in late WBiHP infection. The number of differentially expressed proteins increased overtime in both cell lines. Late protein downregulation of known Giardia virulence genes was evident in WBiHP, but not in WBiCAT. Integrated analyses highlighted a coherent regulation of membrane-associated protein, indicating a significant impact of GLV on the plasma membrane.

Conclusions: These findings demonstrate that GLV subtypes induce distinct molecular responses in *G. duodenalis*, affecting stress response, membrane biology, and virulence. GLVs may therefore represent valuable tools or targets for novel antiparasitic strategies.

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MULTI-OMICS APPROACHES TO UNVEIL HOST-PARASITE INTERACTIONS

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Background and Aims: Schistosomiasis is a vector-borne disease, ranking third only to malaria and COVID-19 in terms of number of infected individuals worldwide. The parasitic worms are capable of adapting to both invertebrate and mammalian hosts, hijacking their immune systems to ensure their own perpetuation. Effective surveillance relies on reliable diagnostic tests and drug prophylactics, as the disease shares early clinical symptoms with several other pathogens. In this regard, the search for suitable biomarkers and new drug targets is of utmost importance. In the last decade, numerous studies on genomics and transcriptomics have emerged to unravel the complexity of the *Schistosoma* parasite.

Methods: We are currently using a multiomics approach, based on in-situ high resolution MS, to caracterise the molecular structure of eggshell, both free and trapped in the mice host liver tissue. In parallel we are detecting and analyzing the secreted/excreted molecules, by producing them as recombinant proteins. The lipidomics and proteomics analysis will be complemented by metabolomics analysis by NMR.

Results: We shall present the preliminary map of molecular composition of eggs from *Schistosoma mansoni* (Belo Horizonte strain) trapped in mouse liver tissue. Their eggshell is rich in complex phospholipids and glycolipids, despite the lack of biosynthesic enzymes. The intersticial space is filled with interesting biomarkers, which are under study by molecular biophysics, HR-MS and IM-MS.

Conclusions: We shall present the first high resolution in situ composition of the molecular interface between *S. mansoni*'s eggs and hepatic tissue.

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PROTEASES AND THEIR INHIBITORS OF ZOONOTIC ANISAKIS PEGREFFII LARVAE

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Background and Aims: *De novo* assembly of the *A. pegreffii* genome, complemented by transcriptomics profiling of the parasite's excretory gland across ontogeny and during invasion of paratenic and accidental host, enabled us to prospect for the nematode's virulence factors within peptidase and peptidase inhibitor (PPI) families.

Methods: Combining, for the first time, long-read PromethION and short-read Illumina sequencing, we propose a draft genome of *A. pegreffii* of 128.84 Mb, 3,070 contigs, with a contig N50 of 1,953 bp and a GC content of 44.11%. The annotation includes 13,914 protein-coding genes and 1,078 non-coding RNA genes. Based on functional annotation, GO terms, specific domains, and BLAST searches against the MEROPS and UNIREF 100 databases, peptidases and peptidase inhibitors were scrutinised.

Results: During moulting of L3 to L4, the expression of 262 proteases and 28 protease inhibitors was recorded in the excretory gland cell, with metalloproteases and serine proteases as predominant types, of which 103 were differentially regulated. Prolylcarboxypeptidases were most strongly upregulated, whereas homologues of astacins, other metallopeptidases, and autophagin were downregulated. In accordance with general transcriptional activity observed during infection of an accidental poikilothermic host (rat) and a paratenic host (seabass), over a hundred peptidases and inhibitors were regulated during the L 3' s attempt to infect a rat, and far fewer (17) in seabass.

Conclusions: This confirms that important virulence factors in *A. pegreffii* are closely connected and play a role in its development and maturation, and sets the ground for a better understanding of its pathogenesis.

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ISOLATION AND ANALYSIS OF TOXOPLASMA GONDII FROM A JAPANESE PATIENT WITH CONGENITAL TOXOPLASMOSIS

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Background and Aims: We have isolated, whole genome-sequenced, and compared *Toxoplasma gondii* strains from Japan and found that *T. gondii* in Japan has evolved in a unique manner (Fukumoto et al. (2020), Matsuzaki et al. *in preparation*). In this study, we have succeeded in isolating a Japanese strain of *T. gondii* from congenital toxoplasmosis and report the results we have been able to analyze to date.

Methods: Isolation was attempted using spinal fluid collected from a 9-day-old patient child. Anti-*T. gondii* antibody was observed during the mother's pregnancy, and the child had intracranial calcification, enlarged ventricles, bilateral choroiditis, and was positive for anti-*T. gondii* IgM. PCR testing was performed on the collected specimens to confirm positivity, and the remaining specimens were inoculated into mice previously treated with dexamethasone as part of the bioassay. Two weeks after inoculation, abdominal lavage fluid was collected and added to human foreskin fibroblast cultures, resulting in the successful establishment of a parasite strain. During this period, the same parasite strain was maintained in mice, but its virulence to mice seemed to be weak. Genomic DNA was purified and the entire genome sequence was determined. Comparison of the obtained sequence with known genomes of isolates from all over the world

Results: The genome analysis suggests that this isolate is not a unique genotype found in Japan, but is identical to Type II, which is widely recognized in the United States and Europe.

Conclusions: We are currently conducting more detailed analyses of the whole genome sequence and pathogenicity against mouse.

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TRANSCRIPTOMIC HETEROGENEITY AND METABOLIC ADAPTATION DURING DIFFERENTIATION OF TRYPANOSOMA CRUZI AXENIC AMASTIGOTES

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Background and Aims: The study of *Trypanosoma cruzi* amastigotes presents major methodological challenges due to their intracellular localization and the heterogeneity of stages within infected cells. To overcome these difficulties, alternative strategies, such as the differentiation of trypomastigotes into axenic amastigotes, have been developed to enable their study under controlled conditions. In this work, axenic amastigotes were evaluated as a model to study the amastigote stage without intracellular constraints, with the objective of validating their suitability through transcriptomic analysis.

Methods: RNA-seq analyses were performed on the main life cycle stages of *T. cruzi* as well as on axenic amastigotes collected at different time points during differentiation. This study represents one of the first transcriptomic comparisons of these stages in the DM28c strain and includes a temporal dynamic characterization of axenic amastigotes.

Results: Progressive transcriptomic and morphological changes revealed 191 genes differentially expressed during differentiation from axenic amastigotes to epimastigotes. Among these genes, increased expression was observed for genes involved in carbohydrate metabolism, together with a decrease in genes related to amino acid metabolism, indicating a metabolic shift in carbon source utilization. In addition, transcriptomic analyses revealed increased expression of retrotransposon multigene families during the differentiation process.

Conclusions: Transcriptomic heterogeneity in *T. cruzi* may provide adaptive advantages by enabling rapid responses to changes in nutrient availability. Moreover, the DM28c strain exhibits an alternative differentiation pathway from amastigotes to epimastigotes, supporting its use as a suitable experimental model for the study of differentiation mechanisms. Collectively, these results suggest coordinated metabolic and regulatory reprogramming during the parasite's life cycle.

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COMPARISON OF PROTEASE ACTIVITIES OF CELLULAR EXTRACT AND EXTRA CELLULAR SECRETIONS OF TRICHOMONAS VAGINALIS ISOLATES BY ZYMOGRAPHY METHOD IN IRAN

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Background and Aims: Trichomonas vaginalis is the most common non-viral sexually transmitted protozoan, responsible for over 270 million new infections annually. Its pathogenicity is closely linked to proteolytic enzymes that degrade host tissues, promote adhesion, and help evade immune defenses.

Methods: Cysteine proteases (CPs) represent more than 90% of T. vaginalis's proteolytic capacity, yet the comparative roles of cellular and secretory enzymes among regional isolates remain poorly defined.Materials and MethodsThree Iranian isolates (Shiraz, Kerman, and Tehran) were cultured axenically in TYI-S33 medium. Proteases were extracted from both cellular extracts (CE) and extracellular secretions (ES) during logarithmic (48 h) and stationary (72 h) growth phases. Gelatin-substrate zymography was applied to characterize molecular weights and activities under acidic conditions (pH 5.5). Band intensities were quantified using ImageJ software to assess relative proteolytic strength

Results: All isolates exhibited typical morphology and similar growth dynamics. Zymographic profiling revealed three main protease groups: 75 kDa (mainly intracellular), 25–30 kDa (shared between CE and ES), and a dominant 17 kDa cysteine protease confined to the ES. The ES/CE enzymatic activity ratio was approximately 4:1, and proteolytic activity slightly increased in the stationary phase

Conclusions: The predominance of the 17 kDa secreted protease suggests its central role in early tissue invasion and adaptation to the vaginal acidic environment. Minimal variations among isolates indicate a conserved enzymatic pattern in Iranian strains. Owing to its abundance and accessibility, the 17 kDa cysteine protease could serve as a diagnostic biomarker and a promising target for anti-protease chemotherapeutic development

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EFFECT OF HYDROXYUREA ON THE MORPHOLOGY, PROLIFERATION, AND PROTEIN EXPRESSION OF TAENIA CRASSICEPS WFU STRAIN

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Background and Aims: In cestodes, a population of totipotent cells with a central role in cell proliferation, parasite development, and regeneration has been identified. One strategy for their molecular and structural characterization has been to use hydroxyurea (HU), an S-phase arrest agent, to inhibit the cell cycle. Previous studies have shown that isolated cells from *Taenia crassiceps* can regenerate into cysticerci when injected into mouse hosts, suggesting a key role for proliferative cells in larval regeneration. The objective of this study was to characterize proliferative cells using HU and to evaluate the impact of this treatment on larval survival and physiology.

Methods: *T. crassiceps* cysticerci were treated for six days with HU at concentrations of 25 mM and 40 mM. Proliferative cells reduction and recovery were assessed using viability assays based on Alamar Blue reduction, morphological analysis, and the detection of positive cells to 5-ethynyl-2'-deoxyuridine. Proteomic analyses were performed to identify proteins with altered expression following treatment.

Results: HU treatment induced changes in the size and shape. A significant reduction in proliferative cells at both concentrations was observed. Partial recovery of proliferative cells was observed on days 3 and 6 after treatment with 25 mM HU, whereas no clear recovery was detected at 40 mM. Proteomic analysis identified proteins with differential expression mainly related to DNA and RNA binding and damage, glycolysis, cytoskeletal, and muscle structure.

Conclusions: The findings of this work highlight the importance of proliferative cells in parasite survival and suggest that proteins involved with proliferative cells will be candidates for the development of new cysticercosis treatment.

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CHEMOSENSATION IN ASCARIS INFECTION

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Background and Aims: Ascariasis, caused by *Ascaris* spp., infects ~819 million people worldwide and poses a major threat to public health and the livestock industry. Current mass-deworming strategies do not prevent reinfection and risk promoting drug resistance. Hepatopulmonary migration (HPM) of *Ascaris* larvae—traditionally considered passive—may instead be actively guided by chemosensation. We aimed to define the molecular basis of *Ascaris* chemosensation and determine whether larvae use host-derived cues to navigate towards liver tissue.

Methods: Initially, larval behaviour was assessed using agar migration assays and a tailored microfluidic device that recreated *in vivo*-like gradients of freshly slaughtered pig liver and lung homogenates. To define the underlying sensory machinery, we combined comparative genomics, phylogenetic analyses, and tissue-specific transcriptomics to annotate sensory signalling components in *Ascaris*. Activation of chemosensory pathways was further assessed by RNA-seq of larvae stimulated with liver or lung homogenates.

Results: Both agar and microfluidic assays revealed robust, directional chemotaxis towards liver homogenate compared with lung or control media. Microfluidic tracking showed increased forward velocity and reduced turning behaviour in liver gradients, consistent with directed navigation. Genomic analyses identified a reduced but highly divergent repertoire of chemosensory GPCRs and lineage-specific signalling molecules, forming a discrete pathway enriched in head and amphidal tissues. Transcriptomic profiling of stimulated larvae revealed differential transcription of genes involved in neuropeptide processing, chemosensory signalling, and fatty-acid metabolism which potentially guide HPM.

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Conclusions: *Ascaris* larvae possess a compact, functional chemosensory system that actively guides HPM towards liver-derived cues. Future studies will aim to identify target-receptor interactions to potentially block chemotaxis and disrupt HPM.

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POSTER ON BOARD SHIFT 02
DIAGNOSTICS, HOST-PARASITE INTERACTION AND EMERGING TECHNOLOGIES / GENOMICS, TRANSCRIPTOMICS, AND PROTEOMICS IN PARASITE RESEARCH

PRESENCE OF THE 26S PROTEASOME IN TROPHOZOITES OF NAEGLERIA GENUS

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Background and Aims: Free-living amoebae of the genus *Naegleria* includes species of clinical and biological relevance. *Naegleria fowleri* is the only species pathogenic to humans, causing primary amoebic meningoencephalitis (PAM) which is a highly fatal disease that develops in the central nervous system, whereas *N. gruberi* and *N. lovaniensis* are non-pathogenic species used as experimental models. The 26S proteasome is the main proteolytic complex in eukaryotic cells and consists of a catalytic 20S core particle and a 19S regulatory particle responsible for recognition and processing of ubiquitinated proteins. The aim of this project was to demonstrate the presence of 19S subunits.

Methods: Additionally, to know whether it is co-localized with the 20S subunits (which have already been described) to form the 26S proteasome in trophozoites of *N. fowleri, N. lovaniensis* and *N. gruberi* through experimental techniques.

Results: The presence of 19S proteasome subunits was confirmed in all three *Naegleria* species. Localization analyses demonstrated co-localization of 19S and 20S subunits, indicating the assembly of the complete 26S proteasome complex in trophozoites.

Conclusions: These results demonstrate that *Naegleria* species possess functional 26S proteasomes composed of both 19S regulatory and 20S catalytic subunits. Our acknowledgement to CONACYT for financial support (CVU: 997938) provided during the project.

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GLYCOSYLATION PATTERNS IN MECCUS (TRIATOMA) PALLIDIPENNIS GUT: IMPLICATIONS FOR THE DEVELOPMENT OF VECTOR CONTROL STRATEGIES

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Background and Aims: Chagas disease remains a major public health problem in Mexico, where *Meccus (Triatoma) pallidipennis* is a key vector of *Trypanosoma cruzi*. The triatomine gut is central to parasite development, yet region-specific glycosylation patterns remain poorly characterised. This study aimed to compare protein expression and glycosylation profiles in the anterior midgut (AMG) and rectum (RE) of fifth-instar nymphs of *M. pallidipennis* to identify potential targets for vector control.

Methods: AMG and RE tissues from unfed fifth-instar nymphs were dissected and analysed by one- and two-dimensional electrophoresis. Glycosylation patterns were assessed using lectin blotting with concanavalin A (ConA), wheat germ agglutinin (WGA) and peanut agglutinin (PNA). Differential protein expression was evaluated by Western blotting for HSP70, α-tubulin and β-actin.

Results: Distinct proteomic and glycosylation profiles were observed between gut regions. The RE displayed a higher diversity of glycosylated proteins, particularly those containing mannose and glucose residues detected by ConA. In contrast, N-acetylglucosamine and sialic acid residues identified by WGA were more frequent in the AMG, although signal intensity was greater in the RE. Differential expression of α-tubulin and β-actin further supported functional specialisation of these regions.

Conclusions: Region-specific glycosylation patterns in the triatomine gut highlight the rectum as a critical target for disrupting *T. cruzi* development. These findings support the potential use of lectin-based strategies in novel vector control approaches.

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THEILERIA PARVA TRANSCRIPTOME ANALYSIS REVEALS POSSIBLE VIRULENCE FACTORS

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Background and Aims: *Theileria parva* infections cause a fatal lymphoproliferative disease that severely impacts cattle production across Africa. Although it is well known that *T. parva* strains vary in virulence, the molecular basis of this remains poorly understood.

Methods: Transcriptome profiles of virulent and mild *T. parva* strains were compared to determine key virulence factors (VFs).

Results: Over 30% of top 25 protein-coding differentially expressed genes (DEGs) upregulated in virulent strains encode members of subtelomeric multigene families, particularly subtelomeric variable secreted proteins (SVSP) and SfiI-subtelomeric fragment–related (FAINT) proteins. Members of subtelomeric gene families are major virulence determinants in apicomplexan parasites, enabling antigenic variation, modulation of host responses, and enhanced survival. Canonical histones were the second major group of VFs upregulated in virulent strains. Given that they are synthesized in synchronization with DNA replication to support chromatin assembly in proliferating cells, histones may support *T. parva* schizonts-induced uncontrolled lymphocyte proliferation resembling oncogenic processes. Pathway associations include NET formation, viral carcinogenesis, and necroptosis, further implying roles in inflammation, immune modulation, and potentially tumour-like phenotypes in infected lymphocytes. Other VFs include the Tp9 antigen, rhomboid protease, a GCC2/GCC3-domain protein, a kinase, and a MACPF-domain protein. Notably, the latter four were exclusively expressed in virulent strains. Each of these VFs have known or putative roles in invasion, immune modulation, host-cell signalling, or intracellular survival. Protein-protein interaction analysis revealed interconnections among VFs.

Conclusions: These findings show that *T. parva* has a multifactorial virulome framework targeting key host pathways. Identified VFs may be possible targets for therapies and vaccines against *T. parva*.

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PRDM1/BLIMP-1 ACTS AS A COREPRESSOR OF FOXA TO SUPPRESS ESOPHAGEAL GLAND CELL FATE IN SCHISTOSOME PARASITES

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Background and Aims: Background: In schistosomes, parasitic flatworms that cause schistosomiasis, the forkhead transcription factor FoxA regulates the development/maintenance of the esophageal gland (EG), an organ essential for immune evasion and survival *in vivo*. In addition to its EG expression, prior single-cell studies show *foxA* expression in a limited number of neural subsets and intestinal progenitors. However, we lack a comprehensive understanding of the cell-type-specific expression and function of FoxA. A pioneer factor such as FoxA works with cofactors to promote or repress lineage-specific gene expression by altering chromatin accessibility. Thus, we hypothesize that schistosome FoxA works with tissue-specific cofactors to regulate the production and/or function of non-EG cell types.

Methods: Methods: To enrich for *foxA*-expressing cell types and to identify potential FoxA cofactors, we performed a head-enriched (HE) single-cell (sc) RNA-seq coupled with *in situ* hybridization (ISH) and RNAi of candidate genes.

Results: Results: HE scRNA-seq shows *foxA* expression in EG, stem cells, intestine, and neurons. Highly associated with *foxA* expression in these non-EG cell types is *prdm1/blimp-1*, encoding a known transcriptional repressor and FoxA cofactor. ISH showed *prdm1/blimp-1* enrichment in neural and intestinal subsets. RNAi-mediated *prdm1/blimp-1* knockdown significantly increased ectopic EG gene expression in the parasite body posterior to the EG, suggesting its role in suppressing the EG fate in non-EG cells. Motif analysis identified both FoxA and Prdm1/Blimp-1 motifs in the *foxA* regulatory region, suggesting this suppression may occur via negative regulation of *foxA*.

Conclusions: Conclusions: We conclude that Prdm1/Blimp-1 acts as a corepressor of EG cell fate in non-EG *foxA* expressing cells.

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ANNOTATION AND PRELIMINARY CHARACTERIZATION OF LNCRNAS IN TRYPANOSOMA CRUZI

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Background and Aims: *Trypanosoma cruzi*, the causative agent of Chagas disease, undergoes extensive gene-expression remodeling across its life cycle, driven largely by post-transcriptional regulation. Although protein regulators have been studied, the contribution of RNA molecules, particularly long non-coding RNAs (lncRNAs), remains poorly understood. In this context, we aimed to develop a reproducible approach to annotate lncRNAs and to assess their genomic context and developmental regulation.

Methods: We developed a bioinformatic pipeline that integrates transcriptome assemblies, coding-potential filtering, and comparative analyses to identify lncRNA candidates in *T. cruzi*. Candidates were classified by genomic context and were clustered by sequence to analyze genomic duplications. Expression was assessed using RNA-seq data from all life cycle stages. Oxford Nanopore Direct RNA-seq was used to support full-length transcript identification and to validate sequences and transcript boundaries for selected lncRNAs.

Results: We identified hundreds of novel lncRNA candidates, many of which originate from transposon-derived sequences. Nanopore data supported full-length transcripts for multiple lncRNAs. Although most lncRNAs were detected at low transcript numbers, several reached expression levels comparable to mRNAs. Differential expression analysis across developmental stages revealed widespread stage-specific regulation.

Conclusions: These findings expand current knowledge of non-coding RNAs in *T. cruzi* and provide a foundation for functional studies of lncRNA-mediated regulation during parasite development and host infection.

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A COMPREHENSIVE PROTEOGENOMIC LANDSCAPE OF MICROPROTEINS IN TRICHOMONAS VAGINALIS

Jhen Wei Syu, Hong Wei Luo, Seow Chin Ong, Chih Yu Tsai, Po Jung Huang, Chi Ching Lee, Yuan Ming Yeh, Rose Lin, Petrus Tang
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Background and Aims: Microproteins (MPs), encoded by small open reading frames (sORFs) shorter than 100 amino acids, are key regulators of fundamental biological processes. However, the microproteome of parasitic protozoa remains largely unexplored. This study aimed to establish the first comprehensive MP atlas of *Trichomonas vaginalis* using an integrated proteogenomic framework.

Methods: We integrated RNA-seq datasets from 22 representative *T. vaginalis* isolates to identify expressed MP-encoding genes and validated their translation using LC–MS/MS. A de novo pipeline combined with homology filtering was applied to identify unannotated MPs arising from intergenic sORFs. Proteomic data were analyzed using Mascot for annotated MPs and FragPipe for novel candidates, both at a 1% false discovery rate. In addition, ultra-deep single-cell RNA sequencing (10x Genomics; 100 Gb) was performed on isolate ATCC30236 to assess MP expression at single-cell resolution.

Results: Transcriptomic profiling identified 786 annotated MP genes consistently expressed across more than 20 isolates and revealed 150 previously unannotated intergenic sORFs. Functional enrichment analysis linked these core MPs predominantly to ribosomal components and organelle-associated functions. Proteomic validation confirmed the translation of 103 core MPs. Single-cell analysis further refined this landscape, identifying 147 annotated MPs and 3 unannotated sORFs expressed in over half of the cells, indicating potential essential roles in parasite biology.

Conclusions: This study presents the most comprehensive MP atlas of *T. vaginalis* to date. Validation at both protein and single-cell levels provides a foundational resource for functional characterization and the development of novel intervention strategies against trichomoniasis.

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ASEXUALLY PROLIFERATING SPIROMETRA MANSONI: CHARACTERIZATION OF A UNIQUE STRAIN EXPANDING WITHIN THE ANIMAL HOST

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Background and Aims: *Spirometra mansoni* is a cestode whose definitive hosts are dogs and cats. Although plerocercoids can infect a wide range of animals, including humans, they do not normally proliferate within intermediate or paratenic hosts. In contrast, we identified a unique strain of *S. mansoni* (T1) that proliferates within the host body. This study aimed to characterize this strain and elucidate the mechanisms underlying its unusual in vivo proliferation.

Methods: The proliferative capacity of *S. mansoni* T1 was examined in vitro (cultured in DMEM) and in vivo, in which mice and hamsters were each infected with 10 larvae. Parasite numbers were quantified at long-term time points. Morphological characteristics were analyzed using histological analysis, scanning electron microscopy (SEM), and transmission electron microscopy (TEM). Gene expression profiles were compared with those of a normal S. mansoni strain using RNA-seq analysis.

Results: No proliferation of *S. mansoni* T1 was observed in vitro during 117 days of culture. In contrast, marked proliferation occurred in vivo: parasite numbers increased from 10 to 26 in mice after 470 days and from 10 to 438 in hamsters after 380 days. *S. mansoni* T1 exhibited a smaller body length (2.1–10.4 mm) than the normal strain and frequently displayed branching body structures. Genes associated with cell proliferation were significantly upregulated in *S. mansoni* T1.

Conclusions: The reduced body size and branching morphology of *S. mansoni* T1 suggest asexual propagation through body fragmentation. This unusual proliferative capacity is likely enabled by physiological states and gene expression programs that differ from the normal *S. mansoni* strain.

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PROTEOMIC CHARACTERIZATION OF EXTRACELLULAR VESICLES FROM ATTENUATED AND VIRULENT LEISHMANIA (LEISHMANIA) AMAZONENSIS REVEALS PROTEASOME-ASSOCIATED VIRULENCE SIGNATURES

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Background and Aims: Extracellular vesicles (EVs) released by *Leishmania* contain antigens, virulence factors, and bioactive molecules that modulate parasite–host interactions, favoring parasite survival. Differences in Leishmania virulence can alter the cargo of their EVs and, consequently, the infection. In this study, we performed an extensive morphological and proteomic characterization of EVs derived from *L. (L.) amazonensis* with attenuated (AEVs) and virulent (VEVs) profiles.

Methods: EVs were obtained from parasite supernatants and purified by size-exclusion chromatography. Cryo-electron microscopy and nanoparticle tracking analysis were used to evaluate purified EVs. EVs were submitted to trypsin digestion, and the single-pot solid-phase sample preparation (SP3 protocol). The LC-MS/MS analyses were performed by the Vanquish Neo/Orbitrap Exploris 480 system.

Results: Morphological analyses using nanoparticle tracking analysis (NTA), cryo-electron microscopy (Cryo-EM), zeta potential measurements, and atomic force microscopy revealed similar morphology between AEVs and VEVs. Proteomic analysis identified 936 proteins (13.49% of the predicted proteome), representing a wide, comprehensive EV proteome for *L. (L.) amazonensis*. Notably, VEVs showed a higher abundance of proteolysis- and proteasome complex–related proteins compared to AEVs.

Conclusions: It is not clear what the function of proteases and proteasome complex proteins is within the parasite VEVs. They can be an alternative mechanism to parasite exocytosis, or they can participate in the initial infection by degrading extracellular matrix and immune components, or by facilitating the delivery of virulence factors. Further studies, including proteolytic activity assays, are ongoing to elucidate the functional role of EVs in *L. (L.) amazonensis*.

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BEYOND THE TRANSCRIPT: CHROMATIN IMPLICATIONS IN TRANS-SPLICING IN TRYPANOSOMATIDS

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Background and Aims: *Trypanosoma cruzi, Trypanosoma brucei* and *Leishmania major*, usually known as TriTryps, are the causal agents of animal and human sickness, and are characterized by having complex life cycles, alternating between a mammalian host and an insect vector. Their genes are organized in long transcriptional units that give rise to polycistronic transcripts which maturate into mRNA by a process known as trans-splicing. Among those genes, an important subset is composed of multicopy genes, which play crucial roles in host invasion and immune evasion.

Methods: We performed a thorough comparison of MNase-seq data publicly available for the stages present in the insect vectors in TriTryps, generated by others and in our laboratory. We also predicted the most likely trans-splicing acceptor sites (TASs). We developed a unified and systematic analysis pipeline and applied it consistently to all datasets.

Results: We found that the average chromatin organization is very similar among the TriTryps with a mild nucleosome depletion at the TASs, and the same layout is observed in most of the genome. A detailed examination of the nucleosome landscapes resulting from different levels of chromatin digestion in *T. brucei* shows that an MNase-sensitive complex is protecting the TASs, and it is at least partly composed of histones. Additionally, comparative analysis for single and multi-copy genes in *T. cruzi* revealed a differential chromatin structure at the TASs.

Conclusions: TriTryps share distinctive chromatin organization at TASs, where NDRs are consistently formed to ensure transcript maturation, while genome-wide accessibility varies, underscoring the importance of assessing digestion levels.

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TOXOCARA CANIS LARVAE RECOGNIZE AND RESPOND TO CANINE SOMATOTROPIN THROUGH HORMONE RECEPTORS

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Background and Aims: The role of hormones in the infection and development of *Toxocara canis* is one of the most interesting aspects of the host-parasite relationship. One of the most important physiological events in puppies is their high levels of serum somatotropin, which coincides with their high capacity to develop adult worms. Therefore, the objective was to demonstrate the presence of the canine somatotropin receptor (CSR) and some effects of its stimulation in *T. canis* larvae.

Methods: To demonstrate the presence of CSR in a protein extract of *T. canis* larvae, a Dot Blot with antibodies against CSR (abcam, ab230996) was used. A fragment of the CSR gene was obtained by nested-PCR from gDNA of *T. canis* larvae, and the fragment was subsequently sequenced. The *in vitro* effect of somatotropin on *T. canis* larvae incubated with different concentrations of the hormone was measured.

Results: A positive Dot Blot for CSR was obtained in the protein extract of *T. canis*. The 420 bp amplicon obtained had a 98.64% similarity to the CSR. In the *in vitro* assay, larvae stimulated with 0.5 ng/mL accelerated their growth and increased their motility on day 5 of exposure to the hormone ($p<0.05$).

Conclusions: Our results strongly suggest that *T. canis* larvae have CSR and are able to recognize and utilize somatotropin for their development. This is likely one reason why the *T. canis* adult worms develop primarily in puppies. Funded by PAPIIT-UNAM, projects IN IN212626 and IN218726. Cátedras FES-C, CI2630 and CI2644.

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NUTRITIONAL MODULATION OF GASTROINTESTINAL TISSUE RESPONSE IN DORPER LAMBS CO-INFECTED WITH HAEMONCHUS CONTORTUS AND TRICHOSTRONGYLUS COLUBRIFORMIS

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Background and Aims: Nutritional management can directly influence the host's ability to mount an effective immune response against gastrointestinal parasites, representing a viable and sustainable strategy for controlling these infections. This study aimed to evaluate the influence of diets with different concentrate levels on the tissue response of the abomasum and small intestine of Dorper lambs artificially infected with *Haemonchus contortus* and *Trichostrongylus colubriformis*.

Methods: Forty-four male Dorper lambs were assigned to four different forage-to-concentrate diets (0%:100%, 25%:75%, 50%:50%, or 75%:25%) and two infection statuses (infected or non-infected). Lambs received 1,000 infective larvae (L3) of each parasite species every three days for 12 weeks, totaling 28,000 L3 of each species. Histopathological evaluation was performed in five different mucosal regions using H&E-stained sections.

Results: A significant effect of diet (p=0.0475) and infection (p=<0.0001) was observed on histopathologic scores in abomasal mucosa, with non-supplemented infected animals showing greater mucosal impairment, characterized by superficial epithelial damage, alterations in gastric crypts, and lymphoid hyperplasia, indicating a more intense local inflammatory response. A similar pattern was observed in the duodenum with infection (p=<0.0001) and diet*infection (p=0.0492) effect, with non-supplemented infected animals exhibiting higher scores of crypt distension, villous lesions and atrophy, whereas supplemented groups showed less severe alterations, particularly those fed with 75% concentrate. In the non-infected animals, lesions were absent or mild.

Conclusions: Adding concentrate to the diet lessened the damage to tissues, especially in the abomasum, and showed a trend of less damage in the duodenum, highlighting the importance of good nutrition in managing worm infections.

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INTERACTION OF TWO ENTAMOEBA HISTOLYTICA CLONES WITH DIFFERENT PATHOGENIC PROPERTIES WITH HUMAN INTESTINAL EPITHELIAL CELL BARRIERS

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Background and Aims: More than 50 million people worldwide are estimated to be infected with *Entamoeba histolytica (Ehis)*, leading to 26,700 deaths in 2016. Transmission of *Ehis* primarily occurs through the ingestion of infectious cysts via contaminated food or water. In the colon, cysts excyst into motile trophozoites, which proliferate by binary fission. Upon reaching the descending colon, trophozoites undergo encystation and are subsequently excreted with the feces, most often without causing clinical symptoms. In approximately 10% of infections, trophozoites penetrate the intestinal epithelium and underlying tissue, causing severe tissue damage and inflammation (amoebic colitis). Via the bloodstream, trophozoites may disseminate to extraintestinal sites, particularly the liver, where they induce abscess formation. Both manifestations can be fatal if left untreated.

Methods: The triggers underlying the invasive behavior of *Ehis* remain incompletely understood. To gain deeper insight into host–parasite interactions during invasion, the interaction between two genetically identical *Ehis* clones differing in pathogenicity, and primary human intestinal spheroid-derived epithelial cells was investigated using transepithelial electrical resistance (TEER) measurements and transcriptome analysis.

Results: Pathogenic (p) $B2^{p}$ trophozoites disrupted epithelial cell barrier integrity more efficiently than non-pathogenic (np) $A1^{np}$ trophozoites. This was accompanied by enhanced expression of pro-inflammatory genes, including *CXCL8, IL6, IL1B,* and *IL1A*, in epithelial cells following contact with $B2^{p}$ trophozoites. In contrast, $A1^{np}$ trophozoites preferentially induced increased expression of genes associated with tissue regeneration.

Conclusions: Overall, the invasion processes of $A1^{np}$ and $B2^{p}$ trophozoites differed substantially, with the $B2^{p}$ clone exhibiting more aggressive behavior and eliciting a pro-inflammatory response in host cells at mRNA level.

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SEX-SPECIFIC CPLA$_2$-DRIVEN GLYCEROPHOSPHOLIPID REMODELING SHAPES HEPATIC IMMUNITY IN VISCERAL LEISHMANIASIS

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Background and Aims: Visceral leishmaniasis (VL), caused by *Leishmania donovani* and *L. infantum*, remains a major global health challenge with limited therapeutic options. Host lipid metabolism plays a critical role in disease outcome, yet sex-specific mechanisms remain poorly defined. Here, we identify glycerophospholipid remodeling and cytosolic phospholipase A_2 (cPLA$_2$) activation as sex-dependent metabolic checkpoints governing hepatic immune responses during VL.

Methods: Untargeted lipidomics was performed on livers from male and female *Leishmania*-infected mice, followed by phospho-protein analysis and functional cPLA$_2$ inhibition. Spatial validation of lipid remodeling and cPLA2 activation was achieved using immunofluorescence and MALDI mass spectrometry imaging (MALDI-MSI).

Results: Infection induced a profound remodeling of glycerophospholipid pathways including phosphatidylcholine and lysophosphatidylcholine species, supporting enhanced cPLA$_2$-driven membrane remodeling. cPLA$_2$ phosphorylation was selectively increased in male mice, whereas females showed limited pathway activation. Pharmacological cPLA$_2$ inhibition significantly reduced hepatic parasite burden in males, but not females, demonstrating a sex-specific enzymatic dependency. Phospho-cPLA$_2$ localized predominantly to CD45$^+$ immune populations within hepatic lesions , with spatial lipidomic mapping by MALDI-MSI confirming lesion-specific glycerophospholipid remodeling.

Conclusions: Our data establish glycerophospholipid turnover and cPLA$_2$ activation as sex-dependent metabolic checkpoints linking hepatic immunity with parasite persistence. These findings underscore the necessity of incorporating sex-informed metabolic paradigms in the development of targeted VL interventions.

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CRITICAL ROLE OF BABESIA BOVIS SPHERICAL BODY PROTEIN 3 IN RIDGE FORMATION ON INFECTED RED BLOOD CELLS

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Background and Aims: *Babesia bovis* leads accumulation of infected red blood cells (iRBCs) in the brain microvasculature that results in severe clinical complications known as cerebral babesiosis. This parasite exports various proteins to the iRBCs that lead to the formation of protrusions known as "ridges" on the surface of iRBCs, which serve as sites for cytoadhesion to endothelial cells. Spherical body proteins (SBPs) are exported into iRBCs, and four proteins (SBP1-4) have been reported to date. In this study, we elucidated the function of SBP3.

Methods: Localization of SBP3 was examined by immunofluorescence assay and immunoelectron microscopy. SBP3 knockdown (KD) was performed using the glmS system and validated by Western blotting and immunofluorescence assay. Parasite growth was assessed on the KD parasites. Surface VESA-1 expression and cytoadhesion to bovine brain microvascular endothelial cells were measured. SBP3-associated host proteins were identified by immunoprecipitation and mass spectrometry (IP-MS).

Results: Localization analysis revealed that SBP3 colocalizes with VESA-1, a major parasite ligand responsible for cytoadhesion, and SBP3 is partially colocalized with SBP4. Immunoelectron microscopy showed SBP3 localized at ridges. Effective knockdown was confirmed in KD parasites and caused severe growth defects and reduced surface VESA-1 expression; however, SBP4 remained intact. Ridges were abolished, and iRBC cytoadhesion to brain endothelium was markedly decreased in KD parasites. IP-MS detected a host Band 3 complex, suggesting SBP3's association with iRBC cytoskeletal proteins.

Conclusions: This study revealed the vital role of SBP3 in ridge formation and its significance in the pathogenesis of cerebral babesiosis.

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INFECTION OF ADIPOCYTES BY LEISHMANIA INFANTUM: EVIDENCE FOR A NOVEL CELLULAR NICHE

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Background and Aims: Leishmaniasis is a parasitic disease caused by protozoa of the genus *Leishmania*. It presents a wide range of clinical manifestations, from localized cutaneous lesions to potentially fatal visceral infections. Although the mechanisms underlying the disease are not yet fully understood, the host's nutritional lipidic status appears to play a major role in the establishment, progression, and severity of infection. We suggest that parasites may exploit adipocytes as alternative reservoirs, thereby promoting their survival and persistence within the host. It is therefore crucial to determine whether, in addition to infecting macrophages, the parasites are able to migrate toward adipose cells. Such cellular plasticity could represent a significant challenge for the treatment and clinical management of leishmaniasis.

Methods: To investigate this hypothesis, macrophages, preadipocytes, and adipocytes were infected with *L. infantum* promastigotes as well as with amastigotes isolated from infected macrophages. A co-culture system using inserts was developed to generate two distinct compartments and to assess the ability of the parasites to migrate from one cell type to another. Infection was evaluated using fluorescence microscopy and electron microscopy, and infection rates were quantified.

Results: Our results demonstrate that *Leishmania* is capable of infecting adipocytes at a rate of approximately 50%, and that amastigotes can circulate between the two cell types.

Conclusions: These findings provide key insights for the development of new therapeutic and preventive strategies against leishmaniasis.

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INTERACTIONAL EXPERIMENTAL DESIGN FOR THE EFFICIENT BLOCKING OF THE PLASMODIUM INHIBITORY PROPERTY OF AN ALPHA ENDOSYMBIONT (ALONE OR IN COMBINATION) IN THE ANOPHELES MOSQUITO UN A CONTEXT OF ENDOSYMBIOTIC DIVERSITY FOR THE SUCCESS OF BIOLOGICAL CONTROL."

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Background and Aims: The biological control currently to fight against malaria holds great promise. However, the remarkable performances of certain endosymbionts, particularly their *Plasmodium*-inhibitory properties and their ability to restore mosquito susceptibility to insecticides remain subject to debate due to the lack of a suitable experimental design specifically tailored to this type of study. Such investigations, given their high level of complexity, require approaches consistent with computational biology, which is often not considered in laboratory evaluations or in natural transmission studies.Integrating then the mosquito microbiota, immune expressions, and its insecticide resistance genes.

Methods: For our expérience, we used two groups of mosquitoes Anopheles(field and control and fed with gametocyteds blood by Direct Membrane Feeder Assay.. Seven and fourteen day post-infection we did the stomaches and salivaries glands dissection to find oocysts and sporozoits infections. We completed this steps by molecular biology analysis(PCR.qPCR and ONGS) to establish interactions Endosymnbonts-Parasite plasmodium-Genes.

Results: As results we make à board with many events of one or many endosymbionts Corralated with mosquitoes immunes genes to détermine interactions mechanisms in the parasite blocking property.This results shows a system with many interactions which could occurs biological control failure.

Conclusions: Our study enable the elucidation of several observed mechanisms and the identification of the specific role of each endosymbiont (whether in association or not) previously characterized as candidate symbionts. It would be the establishment of an endosymbiotic database supporting more informed choices by different scientific teams according to their specific objectives, thereby contributing to the overall success of biological malaria control programs.

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TOXOCARA CANIS: A SILENT THREAT TO LONG-TERM INFECTED HOSTS

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Background and Aims: *Toxocara canis,* a widespread parasite of canids, infects wide range of paratenic hosts, including humans. It is an overlooked agent of nervous system infections, with few but severe human case reports described. Previous studies mainly focused on acute effects of high-dose infections, whereas our study investigates the long-term impact of low to high infection doses. We bring investigation of up to 100 weeks long experimental infections of mice with focus on the pathologies in the brain in combination with their physical condition and motor skills.

Methods: BALB/c mice were infected with 10, 100, and 1000 *T. canis* larvae/mouse and observed within 100 weeks after infection. Successful infection was confirmed by specific anti-*T. canis* excretory-secretory antigens IgG antibody response. Physical condition and motor skills were evaluated, and brain pathology was analyzed histologically along with larvae counts in the brain and selected visceral organs at defined intervals.

Results: Necrosis, cellular infiltrations, and activated microglia/macrophages were observed in moderate- and high-dose groups, while hemorrhages occurred in all infected mice. Alterations in general health and motor performance appeared in all infected groups, with earlier onset and greater severity at higher doses. Specific IgG antibody levels corresponded to infection dose.

Conclusions: Our results demonstrate the negative effect of long-term *T. canis* infection on the paratenic host, particularly at moderate and high doses. Even low-dose infections caused brain pathology, though physical and motor functions remained comparable to controls. This study highlights the persistent and dose-dependent neurological consequences of *T. canis* infection in mice as presented in our paper (Bernardová et al. 2025).

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ULTRASTRUCTURE EXPANSION MICROSCOPY REVEALS TWO NEW CYTOSKELETAL STRUCTURES DURING FLAGELLAR POCKET COLLAR BIOGENESIS IN TRYPANOSOMA BRUCEI

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Background and Aims: *Trypanosoma brucei* causes Human African Trypanosomiasis (sleeping sickness) and animal African trypanosomiasis (nagana), diseases that burden millions of people and livestock in sub-Saharan Africa. The flagellar pocket (FP), the sole endo- and exocytic organelle in trypanosomes, is essential for nutrient uptake, surface protein recycling, and host immune evasion. FP formation depends on a cytoskeletal structure called the flagellar pocket collar (FPC). Despite its critical role, the mechanisms governing FPC biogenesis during cell division remain poorly understood. This study aimed to characterize the spatiotemporal dynamics of FPC assembly across the *T. brucei* cell cycle.

Methods: Ultrastructure expansion microscopy (U-ExM) was applied to *T. brucei* cells at defined cell cycle stages. Immunofluorescence tracked the localization dynamics of key FPC proteins, BILBO1, MORN1, and BILBO2, alongside the hook complex (HC). Formation of the new microtubule quartet (nMtQ) was mapped in relation to new flagellum growth.

Results: FPC biogenesis occurs *de novo* yet remains closely associated with the existing FPC and nMtQ. Two previously undetected transient structures were identified: the proFPC, an early precursor assembly preceding mature FPC formation, and the FPC-interconnecting fiber (FPC-IF), a transient filamentous structure linking the nascent FPC to existing cellular components during cytokinesis. Both structures incorporate known FPC proteins and appear critical for coordinating cytoskeletal organization during cell division.

Conclusions: These findings reveal new steps in FPC biogenesis in *T. brucei*, advancing our understanding of how the parasite assembles and maintains an organelle essential for its survival and immune evasion, and opening new avenues for antiparasitic intervention.

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ATOMIC FORCE MICROSCOPY REVEALS CYTOADHESION MECHANISMS OF PLASMODIUM FALCIPARUM INFECTED RED BLOOD CELLS

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Background and Aims: Malaria remains among the deadliest infectious diseases worldwide, with most fatal cases caused by *Plasmodium falciparum*. Severe disease results from parasite-host interactions, most notably the cytoadhesion of infected red blood cells (iRBCs) to the vascular endothelium of vital organs. This process is mediated by the *P. falciparum* erythrocyte membrane protein 1 (PfEMP1) family, large transmembrane proteins concentrated in membrane protrusions termed knobs. Although cytoadhesion is central to malaria pathogenesis, the biophysical mechanisms linking PfEMP1 binding properties, knob architecture, endothelial cell receptor (ECR) profiles, and disease severity remain poorly understood.

Methods: In this project, the molecular binding properties between iRBCs and ECRs will be quantified using single-cell force spectroscopy (SCFS), including measurements of binding events, rupture forces, bond lifetimes, and dissociation kinetics. Second, the influence of PfEMP1 on iRBC morphology and mechanics will be characterized, focusing on knob size and elasticity. PfEMP1 distribution will be analyzed by molecular recognition imaging with different binding partners. Third, synthetic peptides derived from ECR binding epitopes will be iteratively optimized and tested as potential inhibitors of cytoadhesion.

Results: Preliminary observations revealed distinct adhesion behaviors, with rolling interactions via CD36 and stable adhesion to other ECRs such as ICAM-1. SCFS demonstrated receptor-specific differences in binding behavior, indicating distinct force regimes and bond stabilities. These findings support the hypothesis that PfEMP1 variant, endothelial cell origin, and physical conditions determine cytoadhesion and influence disease severity.

Conclusions: This project provide insight into how cytoadhesion contribute to malaria pathogenesis at molecular level, and aims to generate therapeutically applicable active substances against malaria.

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THE SECRET LIFE OF PARASITES: IN VITRO 3D CELL CULTURE MODELS AND ADVANCED MICROSCOPY PROVIDE UNPRECEDENTED INSIGHTS INTO EARLY HOST:PARASITE INTERACTIONS

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Background and Aims: The earliest migratory stages of the zoonotic trematode *Fasciola hepatica* represent the most clinically and therapeutically important window for intervention. Yet this "early infection gap" remains critically understudied because parasites are tiny (~140 µm) relative to their mammalian hosts, making them difficult to study *in vivo*. We aim to close this gap using *in vitro* hepatic models paired with multimodal imaging to resolve host:parasite interactions and quantify parasite behaviour.

Methods: We have established multiple hepatocyte models, including spheroids derived from HepG2 and Primary Human Hepatocytes, that support the growth and development of *F. hepatica* newly excysted juveniles (NEJ) *in vitro*. Viability, growth, and development were quantified over 21 days and benchmarked against two-dimensional (2D) and cell-free conditions. Parasite development and behaviour were captured using micro computed tomography (microCT), live cell imaging, fluorescent confocal, and scanning electron microscopy. We developed a behavioural ethogram to quantify host:parasite interactions (e.g., activity, feeding, rest), while an intravital microscopy (IVM) workflow was established to benchmark *in vitro* outcomes against *in vivo* invasion and migration.

Results: NEJ exhibited significantly increased survival, growth, and development relative to controls, alongside active exploration, attachment, and feeding, suggesting host cue sensing. Imaging showed parasite interaction with, and uptake of, host material, with maturation of musculature and tegumental features. IVM provided the first real-time visualisation of invasion and migration in a murine model.

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Conclusions: Using a range of advanced technologies, we present the first biologically relevant co-culture systems for any zoonotic helminth, the outcomes of which stand to revolutionise helminth biology and therapeutic discovery.

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ACANTHAMOEBA–FUNGUS INTERACTIONS MODULATE FUNGAL SECONDARY METABOLITE PROFILES AND ANTIPROTOZOAL ACTIVITY

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Background and Aims: *Acanthamoeba* spp. are free-living protozoa capable of causing severe infections and establishing complex trophic interactions with coexisting microbiota. Some microorganisms can evade the amoebal lysosomal system, shifting *amoeba* from predator to reservoir and inducing genotypic and phenotypic changes, including increased virulence and molecular reprogramming of secondary metabolite (SM) production. In this context, this study investigated *Acanthamoeba*–fungus interactions by evaluating key processes, phagocytosis, amoebal cell death, and fungal germination and assessing SM production and the antiamoebal activity of fungal extracts derived from these interactions.

Methods: *Acanthamoeba castellanii* (ATCC 50492) was co-cultured with fungal (*Aspergillus niger, Penicillium* spp., *Fusarium oxysporum, Phialomoniopsis curvata*, and *Paecilomyces lilacinus*). Interactions were monitored by quantitative microscopy over 48 h. Following post-interaction fungal growth, biomass was extracted using methanol, dichloromethane:methanol (1:1), and hexane, yielding polar, apolar, and lipid fractions; non-interacting extracts served as negative controls. Antiamoebal activity against trophozoites was assessed over 96 h using the AlamarBlue® assay.

Results: Interactions indicated fungal resistance to amoebal predation, with amoebal mortality reaching ~7% after 48 h, concomitant with conidial phagocytosis rates of ~30% for *A. niger* and *Penicillium* spp. at 6 h. Germination was strain-dependent, peaking at 12 h for *F. oxysporum* (~2%) and at 48 h for *P. curvata* (~4%). Apolar fractions from *A. niger* and *Penicillium* spp. showed moderate antiamoebal activity (~10% mortality), whereas *F. oxysporum* extracts were more active after interaction. Notably, *P. curvata* produced the most active extracts, reaching up to 15% mortality.

Conclusions: These findings highlight amoeba–fungus interactions as a model for host–parasite interactions and antiprotozoal compound discovery.

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FETAL REVERSION IS ENHANCED IN CO-INFECTED INTESTINE AND IS ASSOCIATED WITH TGFB RECEPTOR SIGNALING

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Background and Aims: Intestinal plasticity is central to the robudt regenerative capacity of the intestinal epithelium upon tissue damage and stress damage or stress. Parasitic worms such as *Heligmosomoides bakeri* (*Hb*), a rodent gastrointestinal nematode(GIN), can influence this process to support host survival. Hb-induced fetal reversion, where adult intestines re-express fetal gut genes, has been shown in short-term intestinal organoid cultures and early infections, but it is unclear whether this occurs in more natural settings or affects infection outcomes. We used two models that better mimic real-world exposure, trickle/grazing model (repeated small doses over time) and co-infection (with *Toxoplasma gondii*), in addition to the standard single-dose bolus model.

Methods: We used qPCR, immunofluorescence and RNAscope to investigate adult/fetal reversion (*Clu, Sca1, Olfm4, Ki67 markers*), its mechanisms (Tgfbr2) and impact on intestinal epithelium and the immune correlates (*IL-13, IL-10,* IFNγ).

Results: *Hb*-only mice showed reduced expression of the adult stem cell marker Olfm4 and Ki67, and *IFNγ* and *IL-22* compared to co-infected mice. In contrast, co-infected mice had higher expression of fetal reversion genes (Clu, Sca1) and *IFNγ* and *IL-10*. RNAscope and immunofluorescence localized fetal reversion and Tgfbr2 near *Hb*, and this was linked to reduced differentiation of secretory lineages (goblet, tuft, and Paneth cells), regardless of infection model or timing.

Conclusions: *Hb* induces fetal reversion, and this effect is amplified during multiple infections. TGFβ signaling appears to play a role in this process. Understanding how GINs affect intestinal stem cells could improve insight into GIN pathology and inform future therapies for inflammatory gut diseases, including Crohn's disease.

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TOXOPLASMA GONDII-DERIVED IST ATTENUATES AB-INDUCED NEURITE DEGENERATION AND NEUROTOXICITY

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Background and Aims: In Alzheimer's disease, excessive Aβ accumulation and aggregation lead to amyloid plaque formation. Aβ is generated by sequential cleavage of amyloid precursor protein (APP) by β-secretase (BACE1) and γ-secretase via the amyloidogenic pathway. Accumulated Aβ activates STAT1/NF-κB signaling, upregulating BACE1 expression and forming a pathogenic positive feedback loop that promotes neuroinflammation, oxidative stress, and neurite degeneration. *Toxoplasma gondii*-derived inhibitor of STAT1 transcription (TgIST) inhibits STAT1 transcriptional activity. In this study, we investigated whether TgIST suppresses BACE1 expression by inhibiting STAT1/NF-κB signaling and protects Aβ-damaged neurons.

Methods: We established a TgIST-expressing SH-SY5Y cell line by retroviral transfection with the pBABE-HAII-TgIST plasmid, and the interaction between TgIST and STAT1 was confirmed by co-immunoprecipitation (Co-IP). Following Aβ treatment, BACE1-related transcription factors and inflammatory mediators were analyzed. Reactive oxygen species (ROS) production was assessed using the DCFDA assay, and neurite degeneration was evaluated by Tubulin Tracker staining.

Results: TgIST was successfully expressed in SH-SY5Y cells and interacted with STAT1. In TgIST-expressing cells, the expression of STAT1 was significantly reduced, and subsequently, BACE1-related transcription factors and inflammatory mediators induced by Aβ were decreased. Furthermore, TgIST markedly attenuated Aβ-induced ROS production and restored neurite beading and fragmentation to baseline levels.

Conclusions: Our findings demonstrate that TgIST downregulates the STAT1/NF-κB signaling axis, suppressing BACE1-related transcription factors and inflammatory mediators. Moreover, TgIST alleviates Aβ-induced oxidative stress and neurite degeneration, leading to reduced neurotoxicity. Collectively, these results suggest that TgIST has a potential therapeutic candidate for Alzheimer's disease through modulation of Aβ pathology.

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EVALUATING THERAPEUTIC INTERVENTIONS AGAINST NAEGLERIA FOWLERI USING A FLOW CYTOMETRY-BASED BEAD UPTAKE ASSAY

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Background and Aims: *Naegleria fowleri*, the brain-eating amoeba, is rare but deadly. Documented cases in the USA include 167 cases with 163 deaths (1962–2025). No treatments are currently FDA or EMA approved, but several candidate drugs have been mentioned by the CDC as potentially effective.

Methods: We evaluated the activity of amphotericin B, azithromycin, fluconazole, miltefosine, and rifampin using a novel flow cytometry-based bead uptake assay. Amoebae were cultured xenically (with HeLa human cervical carcinoma cells as a feeder layer) or axenically, then drug-treated and exposed to 1 µm fluorescent beads, approximately the size of bacterial cells. We evaluated uptake by measuring intracellular fluorescence via flow cytometry and confirmed with confocal microscopy.

Results: The bead uptake assay showed a linear dose-response curve across several drugs, and was compared to control cells treated with cytochalasin D, a phagocytosis inhibitor. Of the drugs tested, amphotericin B and azithromycin performed best, reducing brain-eating amoeba feeding by 40%.

Conclusions: Although the in vivo feeding activity of brain tissue may not be directly phagocytic of large eukaryotic cells, this bead uptake assay can be used as a surrogate measurement of amoebistatic or amoebicidal activity and can be scaled up for high-throughput analysis. It is hoped this technique will lead to additional drug discovery and testing to inform clinical practice against this rare but deadly parasite.

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DEVELOPING AN IN VITRO MODEL OF FEMALE GENITAL SCHISTOSOMIASIS

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Background and Aims: Female genital schistosomiasis (FGS), caused by *Schistosoma haematobium*, affects up to 56 million women in sub-Saharan Africa and leads to significant reproductive morbidity and increased susceptibility to HIV and HPV infection. Despite this burden, no specific treatment recommendations exist, as praziquantel eliminates adult worms but does not resolve egg-induced inflammation and fibrosis. FGS is excessively difficult to study under laboratory conditions because, whilst *S. haematobium* can be maintained in rodents, the worms do not reach the venous plexus of the rodents' bladder and the urogenital lesions are thus not replicated in the animal model. Progress in understanding FGS pathophysiology and identifying therapeutic targets is thus limited by the lack of relevant preclinical models. This study aims to establish a physiologically relevant in vitro model of FGS to recapitulate egg-induced granulomatous inflammation and support drug screening efforts.

Methods: Mouse vaginal organoids were generated using an adapted long-term culture protocol to preserve epithelial architecture and function. Organoids were exposed to schistosome eggs and co-cultured with immune cells to emulate granuloma formation and inflammatory responses associated with FGS.

Results: Vaginal organoids were successfully established and maintained in three-dimensional culture. In co-culture conditions, a cellular layer was observed forming around a subset of schistosome eggs and preliminary cytokine quantification suggests the onset of a Th2-type immune response.

Conclusions: This in vitro organoid-based model represents a promising platform to study FGS-associated pathology and to evaluate therapeutic strategies targeting egg-driven inflammation and tissue damage beyond antiparasitic treatment.

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CHARACTERIZING FUSION-ASSOCIATED STERILITY IN PLASMODIUM BERGHEI

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Background and Aims: Work in *Plasmodium* has identified HAP2 as an ancestral eukaryotic gamete fusogen, revealing a conserved mechanism of sexual reproduction across protists, plants and animals. Expressed on male gametes, HAP2 drives fusion with female macrogametes, enabling zygote formation and parasite transmission. Disruption of HAP2 results in male sterility and complete transmission blockade.

Methods: In search of putative HAP2 interacting proteins we modelled male-sterility genes from our recent functional screens using AlphaFold. We identified PBANKA_1329000 as a *P. berghei* structural counterpart. *In vivo* and *in vitro* assays were conducted to characterise the phenotype of the gene deletion and protein localisation. A co-IP was performed to identify interaction partners of PBANKA_1329000.

Results: Functional studies show that knockout of PBANKA_1329000 microgametes fail to progress from adhesion to fusion, leading to a complete absence of ookinete *in vitro* and oocyst formation in the mosquito midgut. The localization of PBANKA_1329000 seems to be limited to the male gametocyte. A co-IP experiment pulled down HAP2 from inactivated gametocytes, confirming the predicted interaction, along with additional candidates enriched in male gametocytes.

Conclusions: This identifies PBANKA_1329000 as a second essential fusion factor, though its precise function remains unknown. Ongoing work is focused on verifying the interaction partners, with the aim of elucidating its role in the broader fusion machinery. These findings provide new insights into the molecular basis of malaria transmission and highlight the evolutionary convergence of fusion mechanisms.

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DIAGNOSTICS, HOST-PARASITE INTERACTION AND EMERGING TECHNOLOGIES / HOST-PARASITE MOLECULAR AND CELLULAR INTERACTIONS

ELUCIDATING THE MOLECULAR BASIS FOR THE RECOGNITION OF HUMAN BASIGIN BY PLASMODIUM VIVAX TRYPTOPHAN-RICH ANTIGENS (PVTRAGS)

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Background and Aims: *Plasmodium vivax* is the most widespread human-infective malaria parasite. Host reticulocyte invasion by the merozoite is an essential life cycle event. Yet, our understanding of interactions at the merozoite-reticulocyte interface is limited. PvTRAgs are merozoite surface antigens mediating reticulocyte binding. While some PvTRAgs have been shown to bind basigin, the structural basis for basigin recognition is unknown and how this event relates to reticulocyte invasion is unclear. Given the knowledge gap in *P. vivax* biology and the importance of PvTRAgs in merozoite biology, tackling these issues is expected to generate novel findings that may support *P. vivax* vaccine design efforts.

Methods: PvTRAgs and human basigin were recombinantly produced and purified in *E. coli*. The PvTRAg-basigin interactions were further investigated via surface plasmon resonance (SPR) and macromolecular X-ray crystallography (MX). Furthermore, anti-PvTRAg single domain antibodies (sdAbs) were obtained through llama immunization.

Results: Eight different PvTRAgs and basigin have been successfully produced with high yields. Furthermore, SPR experiments reveal PvTRAg38-basigin binding with a µM affinity. Llama immunization resulted in a wide array of anti-PvTRAg sdAbs. Interestingly, some exhibited cross-reactivity to different PvTRAgs. These sdAbs will be further investigated for their interaction (and invasion) blocking properties. Crystallization trials for the PvTRAg38-basigin complex are ongoing but are yet to be successful. These efforts will be aided by non-interaction blocking sdAbs.

Conclusions: This ongoing research will provide pivotal insights into the structure-function relationship of PvTRAgs. The focus now lies on determining the PvTRAg38-basigin structure and the identification and subsequent structural characterization of interaction-blocking sdAbs.

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IDENTIFICATION OF POTENT SINGLE-DOMAIN ANTIBODIES AGAINST THE MALARIA SPOROZOITE THROUGH SYNTHETIC SINGLE-DOMAIN ANTIBODY LIBRARIES CONTAINING UNCONVENTIONAL DIVERSIFICATION STRATEGIES

Line De Vocht[1], Natalia Smiejkowska[1], Yani Sim[1], Yi Li[2], Vincent Geoghegan[3], Gavin Wright[3], Nick Devoogdt[4], Serge Muyldermans[5], Guy Caljon[6], Yann Sterckx[1]
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Background and Aims: Novel malaria intervention strategies are direly needed. Antibodies (Abs) are potent tools for parasite neutralisation. Besides conventional Abs, the mammalian immune repertoire contains so-called unconventional diversification strategies. Prime examples of unconventional Ab fragments are camelid single-domain Abs (sdAbs), and VH CDR3 domain insertions found in human and bovine Abs (LAIR1 and “stalk-and-knob” domains, respectively). Extensive knowledge on the camelid sdAb structure-function relationship enables synthetic library construction. This project aims to harness the potential of synthetic sdAb libraries with unconventional diversification strategies to tackle the malaria sporozoite.

Methods: Two synthetic sdAb libraries will be constructed by grafting human LAIR1 and bovine “stalk-and-knob” domains onto the CDR3 of a camelid sdAb scaffold. As a proof-of-concept, sdAb-LAIR1 and sdAb-KNOB constructs were tested for their recombinant production in and purification from *E. coli*, thermostability by thermal shift assay (TSA), structural integrity by small-angle X-ray scattering (SAXS), and antigen interaction by enzyme-linked immunosorbent assay (ELISA).

Results: The engineered proteins could be obtained from *E. coli* with yields of at least 3 mg/L. TSA indicates a two-step unfolding caused by the inserted domains and the sdAb scaffold, respectively. While SAXS demonstrates that the obtained proteins are well-behaved in-solution and possess the

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expected structural features, ELISA confirms that these bind their target antigens with the same affinity as the isolated LAIR1 and “stalk-and-knob” domains.

Conclusions: The evaluated proteins are properly folded and functional entities capable of productive antigen recognition. Next, synthetic sdAb-LAIR1 and sdAb-KNOB libraries will be constructed and screened against the malaria sporozoite for novel target finding.

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NON-FALCIPARUM SPECIES AND SUBMICROSCOPIC INFECTIONS IN THREE EPIDEMIOLOGICAL MALARIA FACETS IN CAMEROON

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Background and Aims: There are growing reports on the prevalence of non-falciparum species and submicroscopic infections in sub-Saharan African countries but little information is available from Cameroon.

Methods: A hospital-based cross-sectional study was carried out in four towns (Douala, Maroua, Mayo-Oulo, and Pette) from three malaria epidemiological strata (Forest, Sahelian, and Soudanian) of Cameroon. Malaria parasites were detected by Giemsa light microscopy and polymerase chain reaction (PCR) assay. Non-falciparum isolates were characterized and their 18S gene sequences were BLASTed for confrmatory diagnosis.

Results: PCR assay detected malaria parasites in 82.4% (98/119) patients, among them 12.2% (12/98) were asymptomatic cases. Three Plasmodium species viz. P. falciparum, P. ovale curtisi and P. vivax, and two co-infection types (P. falciparum+P. vivax and P. falciparum+P. ovale curtisi) were found. The remaining infections were mono–infections with either P. falciparum or P. ovale curtisi. All non–falciparum infections were symptomatic and microscopic. The overall proportion of submicroscopic infections was 11.8% (14/119). Most asymptomatic and submicroscopic infection cases were self-medicated with antimalarial drugs and/or medicinal plants. On analysis, P. ovale curtisi sequences were found to be phylogenetically closer to sequences from India while P. vivax isolates appeared closer to those from Nigeria, India, and Cameroon. No G6PD-d case was found among non-falciparum infections.

Conclusions: This study confrms our previous work on circulation of P. vivax and P. ovale curtisi and the absence of P. knowlesi in Cameroon. More studies are needed to address non-falciparum malaria along with submicroscopic infections for efective malaria management and control in Cameroon.

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FUNCTIONAL CHARACTERISATION OF RAN GTPASE IN TRYPANOSOMA CRUZI

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Background and Aims: *Trypanosoma cruzi* relies on tightly regulated nucleocytoplasmic transport to coordinate gene expression, cell cycle progression, and differentiation. Ran GTPase is a central regulator of nuclear trafficking in eukaryotes, yet its role in *T. cruzi* remains poorly understood. This study aimed to functionally characterise Ran GTPase through genetic editing and to evaluate its contribution to parasite growth, development, and intracellular organisation.

Methods: Targeted CRISPR Cas9 editing generated hemi-knockout parasite lines carrying a disrupted Ran allele. Growth kinetics were monitored in epimastigote cultures, while in vitro metacyclogenesis assays assessed differentiation efficiency. Subcellular localisation was examined using fluorescence microscopy and tagged constructs. Additional phenotypic analyses evaluated cell morphology, nuclear distribution patterns, and overall parasite fitness compared with wild-type controls.

Results: Edited parasites displayed reduced proliferation rates and significant impairment in differentiation into metacyclic forms. Localisation studies revealed predominantly nuclear and perinuclear signals consistent with roles in nucleocytoplasmic transport. Phenotypic analyses indicated altered cell cycle progression, morphological changes, and decreased viability, suggesting that disruption of Ran-mediated pathways affects essential regulatory networks required for developmental progression.

Conclusions: Functional disruption of Ran GTPase compromises growth, differentiation capacity, and cellular organisation in *T. cruzi*. These findings highlight nucleocytoplasmic transport as a critical process for parasite biology and support Ran as a promising druggable target. The study provides new mechanistic insight into developmental regulation and encourages further exploration of nuclear transport pathways for antiparasitic intervention strategies. Future studies integrating multi omic approaches and in vivo infection models will further validate therapeutic relevance.

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DESCRIBING THE ROLE OF PHOSPHOLIPASE A2 ACTIVITY IN THE LYSOPHOSPHOLIPID METABOLISM AND INFECTIVITY OF TRYPANOSOMA CRUZI

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Background and Aims: The production of lipid mediators, among them lysophosphatidylcholine C18:1 (LPC-C18:1), a structural analogue to the mammalian platelet activation factor (PAF) is thought to favour the development of microvascular thrombosis during chronic *Trypanosoma cruzi* infection. The molecule is also thought to be synthesized by enzymes with phospholipase A2 activity (PLA2), also potentially important for parasite development and infection. We aimed to confirm the expression of two genes with PLA2 activity in *T. cruzi*, and to determine their role as pathogenicity factors during the differentiation and invasion of host cells, and in the production of phospholipid mediators.

Methods: Using a CRISPR-cas9 system, we produced fluorescent versions of two proteins with PLA2 activity present in the *T. cruzi* genome ($PLA2_A$ and $PLA2_B$). The same system was used to produce a parasite line lacking $PLA2_A$ activity, and to determine its role during metacyclogenesis and infection of mammalian cells. An add-back experiment was carried out to confirm a recovery of the wild-type phenotype upon re-introduction of te $PLA2_A$ gene.

Results: We observed that $PLA2_{A/B}$ localized to the posterior end of *T. cruzi* epimastigotes, likely associated with reservosome membranes. Deletion of the $PLA2_A$ gene led to disrupted metacyclogenesis and infection of mammalian cells, but not to reductions in LPC-C18:1 production.

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Transfection with an add-back plasmid containing the $PLA2_A$ gene led to a partial recovery of the parental line phenotype.

Conclusions: Our results demonstrate that PLA2 activity plays a role in metacyclogenesis and in the invasion of host cells through a mechanism independent of LPC-C18:1 production.

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STUDY OF FIBRINOGEN-RELATED PROTEIN (FREP) GENOMIC DIVERSITY USING GENOMES OF BIOMPHALARIA GLABRATA ISOLATES PRESENTING CONTRASTED SUSCEPTIBILITY TO SCHISTOSOMA MANSONI : AN IN SILICO BASED APPROACH

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Background and Aims: Fibrinogen-RElated Proteins (FREP) are multigenic family firstly identified in *Biomphalaria glabrata* (Bg), the main intermediate host in South America of *Schistosoma mansoni*, the parasite responsible for human intestinal schistosomiasis. FREP are lectins that play key role as pathogen recognition receptors involved in parasite detection. Recent advances in next-generation sequencing have enabled the study of FREP genes diversity across different *Bg* strains exhibiting varying levels of compatibility with *S. mansoni*. The aim of this study was to characterize the diversity and evolution of the FREP genes family within and between these genomes.

Methods: FREP sequences were retrieved from publicly available *Bg* genomes using BLASTp, followed by manual curation to identify, extend or correct full-, partial- and pseudo-gene. Comparative phylogenetic analyses were then conducted across Bg isolates (Sanger, Mline and BS90).

Results: Genomes screening led to improve the annotation of 28% to 57% of FREP genes. The less compatible Bg isolate harbors 62% more genes than the most compatible one. Multiple sequence alignments revealed substantial variations in both gene length and sequence for some FREP subfamilies within and between isolates. Phylogenetic analyses indicated isolate-specific expansions of FREP subfamilies, and additional subfamilies were detected exclusively in less compatible isolate.

Conclusions: Overall, the clustering of FREP loci, evidence of gene duplication, and the presence of pseudogenes support the expansion of this multigenic family in *Bg*. The findings suggest a potential link between FREP diversity and host compatibility with *S. mansoni*, although further investigation is required.

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LEISHMANIA AMAZONENSIS INFECTION ALTERS HOST CELL LIPID METABOLISM THROUGH REGULATION OF STEAROYL-COA DESATURASE-1

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Background and Aims: Leishmaniasis, a neglected disease caused by *Leishmania* parasites, manifests from self-healing skin lesions to fatal visceral infections. Current treatments are limited by toxicity and resistance, and no human vaccine is available. The parasites reside inside macrophages and survival relies on manipulation of their functions. Infection has been shown to alter host lipid metabolism to support parasite persistence and modulate inflammatory responses; however, the mechanisms remain poorly understood. Therefore, we set out to identify *Leishmania*-driven changes in the host cell proteome associated with reprogramming of lipid metabolism.

Methods: Comparative proteomics was performed on mouse bone marrow–derived macrophages (BMDMs) infected or not with *Leishmania amazonensis*. Lipid droplets were analyzed by confocal immunofluorescence. SCD1 impact on parasite replication was assessed using BMDM derived from SCD1 knock-out mice and pharmacological inhibition.

Results: Proteomic analysis revealed that *L. amazonensis* infection upregulates a subset of proteins, including stearoyl-CoA desaturases SCD1 and SCD2, key enzymes involved in monounsaturated fatty acid biosynthesis. Confocal imaging showed that infection induces lipid droplet formation in macrophages in an SCD1-dependent manner. Consistently, SCD1 deficiency led to a reduced infection index in BMDMs, an effect phenocopied by an SCD1 inhibitor.

Conclusions: These findings hint at a parasite-driven SCD1-dependent rewiring of host lipid metabolism that promotes infection. Ongoing studies combining proteomics data validation with reverse and forward genetics approaches and gain- and loss-of-function assays will help us test this hypothesis. Elucidating these mechanisms will shed light on the molecular basis of host cell remodelling of lipid metabolism during *Leishmania* infection to identify possible targets for therapeutic intervention.

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BALANCING PERFORMANCE AND COST: EXPLORING HOST-PARASITE INTERACTION OF ENTAMOEBA HISTOLYTICA DURING INTESTINAL INVASION

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Background and Aims: *Entamoeba histolytica* is a unicellular protozoan parasite and the causative agent of amoebic colitis and amoebic liver abscess. The parasite is transmitted through contaminated food or water. Its life cycle contains two main stages: the permanent stage (cyst) and the motile stage (trophozoite). We aim to further understand the mechanism underlying amoebic colitis by investigating how cysteine peptidases of *E. histolytica* contribute to parasite pathogenicity, and by assessing whether the Caco-2 cell model serves as a cost-effective and reliable alternative to organoid systems in terms of data quality.
We established different *E. histolytica* clones in our lab: the non-pathogenic (np) $A1^{np}$ clone and the pathogenic (p) $B2^{p}$ clone. These clones originate from the same isolate but differ in their pathogenicity (amoebic liver abscess formation). Currently, we facilitate a Caco-2 cell monolayer model to investigate the host-pathogen interactions and monolayer disruption.

Methods: Our methods to investigate the colon invasion and pathogenicity include but are not limited to measuring the transepithelial electrical resistance, immunofluorescence staining, transcriptome analysis and cysteine peptidase assays.

Results: We demonstrated that inhibition of cysteine peptidase activity altered monolayer disruption by $A1^{np}$ and $B2^{p}$ trophozoites compared with their non-inhibited counterparts. Moreover, monolayer disruption differed between the pathogenic $B2^{p}$ trophozoites and the non-pathogenic $A1^{np}$ trophozoites, with $B2^{p}$ trophozoites causing more pronounced monolayer disruption. Transcriptome data of Caco-2 cell monolayer showed differentially expressed genes after incubation with the $A1^{np}$ and $B2^{p}$ clones.

Conclusions: The obtained data was compared with previously obtained colon organoid data regarding quality and cost efficiency of the Caco-2 cell monolayer.

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TRYPANOSOMA CRUZI EXTRACELLULAR VESICLES AS KEY MEDIATORS OF INVASION IN THE CONTEXT OF CHAGASIC CARDIOMYOPATHY

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Background and Aims: Chagasic cardiomyopathy, which the most severe form of Chagas disease, impacting one third of chronic patients. T. cruzi trypomastigotes' molecular invasion mechanisms and associated regulatory processes are mediated by parasite release particles such as extracellular vesicles (EVs), that have an impact on immune response and host tissue functions. The present study aims to clarify the interaction of T. cruzi EVs with the host in the context of cardiomyopathy.

Methods: In invasion assay, human cardiomyocyte cells (AC16) were treated using different EV concentrations before infection with T. cruzi trypomastigote parasites. In parallel, AC16 cells had been exposed to EVs labeled with PKH26 for 1, 6, 24, and 48 hours. The interaction between cells and EVs was examined using Confocal Microscope and an Amnis Image Stream Cytometer.

Results: Our results indicates that parasite's EV enhance parasite invasion into host cells. Confocal microscopy images showed that AC16 cells take in EVs at different points, indicating their location near the nuclear membrane. Image Stream analysis revealed the migration of taken in EVs from the plasma membrane to the nuclear membrane, as well as the co-localization of nuclei, EVs, and tagged trans-sialidase.

Conclusions: EVs play an essential function in the pathophysiology of Chagas cardiomyopathy by promoting parasite invasion into cardiomyocytes. EVs influence parasite-host interactions by increasing parasite invasion. Additionally, the co-localization of EVs with trans-sialidase and their location close to the nuclear membrane point to an intracellular mechanism that may affect cellular processes and the development of the disease. Supported by FAPESP 2024/15997-5, CNPq, and CAPES.

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LECTIN-MEDIATED INTERACTIONS IN THE GUT OF MECCUS PALLIDIPENNIS DURING TRYPANOSOMA CRUZI METACYCLOGENESIS

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Background and Aims: The successful transmission of *Trypanosoma cruzi* depends on complex molecular interactions within its triatomine vector. In Mexico, *Meccus pallidipennis* is an epidemiologically relevant species, yet the relationship between gut protein glycosylation and parasite metacyclogenesis remains poorly understood. This study aimed to characterise and compare protein and glycoprotein profiles in the anterior midgut (AMG) and rectum (RE) of male and female *M. pallidipennis*, and to assess their association with *T. cruzi* metacyclogenesis.

Methods: Adult triatomines were experimentally infected with the *T. cruzi* Morelos isolate (TcI). Protein profiles from AMG and RE were analysed by SDS-PAGE and two-dimensional electrophoresis. Glycosylation patterns were evaluated using lectin blotting with ConA, WGA and PNA. Parasite burden and metacyclogenic index were quantified in both sexes.

Results: Marked regional and sex-dependent differences were observed. The RE showed lower protein overlap between sexes than the AMG, indicating functional sexual dimorphism. Infected females exhibited a greater diversity of glycoproteins in the RE and a significantly higher parasite load. Consistently, the metacyclogenic index was higher in females than in males, suggesting a gut environment more permissive to parasite differentiation.

Conclusions: Sex-specific differences in gut protein and glycoprotein profiles of *M. pallidipennis*, particularly in the rectum, are associated with enhanced *T. cruzi* metacyclogenesis in females. These findings highlight the importance of vector sexual dimorphism in parasite development and may inform future strategies to disrupt vector–parasite interactions.

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COLONIC MUC2 MUCUS RICH IN SIALO-FUCOSYLATED GLYCANS ENHANCES ENTAMOEBA HISTOLYTICA VIRULENCE AND MUCOPHAGY

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Background and Aims: The MUC2 mucus bilayer are the first line of innate host defence against colonic pathogens, including the parasite *Entamoeba histolytica* (*Eh*). Alterations in MUC2 production and glycosylation adversely affects epithelial barrier function and protection against disease. Here, we interrogated if mutant MUC2 can alter *Eh* virulence and pathogenicity.

Methods: Two cell lines were used: WT and a hypersecretory *Mut* LS174T goblet-like clone that produce sialylated and fucosylated mucus. Mucus secretion in WT and *Mut* cells was quantified by ^{3}H-metabolically labeled mucus secretion in response to *Eh*. Mucus layer integrity was enumerated by *Eh* adherence to cells and cytotoxicity measured by LDH release. Mucus degradation by *Eh* was quantified by immunoblotting using MUC2 specific antibodies against the protein and O-glycans. *Eh* ingestion of WT and *Mut* mucus (mucophagy) was visualized by confocal microscopy and imaging flow cytometry. Following mucophagy, modulation of *Eh* virulence genes was measured by RT-PCR, proteomics, and enzymatic activity quantified by enzyme specific substrates.

Results: *Mut* cells secreted more mucus constitutively, and in response to *Eh* hyper-secreted mucus enhanced *Eh* attachment, mucophagy, and cytotoxicity of goblet cells. *Mut* mucus was readily degraded and ingested by *Eh* as compared to WT mucus. Increased ingestion of *Mut* mucus upregulated *Eh* virulence with increased enzymatic activity that rapidly degraded *Mut* mucus in a time- and dose-dependent manner.

Conclusions: This study highlights the importance of proper mucus formation in conferring structural and functional integrity of the mucus gel that provides protective innate host defense in the gut against pathogens.

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EXPLORING THE ORIGIN AND EXPANSION OF A MULTIGENIC FAMILY OF IMMUNE RECOGNITION MOLECULES IN THE GASTROPOD PHYLA: THE STORY OF FIBRINOGEN-RELATED PROTEINS (FREP)

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Background and Aims: Fibrinogen-RElated Proteins (FREPs), first described in 1997 in the freshwater snail *Biomphalaria glabrata*, are pattern recognition receptors playing a key role in interacting with trematode parasites. These proteins are characterized by the combination of one or two immunoglobulin (IgSF) domains and one fibrinogen (FBG) domain. A huge diversity of FREPs has been described in some non-vertebrate phyla. The present study aimed to explore the phylogenetic origin and expansion of this multigenic family across mollusk species.

Methods: *In Silico* analyses were carried out to identify and confirm FREP occurrence (Hidden Markov Models, multiple sequence alignments, phylogeny) and their genomic structure (intron/exon) using chromosome-level genome assemblies from 21 mollusk species.

Results: We first detected the presence of FREPs exclusively in gastropods, with an evolutionary radiation originating in the common ancestor of Planorbidae. The highly different occurrences of FREP paralogs (from 37 genes in *Biomphalaria glabrata* to 152 in *Elysia marginata*) revealed dynamic duplication and loss events, consistent with the birth-and-death model. Finally, phylogenetic analyses revealed the evolutionary history of FREP paralogs, with probable origin of duplications arising at the genus level, followed by rearrangement between IgSF and FBG domains. Our results suggested specific structural variations within gastropod families and species, with ancestral directed selection and/or functional convergence of those paralogs.

Conclusions: Taken together, FREPs represent a gastropod-derived immune innovation with an important genomic diversification dynamic, establishing a framework to link FREP genomic variation with functional differences in host-parasite interactions.

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VES1A GENES EXPRESSION IS THE MAJOR DETERMINANT OF BABESIA BOVIS-INFECTED ERYTHROCYTES CYTOADHESION TO ENDOTHELIAL CELLS

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Background and Aims: *Babesia bovis* causes the most severe form of bovine babesiosis, characterized by high mortality in naive adult cattle. This parasite invades red blood cells (RBCs) and exports numerous proteins that alter RBC properties. As a result, infected RBCs (iRBCs) are capable to cytoadhere in the microvasculature of internal organs and brain, leading to respiratory distress, neurologic signs, and mortality. Variant Erythrocyte Surface Antigen 1 (VESA1) is a major virulence factor exported by *B. bovis* that mediates immune evasion through antigenic variation and intravascular parasite sequestration. VESA1 is a heterodimer protein encoded by *ves1α* and *ves1β* multigene family and localized on the ridges, the focal point for cytoadhesion.

Methods: To gain further insights into the molecular mechanisms of cytoadhesion of *B. bovis*, we panned the parasites with bovine brain microvasculature endothelial cells, which resulted in obtaining several clones with different cytoadherence abilities.

Results: The transcriptome analysis of 2 high and 2 low cytoadherent clones revealed that *ves1α* sequences were diversified, likely resulting from genomic recombination. On the other hand, *ves1β* sequences were almost identical among these 4 clones. Insertion and expression of *ves1α* of a clone with high binding into *ef-1α* locus of a low binding clone increased cytoadherence, confirming the role of *ves1α* suggested by our transcriptome data. Whole genome sequencing of cytoadherent clones revealed an active locus of *ves1* on chromosome 2.

Conclusions: These results suggest that VESA1a proteins encoded by *ves1α* genes determine the cytoadherence strength of *B. bovis* and they are in the active site for recombination.

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IDENTIFICATION AND CHARACTERIZATION OF A DNA-BINDING PROTEIN FROM STARVED CELLS (DPS) HOMOLOGUE IN ACANTHAMOEBA CASTELLANII: IMPLICATIONS FOR ENCYSTMENT-INDUCED DNA PROTECTION

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Background and Aims: *Acanthamoeba* spp. are opportunistic human pathogens that transition from motile trophozoites to environmentally resistant cysts under adverse conditions. Although significant degradation of internal structures and macromolecules occurs during encystment, the mechanisms underlying DNA protection in protozoa remain unclear. This study aimed to identify and characterize a bacterial Dps homologue (AcDps) in *Acanthamoeba castellanii* and investigate its role in DNA stabilization

Methods: AcDps was cloned and expressed as a recombinant protein (rAcDps). Expression profiles were analyzed using qRT-PCR and Western blotting. Intracellular localization was determined via EGFP-fused protein expression and confocal microscopy. The structural dynamics and DNA-binding capacity of rAcDps were evaluated using Electrophoretic Mobility Shift Assay (EMSA) and Atomic Force Microscopy (AFM) under various pH and divalent cation conditions.

Results: AcDps expression was minimal in trophozoites but surged 12-fold by 72 hours post-encystment induction. The protein localized to both the nucleus and cytoplasm . rAcDps formed stable, quasi-spherical oligomeric complexes, with assembly significantly enhanced at pH 5.0 and 9.0. In the presence of Zn^{2+} ions, AcDps bound non-specifically to linear and supercoiled DNA, forming quasi-DNA complexes that effectively protected DNA from DNase I degradation.

Conclusions: This study reports the first identification of a Dps homologue in a eukaryotic organism. AcDps plays a critical role in DNA stabilization under encystment-induced stress. Its structural stability and protective function highlight its evolutionary significance and suggest its potential as a selective therapeutic target.

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TRYPANOSOMA CRUZI INFECTIVITY AND SIGNALING THROUGH HOSTS AND STRAINS

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Background and Aims: Chagas disease, caused by the intracellular parasite *Trypanosoma cruzi,* is still an unresolved public health challenge. Strains seem to have distinct tropisms, drug resistances and pathologies. There are also a variety of internalization mechanisms and a considerable ambiguity in the signaling pathways that are triggered during this intracellular stage, suggesting an interaction between the particular pairs of parasite strain and host cell type. Using relevant human cell lines in combination with strains from different Discrete Typing Units (DTUs), we aim to find common signaling pathways and unique mechanisms in each cell line and strain.

Methods: We employed the AC16, Caco2 and HeLa cell lines together with Dm28c (DTU I) and Tulahuen (DTU VI) trypomastigotes. The infection level of each host-parasite pair was evaluated at different multiplicities of infection (MOI) through fluorescence microscopy. Additionally, we extracted RNA from each pair to perform a dual transcriptome analysis (in progress). To consider culture conditions in the statistical model, these samples were also imaged with confocal microscopy.

Results: Dm28c was more infective overall, with 33% more parasites in infected cells and 58% more infected cells. AC16 was the most receptive to parasites, while HeLa was the least infected, particularly with Tulahuen. In general, higher MOIs showed increased parasite burden and a higher proportion of infected cells, which were utilized for the sequencing samples.

Conclusions: We observed differences in the infectivity of both parasite strains and the receptivity of the 3 human cell lines. The information obtained with these analyses is crucial for modelling our transcriptomics data.

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EVALUATION OF HEMATOLOGICAL AND CLINICAL CHANGES IN BABESIA-INFECTED INDIGENOUS CATTLE OF BANGLADESH

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Background and Aims: Babesiosis is an economically important tick-borne hemaprotozoan disease of cattle caused mainly by *Babesia bigemina* and *Babesia bovis*. The disease is widely prevalent in tropical and subtropical regions and leads to substantial production losses due to reduced milk yield, poor growth, and high mortality. Clinically, babesiosis is characterized by fever, anemia, jaundice, and hemoglobinuria. Hematological alterations play a crucial role in disease pathogenesis and may provide valuable diagnostic and prognostic information.

Methods: A total of 30 blood samples were collected, including 20 samples from cattle showing clinical signs suggestive of babesiosis (fever, weakness, pale or icteric mucous membranes) and 10 samples from apparently healthy cattle. Thin blood smears were prepared from all samples, stained with Giemsa, and examined microscopically for the detection of *Babesia* spp. Hematological parameters—Total Erythrocyte Count (TEC), Hemoglobin concentration (Hb), Packed Cell Volume (PCV), Total Leucocyte Count (TLC), and Differential Leukocyte Count (DLC)—were analyzed using an automated hematology analyzer.

Results: Microscopic examination revealed that 12 (60%) of the clinically suspected cattle were positive for *Babesia* infection. Infected cattle showed significant reductions in TEC, Hb, and PCV compared to healthy controls, indicating severe anemia due to intravascular hemolysis and erythrocyte destruction. In contrast, increased percentages of TLC, neutrophils, eosinophils, and monocytes, along with decreased lymphocyte counts, were observed, reflecting inflammatory and immune responses.

Conclusions: *Babesia* infection induces pronounced hematological disturbances in cattle, particularly anemia and leukocyte profile alterations. Routine hematological examination, together with microscopic diagnosis, can serve as an effective tool for the diagnosis and management of bovine babesiosis.

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ASSESSMENT OF EXOVESICLES (EVS) AND PSLG1 EXPRESSION AS PROTHROMBOTIC BIOMARKERS IN PATIENTS WITH CHAGAS CARDIOMYOPATHY IN COLOMBIA

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Background and Aims: Chagas cardiomyopathy (CCC) is closely linked to coagulation abnormalities that often lead to thrombotic events, increasing mortality in affected patients. Given the regulatory role of extracellular vesicles (EVs) and the PSGL1 gene in inflammation, coagulation, and angiogenesis, this study aimed to characterize EV subtypes, PSGL1 expression, and their association with coagulation alterations in individuals with CCC.

Methods: A total of 78 participants were included: 46.1% (36/78) with CCC, 8.9% (7/78) with non-Chagas cardiomyopathy (CM), and 44.8% (35/78) healthy controls. EV concentration (EVs/mL) was quantified by flow cytometry, along with the proportion of EVs expressing PSGL-1 (PSGL-1+ EVs), Tissue Factor (TF+ EVs), and CD41a (CD41a+ EVs). The ability of EVs to induce platelet aggregation was assessed by spectrophotometry. Prothrombotic biomarkers including platelet count, activated partial thromboplastin time (PTT), prothrombin time (PT), and D-dimer were also evaluated.

Results: The CCC group showed elevated D-dimer levels, reduced EV counts per mL of plasma, and a significant increase in PSGL-1+ EVs ($P < 0.05$) compared with controls. No significant differences were observed among groups for the other biomarkers, including TF+ and CD41a+ EVs. Based on the increase in PSGL-1+ EVs, *PSGL-1* gene expression was evaluated by qRT-PCR, revealing a 1.2-fold increase compared with the control group.

Conclusions: The higher proportion of PSGL-1+ EVs in CCC patients, together with the increased expression of the PSGL-1 gene, may be associated with myocardial inflammation in CCC. The interaction of these EVs with platelet-derived P-selectin could promote thrombus formation, consistent with the elevated D-dimer levels observed in the CCC group.

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FROM PARASITE TO REMEDY: USING TOXOCARA CANIS RECOMBINANT C-TYPE LECTIN AS A NEW IMMUNOTHERAPY FOR AUTOIMMUNE DISEASE

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Background and Aims: Background and aims: *Toxocara canis* causes chronic toxocariasis in humans as an accidental paratenic hosts. The C-type lectin (CTL) family, a key component of the parasite's excretory-secretory products, regulates immune responses. This study evaluated the immunomodulatory and therapeutic potential of recombinant *T. canis* CTL (rCTL) in two murine models of autoimmune disorders: experimental autoimmune encephalomyelitis (EAE) and atopic dermatitis (AD).

Methods: Methods: Recombinant r-CTL was expressed in prokaryotic systems and purified using Ni-NTA spin columns. For the EAE model, Balb/C57 mice were divided into six groups: post-EAE r-CTL treatment (Group I), EAE only (II), E. coli lysate control (III), PBS control (IV), healthy control (V), and r-CTL only (VI). For the AD model, 35 BALB/c mice were divided into seven groups. All except normal controls received DNCB to induce AD-like symptoms. Treatment groups received r-CTL, dexamethasone, or bacterial lysate. Assessments included dermatitis severity, scratching behavior, serum IgE (ELISA), and histopathology of skin changes.

Results: Results: In the EAE model, rCTL reduced disability scores, delayed onset, and increased Foxp3 and TGF-β. In the AD model, rCTL decreased dermatitis, itching, and IgE levels, with histologic improvement.

Conclusions: Conclusions: Recombinant *T. canis* C-type lectin shows promising immunomodulatory effects in EAE and atopic dermatitis models, improving clinical and histopathological outcomes. These findings support rCTL as a potential immunotherapeutic agent for autoimmune diseases. However, further studies and long-term follow-up are still needed.

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DIRECT MECHANICAL STIMULATION TRIGGERS HATCHING IN TRICHURIS SUIS EGGS

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Background and Aims: Hatching of *Trichuris suis* eggs within the host intestine is essential for infection establishment, yet the mechanisms triggering this process remain incompletely understood. Although intestinal microbiota and chemical factors have been implicated, the contribution of physical cues has not been systematically evaluated. This study aimed to determine whether mechanical stimulation plays a direct role in inducing egg hatching.

Methods: An in vitro hatching system was employed to assess environmental signals required for egg hatching. Embryonated eggs were incubated at 38°C under anaerobic conditions with or without mechanical stimulation. Different types of mechanical stimuli were applied, including agitation with glass beads, ultrasonic treatment, pipetting, fluid flow, and direct compression using a blunt glass needle. Eggshell integrity was manipulated using sodium hypochlorite treatment to evaluate its role in mechanical responsiveness.

Results: Hatching was not induced by incubation at 38°C in the absence of mechanical stimulation. Mechanical stimuli that directly acted on the egg membrane, such as glass bead agitation and ultrasonic treatment, effectively induced hatching. In contrast, indirect mechanical forces generated by pipetting or fluid flow did not trigger hatching. Eggshell weakening enhanced sensitivity to mechanical stimulation, and direct local compression of the egg membrane induced hatching, indicating that localized mechanical deformation serves as the hatching trigger.

Conclusions: These findings demonstrate that direct mechanical stimulation is essential for *T. suis* egg hatching, with localized egg membrane deformation serving as the trigger. These findings provide new insight into the mechanisms underlying whipworm infection establishment.

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COMPREHENSIVE CHARACTERIZATION OF TAENIA SOLIUM NEUROPEPTIDES REVEALS DIVERGENT IMMUNOMODULATORY EFFECTS ON IMMUNE CELLS AND NEURONS

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Background and Aims: Neurocysticercosis, caused by larvae of *Taenia solium*, is the leading helminthic infection of the central nervous system and a major cause of acquired epilepsy globally. Antigens coming from a degenerating cyst interact with host nervous and immune cells and contribute to its pathology. While neuropeptides of other helminths are studied, ***T. solium* neuropeptides** and their biological effects remain largely unknown. This study aimed to identify and annotate *T. solium* neuropeptides for the first time using an in-silico peptidomics pipeline, validating their presence experimentally and assessing their immunomodulatory and neuronal effects.

Methods: We employed an **in-silico approach** to identify and annotate precursor and mature neuropeptides of *T. solium*. Neuropeptides were purified from viable *T. solium* cyst fluid using **acidified methanol extraction and tricine SDS-PAGE** was performed to confirm their presence. Using **LC-MS/MS**, the presence of multiple experimentally detected peptides was validated. Their immunomodulatory roles were then assessed via functional assays such as RT-qPCR and ROS analysis on PBMCs and LPS primed differentiated Shsy5y cell line.

Results: A total of **194 mature neuropeptides from 134 precursors** were identified and classified. Tricine SDS-PAGE confirmed that the peptides predominantly ranged from **1–6 kDa**, which aligned with known neuropeptide profiles. Functional assays on PBMCs, demonstrated anti-inflammatory activity while on LPS primed neurons these neuropeptides showed a pro-inflammatory profile demonstrating a critical role in neuronal excitation.

Conclusions: Together, these findings reveal a previously unrecognized cell-type-specific dual role of *T. solium* neuropeptides. These neuropeptides, can therefore, act as promising novel targets for therapeutic strategies against neurocysticercosis associated seizure occurence.

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ESTABLISHMENT AND CHARACTERIZATION OF A CRYPTOSPORIDIUM PARVUM ISOLATE FROM CATTLE IN THE REPUBLIC OF KOREA

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Background and Aims: *Cryptosporidium parvum* strains may vary in genotype and pathogenicity even within the same species. Therefore, this study aimed to establish a Korean cattle-derived *C. parvum* isolate and to assess its pathogenicity.

Methods: Diarrheic fecal samples were collected from calves in the Republic of Korea, and the presence of *Cryptosporidium* oocysts was confirmed by microscopy. For molecular characterization of the isolate, fecal DNA was extracted and PCR was performed targeting the 18S rRNA, *Cryptosporidium* oocyst wall protein (*COWP*), *gp60*, and actin genes. Phylogenetic analysis was conducted for species identification, and *gp60* sequence analysis was used for subtype determination. To assess pathogenicity, six male Ifng KO mice were orally infected with oocysts obtained via ether extraction. Oocyst shedding was monitored at 7, 14, and 28 days post-infection (dpi), and body weight was recorded at 0, 7, 14, and 28 dpi.

Results: Phylogenetic analysis confirmed the isolate as *C. parvum*, and *gp60* sequence analysis identified the subtype as IIaA17G4R1. The results showed that oocyst shedding peaked at 14 dpi (3.6 × 10^4 oocysts/10 g). Infected mice showed reduced activity and a decreasing trend in body weight, and one mouse died at 27 dpi.

Conclusions: In conclusion, the present study established a Korean cattle–derived *C. parvum* isolate through molecular characterization and pathogenicity assessment. This isolate can serve as a domestic reference strain for standardized experimental work and molecular epidemiological studies of *C. parvum* in Korea.

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MODULATION OF P53-CENTERED SIGNALING BY TOXOPLASMA GONDII EFFECTOR PROTEINS IN COLORECTAL CANCER CELLS

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Background and Aims: The tumor suppressor p53 plays a central role in colorectal cancer by regulating the cell cycle , DNA damage responses, and apoptosis. In colorectal cancer, p53 is frequently attenuated through dysregulation of upstream regulatory pathways, and this results in impaired tumor suppressive activity despite the presence of intact p53 protein. *Toxoplasma gondii (T. gondii)* secretes a range of effector proteins that modulate host transcriptional and signaling axes during infection. Among these, rhoptry (ROP) and dense granule (GRA) protein families have been reported to interfere with host signaling pathways involved in cellular stress responses and oncogenic regulation. In this study, we investigated whether representative *T. gondii*-derived effector proteins modulate p53-centered anticancer signaling in colorectal cancer cells.

Methods: Human colorectal cancer cells (HCT116) stably expressing *T. gondii*-derived effector proteins were established using a lentiviral expression system. All experiments were performed under identical conditions. p53 protein expression and activation were assessed by Western blot analysis, and p53-dependent transcriptional responses were examined using quantitative real-time PCR (qRT-PCR).

Results: Expression of T. gondii-derived effector proteins increased p53 stability and transcriptional activity in colorectal cancer cells, accompanied by reduced upstream inhibitory signaling. This led to induction of p53 target genes, suppressed cell proliferation, and enhanced apoptotic responses, reinforcing p53-dependent tumor suppressive signaling.

Conclusions: Collectively, these results show that T. gondii–derived effector proteins enhance p53 stability and transcriptional activity in colorectal cancer cells, suppressing negative p53 regulation and thereby inhibiting cell proliferation while promoting apoptosis through reinforced p53-dependent tumor suppressive signaling.

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MOLECULAR DETECTION OF RICKETTSIA AFRICAE AND CANDIDATUS MIDICHLORIA MITOCHONDRII IN CATTLE TICKS FROM CAMEROON

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Background and Aims: Background: Ticks spread pathogens that affect both human and animal health and often cause significant economic losses to the livestock industry. However, there is limited data on the distribution of ticks and tick-borne pathogens, particularly those affecting cattle in Cameroon. In this study, ticks were collected from cattle in Buea, the capital of the South West Region of Cameroon. **Aims:** to determine their diversity and identify tick-borne pathogens through PCR and sequencing.

Methods: The tick samples were screened for pathogens using assays that target the rOmpA gene (ompA) of *Rickettsia*, the ssrRNA gene of *Babesia* and *Theileria* and the *Ehrlichia* genus 16SrRNA gene. A total of 458 ticks were collected with *Rhipicephalus annulatus* (65.6%) as the predominant species.

Results: From 68 tick pools screened, 26 (38.2%) were positive for pathogen / symbiont DNA. The pathogen identified was *Rickettsia africae* (22.1%). This study reports the first molecular detection of the symbiont *Candidatus Midichloria mitochondrii* (22.1%) in the sampled tick species. It was observed that male ticks were significantly more likely to test positive for *R. africae* (OR = 208, 95% CI = 28.6 - 4553, $p<0.001$).

Conclusions: Animal handlers may face the risk of these zoonotic infections and as such, there is a need to employ protective measures to prevent or reduce pathogen spread.

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ENDOSYMBIOTIC PARTNERS OF TRICHOMONAS VAGINALIS: MYCOPLASMA HOMINIS, TVV, AND OTHER POTENTIAL CO-INHABITANTS

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Background and Aims: *Trichomonas vaginalis* (TV) is the most common non-viral sexually transmitted pathogen worldwide and is known to establish assumedly symbiotic relationships with *Trichomonas vaginalis* virus (TVV) and the bacterium *Mycoplasma hominis*. These „endosymbionts“ have been proposed to modulate the pathogenicity of TV, yet these relationships remain poorly understood. The aim of this study was to characterize TV isolates from Austrian patients with respect to their symbiotic composition and to investigate whether TV can also harbour *Ureaplasma* spp.

Methods: TV isolates were collected at an Outpatients ´ Centre in Vienna and screened for TVV, *M. hominis* and *Ureaplasma* spp. by PCR. To achieve this, subcultures were installed from the isolates and subjected to RNA, and DNA extraction, respectively. All amplified products were sequenced and compared with reference sequences from GenBank.

Results: Altogether, 82 TV strains were isolated from Austrian patients: 10 isolates derived from men and 72 from women. We confirmed the presence of TVVs (TVV1, TVV2, or TVV3) in 29 of them (35%); no TVV4 was detected. *M. hominis* DNA was found in 28% of the TV isolates by PCR. Preliminary PCR-based results did not reveal the presence of *Ureaplasma* spp. in the tested TV isolates, experiments to infect TV with *Ureaplasma* are currently underway.

Conclusions: Our study provides insight into the symbiotic relationships of Austrian TV isolates. Ongoing work will determine whether *Ureaplasma* can persist as an endosymbiont in TV and investigate the impact of endosymbionts on TV pathogenicity.
This research is supported by the Austrian Science Fund Grants No. 10.55776/KLI751 and No. 10.55776/KLP6210324.

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PROTEOMIC PROFILING OF ACANTHAMOEBA EXTRACELLULAR VESICLES AFTER CONTACT WITH HUMAN CORNEAL EPITHELIAL CELLS

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Background and Aims: Free-living amoebae of the genus *Acanthamoeba* are protists widely distributed in water, soil, air, and anthropogenic environments such as swimming pools and air-conditioning systems. These amoebae can act as opportunistic pathogens, causing partly severe diseases in humans. The most common one is *Acanthamoeba keratitis* (AK), a painful and vision-threatening ocular infection frequently associated with contact lens use. The course of infection mainly depends on amoebic load, virulence, and host susceptibility. Beyond direct cell-to-cell interactions, extracellular vesicles (EVs) have been suggested to play a critical role in pathogenesis.

Methods: In this study, we explored the proteome dynamics of EVs secreted by *Acanthamoeba* during interaction with human corneal epithelial cells (HCECs), using mass spectrometry-based approaches. A pathogenic strain of *Acanthamoeba castellanii* was incubated with HCECs for different periods of time and the proteomic profiles of *Acanthamoeba* extracellular vesicles before and after co-incubation with HCECs were analysed using Trapped Ion Mobility Spectrometry-Time of Flight (timsTOF) mass spectrometry.

Results: We identified and quantified nearly 3,500 proteins from enriched *Acanthamoeba* EVs. Statistical analysis reveals that the presence of HCECs induces notable changes in the quantity of certain proteins within EVs, detectable as early as 30 minutes after co-incubation. The affected proteins include those associated with ribosomes, peroxisomes, and various metabolic pathways.

Conclusions: These detailed proteomic profiles of *Acanthamoeba* EVs in the presence of HCECs provide novel insights into their structural integrity and importance during cell-cell interactions. This research was supported by the Austrian Science Fund Grant No.10.55776/ESP5824124 and grants from the French National Research Agency (ProFI projects: ANR-10-INBS-08 & ANR-24-INBS-0015).

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CHARACTERISATION OF THE MOLECULAR IMMUNOBIOLOGY OF RESISTANCE OR SUSCEPTIBILITY TO SCHISTOSOMA HAEMATOBIUM AND PLASMODIUM FALCIPARUM CO-INFECTION

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Background and Aims: Single-nucleotide polymorphisms (SNPs) within the promoter and intronic regions of cytokine genes have been associated with altered levels of circulating cytokines. The SNPs in the cytokine genes may influence immune response and susceptibility to schistosomiasis and malaria. The study aimed to investigate the association of the cytokine SNPs, which are involved in differential cytokine production among co-infected children aged between 6 –13 years.

Methods: A school-based epidemiological study was undertaken, which included the collection of urine, stool and blood. Whole blood was collected for antibody and cytokine assays and the extraction of DNA for the genotyping of SNPs using ARMS-PCR.

Results: Of the 986 samples analysed, 38.3 % were found to be infected with *S. haematobium*. The mean intensity of infection was heavy, 134 eggs/10 mL urine. Among the children, 27 % were infected with *P. falciparum*, while *S. haematobium* and *P. falciparum* co-infection was 9.9 %. No cases of *S. mansoni* were recorded from the study areas. IgG4 levels were elevated in infected children. IgG1 was highly elevated in the 11 to 13-year age group, which had the least infection. IgE was highly elevated in the uninfected children. IgG1 and IgE are protective antibodies, whereas IgG4 is anti-protective. The GG genotypes were associated with high levels of IL-10 and TNF-α and, therefore, were resistant to infection

Conclusions: The study highlighted that infections in Africa do not exist singly; there is co-existence of *S. haematobium* with *P. falciparum*. SNPs in the cytokine genes do affect resistance or susceptibility to parasitic infections.

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PROTEOMIC CHARACTERIZATION OF INTESTINAL CELL-DERIVED EXTRACELLULAR VESICLES FOLLOWING IN VITRO EXPOSURE TO FASCIOLA HEPATICA JUVENILES

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Background and Aims: A key step in the *Fasciola hepatica* (*Fh*) life cycle is the passage of newly excysted juveniles (NEJs) through the duodenal wall of the definitive host, which requires finely coordinated host-parasite interactions. Extracellular vesicles (EVs) play key roles in host-parasite interplay and the crosstalk among host's cells. Herein, we characterize the protein cargo of EVs released by intestinal cells (ICs) following in vitro exposure to *Fh*NEJs.

Methods: *Fh*NEJs were added to a co-culture of ICs (Caco-2 + HT-29) and maintained for 6 h. Then, worms were removed and cells were thoroughly washed and maintained in culture for 12 h in fresh medium. Untreated IC cultures were used as control. EVs were isolated from the medium by centrifugation and size exclusion chromatography (SEC), and their protein cargo characterized via LC-MS/MS. Differentially abundant proteins were analyzed using Panther GO and STRING.

Results: Proteomic analysis revealed 56 differentially abundant proteins: 43 underrepresented and 13 overrepresented in EVs released by ICs exposed to *Fh*NEJs *vs* control cells. Gene ontology (GO) analyses showed upregulation of routes involving DNA repair and response to stimulus in parasite-exposed cells. Moreover, seven *Fh* proteins were detected in the cells 12h after the NEJs removal including cathepsins and legumains.

Conclusions: Our results suggest that the EV protein cargo reflects intestinal host responses to *Fh*NEJ contact, at least partially. Parasite proteins detected 12 h after worm removal might had been internalized by host cells during the contact phase and released via EVs afterwards. **Funding** Grants: PID2023-146116NB-I00; CIPROM/2023/054; FPU23/00936 (M.P.R.U.).

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PARASITE-DRIVEN AMYLOID FIBRIN MICROCLOTS IN CEREBRAL MALARIA INDICATE PARALLELS WITH VASCULAR MECHANISMS OF NEURODEGENERATION.

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Background and Aims: Cerebral malaria (CM) is a life-threatening neurological complication of *Plasmodium falciparum* infection, characterised by microvascular pathology, coagulopathy, and brain swelling. While parasite sequestration and inflammation are implicated in its pathogenesis, the mechanisms linking them with microvascular coagulopathy and brain swelling seen in fatal disease remains unclear, as do drivers of neurocognitive sequelae. Amyloidogenic processes have been implicated in barrier disruption and cognitive dysfunction in neurodegenerative conditions, and more recently in sepsis-associated coagulopathy, but their role in CM pathogenesis has been little explored.

Methods: Post-mortem brain tissues from fatal paediatric CM cases (n=6) were examined by immunofluorescence for *Plasmodium*-derived factors, fibrinogen, and amyloid. Plasma microclots (n=100) were detected using Thioflavin T and quantified (number and size/field). Co-localisation analyses employed antibodies against fibrinogen, parasite factors, and markers of neuroaxonal injury.

Results: Amyloid aggregates were identified in postmortem brain tissue and were associated with deposition of *Plasmodium*-derived material and vascular damage consistent with Blood-Brain-Barrier breakdown. Circulating amyloid–fibrinogen microclots were significantly increased in number and size in admission plasma samples from Malawian children with CM (n=46). Supporting that they are formed at sites of neurovascular damage, circulating microclots contained adherent neuroaxonal proteins (tau, GFAP, S100B) and caused barrier disruption *in vitro*. Circulating microclots contained parasite-associated factors and *in vitro*, parasite material promoted microclots formation in the presence of endothelial cells and coagulation factors.

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Conclusions: These findings indicate that hyperacute amyloidogenic coagulopathy may contribute to CM neurovascular pathogenesis. Parallels with a role of chronic amyloidogenic coagulopathy in neurodegenerative diseases highlight areas for further research and potential therapeutic targets.

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STEM-LOOPS STRUCTURES FROM GIARDIA DUODENALIS AND IRON REGULATORY PROTEINS INTERACTIONS BY IN SILICO DOCKING

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Background and Aims: *Giardia duodenalis* is the first cause of waterborne diarrhea. The importance of iron in the biology of this and other protozoa has been previously discussed by our research team as well as the presence of stem-loops structures or iron responsive element-like (IRE-like) and iron regulatory proteins (IRP), in some iron regulated mRNAs encoding virulence proteins. The canonical IRE/IRP system involves IRP that can bind these IREs and perform a regulatory effect depending on the location of the IRE. Even though iron is very important for protozoa virulence further investigation is necessary in its role in *Entamoeba histolytica* and *G. duodenalis*. Therefore the aim of this study is the IRE/IRP regulation system in protozoa; our research group performed *in silico* analysis interacting potential IRPs and IRE-like structure of *G. duodenalis´*s Pyruvate Flavodoxin Oxidoredutase (*pfo*), an important target in the biology of the parasite.

Methods: To have a better understanding of the IRE-IRP-like interactions a docking using the software cluspro2.0 was performed. Crystal structures reported in the protein data bank were used for IRPs and the IRE-like structures were modeled using the software simRNA2.0. Then the bonds formed between RNA and protein were obtained with PDBsum and the bond distances were determined manually using VMD.

Results: In our findings there was an interaction between *G. duodenalis´*s IRE-like structure and IRP in the stem and the loop region.

Conclusions: RNA-Protein bonds were found between RNA phosphate groups and positively charged aminoacids. These findings may lead us to getting better predictions of this IRE/IRP-like iron regulatory mechanism.

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RETINOIC ACID TREATMENT IN TOXOPLASMA GONDII-INFECTED FEMALE RATS ATTENUATES ESTROUS CYCLE-DRIVEN BEHAVIORAL VARIABILITY: A POSSIBLE MODEL FOR FEMALE INCLUSION IN PARASITOLOGICAL STUDIES

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Background and Aims: Estrous cycle-driven hormonal fluctuations introduce uncontrolled variability in behavioral assays, often leading to exclusion of female rodents from parasitological studies. To overcome this limitation, we tested a methodological strategy using retinoic acid (RA) in *Rattus norvegicus*, testing whether T*oxoplasma gondii*-infected females, RA-treated, maintained in a cycle stage, exhibit a behavioral profile equivalent to infected females in different stages.

Methods: Female Wistar rats were inoculated orally with *T. gondii* ME-49 and treated with RA (0.3 mg/kg, intraperitoneal). Open field behavior was assessed at 65 days post-infection. Estrous phase was determined by post-test vaginal cytology. Groups: G1 (*T. gondii* + RA, metestrus, n=8); G2 (*T. gondii* only, estrus n=5, metestrus n=5); G3 (uninfected controls, estrus/proestrus n=6, diestrus n=4). ANCOVA tested immobility with group as factor and estrous phase as covariate.

Results: *T. gondii* infection reduced immobility in estrus (G2_estrus: 14.2% vs. G3_estrus: 44.6%, $p<0.001$) and metestrus (G2_metestrus: 27.4% vs. G3_diestrus: 39.3%, $p=0.048$). RA-treated infected metestrus females (G1: 17.8%) were indistinguishable from infected estrus females (14.2%, $p=0.358$) and showed lower immobility than untreated infected metestrus females (27.4%, $p=0.012$). ANCOVA confirmed a significant group effect ($p=0.0387$), while estrous phase was not significant ($p=0.2349$).

Conclusions: RA treatment stabilized *T. gondii*-infected female rats in metestrus, replicating the estrus locomotor profile, suggesting similarity with male behavior, thus attenuating cycle-driven variability. These findings support the inclusion of female subjects in parasitological behavioral studies, overcoming a historical bias that has favored male-only experimental designs.

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COLD STRESS RESTRICTS MALARIA PARASITE GROWTH BY AFFECTING ERYTHROCYTE INVASION

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Background and Aims: Malaria remains a major global infectious disease, and Plasmodium falciparum is the leading cause of severe falciparum malaria. Although P. falciparum is highly sensitive to temperature fluctuations, the intraerythrocytic processes affected by cold stress are not fully defined. We investigated how cold stress influences parasite development and erythrocyte invasion.

Methods: Highly synchronized P. falciparum cultures were exposed to 27, 30, or 33°C for defined periods and compared with standard 37°C conditions. Parasite development was assessed by microscopy. Erythrocyte invasion efficiency following cold exposure was quantified using reinvasion assays. Temperature-dependent transcriptional changes were examined by transcriptomic profiling, and ama1 expression was validated by qPCR at schizont and ring stages. In parallel, infected mice were exposed to 4°C to evaluate effects on parasitemia in a rodent malaria model.

Results: Culture at 27–33°C suppressed intraerythrocytic growth and differentiation in a temperature-dependent manner and was associated with an overall delay in stage progression. Cold-exposed parasites showed reduced erythrocyte invasion efficiency, accompanied by broad downregulation of erythrocyte invasion–associated genes. Notably, ama1 expression was decreased under 33°C exposure at both schizont and ring stages. In vivo, experimental cold exposure of infected mice reduced parasitemia compared with controls.

Conclusions: Cold stress constrains malaria parasite expansion by impairing erythrocyte invasion and repressing invasion-related transcription, including ama1. These findings highlight temperature as a critical environmental regulator of parasite development in warm-blooded hosts and support further evaluation of host temperature modulation as a complementary strategy to limit malaria infection.

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AIRWAY EPITHELIAL SIGNALLING GOVERNS HOST SPECIES-SPECIFIC TYPE-2 RESPONSE TO TISSUE-MIGRATORY HELMINTH LARVAE IN CANIDS

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Background and Aims: Tissue-migratory helminth larvae that migrate through the lungs, including *Angiostrongylus vasorum*, induce distinct pulmonary responses in canid hosts. Evidence suggests that foxes as important wildlife reservoir hosts have developed mechanisms to tolerate and regulate helminth infections whereas dogs appear to mount more destructive responses. Using an air-liquid interface (ALI) *in vitro* model of the respiratory tract, our aim was to determine whether airway epithelial signalling contributes to host species–specific responses.

Methods: Primary airway epithelial cells from foxes and dogs were differentiated into fully functional respiratory epithelia at the ALI and exposed to *A. vasorum* first-stage larval antigens. Epithelial responses were assessed using RNA sequencing, paracellular permeability assays, immunostaining of epithelial differentiation integrity, and targeted qPCR of antigen-presentation and eosinophil-associated markers. Findings were integrated with *in vivo* lung histology to contextualise epithelial responses.

Results: Fox epithelia responded with induction of epithelial alarmins, antigen-presentation pathways, and chemokine signals associated with type-2 immunity. Antigen exposure caused an increase in paracellular permeability in fox cultures, consistent with regulated and reversible epithelial barrier modulation. These responses aligned with eosinophil accumulation in fox lung tissue. In contrast, dog epithelia showed no change in barrier permeability or activation of type-2–associated epithelial programmes following antigen exposure.

Conclusions: These data identify airway epithelial signalling as central determinant of host species–specific responses to tissue-migratory helminth larvae. Regulated epithelial barrier modulation in foxes may facilitate effective type-2 immune orchestration and tolerance, whereas its absence in dogs may contribute to inappropriate immune responses and disease susceptibility.

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B-CELL REPROGRAMMING DURING VISCERAL LEISHMANIASIS IN RHESUS MACAQUES

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Background and Aims: Visceral leishmaniasis (VL) is a neglected tropical disease caused by protozoan parasites. Clinical manifestations of VL include hepatosplenomegaly and hypergammaglobulinemia. We previously reported the reprogramming of myeloid cells associated with parasite persistence despite miltefosine administration. However, little is currently known about how parasite persistence affects B-cells. This study aimed to analyze the differentiation and reprogramming of B-cell subsets in relationship to *Leishmania infantum (L.i)* infection.

Methods: In this study, we examined the differentiation of splenic B cell subsets by flow cytometry and single-cell transcriptomic analyses in a rhesus macaque (RM) model of VL. Furthermore, using a *NoNo* assay, we monitored the impact of *L.i* infection on primary human cells.

Results: Our results reveal abnormal and reprogramming of splenic B cell populations that persist post-treatment in *L.i*-infected RM. Thus, atypical tissue-like memory B cells are observed in treated RM. Furthermore, we identified a subset of splenic B cells expressing PD-1 and CD95. *In vitro* infection of peripheral blood mononuclear cells with *L.i* promastigotes induces CD95/PD-1 expression in memory B cells. Quantitative analysis of the *amastin* gene suggests the occurrence of parasite differentiation. Further analyses are ongoing to clarify the process of B cell differentiation.

Conclusions: Our results highlight splenic B cell reprogramming despite miltefosine therapy. Our findings uncover a novel immune impairment and parasite persistence, highlighting B cells as potential therapeutic targets in visceral leishmaniasis.

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ANTHELMINTIC ACTIVITIES OF DIATOMACEOUS EARTH IN SHEEP

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Background and Aims: Assessed low-cost diatomaceous earth (DE) vs. ivermectin/control for sheep parasites (Moniezia, Haemonchus). Evaluated DE's fecal egg reduction, influence on fecal consistency/feeding, and growth/mortality impact. Supports DE as sustainable, affordable anthelmintic alternative.

Methods: 12 sheep; 3 groups (control, ivermectin 0.2mg/kg, 2% DE feed); 12-wk trial. Biweekly fecal egg counts (FEC) & post-mortem worm counts (TWC) for M. expansa/H. contortus. Weight/mortality/feed tracked. Stats compared FEC/TWC/weight gain/mortality.

Results: Results: Significant Parasite Reduction: DE-treated sheep showed a 71% reduction in *Haemonchus contortus* burden ($p < 0.01$) and complete elimination of *Moniezia expansa* tapeworms, matching ivermectin's efficacy against cestodes. Superior Clinical Outcomes: DE group achieved 27% higher weight gain vs. controls ($p < 0.05$) and reduced mortality by 40%, confirming improved host health. Mechanistic Validation: Microscopy revealed DE caused severe cuticular damage in *H. contortus* (correlating with reduced fecundity) and disrupted *M. expansa* tegument integrity. Cost-Effectiveness: DE lowered treatment costs by 64% compared to ivermectin, with no adverse effects on feeding behavior or wool quality.

Conclusions: This study demonstrates that diatomaceous earth (DE) is a viable, non-chemical anthelmintic alternative for controlling parasitic infections (*Moniezia expansa* and *Haemonchus contortus*) in pasture-raised sheep. DE significantly reduced parasite burden and fecal egg counts, with efficacy comparable to ivermectin. Critically, DE-treated sheep exhibited improved weight gain and reduced mortality rates, underscoring its positive impact on host health. Given its low cost, minimal environmental impact, and absence of chemical residues, DE offers a sustainable solution for parasite management in resource-constrained agricultural systems, particularly where anthelmintic resistance is a growing concern.

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INVESTIGATION OF THE UPTAKE OF EXOGENOUS SIALIC ACID IN SCHISTOSOMA MANSONI: POTENTIAL IMMUNE EVASION STRATEGY

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Background and Aims: Schistosomiasis affects over 240 million people worldwide. Reliance on praziquantel raises concerns over drug resistance, highlighting the need for new therapeutics. *Schistosoma mansoni* releases extracellular vesicles (SmaEVs) that modulate host immune responses. Surprisingly, we identified sialic acid (SA) on SmaEVs, a terminal glycan which the parasite cannot synthesize as it lacks *de novo* SA biosynthetic pathway. SA regulates immune responses in viruses and protozoa, suggesting similar functions in schistosomes. Therefore, we hypothesized that SA is host-derived and investigated its presence and origin in schistosomes.

Methods: SmaEVs from adult worms cultured with or without fetal bovine serum (FBS) were isolated via size-exclusion chromatography and characterized by nanoparticle tracking analysis, cryo-electron microscopy and western blotting. Glycan profiles were assessed using lectin microarrays, with or without neuraminidase (Neu). In parallel, adults cultured with or without FBS were treated with or without Neu and alternatively with exogenous SA or FBS-derived EVs. Glycan profiles of worm lysates were analyzed using lectin microarrays.

Results: SmaEVs had a size of 90 nm and were detected by EV markers CD63 and HSP70. SmaEVs showed strong binding to SA recognizing lectins using lectin microarrays. Neu treatment decreased this binding, confirming SA presence. Moreover, adults cultured in media supplemented with exogenous SA showed no difference in SA binding, whereas exposure to serum or serum-derived EVs increased SA signals, suggesting SA acquisition by schistosomes from host glycoproteins.

Conclusions: *S. mansoni* may exploit host-derived SA as a new strategy to evade host immunity and pathways involved in SA acquisition could be potential targets for therapeutic intervention.

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EXOSOMAL PHOSPHATASE: THE NOVEL VIRULENCE FACTOR OF TRICHOMONAS VAGINALIS

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Background and Aims: *Trichomonas vaginalis* is a human sexually transmitted protist that infects the urogenital tract and causes the disease trichomoniasis. It is estimated that trichomoniasis affects over 150 million people worldwide annually. During infection, the virulence of *Trichomonas vaginalis* depends on the cell shape transformation, cytoadherence, phagocytic activity, presence of endobiotic *Mycoplasma* and viruses, secretion of cysteine proteases, and release of small extracellular vesicles called exosomes. Exosomes deliver various proteins and small RNAs into host cells, modulate host immune response, and host-parasite interactions. We aimed to extend our current knowledge on the role of exosomes in trichomonads' virulence.

Methods: Virulent and avirulent clones were derived from endosymbiont-free *Trichomonas vaginalis* strain 172MI. Exosomes were isolated by tangential filtration and their proteomes and RNA content were compared. Phosphatase activity was determined spectrophotometrically.

Results: Exosomes from the virulent strain contained 18 enriched and 25 unique proteins, including three putative acid histidine phosphatases, and displayed significant increase in the small regulatory tsRNA content. The presence of phosphatases within exosomes was verified by immunoblotting, protein protection assay, and measurement of their enzymatic activity. Exosomes of the trichomonad virulent strain displayed higher phosphatase activity than those of the avirulent strain, which is consistent with the proteomic data.

Conclusions: The delivery of active phosphatases via the exosomal pathway into host cell may cause dephosphorylation of proteins inside host cells and affect the host immune response to the parasite infection. Collectively, phosphatases delivered via exosomes to target cells may represent a novel virulence factor.

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COMPLEXITY OF PLASMODIUM VIVAX MEROZOITE INVASION INTO RETICULOCYTES

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Background and Aims: Vivax malaria is deservedly receiving more attention than in the past. However, research into the causative agent of relapsing malaria: *Plasmodium vivax*, lags considerably behind that of P. falciparum. Aside from a general lack of funding, a key reason for the research effort disparity between these two important malaria parasite species is that a robust continuous culture method for *P. falciparum* has been developed in the late 1970s; whereas the continuous culture of *P. vivax* erythrocytic stages still eludes us to this day, principally due to the fact that *P. vivax* only invades and grows in reticulocytes, the immature erythrocytes.

Methods: We describe the use a sets of biochemical approaches to determine ligands/receptors interactions and improved *ex vivo* methods and tools to study fresh and frozen/thawed isolates from vivax malaria patients.

Results: Here, we will present data on the molecular pathways involved the receptor CD98 used by *Plasmodium* merozoites proteins to enter reticulocytes.

Conclusions: CD98 is an important reticulocyte receptor recognises by the parasite ligands, PvRBP2a and Erythrocyte Binding Protein (EBP).

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LIPOPROTEINS TRIGGER ASEXUAL REPRODUCTION IN THE CESTODE MESOCESTOIDES VOGAE

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Background and Aims: Asexual reproduction is essential for life-cycle completion in many cestodes and trematodes; however, the mechanisms initiating this process remain poorly understood in these parasitic flatworms. *Mesocestoides vogae* is a cestode capable of asexual reproduction; its larval stage, the tetrathyridium, proliferates within the peritoneal cavity of the intermediate host. This process begins with duplication of the scolex, followed by fission of the body axis, ultimately generating two individuals and enabling massive proliferation within the host (Fig.1).



Figure1. Scolex duplication in *Mesocestoides vogae* tetrathyridium

This study aimed to identify host-derived factor(s) that trigger asexual reproduction in *M. vogae* and the parasite receptor(s) involved in their recognition.

Methods: RNA interference was performed using a modified method originally developed for *Fasciola hepatica* (Dell'Oca et al., 2014).

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Results: Because tetrathyridia reside in ascitic fluid derived from host blood exudates, host blood components were hypothesized to contain the triggering factor(s). *In vitro* culture experiments showed that supplementation with serum from the intermediate host, mouse, induced scolex duplication, whereas heat-treated serum did not. Ultrafiltration-based fractionation of mouse serum revealed that scolex duplication was induced exclusively by fractions larger than approximately 60 kDa. Based on these properties, lipoproteins were considered candidate molecules. Consistently, purified mouse lipoproteins triggered scolex duplication when added to the culture medium. Furthermore, RNA interference–mediated knockdown of a gene predicted to encode a lipoprotein receptor significantly reduced the frequency of scolex duplication.

Conclusions: These findings indicate that host-derived lipoproteins trigger asexual reproduction in *M. vogae* and strongly suggest that this parasite recognizes lipoproteins in host ascitic fluid via the

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receptor identified in this study (Fig.2).

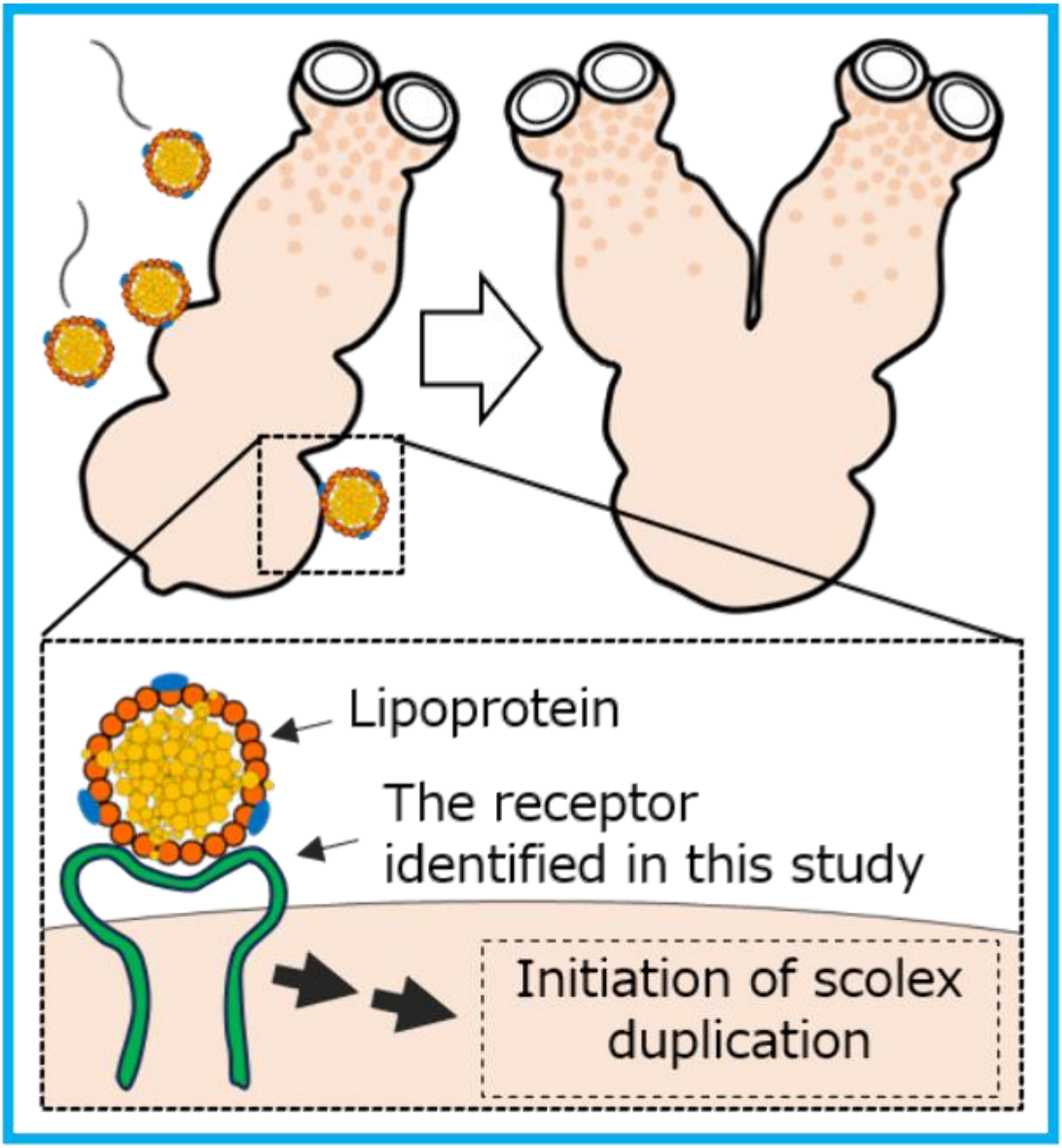


Figure 2. Proposed model for the initiation of scolex duplication

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DIAGNOSTICS, HOST-PARASITE INTERACTION AND EMERGING TECHNOLOGIES / HOST-PARASITE MOLECULAR AND CELLULAR INTERACTIONS

DECIPHERING THE METABOLIC REGULATION OF GLYCOLYSIS IN INTRAERYTHROCYTIC MALARIA PARASITES

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Background and Aims: Liquid-liquid phase separation (LLPS) is a physical phenomenon in which a homogeneous liquid phase separates into two distinct liquid phases due to changes in intermolecular interactions. The glycolytic body (G-body), formed through LLPS of glycolytic enzymes, arises during hypoxic stress in cancer cells and yeast, enhancing glycolytic efficiency under low-oxygen conditions. Glycolysis is a major energy source in intraerythrocytic malaria parasites, operating at high flux for parasite proliferation. We are investigating whether G-bodies form in these malaria parasites.

Methods: Glycolytic enzymes of *Plasmodium falciparum* were labeled with a fluorescent protein using selected linked integration (SLI) and CRISPR/Cas9 strategies. Total internal reflection fluorescence (TIRF) and confocal microscopy were used to visualize cytosolic protein localization under different culture conditions.

Results: We demonstrated that phosphofructokinase (PFK9) forms G-body-like condensates under low-glucose conditions. TIRF analysis suggested the existence of larger PFK9 clusters after 6 h of low-glucose culture. Phosphoglycerate kinase (PGK) also forms condensates under low-glucose conditions; however, these condensates are distinct from those in which PFK9 is localized. There was no clear difference in the transcriptome or metabolome between the standard and low-glucose-cultured parasites.

Conclusions: Since PFK9 is rate-limiting and PGK initiates ATP generation in glycolysis, we hypothesized these condensates regulate glycolysis in intraerythrocytic malaria parasites. Glycolytic metabolite levels remained unchanged after low-glucose stress, suggesting PFK9 condensation maintains glycolytic activity under low substrates. To decipher the physiological significance of G-

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body formation, we plan to conduct live imaging analysis using FRET-based biosensors to quantify the metabolic flux within G-bodies.

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NCRNAS: INVESTIGATING FUNCTIONS AND BIOGENESIS

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Background and Aims: *Leishmania* present an unusual genome organization in which protein-coding genes are arranged in directional polycistronic transcription units, lack introns, and are transcribed in the absence of canonical RNA polymerase II promoters. In several eukaryotes, non-coding RNAs play diverse and essential roles, and although different classes of regulatory ncRNAs have been described in trypanosomatids, knowledge of their features and functions in *Leishmania* remains limited. Transcriptomic analyses identified ~11,000 putative ncRNAs in *L. braziliensis*, ~3,000 of which are stage-regulated. Based on expression and genomic context, 5 lncRNAs enriched in metacyclic promastigotes or amastigotes were selected.

Methods: These lncRNAs were targeted using CRISPR/Cas9, and overexpression lines were generated by plasmid transfection. Growth curves were used to assess promastigote proliferation, while ongoing assays evaluate roles in metacyclogenesis and amastigogenesis. RNA circularization assays with enzymatic treatments were performed to characterize transcript processing. RNA FISH and S1m pulldown assays are planned to determine subcellular localization and protein interactions.

Results: The lncRNA.22.152 was successfully knocked out without affecting promastigote growth. In contrast, complete removal of lncRNA.12.36 required episomal complementation, suggesting potential essentiality, In contrast, the associated protein-coding gene and a neighboring lncRNA within the same 3' UTR were successfully knocked out. Overexpression lines were generated for lncRNA.29.124 derived from the 5' UTR, but knockout remains unsuccessful.

Conclusions: The inability to completely remove lncRNA.12.36 from the genome without episomal complementation suggests that this lncRNA may be essential for parasite. In contrast, the successful knockout of other selected lncRNAs without detectable effects on promastigote growth indicates that their functions may be stage-specific.

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TRANSCRIPTOMIC INSIGHTS INTO METALLOPEPTIDASE ACTIVATION, ENDOTHELIAL DYSFUNCTION, AND IMPAIRED PATHOGEN RECOGNITION IN HYPOCOAGULABLE DOGS INFECTED WITH ANGIOSTRONGYLUS VASORUM

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Background and Aims: Infections with the cardiopulmonary nematode *Angiostrongylus vasorum* in dogs are associated with a high risk of fatal bleeding complications, prompting further investigation of the underlying pathologies.

Methods: We analyzed transcriptomic profiles from i) *A. vasorum*–infected dogs presenting with hypocoagulability (Inf_{hypo}; n = 5), ii) infected dogs with normal coagulation values (Inf_{norm}; n = 5), and iii) uninfected controls (UC; n = 12). Alongside classical hematology and coagulation parameters, including rotational thromboelastometry, we generated a global transcriptomic profile of circulating blood cells, enabling the identification of differentially expressed genes following infection.

Results: Fibrinogen levels were significantly lower in Inf_{hypo} than in Inf_{norm}. All Inf_{hypo} dogs showed out-of-range prothrombin times. Matrix metalloprotease transcripts were strongly upregulated among infected dogs, regardless of their coagulation status, which might amplify vascular disruption and inflammation. Analysis of downregulated transcripts in infected dogs compared to UC indicate a loss of endothelial integrity, especially at the level of the lungs, and of inflammatory processes contributing to fibrinolysis and coagulopathies. Endothelial injury is a known trigger of disseminated intravascular coagulation and consumption of coagulation factors, which could explain the observed coagulation disorders induced by *A. vasorum* infection.

Conclusions: The multifaceted alterations observed in hematologic and coagulation parameters and transcriptomic profiles of *A. vasorum*-infected dogs point to a dynamic and evolving disruption of hemostatic balance. While some animals show signs of early compensatory hypercoagulability,

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others seem to progress toward a state consistent with DIC, marked by factor consumption, endothelial dysfunction, and impaired fibrinogen-dependent clotting.

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TOXOPLASMA GONDII–DERIVED EFFECTORS SUPPRESS PANCREATIC AND HEPATOCELLULAR CARCINOMAS VIA REDOX DYSREGULATION AND P53-DEPENDENT SIGNALING

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Background and Aims: Pancreatic ductal adenocarcinoma (PDAC) and hepatocellular carcinoma (HCC) are hepatobiliary–pancreatic malignancies in which metabolic rewiring and stress-adaptive signaling contribute to limited therapeutic efficacy. *Toxoplasma gondii (T. gondii)* secretes multiple effector proteins capable of reprogramming host cell signaling, but their potential to complement anticancer therapy remains poorly defined. Therefore, we investigated whether functionally distinct *T. gondii*-derived effector proteins can enhance anticancer responses by increasing chemosensitivity and altering tumor cell fate in PDAC and HCC.

Methods: In PDAC models, a nuclear-localizing *T. gondii* effector (dense granule protein) was expressed in PANC-1 cells and xenograft mice, followed by oxaliplatin treatment. Oxaliplatin chemosensitivity, apoptotic signaling, nitric oxide (NO) signaling, mitochondrial reactive oxygen species (ROS), AKT/ERK/mTOR pathways, and tumor formation were assessed. In HCC models, a distinct kinase-type *T. gondii* effector (rhoptry protein) was examined in HepG2 cells to determine its effects on AKT/ERK/mTOR signaling, p53 activation, DNA damage responses, cell-cycle progression, apoptosis, and clonogenicity.

Results: In PDAC, the dense granule protein increased oxaliplatin sensitivity by enhancing iNOS/eNOS-associated NO production and mitochondrial ROS, accompanied by suppression of AKT/ERK/mTOR signaling and reduced expression of mitochondrial metabolic enzymes. These *T. gondii* effector-driven changes resulted in decreased migration, invasion, and tumor growth in xenograft models. In HCC, the rhoptry protein suppressed AKT/ERK/mTOR signaling and robust p53 activation, resulting in G_2/M cell-cycle arrest, increased apoptosis, and inhibition of clonogenic growth.

Conclusions: Functionally distinct *T. gondii*-derived effector proteins exert complementary anticancer effects in hepatobiliary-pancreatic malignancies through redox stress and tumor suppressor activation, augmenting the efficacy of existing anticancer therapies in solid tumors.

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A MULTI-MODAL SPATIAL ANALYSIS OF FASCIOLA HEPATICA INFECTION IN THE NATIVE AUSTRALIAN LYMNAEID HOST AUSTROPEPLEA BRAZIERI.

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Background and Aims: Freshwater snails serve as intermediate hosts for *Fasciola hepatica*, a globally significant zoonotic parasite. Studies have largely focused on disease pathology in the definitive host, while pathology within intermediate hosts, particularly in native Australian lymnaeid species, remains relatively unknown. This project aimed to generate a novel whole-organism, multi-dimensional resource of trematode pathology within a snail host, integrating high-resolution imaging, histopathology, and spatial transcriptomics in a pipeline adaptable to other host–parasite systems.

Methods: This study incorporates four key methodologies: high-resolution X-ray microcomputed tomography (microCT), digitised serial-sectioned histopathology, bulk RNA sequencing, and spatial transcriptomics. Together, these approaches enabled spatially resolved analysis of gene expression within infected organs and at host–parasite interfaces.

Results: MicroCT revealed parasite proliferation along the digestive system and clustering around reproductive organs. Histopathological analysis showed disruption of digestive gland architecture, including focal necrosis and tissue loss. Differential gene expression analysis using bulk RNA sequencing showed clear clustering of infected individuals relative to healthy controls. Infected snails exhibited widespread downregulation of genes associated with digestion and reproduction, alongside selective upregulation of metabolic pathways. Spatial transcriptomic analyses localised transcriptional changes at host–parasite interfaces within digestive and reproductive tissues. This represents a rare application of spatial transcriptomics to simultaneously resolve host and parasite gene expression from two complex multicellular organisms within the same tissue environment.

Conclusions: This study presents the first integration of two- and three-dimensional imaging approaches underpinned by molecular data to form a comprehensive atlas that spatially characterises host–parasite interactions in an intermediate host.

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SUSPECTED DIOCTOPHYME RENALE (GIANT KIDNEY WORM) IN A SHELTER CAT: DIAGNOSTIC BARRIERS AND RENAL PATHOLOGY IN A COMMUNITY-WELLNESS SETTING

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Background and Aims: Dioctophyme renale is a rare renal parasite in domestic animals, with only sporadic feline reports. Diagnosis is challenging in shelter and community-wellness settings where advanced imaging is often inaccessible. This case describes a cat that expelled a segmented larva during vomiting and was later found to have severe unilateral hydronephrosis. The aim is to highlight diagnostic challenges and raise awareness of unusual parasitic presentations in low-resource environments.

Methods: Initial evaluation included physical examination, CBC, serum biochemistry, urinalysis, and three-view abdominal radiographs. The expelled larva was photographed and compared with known nematode larvae. Due to financial limitations, the patient was referred to a full-service hospital for ultrasound, followed by exploratory laparotomy with attempted nephrectomy. Diagnostic interpretation integrated clinical signs, imaging findings, laboratory changes, larval appearance, and surgical observations.

Results: Ultrasound confirmed marked left-sided hydronephrosis with a thin, nonfunctional cortex and no discrete mass. Exploratory surgery revealed extensive vascularization and perirenal inflammation preventing safe nephrectomy. The kidney was left in situ. The patient recovered well on analgesics and a renal diet, with resolution of vomiting and normal activity at follow-up. The combination of larval expulsion, destructive unilateral hydronephrosis, and intra-operative findings supports strong suspicion for renal nematode migration, including possible D. renale.

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Conclusions:
This case emphasizes the need to consider renal parasitism in cats with unilateral renal enlargement and larval expulsion. It highlights diagnostic limitations in community-wellness clinics and reinforces the value of collaborative approaches between shelter veterinarians and full-service hospitals.

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THE STRUCTURAL PLASTICITY OF THE MALARIA PARASITE'S CIRCUMSPOROZOITE PROTEIN IS MEDIATED BY INTRINSIC DISORDER

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University of Antwerp (UAntwerp), Laboratory Of Medical Biochemistry (lmb) And The Infla-med Centre Of Excellence, Antwerp, Belgium

Background and Aims: Malaria remains a significant global health burden. Its etiological agents are *Plasmodium* parasites, of which *P. falciparum* and *P. vivax* are the most notorious human-infective members. The circumsporozoite protein (CSP) is the malaria sporozoite's (SPZ) major surface antigen. Despite CSP's central role in SPZ immunobiology, many aspects of its structure-function relationship remain unknown. While full-length CSP displays features typical of an intrinsically disordered protein (IDP), it hasn't been thoroughly experimentally investigated as such.

Methods: We adopted an integrative approach encompassing various methods (e.g., in silico prediction, SEC-MALS, CD spectroscopy, DLS, SAXS, NMR, and native MS) to thoroughly characterize the structural features of *P. falciparum* and *P. berghei* CSP (PfCSP and PbCSP, respectively) and provide a detailed description of their conformational ensembles.

Results: First, we show that PfCSP and PbCSP possess typical IDP features and suggest that these are conserved among different *Plasmodium* species. By adopting the premise that intrinsic disorder is a central feature in the CSP structure-function relationship, we show that the CSP linker region adopts a plethora of interchangeable conformations (ranging from completely disordered to retaining a spiral-like architecture) in solution. Second, our work suggests that both the number and nature of possible conformations adopted by CSP appear to be heavily context-dependent. We interpret these findings within the broader context of functional data supporting the role of CSP in SPZ biology.

Conclusions: We propose that intrinsic disorder is a central molecular mechanism that underpins CSP's conformational landscape at the parasite surface during SPZ development and infectivity.

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STRUCTURAL PROTEIN OR A PILLAR SUPPORTING PARASITE HEALTH? THE ROLE OF EPSIN IN LEISHMANIA BIOLOGY

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Background and Aims: Upon transmission to the mammalian host, *Leishmania* is phagocytosed by macrophages where it creates parasitophorous vacuoles. Inside, parasites rely on endocytic mechanisms to acquire essential nutrients from the host cell; however, this aspect remains poorly understood and uncharacterized. To deepen our understanding of the *Leishmania* endocytic pathway and its importance for the parasite's replication, we studied Epsin; an adaptor protein of the clathrin-mediated endocytic pathway.

Methods: Using a *L. mexicana* Cas9-T7RNP engineered parasite, we introduced a fluorescent tag on Epsin to monitor its localization during infection in murine bone marrow-derived macrophages. We then generated Epsin knockout parasites by homologous recombination to assess parasite growth and other biological parameters.

Results: The localization of Epsin during infection in murine bone marrow derived macrophages remained within the parasite and did not relocate within the macrophage. Epsin knockout parasites presented growth defects in culture and infected macrophages. We observed that the surface virulence glycolipid lipophosphoglycan (LPG) and Epsin colocalize in the parasite. We showed that Epsin disruption severely affects the distribution of LPG both on the parasite's surface and its release within infected macrophages in the initial hours of infection thus further supporting the notion that Epsin affects its distribution.

Conclusions: Further work will explore the role of Epsin disruption on the endocytic capabilities of the parasite and focus on uncovering its specific mechanism. Generally, this research aims to enrich our knowledge on parasite biology which will deepen our knowledge on the complex host-pathogen interactions. *Supported by the Canadian Institutes of Health Research*

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FROM CHAGAS' LENS TO NANOSCALE VISION: REVISITING TRYPANOSOMA CRUZI VECTOR FORMS WITH U-EXM

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Background and Aims: More than a century after Carlos Chagas first described the developmental forms of Trypanosoma cruzi within its insect vector, many aspects of their ultrastructure remain strikingly consistent with his original observations. In this study, we revisit these vector-associated stages using ultrastructure expansion microscopy (U-ExM) combined with our confocal microscopy setup, enabling nanoscale visualization of parasite organization using conventional fluorescence imaging.

Methods: By integrating U-ExM with specific markers for cytoskeletal components and nucleic acids, we analyzed the architecture of T. cruzi forms obtained from axenic culture and systematically compared them with more than 100 morphological variants described by Chagas in his seminal early-twentieth-century work. We focused on key structural parameters, including the relative positions of the basal body, kinetoplast, and nucleus, as well as flagellar length and overall cell shape.

Results: Our analyses reveal a remarkable concordance between Chagas's detailed drawings and the ultrastructural organization resolved using modern imaging approaches. Despite the technical limitations of the microscopy available at the time, many of his descriptions accurately captured fundamental features of parasite morphology. U-ExM combined with confocal microscopy provides an effective resolution improvement of approximately 20-fold in the lateral (XY) dimension and 30-fold in the axial (Z) dimension compared with the instruments likely used by Chagas, allowing these classical observations to be validated and refined at the nanoscale.

Conclusions: Together, our findings highlight both the enduring value of early protozoological studies and the power of expansion microscopy to bridge historical and contemporary views of parasite cell biology.

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EXPERIMENTAL INFECTION OF PUREBRED SAANEN GOATS HIGH PATHOGENICITY AND VIRULENCE OF BABESIA AKTASI

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Background and Aims: *Babesia aktasi*, recently discovered in goats from the Mediterranean region of Türkiye, has been shown in experimental infections to cause mild clinical symptoms in indigenous goats.Host breed is a significant factor influencing the clinical infection of babesiosis; however, host resistance remains less explored in small ruminants. Differences in susceptibility to *Babesia* parasites may exist among breeds within the same host species. This study aimed to investigate the pathogenesis and virulence of *B. aktasi* in purebred Saanen goats.

Methods: The experimental groups consisted of immunocompetent Saanen goats (n = 7) and immunosuppressed Saanen goats (splenectomy and dexamethasone) (n = 5). The goats were infected with fresh *B. aktasi*–infected blood obtained from donor goats with parasitemia levels of 9.2%–13.5%. They were examined for clinical, parasitological, hematological findings.

Results: Piroplasm forms were detected from the 1st day post-infection, followed by an increase in rectal temperature and parasitemia. Parasitemia ranged from 4.3% to 33.5% in the immunosuppressed group and from 2.1% to 7.6% in the immunocompetent group. Severe clinical symptoms, including anemia, jaundice, and hemoglobinuria, developed in both groups. A significant inverse correlation was observed between parasitemia and RBC, WBC, HCT values. All immunosuppressed goats died on the 4th and 7th days post-infection, while four of seven immunocompetent goats died on between 6-8th days.

Conclusions: Clinical symptoms and hematological parameters revealed that *B. aktasi* is highly pathogenic to Saanen goats, causing severe clinical infections leading to death. This work was financially supported by the Scientific and Technological Council of Türkiye (TUBITAK) (project number: 221O119).

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PHENOTYPIC ANALYSIS OF LESION-INFILTRATING CELLS FOLLOWING CD200 BLOCKADE IN LEISHMANIA AMAZONENSIS INFECTION

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Background and Aims: *Leishmania amazonensis* (*L.a*) causes chronic progressive cutaneous leishmaniasis through mechanisms that remain incompletely understood. Previous studies showed that *L.a* modulates CD200 expression, which binds CD200R and promotes an inhibitory checkpoint signal that maintains immune homeostasis. Parasite-induced CD200 triggers an autoinhibitory response in infected macrophages, blocking the host iNOS/NO-mediated microbicidal pathway, and enabling parasite survival and proliferation. This study characterized the cellular infiltrate in lesions after CD200 blockade and assessed its effect on parasite control.

Methods: C57BL/6 mice were infected with RFP-expressing *Leishmania* and treated with either anti-CD200 or isotype control. At day 16 post-infection, lesion size and parasite burden were assessed. The phenotypic and functional profiles of infiltrating myeloid cells were characterized by spectral flow cytometry.

Results: CD200 blockade significantly reduced lesion size and parasite burden. Phenotypic analysis revealed a decrease in the absolute numbers of infected myeloid cells and $CD4^+$ T cells, and a restricted neutrophil reservoir. Anti-CD200 treatment upregulated CD36, CCR5, and Mannose Receptor expression while boosting IL-1β frequency in monocyte-derived macrophages. Furthermore, anti-CD200-treated mice evidenced a massive predominance of Arginase-1 over iNOS in both macrophages and neutrophils. Notably, this innate control also resulted in a significant reduction of infiltrating IFN-γ^+ $CD4^+$ T cells at the lesion site.

Conclusions: Our data support a critical role for CD200 during *L.a* infection, impacting both lesion development and parasite burden. CD200 blockade enhanced resistance to the parasite and promoted a heterogeneous, resolution-associated immune phenotype that limited inflammatory tissue damage, highlighting CD200 as a potential therapeutic target for leishmaniasis.

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THE ANTI-PARASITIC ACTIVITY OF A NOVEL DEAD-BOX RNA HELICASE INHIBITOR IS ASSOCIATED WITH A TYPE I INTERFERON-RELATED PROTEIN SIGNATURE IN TOXOPLASMA GONDII-INFECTED MACROPHAGES

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Background and Aims: *Toxoplasma gondii* is a widespread intracellular parasite that poses significant risks to immunocompromised and pregnant individuals. Existing treatments are potentially teratogenic and toxic. This study aims to evaluate and characterize the impact of a novel type of weak-clamping rocaglates (i.e., DEAD-box RNA helicase (DDX) inhibitors with low affinity for eukaryotic initiation factor 4A1 (eIF4A1)) on *T. gondii* replication in Bone Marrow Derived Macrophages (BMDM).

Methods: The effect on parasite replication of a library of rocaglates was screened by qPCR. A lead compound, C6, was further characterized. Proteome integral solubility alteration (PISA) assays were used to identify C6-interacting proteins. Its toxicity was evaluated by resazurin assays. Host and parasite *de novo* protein synthesis was assessed through puromycin incorporation. Proteomics data were analyzed using Gene Ontology and Ingenuity Pathway Analysis.

Results: C6 significantly reduced parasite burden without compromising host cell viability. PISA assays identified eIF4A2, eIF4A3, DDX3, and DDX21 as interacting partners, with no detectable parasite targets. These helicases can regulate mRNA translation. C6 counteracted infection-induced *de novo* protein synthesis in the host but not on extracellular tachyzoites, indicating a host-directed mechanism. Proteomic analyses revealed that C6 upregulates proteins associated with type I interferon responses, including RIG-I, MAVS, IRF9, and interferon-stimulated genes (e.g., IRGQ, OAS3, IFITM3, IFI204, etc.).

Conclusions: C6 reduces *T. gondii* replication in BMDM through a host-directed mechanism. Targeted knockout or knockdown of PISA-identified DDX proteins and proteome-identified Type I interferon-associated proteins will further clarify its mechanism of action. Completion of this study will expand understanding of rocaglate activity and host-directed antiparasitic strategies.

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A SINGLE-CELL METHODOLOGY FOR SIMULTANEOUS SUBPOPULATION AND ENERGY METABOLISM ANALYSIS OF TRYPANOSOMA CRUZI DEVELOPMENTAL STAGES AND T. CRUZI INFECTED CELLS

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Background and Aims: The metabolic adaptability of *Trypanosoma cruzi*, the causal agent of Chagas disease, is a key feature of its biology and pathogenicity. Studying parasite and host cell energy metabolism (EM) profiles is crucial for understanding disease progression and developing targeted therapies.

Methods: The recently developed method "Scenith" (single-cell energetic metabolism by profiling translation inhibition) enables the simultaneous identification and metabolic assessment of different cell subpopulations via flow cytometry. This method quantifies puromycin incorporation, reflecting ATP and protein synthesis levels, after blocking various energy-generating pathways. It determines four EM parameters: Glucose Dependency (GD), Mitochondrial Dependency (MD), Glycolytic Capacity, and Fatty Acid/Amino Acid Oxidation (FAO/AAO) Capacity. We adapted Scenith to evaluate the EM profile of *T. cruzi* forms and infected mammalian cells.

Results: Epimastigotes of all evaluated strains (Dm28c, Tulahuen, and K98) showed high MD (65.79–83.22%) and moderate GD (32.76–50.81%). FAO/AAO capacity of epimastigotes was larger than their glycolytic capacity. In contrast, Dm28c trypomastigotes relied predominantly on glucose for energy (GD 97.86% vs MD 73.85%), similarly to intracellular amastigotes. Trypomastigotes displayed low FAO/AAO capacity, whereas amastigotes retained some. Amastigotes with lower dividing rates showed lower translation activity and lower MD. We also assessed EM profiles of infected and uninfected HeLa cells. In all conditions, GD was prominent, but parasite-bearing cells had slightly higher MD than controls. Notably, uninfected cells from infected cultures shared the EM profile of amastigote-bearing cells.

Conclusions: These findings demonstrate that Scenith enables rapid, sensitive evaluation of the EM profile across *T. cruzi* developmental stages and cell subpopulations in infected cultures.

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SHIFT02-398

POSTER ON BOARD SHIFT 02
DIAGNOSTICS, HOST-PARASITE INTERACTION AND EMERGING TECHNOLOGIES / HOST-PARASITE MOLECULAR AND CELLULAR INTERACTIONS

ORGAN-SPECIFIC ENDOTHELIAL RECEPTOR LANDSCAPES IN PFEMP1-MEDIATED CYTOADHESION

Lara Zirwes[1], David Danicic-Rauchberger[1], Johannes Allweier[1], Tobias Spielmann[2], Susann Ofori[1], Rieke Bruns[3], Thomas Gutsmann[3], Iris Bruchhaus[1]
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Background and Aims: Vaccine and therapy development against *Plasmodium falciparum (P. falciparum)* malaria is hindered by the parasite's ability to vary antigens expressed on the surface of infected erythrocytes, thereby promoting immune evasion and cytoadhesion to the vascular endothelium. This process is largely mediated by the *P. falciparum* erythrocyte membrane protein 1 (PfEMP1) family, encoded by ~ 60 *var* genes and expressed in a mutually exclusive manner. To date, at least 24 endothelial cell receptors (ECRs) have been identified as PfEMP1 binding partners, including CD36, ICAM-1 and EPCR. We hypothesised that endothelial cells from different organs exhibit distinct receptor expression profiles that may contribute to organ-specific cytoadhesion. The aim was to define these organ-specific receptor landscapes to enable physiologically relevant analyses of PfEMP1-endothelium interactions.

Methods: To overcome antigenic variation, we employed a selection-linked integration strategy to generate parasite transfectants expressing a single, defined *var* gene. Cytoadhesion was initially studied using transgenic CHO cells expressing individual ECRs under static conditions, flow-based adhesion assays, and single-cell force spectroscopy. To advance towards a more physiologically relevant model, primary endothelial cells from five human organs (brain, dermis, intestine, lung, kidney) were characterised by flow cytometry to assess basal expression of a defined receptor panel.

Results: Flow cytometric analysis revealed organ- and receptor-specific basal expression patterns, differential receptor abundance across endothelial cell types, and TNF-α induced upregulation of selected adhesion receptors.

Conclusions: These characterised endothelial cells will be used in future static and flow-based binding assays with defined PfEMP1 variants to investigate organ-specific cytoadhesion under basal and inflammatory conditions.

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SHIFT02-399

POSTER ON BOARD SHIFT 02
DIAGNOSTICS, HOST-PARASITE INTERACTION AND EMERGING TECHNOLOGIES / CIRCADIAN CLOCK AND PARASITIC INFECTION

CIRCADIAN CONTROL OF LEISHMANIA INFECTION BY THE MACROPHAGE CLOCK

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Background and Aims: Leishmaniasis is a neglected tropical disease caused by the protozoan parasite *Leishmania*. Previous work from our group has shown that *Leishmania major* infection displays a circadian rhythm, with disease outcome in mice depending on the time of infection. This indicates that circadian regulation within immune cells is critical for parasite control. The aim of this study is to identify the molecular mechanisms by which the circadian clock regulates the host response to *Leishmania* infection.

Methods: Bone marrow–derived macrophages (BMDMs) were synchronized and infected with *L. major* at distinct circadian times. Parasite attachment and internalization were quantified, alongside assessment of phagolysosome formation, reactive oxygen species (ROS) production, and cytokine secretion. Furthermore, mice were infected at different times, and immune cell recruitment was assessed by flow cytometry. Circadian regulation was examined using wild-type (WT) and clock-impaired *Bmal1* or *Rev-erbα* knockout (KO) models.

Results: In WT mice, infection magnitude and parasite internalization varied according to the time of infection both in vivo and in vitro. In vitro, phagolysosome formation was under circadian control, with rhythmic expression of the early endosome marker EEA1 observed in WT BMDMs but abolished in *Bmal1* KO cells. ROS production and cytokine secretion also varied by infection time. Preliminary results indicate that *Rev-erbα* KO BMDMs exhibit increased parasite attachment and internalization compared to WT cells, accompanied by loss of circadian rhythmicity.

Conclusions: These findings demonstrate that the macrophage circadian clock regulates key cellular processes controlling *Leishmania* infection. Understanding these mechanisms may inform the development of chronotherapy-based strategies to improve treatment outcomes.

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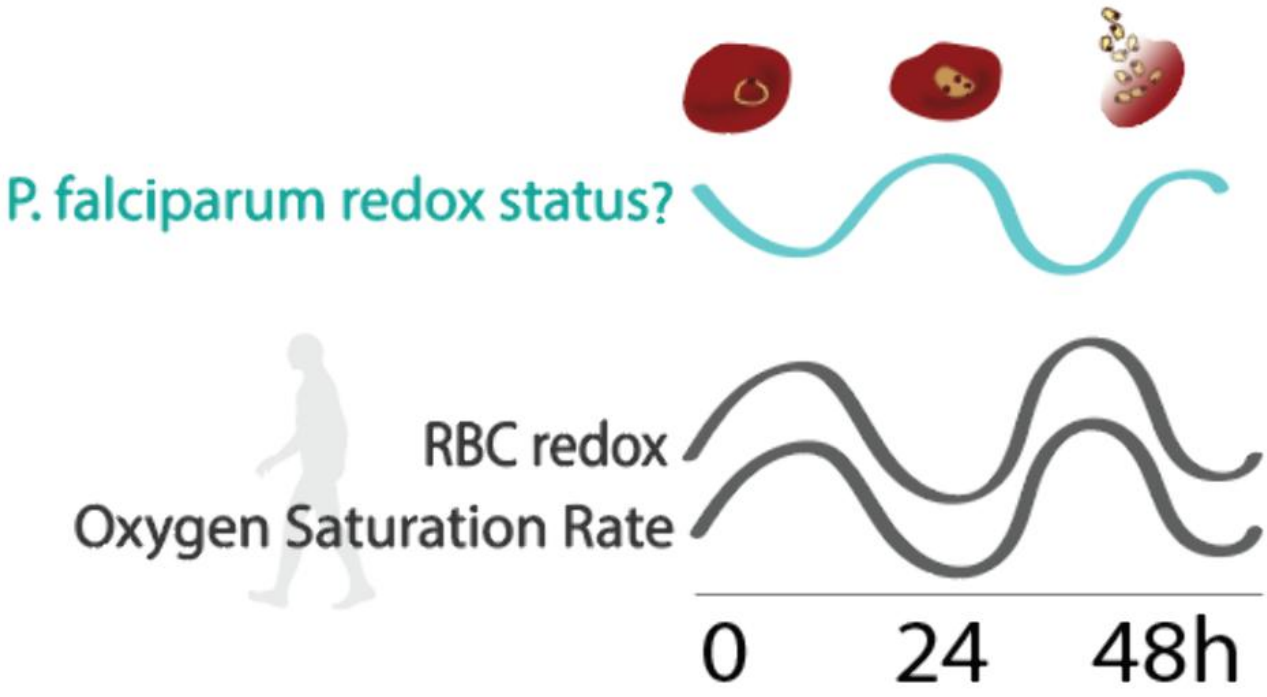
SHIFT02-400

POSTER ON BOARD SHIFT 02
DIAGNOSTICS, HOST-PARASITE INTERACTION AND EMERGING TECHNOLOGIES / CIRCADIAN CLOCK AND PARASITIC INFECTION

IMPACT OF ENVIRONMENTAL OXYGEN TENSION ON PLASMODIUM FALCIPARUM

Yunhung Tu[1], Paul Tyagi[1], Yihui Liang[1], Taiki Yamamoto[1], Filipa Rijo-Ferreira[1,2]
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Background and Aims: Malaria remains a global health issue, exacerbated by the rise of artemisinin resistance. While the rhythmic paroxysmal fever characteristic of malaria results from the synchronous erythrocytic growth cycle of *Plasmodium* parasites, the mechanisms governing this synchrony remain elusive. Most antimalarials, such as quinolines, artemisinin and atovaquone, kill parasites by disrupting **redox homeostasis**. Endogenous reactive oxygen species (ROS) primarily arise from hemoglobin digestion and mitochondrial electron transport—processes essential for parasite survival. Given that host erythrocytes and blood oxygen levels exhibit circadian oscillations, we aim to investigate whether the parasite's internal redox state follows a similar rhythm and how environmental oxygen tension affects
growth.

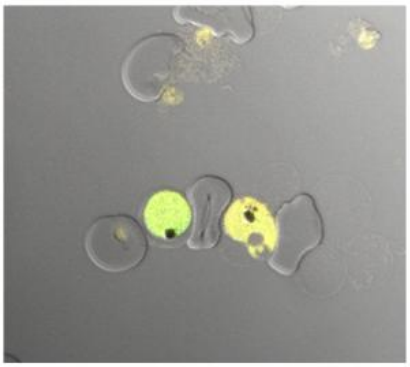


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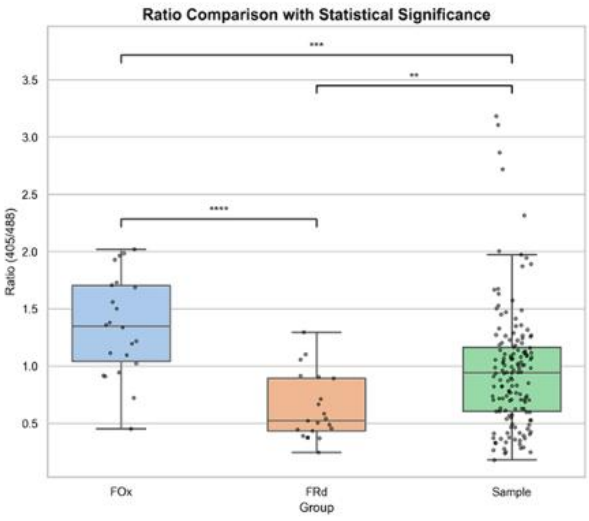
Methods: We developed a pipeline using a novel hGrx-sfroGFP2 *Plasmodium* redox-probe reporter line to measure redox status across intraerythrocytic stages. In parallel, we engineered an automated continuous culture system to maintain stable, long-term parasite growth under precise environmental control.

Results: The sensitivity of the Python-based redox reporter image analysis pipeline was validated by comparing cells treated with oxidizing and reducing agents, which exhibited significantly distinct fluorescence ratios compared to untreated controls (Figure 1).

Figure 1.



Confocal image of redox reporter cells.

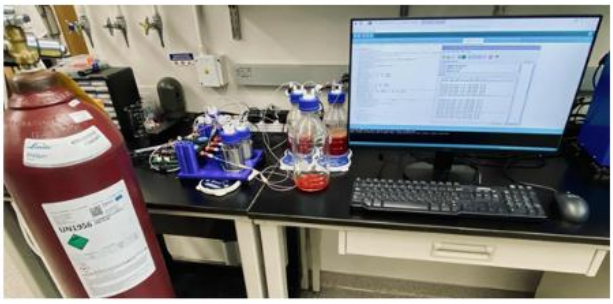


Significant difference of average oxidized level in oxidant treated, reductant treated, and control group.

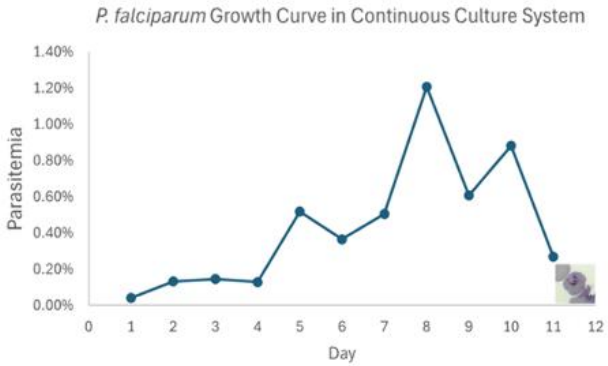
The automated continuous culture system demonstrated high reliability, maintaining constant culture conditions and stable parasitemia for over 10 consecutive days (Figure 2).

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Figure 2.



Plasmodium automated continuous culture system.



P. Falciparum growth curve in the automated continuous culture system.

By integrating these tools we will investigate how environmental oxygen tension affects cellular redox status and growth.

Conclusions: We have successfully developed a novel automated continuous culture platform coupled with a specialized hGrx1-sfroGFP2 reporter line to map the redox dynamics of *P. falciparum* intraerythrocytic satges for future environmental oxygen tension studies.

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SHIFT02-401

POSTER ON BOARD SHIFT 02
DIAGNOSTICS, HOST-PARASITE INTERACTION AND EMERGING TECHNOLOGIES / ARTIFICIAL INTELLIGENCE AND MACHINE LEARNING IN PARASITOLOGY
08-19-2026 9:00 AM - 7:00 PM

INTEGRATING ARTIFICIAL INTELLIGENCE INTO AVIAN PARASITOLOGICAL DIAGNOSIS: OPPORTUNITIES AND LIMITATIONS

Cristina Almeida[1,2], João Lozano[1], Luís De Carvalho[1], Daniela Sebastião[3]
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Background and Aims: Intestinal parasitism is a major health concern in avian species maintained in domestic, ornamental, and captive environments. Protozoa and nematodes are the most frequently detected intestinal parasites, with *Eimeria* spp., *Isospora* spp., *Capillaria* spp., and strongylid-type nematodes commonly reported. Conventional coprological techniques such as Mini-FLOTAC are widely used but are time-consuming and dependent on operator expertise. This study aimed to evaluate the performance of an artificial intelligence (AI)–based system for the identification of intestinal parasites in birds and to compare its diagnostic accuracy with the Mini-FLOTAC method.

Methods: 73 fecal samples were collected from captive birds, including 40 peafowl (*Pavo cristatus*), 13 psittacines and 20 chickens (*Gallus gallus domesticus*). All samples were analyzed using the Mini-FLOTAC technique as the reference method and in parallel by AI-based image analysis system. Twenty percent of the samples were evaluated blindly by an experienced investigator, all samples were analyzed by the principal investigator. Information on hygiene and previous antiparasitic treatments was obtained through questionnaires.

Results: Eighty percent of peafowl samples were positive for intestinal parasites, with strongylid-type nematodes, *Capillaria* spp., and coccidia detected; similar findings in chickens. In psittacines, diagnostic concordance between AI and Mini-FLOTAC reached 100%. In peafowl and chickens, human evaluation demonstrated higher accuracy in parasite species classification, with all diagnostic discrepancies attributable to AI misclassification.

Conclusions: AI-based diagnosis reduced parasite detection time and showed high agreement with Mini-FLOTAC, particularly in psittacines. While human expertise remains essential for species-level identification, AI represents valuable complementary tool for rapid screening and parasitological surveillance in avian populations.

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08-19-2026 9:00 AM - 7:00 PM

AI-DRIVEN HOTSPOT DETECTION AND PROGRAM PERFORMANCE ANALYSIS OF SCHISTOSOMIASIS IN AFRICA

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[1]Newcastle University, Biomedical Informatics, Newcastle Upon Tyne, United Kingdom, [2]Tai Solarin University, Biology, Ijebu-Ode, Nigeria, [3]Lead City University, Molecular Biology And Genomics, Ibadan, Nigeria, [4]Federal University of Agriculture, Abeokuta, Veterinary Medicine, Abeokuta, Nigeria

Background and Aims: Schistosomiasis remains a persistent neglected tropical disease in sub-Saharan Africa, with substantial subnational heterogeneity and continued transmission despite repeated mass drug administration. This study developed an AI-operated analytical framework to identify transmission hotspots across endemic African countries.

Methods: A multi-component analytical architecture was implemented, integrating machine-learning clustering, supervised hotspot prediction, geographic risk stratification, and spatiotemporal trend modelling. K-means clustering (k=4) was used to detect structural epidemiological patterns. Random forest models were applied to predict hotspot districts. Combined risk stratification assessed district-level priority categories. Spatiotemporal endemicity trends were evaluated to quantify temporal change and inter-district variability.

Results: Clustering revealed structurally imbalanced epidemiological patterns, with one dominant cluster comprising 65.9% of districts, indicating widespread similarity in coverage and endemicity, alongside smaller atypical clusters suggestive of extreme-risk or high-performance contexts. The random forest model predicted hotspots with 88% accuracy and an AUC of 0.80, demonstrating strong discriminatory ability; however, extreme class imbalance produced very high recall for hotspots (0.99) but near-zero recall for non-hotspots, highlighting methodological challenges in elimination-phase modelling. Geographic stratification showed that most districts fell into moderate-priority categories rather than extreme high-risk groups. Spatiotemporal analysis indicated an overall negative mean endemicity trend (p = –7.59), but a median slope of zero, reflecting widespread stability with substantial inter-district variability.

Conclusions: The findings demonstrate that schistosomiasis eradication progress is discontinuous and geographically embedded. The proposed AI-driven hybrid stratification framework offers a scalable approach for targeted intervention planning and optimal resource allocation. The study underscores the need for modular, evidence-based, and geographically tailored eradication strategies across Africa.

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08-19-2026 9:00 AM - 7:00 PM

AI-ASSISTED DIAGNOSTIC TOOL FOR COMPANION ANIMAL PARASITOLOGY

Helena Bonar, Kerrie Duffy
Micron Agritech, Dublin, Ireland

Background and Aims: Parasitic infections can have a significant impact on the health and welfare of companion animals. Detection currently relies on fecal floatation methods using microscopy performed by trained professionals; however, sensitivity and specificity can vary widely depending on the method used. Micron Agritech aims to leverage machine learning alongside traditional techniques to improve the identification of gastrointestinal parasites in companion animal fecal samples.

Methods: Canine fecal samples were collected from across Ireland. A true egg count value was established for each sample and compared across four methods: passive flotation, centrifugal flotation, McMaster, and Micron Agritech Pet Plus™. For the Pet Plus™ method, multiple prototype extraction tools were evaluated for their ability to consistently recover the top layer of sample following centrifugation.

Results: The Micron Agritech Pet Plus™ method performed similarly to the gold standard method , centrifugal floatation, while the modified McMaster method also demonstrated high recovery. Passive floatation was not as sensitive as the centrifugal floatation, highlighting the benefit in centrifuging canine fecal samples in parasite egg recovery. In contrast, the McMaster yielded the lowest counts , again suggesting the need to use the centrifuge.

Conclusions: The Micron Agritech Pet Plus™ method shows promise as a rapid and user-friendly diagnostic tool for detecting parasitic infections in companion animals. Current outputs are presented as burden categories, supporting timely treatment decisions in veterinary practice.

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08-19-2026 9:00 AM - 7:00 PM

UNSUPERVISED MACHINE LEARNING IDENTIFIES BIOMARKERS OF DISEASE PROGRESSION IN POST KALA AZAR DERMAL LEISHMANIASIS

Eugenia Carrillo[1], Ana Torres[1], Brima Musa Younis[2], Jose Carlos Solana[3], Samuel Tesema[4], Javier Moreno[1], Antonio Javier Martín-Galiano[5], Ahmed Mudawi Musa[2], Fabiana Alves[6]
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Background and Aims: Post kala azar dermal leishmaniasis (PKDL) is a neglected complication of visceral leishmaniasis with heterogeneous clinical outcomes. Early identification of patients at risk of disease progression remains challenging due to the lack of reliable prognostic biomarkers. This study aimed to identify biochemical, haematological and immunological factors associated with PKDL progression using an unsupervised machine learning approach.

Methods: Baseline clinical and laboratory data from 110 Sudanese PKDL patients were analysed, including 31 biochemical, haematological and immunological variables. Patients were classified as having stable or progressive disease based on clinical evolution over six months. Conventional statistical analyses were complemented with unsupervised machine learning, including data normalization, principal component analysis and k-means clustering, to identify multivariate patterns associated with disease progression.

Results: Univariate analyses showed limited ability of individual variables to discriminate progressive PKDL. In contrast, unsupervised clustering identified a patient subgroup enriched in progressive cases, with a 3.1-fold higher risk of disease worsening. This cluster was defined by reduced albumin levels, haematocrit and *Leishmania*-specific IFN-g production, together with additional alterations in haematological and immunological parameters. Overall, a combination of 14 variables was associated with a worsening PKDL phenotype, reflecting anaemia, impaired Th1 immune responses and systemic physiological alterations.

Conclusions: Unsupervised machine learning enabled the identification of a multivariate biomarker signature associated with PKDL progression. This approach may support early risk stratification and inform future development of predictive tools for improved clinical management of PKDL.

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DEEP WORM: MACHINE LEARNING-BASED DETECTION AND SPECIATION OF MICROFILARIAE IN PERIPHERAL BLOOD

Maria Diaz De Leon Derby, Zihao Zhao, Mingxi Tang, Meixin Ma, Daniel Fletcher
University of California, Berkeley, Berkeley, United States of America

Background and Aims: Automated analysis algorithms can accelerate clinical decision making, particularly for point-of-care devices such as the NTDscope, a mobile microscope used to diagnose Neglected Tropical Diseases (NTDs). The NTDscope is used to quantify *Loa loa* microfilarial loads in peripheral blood for onchocerciasis Test-and-Not-Treat campaigns using conventional image processing methods and biophysical priors to detect microfilarial motion. However, the baseline algorithm cannot differentiate microfilarial species. Here, we applied deep learning to improve microfilariae quantification and enable species differentiation.

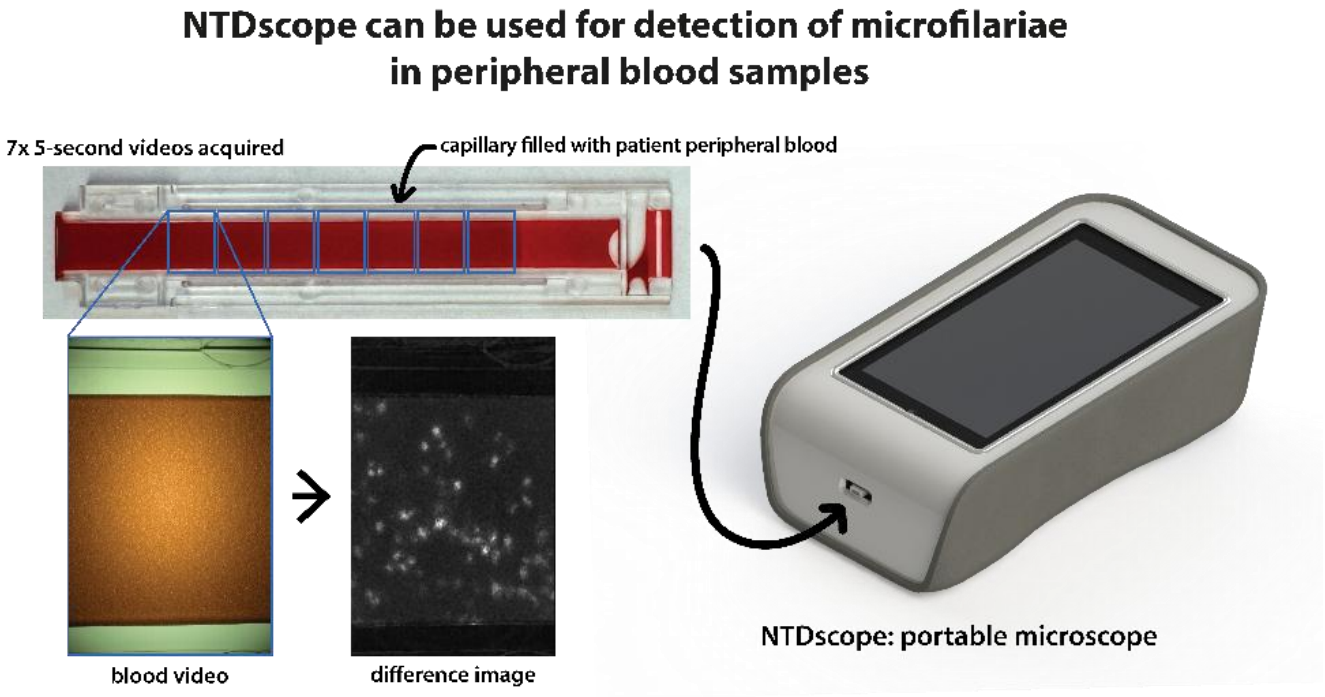


Figure 1: NTDscope workflow, including blood collection, video acquisition, and difference image generation.

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Methods: We trained YOLOv8 object detection models using difference images generated from NTDscope videos of patient blood samples. A dataset of 2,469 manually annotated images was used to train an initial model, which was then used to generate annotations for 9,000 additional images. Two models were trained on this bootstrapped dataset: YOLO large and YOLO large no neg, which excluded samples negative for *L. loa* by thick smear. Additionally, a YOLO11-based classification model was trained to distinguish microfilariae-free samples from those containing *L. loa* or *Brugia malayi*.

Results: All YOLO-based object detection models outperformed the baseline algorithm for microfilariae quantification, with bootstrapped models performing best. The classification model achieved >99% sensitivity and specificity for positive versus negative samples and 93% sensitivity and 99.7% specificity for distinguishing *L. loa* from *B.*
malayi.

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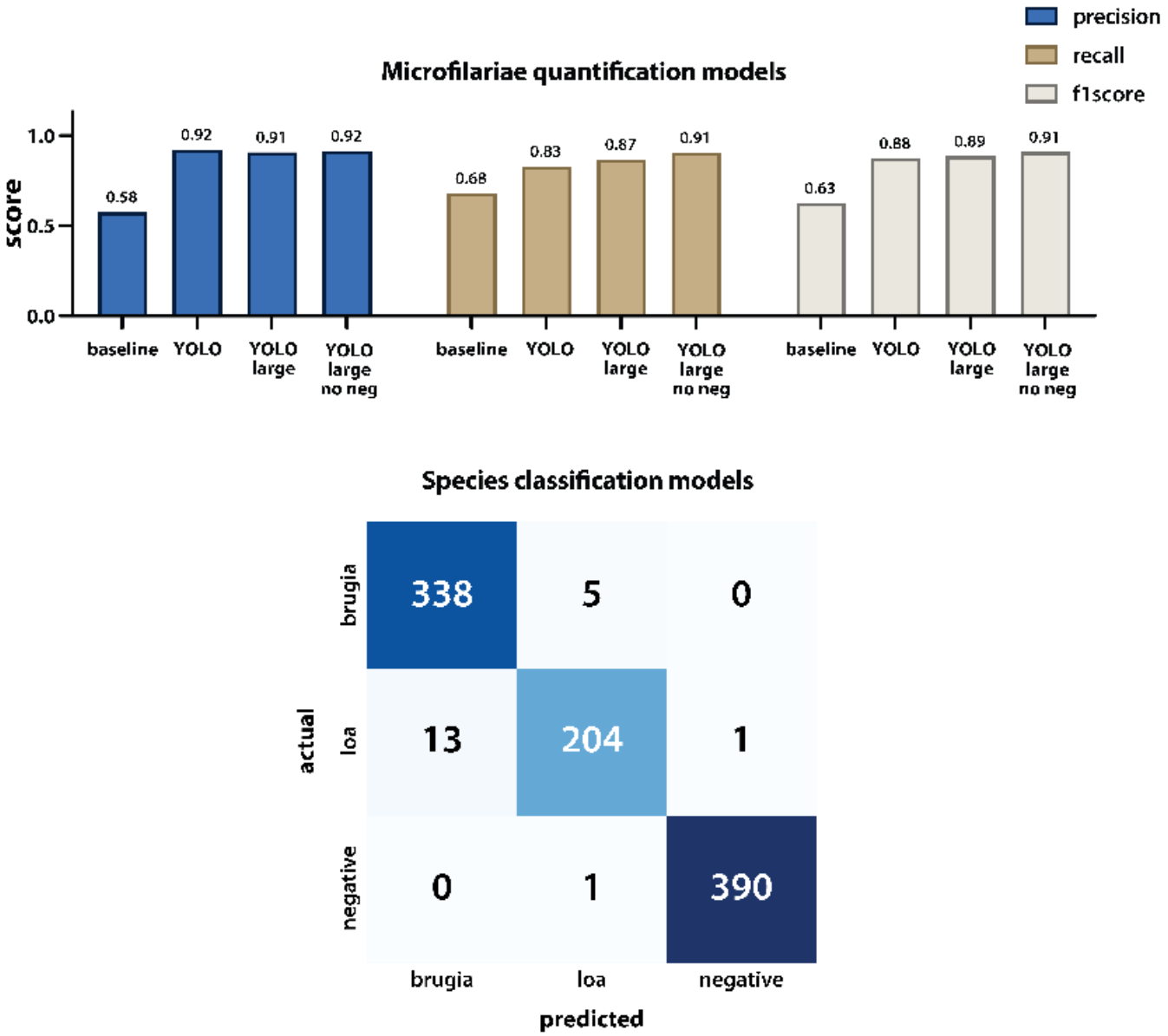


Figure 2: Performance of microfilariae quantification (top) and species classification models (bottom).

Conclusions: Our work demonstrates that deep learning can improve microfilariae quantification and enable species differentiation, supporting enhanced point-of-care diagnostics for NTD control.

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POSTER ON BOARD SHIFT 02
DIAGNOSTICS, HOST-PARASITE INTERACTION AND EMERGING TECHNOLOGIES / ARTIFICIAL INTELLIGENCE AND MACHINE LEARNING IN PARASITOLOGY
08-19-2026 9:00 AM - 7:00 PM

AUTOMATED IMAGE-BASED FLOW CYTOMETRY FOR HIGH-THROUGHPUT COPROSCOPICAL PARASITE DETECTION AND QUANTIFICATION USING MACHINE LEARNING ALGORITHMS

Alexander Hämmerli[1], Philipp Kronenberg[2], Lukas Neutsch[1], Ramon Eichenberger[2]
[1]Zurich University of Applied Sciences, Bioprocess Technology, Wädenswil, Switzerland, [2]Zurich University of Applied Sciences, Medical Micro- And Molecular Biology, Wädenswil, Switzerland

Background and Aims: Traditional coproscopical diagnostics by microscopic examination is labor-intensive, time-consuming, and prone to human error by misinterpretation, generating costs in the healthcare sector. Furthermore, in the livestock industry, anthelmintic resistance of parasites is alarming increasing and targeted anthelmintic treatment by preceding diagnostics should become standard. The aim of this work was to develop a novel diagnostic workflow for accurate parasite-egg detection in fecal samples.

Methods: A detection and quantification of parasite stages via image-based flow cytometry and subsequent analysis via different machine learning (ML) algorithms was validated.

Results: We demonstrate a specific and sensitive detection of different ruminant parasite- and 15 pollen species, which are representing potential contaminations and mis-diagnosis reagents. Three ML tools (logistic regression, kNN-DTW and MiniROCKET) were compared based on the list mode and pulse shape parameters obtained by the flow cytometer, all of them achieving an accuracy of over 95 % and high reproducibility, but significant different computing time (12, 290, and 0.6 min, respectively). The approach was successfully translated to stool samples from humans, differentiating parasite stages from matrix particles and spores from edible mushrooms (a common contamination in human samples which entail a high risk of false positive categorization).

Conclusions: The data indicates that automated approaches for parasitic egg detection and classification outperform manual microscopic examination in time, costs, and detection accuracy. Therefore, an optimized diagnostic workflow will reduce the level of specialization and time needed for coproscopical examination and subsequently reduce the associated costs for diagnostics.

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08-19-2026 9:00 AM - 7:00 PM

EVALUATION OF ARTIFICIAL INTELLIGENCE–ASSISTED AUTOMATED FAECAL ANALYSIS FOR DETECTION OF INTESTINAL PARASITES

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[1]Faculty of Medicine, University of Ljubljana, Institute Of Microbiology And Immunology, Ljubljana, Slovenia, [2]Axon Lab d.o.o., Slovenska Bistrica, Slovenia

Background and Aims: Gastrointestinal parasites remain a major global public health concern. Microscopy is the diagnostic gold standard but is labour-intensive, time-consuming, and reliant on expert microscopists, potentially affecting accuracy. AI-assisted faecal analysis offers a faster, scalable, and cost-effective alternative. This study compared AI-assisted faecal analyser for parasite detection in stool samples with microscopy.

Methods: Stool samples from 155 patients were analysed. Routine diagnostics included sedimentation-flotation, microscopy of iodine-stained slides, and *Cryptosporidium*/*Giardia* direct immunofluorescence (DIF) for diarrhoeal samples. Results were compared with analysis using the FS-30 Feces Analyzer (Keyu, China), followed by user audit of positive images. Based on study findings, the manufacturer updated the FS-30 software and samples were reanalysed.

Results: Routine diagnostic methods identified 41 patients as positive and 114 as negative and FS-30 identified 36/41 positives and 92/114 negatives. Twenty-four patients were enrolled after the software update, and analysed using the improved software version only. Of the microscopy-positive patients, 29 were analysed using both versions of the FS-30 software. Of these, 24 were positive with the first version, and one additional after software improvement. Among microscopy-negative samples, 102 were analysed using both versions of the FS-30 software: 68 were negative initially and 82 with the improved software. After user audit and software update, false positivity decreased.

Conclusions: The FS-30 Feces Analyzer is useful for identifying parasites in stool. It can be used as an alternative to microscopy of iodine-stained native samples in combination with an user audit to help rule out false positives. Collaboration with manufacturers is essential to optimise AI-based diagnostic performance.

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DIAGNOSTICS, HOST-PARASITE INTERACTION AND EMERGING TECHNOLOGIES / ARTIFICIAL INTELLIGENCE AND MACHINE LEARNING IN PARASITOLOGY
08-19-2026 9:00 AM - 7:00 PM

THE USE OF ARTIFICIAL INTELLIGENCE COMBINED WITH TRADITIONAL QUESTIONNAIRES, COPROSCOPIC AND SEROLOGICAL METHODS IN THE DIAGNOSIS OF HUMAN INTESTINAL PARASITES.

Montserrat Gras Graupera, Joan Montlló Ribó
Gras Clinical Services & Research, R&d Research, Warsaw, Poland

Background and Aims: Intestinal parasitic infections have posed a persistent public health challenge since antiquity and continue to affect vulnerable populations worldwide. Although traditional diagnostic techniques, such as microscopic examination and molecular assays, remain widely used, they are limited by variable sensitivity, reliance on expert interpretation, and time-consuming procedures. Recent advances in artificial intelligence (AI) offer new opportunities to improve diagnostic accuracy and efficiency. The aim of this study was to assess the applicability and effectiveness of an integrated diagnostic approach combining AI with a specifically designed clinical questionnaire to enhance the detection and management of intestinal parasitic infections.

Methods: A dichotomous clinical questionnaire comprising 112 items was developed following an extensive literature review and expert consensus using the Delphi method. Questionnaire items addressed symptoms, risk factors, and exposure history relevant to parasitic infections. Collected data were integrated into an AI framework based on a random forest algorithm. To address class imbalance, the Synthetic Minority Oversampling Technique (SMOTE) was applied. Diagnostic performance, processing time, and concordance with conventional diagnostic methods were assessed using statistical analysis.

Results: The AI-based approach demonstrated improved diagnostic accuracy and significantly reduced diagnostic time. The model efficiently identified relevant clinical patterns and performed particularly well in excluding negative cases. However, reduced sensitivity for positive cases was observed, highlighting the need for further optimisation.

Conclusions: The integration of AI with a clinical questionnaire represents a promising diagnostic support tool in parasitology. Further refinement is required to improve sensitivity and ensure robust application in routine clinical practice.

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DIAGNOSTICS, HOST-PARASITE INTERACTION AND EMERGING TECHNOLOGIES / ARTIFICIAL INTELLIGENCE AND MACHINE LEARNING IN PARASITOLOGY
08-19-2026 9:00 AM - 7:00 PM

AI ONCHO: A DEEP LEARNING SYSTEM FOR AUTOMATED HISTOLOGICAL EVALUATION OF ONCHOCERCA VOLVULUS NODULES IN CLINICAL TRIALS

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Background and Aims: Histological analysis of *Onchocerca volvulus* nodules is a critical bottleneck in clinical trials due to limited expert availability and time-intensive manual assessment. We developed “AI Oncho,” a computer vision-based deep learning tool designed to provide rapid, standardized results and reduce human workload in phase II/III clinical trials for macro-filaricidal and anti-*Wolbachia* regimens.

Methods: The system utilizes a multi-stage pipeline to process digitized whole-slide images. Separate deep learning models were trained to detect worm sections and classify vitality, embryogenesis, *Wolbachia* presence, and free microfilariae (Mf). To improve nodule-level assessment, we implemented a data-driven multiple threshold (MT) aggregation model. The system was validated against human evaluation using data from historical and recent clinical trials.

Results: The MT aggregation model achieved high accuracies across all trials: vitality (96.4–97.5%), embryogenesis (91.0–95.0%), and *Wolbachia* presence (93.4–95.9%). Mf detection accuracy ranged from 77.7% to 93.1%; notably, the models identified true Mf positives that human experts had missed. Workflow simulations demonstrated that our tool allows 80% of nodules to be analyzed by only one human expert plus “AI Oncho”. This reduces dual expert review requirements to 20% of nodules, saving 45–50% of the total expert workload.

Conclusions: Achieving >90% accuracy for most parameters while significantly reducing analysis time, this validated automated system is ready for deployment in upcoming clinical trials. The tool aligns with the WHO 2030 Roadmap for Neglected Tropical Diseases by mitigating the global shortage

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of specific histopathology expertise, thereby accelerating the development of the macrofilaricidal treatment regimens that are essential for onchocerciasis elimination.

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MOLECULAR ORGANISATION OF SUBPELLICULAR MICROTUBULES IN LEISHMANIA MAJOR

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Background and Aims: *Leishmania major*, one of the causative agents of cutaneous leishmaniasis, cycles between its sandfly vector and its mammalian host. To survive these very different environments, it relies on a robust cytoskeleton to maintain its shape and structural integrity. Central to this are the subpellicular microtubules (SPMTs), which are organized into a highly crosslinked array located just beneath the plasma membrane and are essential for parasite viability. However, despite their central role, the precise organisation of the SPMT array and its components remain poorly understood. Here, we aim to define the molecular organisation of the SPMT array by identifying its associated proteins and to determine how these components contribute to cytoskeletal integrity.

Methods: We developed a protocol to isolate the microtubule cytoskeleton from *Leishmania major* promastigotes. We then used cryo-electron tomography to generate three-dimensional reconstructions of the SPMT array. To further improve resolution, we combined deep learning–based approaches with subtomogram averaging to enhance the signal-to-noise ratio and resolve the repeating structural features of the SPMTs.

Results: We resolved a previously uncharacterised repeating density decorating the SPMTs with a periodicity of 24 nm along the microtubule axis. This density consistently binds three adjacent protofilaments simultaneously thereby likely contributing to lateral stabilisation of the microtubule and crosslinking within the array.

Conclusions: Together, these findings provide new structural insights into the organisation and stabilisation of the SPMT array in *Leishmania major*.

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A MULTIMODAL ARTIFICIAL INTELLIGENCE TOOL FOR THE ENHANCED DETECTION OF FEMALE GENITAL SCHISTOSOMIASIS

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Background and Aims: Female genital schistosomiasis (FGS) is a gynaecological disease found primarily in sub-Saharan Africa. It can be diagnosed based on the presence of lesions on the cervix and surrounding tissue caused by *Schistosoma haematobium* egg deposition and associated inflammatory response. These lesions are often subtle and heterogeneous, leading to misclassification and poor diagnostic accuracy when relying on clinician visual assessment alone. Artificial intelligence (AI) provides the opportunity to enhance detection and scalability in areas with few trained reviewers. This study aimed to develop an AI-based approach, using both image and non-image data (i.e. multimodal computer vision), to enhance visual diagnostics for FGS.

Methods: A two-stage multimodal pipeline was developed to classify cervical images as FGS-positive or FGS-negative. First, an unsupervised autoencoder-based anomaly detector was developed to exclude images containing no evidence of FGS-related abnormalities. Remaining images were processed through a supervised convolutional neural network (CNN) classifier with multimodal feature (cervical images, FGS and STI test results, symptom and sociodemographic data) fusion and 5-fold cross-validation. Models were trained and internally validated using baseline results of 1,691 women enrolled in a longitudinal (2021-2026) study in Zambia.

Results: The supervised classifier achieved a specificity of 80.9% (95% CI 77.6%-83.8%) and sensitivity of 70.2% (95% CI 65.5%-74.5%). The filtering autoencoder demonstrated high performance (accuracy 86.5%) in detecting FGS-related abnormalities in cervical images, improving downstream classification capabilities.

Conclusions: This novel two-stage multimodal approach to FGS diagnosis achieved high diagnostic performance upon internal validation. These findings support the continued development of AI-assisted tools to enhance visual diagnostics.

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ARTIFICIAL INTELLIGENCE FOR ENTERIC PARASITE DETECTION: TWO PHASE EVALUATION OF THE KU F40 ANALYZER BEFORE AND AFTER DATABASE UPGRADE

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LHUB-ULB, Microbiology, Brussels, Belgium

Background and Aims: Microscopy remains the reference method for detecting intestinal parasites but is increasingly challenged by technical complexity and limited specialized staff. Automated systems integrating artificial intelligence (AI) may support routine workflows. This study prospectively evaluated the performance of the KU-F40 automated fecal analyzer, combining microscopic imaging and AI-based recognition algorithms, before and after a database upgrade.

Methods: Triple-Faeces-Test sets were prospectively collected during two one-month periods. In phase one, 131 sets were analyzed, each including one fresh and one SAF-preserved specimen. Images obtained were used to retrain the reference database. In phase two, 122 additional sets were processed using the upgraded system. KU-F40 results were compared with microscopy, and discordant findings were independently reviewed by two expert technologists.

Results: In phase one, microscopy confirmed parasites in 19.8% of sets. The KU-F40 showed a sensitivity of 50%, specificity 77%, positive predictive value (PPV) 37%, and negative predictive value (NPV) 85%. Frequent false positives involved *Blastocystis hominis*, *Clonorchis sinensis*, and *Ascaris lumbricoides*. In phase two, microscopy detected parasites in 12% of sets, whereas the analyzer reported parasites, mainly *Blastocystis hominis,* in more than 95% of samples. Sensitivity was 36%, specificity 2.8%, PPV 5.4%, and NPV 41%. Several species, including *Dientamoeba fragilis*, *Endolimax nana*, and *Pseudolimax butschlii*, were consistently missed by the KU-F40.

Conclusions: Despite its potential, the KU F40's performance before and after the upgrade remains inadequate for routine use. Significant optimization is required to improve specificity and reduce systematic overcalling.

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MACHINE LEARNING PREDICTION OF ANTHELMINTIC TREATMENT REQUIREMENT BASED ON ANTI-ES24 OF HAEMONCHUS CONTORTUS ANTIBODY LEVELS IN SANTA INÊS, TEXEL AND WHITE DORPER LAMBS

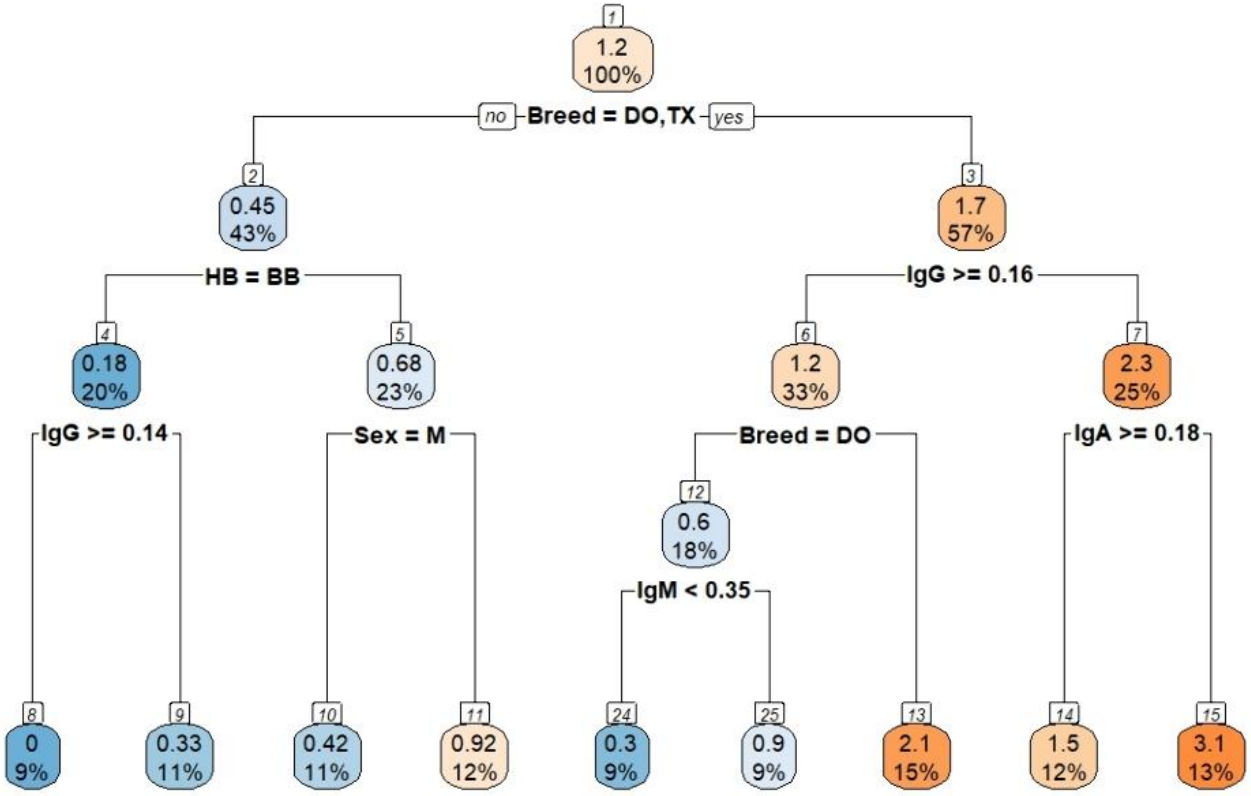
Simone Niciura[1], Emanuelle Campos[2], Rafaela Kapritchkoff[3], Ana Carolina Chagas[1], Cintia Okino[1]
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Background and Aims: Anthelmintic requirement was predicted in sheep using machine learning based on sex, breed, β-globin gene haplotype (HB), and IgA, IgM, and IgG antibodies against excretory/secretory 24kDa protein (ES24) of *Haemonchus contortus*.

Methods: Under natural infection, 147 Santa Inês (SI), Texel (TX), and White Dorper (DO) lambs were evaluated for fecal egg counts (FEC) and packed cell volume (PCV) every 21 days from D63 to D189. Lambs with FEC≥10,000 and PCV≤24% or PCV≤22% were subjected to anthelmintic treatment (numAH). Plasma ES24 antibodies were assessed on D63 by indirect ELISA. A regression tree using the rpart package in R predicted the numAH and its accuracy was calculated by the root mean square error (RMSE).

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Results:



The numAH varied from 0 to 6 (mean=1.2). In the best tree (R^2=0.44, training RMSE=1.10, and test RMSE=0.83), the upper node split SI from DO and TX. Higher numAH was observed in DO and TX with IgG≥0.16 and IgA≥0.18 (3.1) or IgA<0.18 (1.5) and in DO with IgG<0.16 (2.1), and lower numAH in TX with IgM<0.35 (0.9) or IgM≥0.35 (0.3). In SI, AA/AB lambs with IgG<0.13 showed lower numAH (0) than IgG≥0.14 (0.33), females (0.42), and males (0.92).

Conclusions: Antibody plasma levels, breed, sex, and HB affected anthelmintic requirement in lambs, and SI is naturally more resistant to gastrointestinal nematode infection and require fewer anthelmintic treatments. Anti-ES24 of *H. contortus* levels were able to predict the numAH in lambs, being useful to identify animals for more frequent monitoring and predict infection progression.
Financial support: FAPESP (2021/02535-5 and 2024/09500-0).

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INTERPRETABLE MACHINE LEARNING AND SIGNAL PROCESSING FOR AUTOMATED READING AND QUALITY CONTROL OF LATERAL FLOW TESTS FOR SCHISTOSOMIASIS

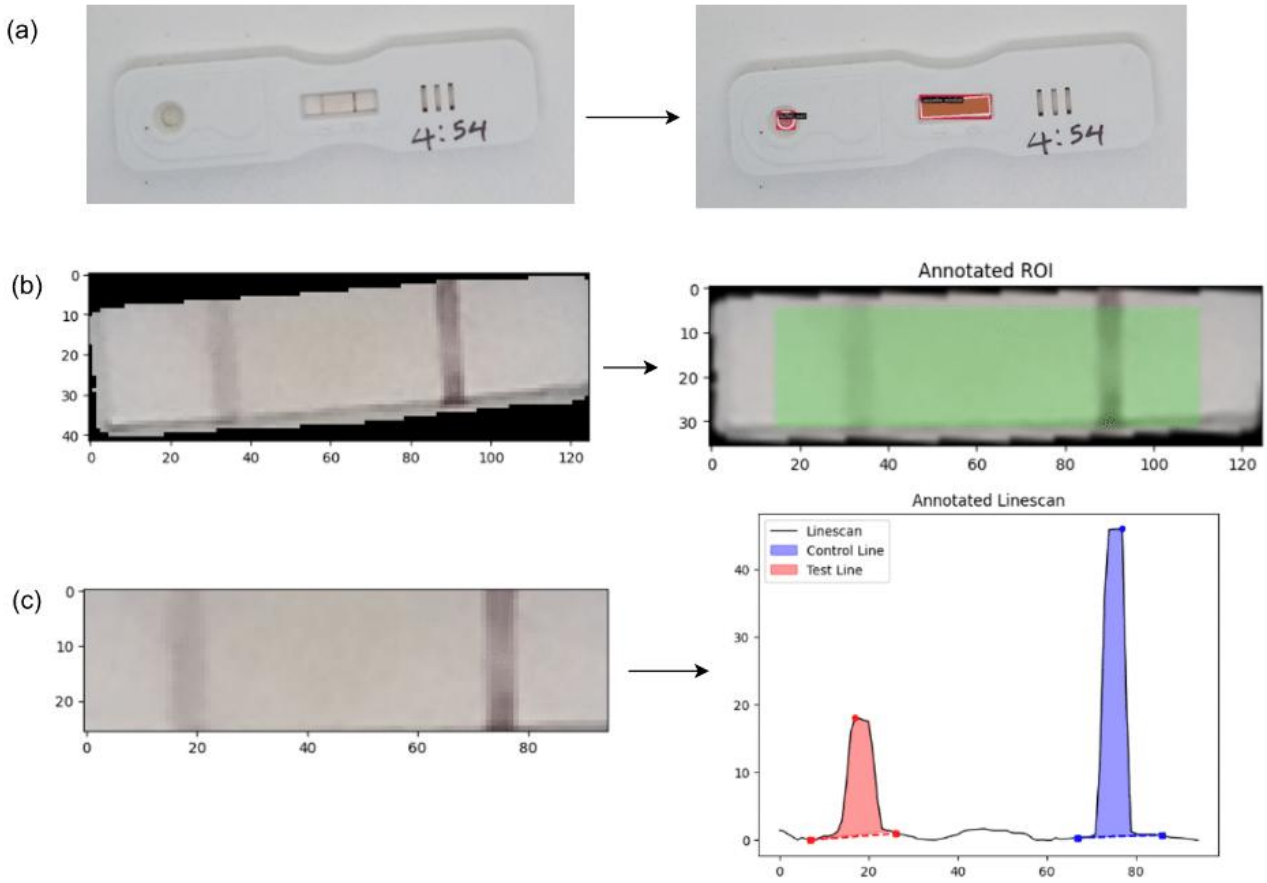
Chris Ho[1], Christin Puthur[2], Betty Nabatte[3], Carson Moore[4], Theresia Abdoel[5], Rene Paulussen[5], Pafue Nganjimi[6], Pytsje Hoekstra[7], Narcis Kabatereine[3], Kawesa Bumali[3], John Odea[3], Ronald Bogere[3], Rosette Katushabe[3], Govert Van Dam[7], Thomas Scherr[4], Goylette Chami[8]
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Background and Aims: There is a lack of automated pipelines for diagnostic classification of point-of-care tests for neglected tropical diseases. Here we present an end-to-end automated pipeline for the analysis of point-of-care circulating cathodic antigen tests for schistosomiasis.

Methods: We incorporated deep learning for cassette segmentation with signal processing. Automated classifications were compared to quantitative readings from calibrated antigen samples examined in lateral flow readers, and visual readings from highly trained field and senior technicians. We also trained commonly used deep learning classification models to compare performance with the signal processing pipeline. The automated pipeline was evaluated for 3188 individuals within the

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SchistoTrack cohort in rural Uganda.



Results: Our quantitative classifications were on par with a lateral flow reader, and showed 86.6% sensitivity and 96.5% specificity with visual readings from a senior technician, which was an improvement on the visual readings from field technicians. Automated classifications were possible in as little as five minutes after test preparation for high antigen concentrations. We showed visual trace uncertainty can be resolved with signal processing, indicating visual traces should be classified as negative. Performance of the interpretable signal processing-based pipeline was comparable to deep learning classification models.

Conclusions: Our pipeline will aid in advancing diagnostics to meet the World Health Organization target product profile for schistosomiasis, provide quantitative assessments for other diagnostics, enable large-scale surveillance in areas targeting elimination, and provide real-time quality control for diagnostics introduced into primary healthcare facilities.

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TITLE AI-AUGMENTED MICROSCOPY FOR RAPID DETECTION OF ENTAMOEBA, GIARDIA, AND CRYPTOSPORIDIUM IN CLINICAL STOOL SMEARS USING DEEP LEARNING MODELS

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Background and Aims: Diagnosis of enteric protozoa relies largely on microscopy, which is limited by low sensitivity, observer dependence, and poor scalability in high-burden settings. Although molecular assays (qPCR/LAMP) improve detection, their cost and turnaround time restrict routine use. We developed and validated an AI-based object-detection system using YOLO models (v8–v11) to enable rapid, reproducible detection of *Entamoeba*, *Giardia*, and *Cryptosporidium* from routinely trichrome and modified Ziehl–Neelsen stained stool smears.

Methods: Digital micrographs of trichrome-stained (*Entamoeba/Giardia*) and modified Ziehl–Neelsen–stained (*Cryptosporidium*) smears were annotated and trained using YOLOv8–v11 models under identical settings. Performance was evaluated using precision, recall, F1-score, mAP@0.5, IoU, cross-validation stability, and inference speed. External validation used 200 unseen images, with comparisons to expert microscopy and multiplex real-time PCR.

Results: For trichrome-stained ***Entamoeba* and *Giardia***, **YOLOv11m** achieved 97.34% accuracy, precision 98.17%, recall 98.92%, F1-score 98.54%, and mAP@0.5 of 99.02%, with high spatial localisation (IoU 0.93), low cross-validation variance (<1%), and real-time inference (0.19 s/image). For modified ZN–stained ***Cryptosporidium***, YOLOv11n achieved the highest cyst mAP (97.7%), while **YOLOv10n** demonstrated superior stability and generalisability across heterogeneous stool

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backgrounds, enabling reliable real-time inference (≤0.26

Figure 1. Workflow for dataset preparation and annotation for YOLO-based AI model training: Sequential process showing trichrome staining of clinical stool samples, microscopic imaging at 100×, image pre-processing, manual annotation of cysts and trophozoites in LabelImg (YOLO format), and dataset splitting (80% training, 20% validation) with augmentation for model optimization.

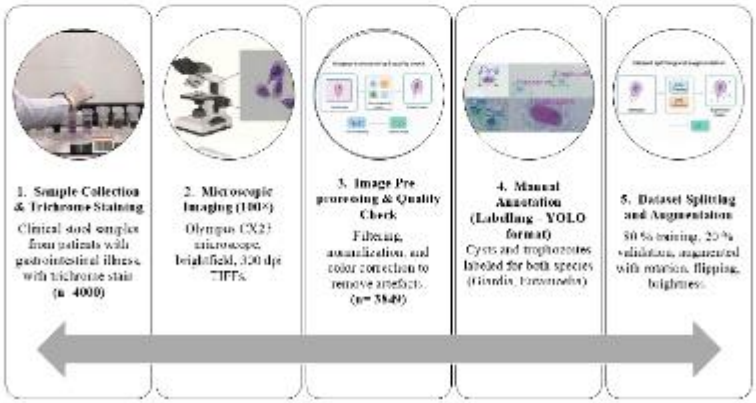
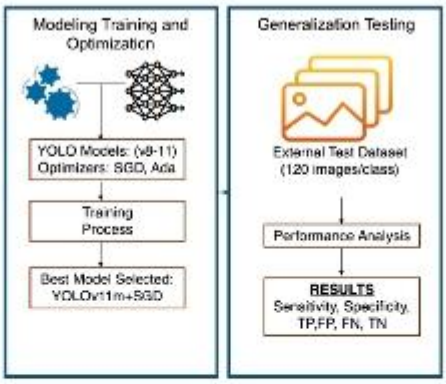


Figure 2. Model training, optimization, and generalization testing workflow



s/image).

Conclusions: Conclusion:

This first-of-its-kind AI-assisted microscopy platform enables fast, reproducible, and highly accurate detection of *Entamoeba*, *Giardia*, and *Cryptosporidium* directly from routine stained smears. By

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overcoming key limitations of conventional microscopy and enabling rapid triage before molecular confirmation, it offers a scalable, low-resource diagnostic solution with significant implications for parasitic disease control in high-burden settings. **Acknowledgement:**
We thank the participating patients, clinical departments and PGIMER for their support & cooperation.

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OCTOPI: AI-POWERED MICROSCOPY FOR DETECTING MALARIA AND OTHER PARASITIC DISEASES

Pranav Shrestha[1], Heguang Lin[2], You Yan[1], Emily Chu[3], Oswald Byaruhanga[4], David Mcilwain[5], Prabha Chandrasekaran[6], Misago Seth[7], Deus Ishengoma[7], Charles Delahunt[8], Joaniter Nankabirwa[9], Prasanna Jagannathan[10], Hongquan Li[1], Manu Prakash[3]
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Background and Aims: Microscopy remains the gold-standard diagnostic for many parasitic diseases (malaria, schistosomiasis, soil-transmitted helminths, enteric protozoa), yet it is labor-intensive, dependent on scarce expert microscopists, and inaccessible in many low- and middle-income country (LMIC) primary care facilities. Automation and artificial intelligence (AI) can help address these limitations. We present Octopi, an open, AI-powered automated microscopy platform designed for co-development of diagnostic AI models across parasitic and hematological diseases.

Methods: Octopi performs high-throughput slide scanning (~1 million blood cells/minute) with on-board AI-assisted disease detection. For malaria, DAPI staining combined with a spectral shift in fluorescence emission enables rapid screening at low magnification (20× objective), with detection via blood cell segmentation, fluorescent spot detection, and machine learning classification. To support adaptation to new diseases, we built a web-based annotation and training pipeline, used for malaria and easily adapted to other pathogens and conditions.

Results: Proof-of-concept validation on 213 clinical samples from Uganda and the United States (905 million red blood cells, ~1.4 million parasites) achieved preliminary patient-level sensitivity and specificity above 97%, with an estimated limit of detection around 12 parasites/µL. Extended evaluation is ongoing across community surveillance in Tanzania (~500 samples) and hospital and rural clinic deployments in Liberia (~1,000 samples), spanning broader parasitemia ranges and field conditions.

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Conclusions: Octopi is an open platform for AI-powered diagnostics in low-resource settings, with malaria as a validated application and a straightforward path to additional parasitic diseases (schistosomiasis, enteric parasites, helminths) and hematological conditions. It anchors ODION (odion.org), a co-development network for open, locally-driven diagnostic AI across LMICs.

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A MULTI-PROTEIN BIOINFORMATICS PIPELINE TO IDENTIFY SPECIFIC B-CELL EPITOPES FOR TOXOCARIASIS DIAGNOSIS

Camila Vieira Macedo[1], Richard Ward[2], João Setubal[3], Francislon Silva[3], Aline Diniz Cabral[1], Sergio Vieira Dos Santos[1], Pedro Chieffi[1], Gabriela Rodrigues E Fonseca[1]
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Background and Aims: Human toxocariasis, a neglected zoonosis caused by Toxocara canis or T. cati, remains a public health challenge. Current immunodiagnostics using Toxocara excretory-secretory antigen suffer from significant cross-reactivity with Ascaris spp., compromising specificity in polyparasitic regions. Previous work focusing on predicted peptides from the Tc-CTL-1 protein demonstrated high immunogenicity; however, it failed to eliminate cross-reactivity with Ascaris. These findings underscored the need for a more comprehensive, multi-protein comparative approach. The aim of this study is to validate an integrated bioinformatics pipeline for identifying Toxocara-specific peptide epitopes derived from multiple proteins, serving as an initial step to enhance the specificity of Toxocara immunodiagnosis through both linear and structural analyses

Methods: Larval T. canis, adult Ascaris suum and Ascaris lumbricoides sequences were retrieved from the NCBI database. Secreted T. canis proteins were filtered via SignalP 6.0 and Outcyte. T. canis sequences were analysed against Ascaris spp. through BLASTp, and selected proteins were modelled with AlphaFold 3. B-cell epitopes were predicted using BEPIPRED 3.0 and Discotope 3.0, prioritising candidates based on number of amino acids and inter-tool agreement.

Results: The pipeline successfully identified 126 unique peptides with high potential for diagnostic discrimination.

Conclusions: By shifting focus from single proteins to an extensive comparative analysis of the least conserved regions, it was possible to identify specific markers that can surpass common setbacks. This is the first study to employ extensive comparative bioinformatics across multiple Toxocara proteins, providing a robust and scalable tool for improved diagnosis and global seroprevalence mapping.

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CAUSOBIOME: A GENERALIZABLE CAUSAL INFERENCE FRAMEWORK TO IDENTIFY MICROBIAL AND PARASITIC DRIVERS OF DISEASE PROGRESSION

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Background and Aims: Identifying the microbial features driving disease progression requires distinguishing true causal effects from mere correlations. Current microbiome analysis methods rarely account for causal dynamics, limiting their diagnostic and therapeutic potential. We aimed to develop a robust causal inference pipeline capable of identifying microbial and parasitic factors causally involved in human disease progression.

Methods: We developed **CausoBiome** (github.com/AscanOfficiel2/CausoBiome), a modular, stage-aware pipeline that integrates genome-resolved metagenomics, Double Machine Learning-based causal inference, robust biomarker selection, and external validation. The pipeline was initially applied to analyze the microbiome of over 500 colorectal cancer (CRC) patients, utilizing more than 800 faecal metagenomic samples representing healthy, adenoma, and carcinoma stages. Identified biomarkers underwent independent external validation to ensure robustness and reproducibility.

Results: We reconstructed 2,340 high-quality microbial genomes and discovered clear, disease-stage-specific microbial shifts: *Streptococcus vestibularis* enriched at adenoma stages, while *Clostridium innocuum*, *Bacteroides ovatus*, and *Serratia marcescens* dominated carcinoma stages. CausoBiome identified a robust, causally influential 26-gene module of antimicrobial resistance genes (ARGs) and virulence factors (VFs), notably centered around *ErmF*, *dfrF*, and *cps4L*. These biomarkers consistently showed significant causal effects on CRC progression, independently replicated with high statistical confidence (FDR < 0.05). Functional analysis revealed predominant roles in capsule synthesis and bacterial adhesion, consistent with mucosal biofilm persistence.

Conclusions: CausoBiome reliably identifies microbial biomarkers causally associated with disease progression, significantly advancing diagnostics and therapy. Its generalizable framework can readily adapt to parasite-host interactions, enabling causal inference on parasitic virulence and disease dynamics, providing actionable insights across microbiology and parasitology.

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TO RECEIVE BOARD # ONSITE

POSTER ON BOARD SHIFT 02
DIAGNOSTICS, HOST-PARASITE INTERACTION AND EMERGING TECHNOLOGIES / HOST-PARASITE MOLECULAR AND CELLULAR INTERACTIONS

IMMUNOMODULATORY POTENTIAL OF SOLUBLE HUMAN LEUKOCYTE ANTIGEN G ON IGG RESPONSE TO MALARIA VACCINE CANDIDATE ANTIGENS FOR PREGNANT GHANAIAN WOMEN.

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Background and Aims: Human leukocyte antigen G (HLA-G) is a non-classical molecule of the major histocompatibility complex class Ib. HLA-G downregulates the mother's immune response to the foetus, thereby generating immune tolerance involved with *Plasmodium falciparum* antigens *in utero*. We determined the association of vaccine induced IgG responses and sHLA-G levels at ANC and delivery. sHLA-G levels of mother and cord were compared. Factors that could affect sHLA-G levels in *P. falciparum* and *Schistosoma haematobium* infected pregnant women were identified.

Methods: This cross-sectional study in Adidome and Battor Hospitals in the Volta Region of Ghana recruited 157 pregnant women, 81 at ANC and 76 at delivery. *P. falciparum* infections at ANC and delivery were detected by qPCR. Urine samples were filtered and microscopy was done to identify *S. haematobium* infections. Plasma IgG response to five malaria vaccine candidates and sHLA-G was quantified by ELISA. An association between sHLA-G levels and IgG response to malaria vaccine antigens was determined by a multivariable regression model.

Results: Mean sHLA-G levels at ANC was 25 ± 2 ng/µl and at delivery, 30 ± 2 ng/µl. A negative linear correlation of sHLA-G and anti-GLURP-R0 vaccine antibody response was seen at ANC. A significant positive correlation was observed for maternal and cord plasma sHLA-G. At delivery, a positive association between *P. falciparum* infection and sHLA-G was observed. sHLA-G levels were found to decrease with IPTp and ITN use.

Conclusions: The association between sHLA- G and deceased GLURP-R0 response is crucial for designing and testing of malaria vaccine candidates in semi-immune individuals.

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POSTER ON BOARD SHIFT 02
DIAGNOSTICS, HOST-PARASITE INTERACTION AND EMERGING TECHNOLOGIES / HOST-PARASITE MOLECULAR AND CELLULAR INTERACTIONS

GLYCOSOMAL AQUAGLYCEROPORIN 1 DUAL ROLE IN IRON HOMEOSTASIS AND ANTIMONY SUSCEPTIBILITY IN LEISHMANIA AMAZONENSIS

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Background and Aims: One critical condition found by *Leishmania* in the macrophage is the lack of various nutrients, such as iron, which is an essential cofactor for several enzymes. Using immunofluorescence, we confirmed the glycosomal localization of the protein aquaglyceroporin 1 (AQP1), a porin allowing the transport of trivalent antimony (SbIII), water, glycerol, urea, dihydroxyacetone, methylglyoxal, and polyols, reported here for the first time to be associated with glycosomes.

Methods: Full aqp1 knockouts and add-back strains were generated using CRISPR/Cas9 and validated by PCR. Promastigote growth was assessed in both regular and heme-depleted medium, followed by evaluation of trivalent antimony (SbIII) resistance using MTT assays. Intracellular iron and antimony levels were quantified by Inductively Coupled Plasma Mass Spectrometry (ICP-MS), while Reactive Oxygen Species (ROS) levels and superoxide dismutase (SOD) activity were measured to assess oxidative stress. Finally, *in vitro* and *in vivo* infection experiments were performed to evaluate parasite virulence.

Results: The knockout of *aqp1* impacted the growth of promastigotes in vitro, particularly under heme-depleted conditions. Moreover, phenotypic analyses revealed that *aqp1* deficiency contributes to partial resistance to SbIII, increases superoxide dismutase activity and ROS levels, reduces osmoregulatory capacity, and decreases intracellular iron levels. Furthermore, its absence significantly reduced both *in vitro* and *in vivo* virulence.

Conclusions: These findings demonstrate that AQP1 plays a key role in *Leishmania amazonensis* growth, osmoregulation, and SbIII susceptibility. Its deficiency reduces intracellular iron and virulence. In-depth characterization of these mutant strains will contribute to elucidating the relationship between AQP1 function and the iron metabolism
into *Leishmania amazonensis* glycosomes.

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POSTER ON BOARD SHIFT 02
RESISTANCE AND DEVELOPMENT OF DRUGS AND VACCINES / PARASITE EXTRACELLULAR VESICLES (EVS) FOR THERAPEUTICS AND DIAGNOSTICS

LEISHMANIA EXTRACELLULAR VESICLES AND THEIR IMPACT ON EXPERIMENTAL INFECTION AND PROTECTIVE IMMUNIZATION

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Background and Aims: Extracellular vesicles (EVs) released by *Leishmania* spp. contribute to host–pathogen communication, participating in the initial infection, parasite survival, immune modulation, and disease progression. These EVs deliver their cargo, interfering with innate and adaptive immune responses. *Leishmania* spp. EVs can exacerbate infection by favoring a regulatory or anti-inflammatory immune profile, depending on the parasite species and host context. Conversely, there is evidence that these vesicles also hold significant potential as immunogenic components when strategically exploited.

Methods: Thus, using purified EVs obtained from *Leishmania* parasites is an interesting approach to explore their role in pathogenesis, protection, and therapeutics.

Results: Our group demonstrated that intraperitoneal immunization with these EVs provided partial protection in an experimental model of cutaneous leishmaniasis. Furthermore, our data showed that intranasal immunization with *L. amazonensis* EVs induced a protective response in experimentally infected animals, with a significant reduction in parasite load, decreased inflammation, and induction of a Th1 response. Exploring the immunomodulatory activity of EVs in a treatment model is also worth evaluating. Our group observed that treatment with EVs stabilized skin lesions and parasite load in a cutaneous leishmaniasis model. Proteomic characterization of these EVs revealed the presence of immunogenic parasite-derived molecules. Furthermore, comparing the protein content of EVs from parasites with distinct virulence profiles revealed insights into the virulence factors present in these EVs.

Conclusions: Overall, understanding the role of *Leishmania* extracellular vesicles in disease pathogenesis, immunization, and treatment is essential for the rational design of innovative therapeutic and prophylactic approaches to control leishmaniasis.

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