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ABSTRACTS

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Neuronal control of ant navigation

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Spatial orientation is crucial for many animal species, and parasites have been shown to interfere with related behaviors in insects. Social insects like ants highly depend on efficient navigation, as they venture out on long-ranging foraging trips and must find back to their common nest for cooperative food provisioning to their offspring. *Cataglyphis* desert ants are excellent experimental models for studying neuronal mechanisms underlying behavioral development into successful navigators [1]. I will highlight our research on the neuroethology of ant navigation to trigger potential bridges for investigating brain control by parasites in the emerging field of neuroparasitology. To promote neuroethological research in the ant model, we performed 3D confocal and multiphoton microscopy combined with mass-spectrometric imaging to develop a comprehensive high-resolution anatomical and neurochemical atlas of the desert ant brain [2, 3]. Experimental analyses during the natural behavioral transition of the ants from tasks in the dark nest interior to performing first foraging trips revealed specific neuropeptide modulators involved in triggering this important behavioral transition. By manipulating the sensory input provided by the sun and the earth"s magnetic field, we could show that the ants use geomagnetic information to calibrate their sun-compass system during an early sensitive period when the ants perform systematic learning walks [4]. The underlying calibration of neurocircuits leads to structural synaptic plasticity within distinct neuronal pathways to the central complex and the mushroom bodies, sensory integration centers in the insect brain involved in spatial orientation and memory formation. I will discuss the relevance of our results on navigation and behavioral plasticity in the ant model for potential future efforts towards understanding insect brain manipulations by parasites affecting spatial orientation. Supported by DFG project numbers 405620408 and 272768235.

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2. Habenstein J, Amini E, Grübel K, el Jundi B, Rössler W (2020) The brain of *Cataglyphis* ants: neuronal organization and visual projections. *J Comp Neurol* 528:3479–3506. https://doi.org/10.1002/cne.24934
3. Habenstein J, Schmitt F, Liessem S, Ly A, Trede D, Wegener C, Predel R, Rössler W, Neupert S (2021) Transcriptomic, peptidomic and mass spectrometry imaging analysis of the brain in the ant *Cataglyphis nodus*. *J Neurochem* 158:391-412. https://doi.org/10.1111/jnc.15346
4. Grob R, Müller VL, Grübel K, Rössler W, Fleischmann P (2024) Importance of magnetic information for neuronal plasticity in desert ants. *PNAS* 121 (8) e2320764121. https://doi.org/10.1073/pnas.2320764121

SE01.02

Discovery of *Leishmania* secretory factor that targets macrophage miR-122–hepcidin pathway to downregulate Nramp1 levels and facilitate phagolysosomal iron acquisition

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Introduction: Nramp1 (Natural resistance-associated macrophage protein 1) is a crucial phagolysosomal iron exporter in macrophages, playing a pivotal role in resistance against various intracellular pathogens, including *Leishmania*. Previously, we showed that *Leishmania major* infection downregulates Nramp1, increasing phagolysosomal iron and promoting pathogen survival. In this follow-up study, we observed that Nramp1 is also downregulated in uninfected macrophages located adjacent to *L. major*-infected macrophage cells. These findings suggest the possible involvement of a *Leishmania* secretory factor in mediating Nramp1 downregulation.

Objectives: This study aimed to identify the secretory factor responsible for downregulating Nramp1 levels and to elucidate the molecular mechanisms by which it downregulates Nramp1 expression in macrophages.

Materials & Methods: *L. major* promastigote conditioned media (Lm-CM) were prepared from late log phase culture and used this conditioned medium to treat macrophages. Exosomes were also isolated from LmCM. CRISPR-Cas9 was technology employed to generate LmGP63 knockout strain. For in vivo experiments, LmWT, LmCas9, and LmGP63-/- strains were used to infect the footpads Balb/C mice.

Results: We found that macrophage cells treated with LmCM showed downregulation of Nramp1 level, resulting in elevated phagolysosomal iron levels. This effect was abolished when LmCM were subjected to heat and trypsin treatments, suggesting the involvement of a proteinaceous factor. Further pharmacological studies using EDTA and 1,10-phenanthroline, known inhibitors of GP63, indicated that GP63 may be responsible for Nramp1 downregulation. To validate this, we generated LmGP63-/- strain and LmGP63-/- CM failed to downregulate Nramp1. Additional studies with LmGP63-/- CM revealed that (i) GP63 induces ubiquitine-proteasome mediated degradation of Nramp1 by upregulating the iron regulatory protein hepcidin; ii) GP63 modulates hepcidin mRNA levels by altering pre-miRNA-122 levels, which is known to negatively regulate hepcidin expression. In vivo, BALB/c mice infected with wild-type *L. major* showed Nramp1 downregulation, whereas LmGP63-/- infections did not.

Conclusion: In this study, we identify GP63 as the key secretory factor that downregulates Nramp1. GP63 promotes ubiquitin-proteasome-mediated degradation of Nramp1 by upregulating hepcidin, which is linked by the downregulation of DICER1, that alters the expression of Pre-miRNA-122.

SE01.03

Digital PCR reveals high *PvDBP*1 copies in the majority of duffy-negative, *Plasmodium vivax* infected Individuals from Central Africa

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The interaction between *Plasmodium vivax* (Pv) Duffy Binding Protein 1 (PvDBP1) and the Duffy antigen receptor for chemokines (DARC) is crucial to human erythrocyte invasion. Additionally, recent studies have demonstrated that PvEBP/DBP2 and PvRBP2b play a role in reticulocyte invasion. Vivax malaria, once thought to be rare in Duffy negative Africans, is now reported in various parts of Africa, suggesting potential alternate infection mechanisms. One hypothesis is that copy number variation (CNV) of genes involved in erythrocyte invasion may impact parasite invasion capability and/or host immune evasion particularly in Duffy negative individuals. This study utilized dPCR, a novel high throughput approach which provides more precise and improved assessment of CNVs than quantitative and conventional PCR. We assessed CNV of PvDBP1, PvEBP/DBP2, and PvRBP2b in 81 *Pv* samples isolated from Duffy negative individuals from three eco-epidemiological sites across Cameroon. For a subset of samples, we compare dPCR results with qPCR and PCR diagnostic approaches. 86.3% of 81 Pv patients had 2-5 copies of PvDBP1, compared to 90% one copy in PvEBP/DBP2 and PvRBP2b. The dPCR results were consistent with PCR results. In Bamenda, both Malagasy and Cambodian duplication types were detected in 22 of 31 (70%) individuals possibly due to polyclonality within a sample. In Bertoua and Buea, the Cambodian duplication was more prevalent. Parasitemia was significantly higher in samples with both duplications and those with Cambodian type than in those without duplications (p=0.0474 and 0.0143). Though no significant difference was observed in PvDBP1 CNV among the three sites, highest copies were observed in Buea and Bertoua, which are considered as regions of high malaria transmission due to their ecological settings and climate. Further, our data showed that CNV analysis by qPCR may not be as precise as dPCR particularly for low-parasitemia Duffy negative Pv samples. Our earlier findings revealed high PvDBP1 copies in 25% of Duffy negative infections in East Africa, and 90% in Central Africa. Such contrast may imply different magnitudes of selection pressure among the African populations and functional advantages in erythrocyte invasion through increase in PvDBP1 dosage. On the other hand, predominantly low PvEBP/DBP2 and PvRBP2b copy raise question to their role in Duffy negative erythrocyte invasion.

Fig. 1

Table 1: Prevalence of PvDBP1, PvDBP2 and PvRBP2b CNV

Study site	Sample size	PvDBP 1		PvDBP2/PvDBP 2		PvRBP2b	
		Single copy	Multi-copy	Single copy	Multi-copy	Single copy	Multi-copy
Gamasira	35	2 (5.7%)	33(94.3%)	32 (91.4%)	3 (8.6%)	32(91.4%)	3 (8.6%)
Gelemso	29	2 (6.9%)	27 (93.1%)	29 (100%)	0 (0.0%)	29 (100%)	0 (0.0%)
Guso	26	3(11.5%)	20 (87.1%)	24 (92.3%)	2 (7.7%)	23 (88.5%)	3 (11.5%)
Total	92	7 (7.6%)	85(77.39%)	87 (85.6%)	5 (5.4%)	85 (93.5%)	6 (6.5%)

Table 2: Distribution of PvDBP duplication types in study sites

Study site	Sample size	PvDBP multiplication type		
		Malagasy	Cambodian	Malagasy + Cambodia
Gamasira	35	3 (8.6%)	9 (25.7%)	21 (60.0%)
Gelemso	29	0 (0.0%)	29(100.0%)	0 (0.0%)
Guso	26	0 (0.0%)	22 (84.6%)	0 (0.0%)

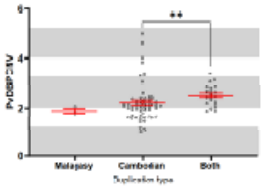
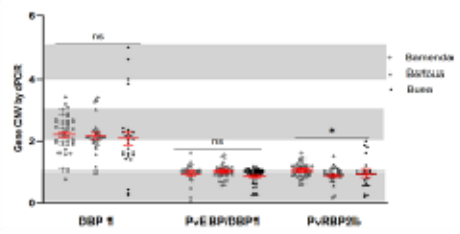
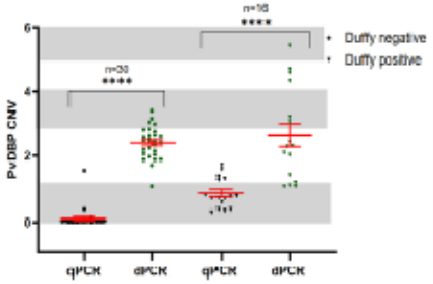


Figure 1: Patients with Cambodian and those with both Cambodian and malagasy-type duplications tend to have higher PvDBP duplications compare to those with just Malagasy-type duplications. Pair wise comparison using the Non parametric Mann Whitney test reveal that patients with just the Cambodian type duplications have higher CNVs compared o those with both duplication types.

Fig. 2





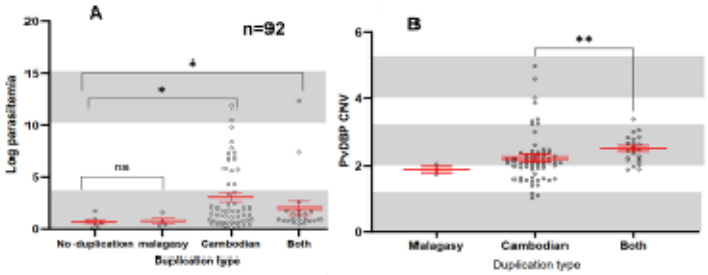


Figure 2: PvDBP duplications results in higher parasitemia. A significantly higher parasitemia was observed in patients with Cambodian type (p = 0.0143) and those with both malagasy and cambodian-type duplications (p = 0.0474) but not in those with only Malagasy-type duplication (p = 0.7824)

SE01.04

Intracellular *Eimeria bovis* macromeront formation induces bystander cell accumulation and TNT-based mitochondria transfer

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Introduction *Eimeria bovis* first merogony is an intracellular process (~ 3 weeks) resulting in the formation of large macromeronts (≤ 400 µm) containing up to 140,000 merozoites I, each. The production of merozoites I poses critical metabolic stress on bovine endothelial host cells, leading to mitochondrial dysregulation and premature senescence. In this context, an accumulation of non-infected bystander cells (BCs) around *E. bovis* macromeront-carrying host cells (MCHCs), eventually supporting MCHCs, was observed.

Objective We intend to study whether BC accumulation is driven by *E. bovis* infection and aiding MCHCs to recover to a normal operating state via tunneling nanotube (TNT)-based organelle transfer.

Materials & Methods BC accumulation was quantified by 3D confocal microscopy. A meront-transfer-system was established to evaluate the supportive BC capacity of different cell types. Since healthy cells might support stressed cells by transferring cargo like mitochondria via TNTs, we studied if *E. bovis* infection affected cellular TNT formation. By utilizing the meront-transfer-system, recipient non-infected BCs were pre-treated with stimulators or inhibitors of TNT formation (H89, Y26732, cytochalasin B) and their effects on *E. bovis* development was estimated in BC-MCHC-cocultures. To study the transfer of mitochondria via TNTs, non-infected and *E. bovis*-infected cells where stained with respective dyes and cargo transfer was illustrated.

Results Within *E. bovis*-infected cell layers, an increase of BCs at all sides of MCHCs was stated, thereby correlating with meront sizes and maturation. When using different cell types as BCs, we showed that macromeront development was best supported by human fibroblasts, followed by human and bovine endothelial cells. Overall, TNT numbers were increased in *E. bovis*-infected cell layers. The relevance of TNTs for parasite development was underlined by selective BC cytochalasin B treatments, which blocked both TNT formation and merozoite I production. However, stimulation of TNT formation by H89 or Y26732 treatments failed to affect macromeront formation. Given that TNT-based transfer may improve the energetic status of *E. bovis*-infected cells, we here additionally demonstrated that non-infected cells donated mitochondria to *E. bovis*-infected cells.

Conclusion BC-based TNT-mediated mitochondria transfer may evidence a new mechanism of parasite-induced host cell modulation, aiding MCHCs to support parasite proliferation.

SE01.05

Biophysical and immunological insights into malaria pathogenesis: From merozoite invasion to vasculature sequestration

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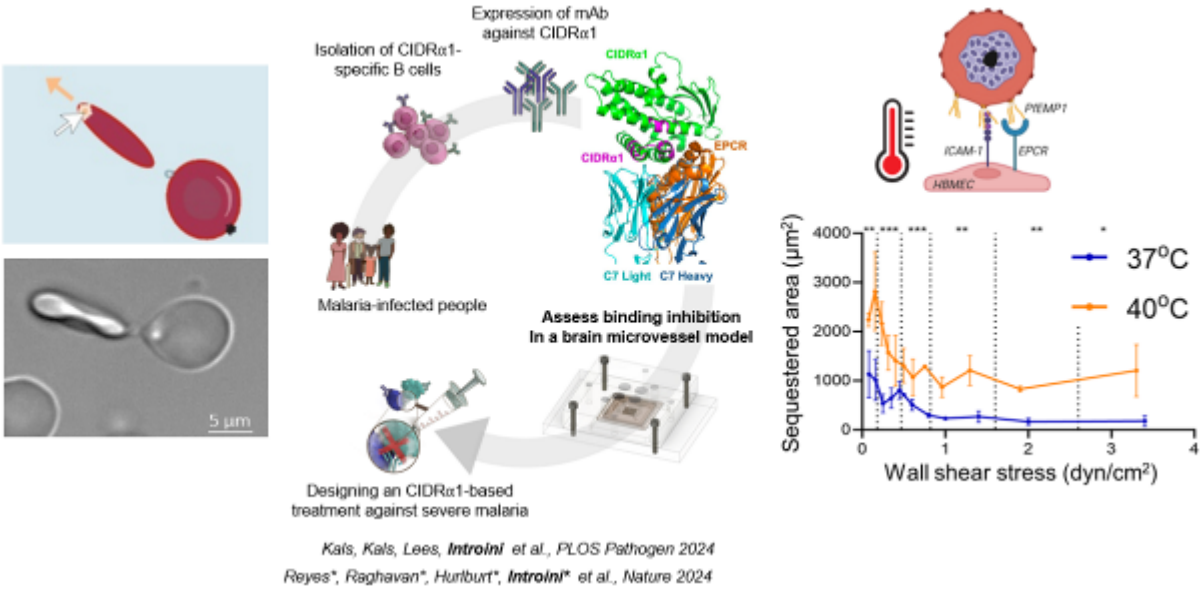
Introduction: Clinical symptoms and pathogenesis of Plasmodium falciparum infection arise during the blood stage, involving rapid erythrocyte invasion and sequestration of infected cells in the microvasculature. While invasion ensures parasite survival, sequestration underlies severe pathologies, including cerebral malaria. The rapid nature of invasion and complex host-parasite interactions during sequestration have limited mechanistic understanding, hindering targeted therapy development. Objectives: 1. Quantify attachment forces and kinetics of P. falciparum ligands during merozoite-erythrocyte interaction. 2. Identify broadly reactive monoclonal antibodies inhibiting PfEMP1, a key virulence protein in severe malaria. 3. Investigate the effects of febrile temperatures on endothelial function and parasite sequestration. Methods: Optical tweezers quantified real-time attachment frequency, kinetics, and adhesion forces of merozoite-erythrocyte interactions. Using inhibitors, antibodies, and genetically modified P. falciparum strains, we dissected the contributions of specific ligands to invasion. A 3D bioengineered human brain microvessel model replicating physiological flow conditions was used to study endothelial binding. Monoclonal antibodies targeting PfEMP1 from Ugandan adults were tested, alongside endothelial responses at 40°C using structural and functional assays. Results:1. The major P. falciparum merozoite surface protein PfMSP1, long considered key to attachment, showed no impact on adhesion forces. In contrast, knockouts of PfEBA and PfRH ligand families significantly reduced adhesion. 2. Two monoclonal antibodies targeting the CIDRα1 domain of PfEMP1 effectively blocked Endothelial Protein C Receptor (EPCR) binding, preventing sequestration. 3. Hyperthermia enhanced parasite binding to endothelial cells in a flow and receptor-dependent manner, associated to disruption of glycocalyx integrity, increasing endothelial vulnerability to damage. Conclusions: By integrating biophysical and tissue-engineering approaches, this study advances fundamental understanding of P. falciparum invasion and sequestration. These findings inform innovative therapeutic strategies, including antibody-based interventions, to combat severe malaria.

Fig. 1



SE01.06

The proteasome as an anti-parasitic drug target

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The proteasome is a complex molecular machine crucial for maintaining proteostasis in the cell. Once considered a challenging drug target due to its evolutionary conservation, the past 15 years have seen remarkable progress in the design of specific small molecules that inhibit 20S proteasome function. This progress has led to improved outcomes in certain cancers and the potential to treat infectious diseases. The Center for Discovery and Innovation in Parasitic Diseases at the University of California San Diego is focused on developing inhibitors of the 20S proteasome for a number of parasites, including the African trypanosome and the schistosome. I will highlight the molecular, biochemical and chemical tools we utilize to advance our research in this field.

SE01.07

The ace in the game: GP63 as a genetically divergent and multifaceted protease regulating the various stages of *Leishmania donovani* infectivity

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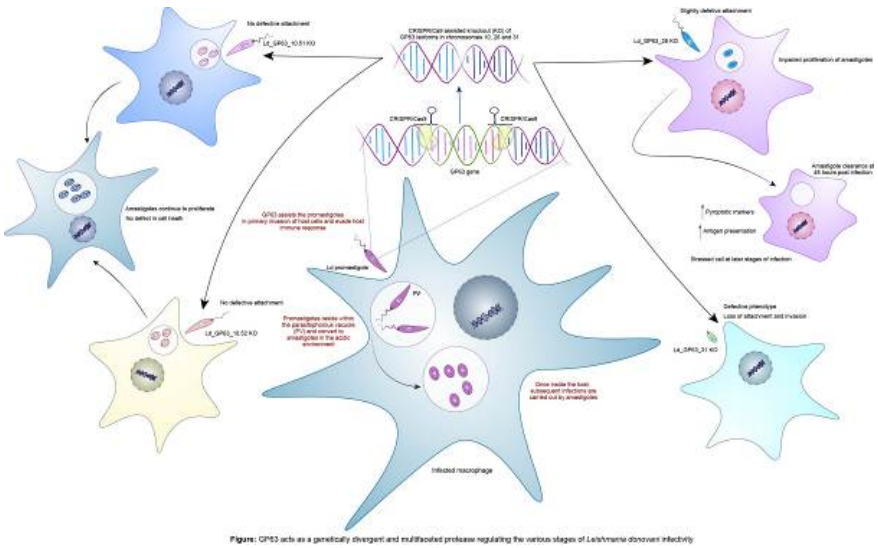
The rising incidence of drug unresponsiveness among clinical isolates of *Leishmania donovani* (Ld), responsible for causing fatal visceral leishmaniasis (VL), underscores urgent need of identification of novel chemotherapeutic targets. One promising target is the zinc metalloprotease Glycoprotease63 (GP63), crucial at all stages of *Leishmania* infection. While the function of GP63 has been extensively studied in *Leishmania major* (the causative agent of cutaneous leishmaniasis), its role in Ld remains poorly characterized. Metaphylome analyses have identified GP63 as one of the most heterogeneous genes in *Leishmania*, with the Ld genome encoding four distinct GP63 isoforms from three chromosomal origins: 10, 28, and 31. To elucidate the roles of these GP63 isoforms during Ld infection, CRISPR-mediated knockouts (KOs) of individual isoforms were performed. The findings revealed that two GP63 isoforms from chromosome 10 were fragmented and exhibited no observable phenotypic defect. Interestingly, GP63_28 KO although did not result in a major defect in host invasion, they failed to persist as intracellular amastigotes by clearing out from infected macrophages at 48Hrs post infection (p.i). This intracellular clearance of GP63_28 KO amastigotes is accompanied with increased expression of pyroptotic markers, viz. NLRP3, NLRP1, AIM2, IL1B and Caspase1, suggesting GP63_28 might play a vital role in nullifying host immune response allowing proliferation of intracellular Ld amastigotes. Furthermore, Ld_GP63_28 KO infected macrophages exhibited increased propensity of antigen presentation as compared to wild-type parental infection. In contrast, GP63_31 KO promastigotes showed a drastic change in morphology, and their infection resulting in a significant defect in attachment and host invasion. These observations align with localization studies, which identified GP63_31 at both promastigote surface and cytoplasm, unlike GP63_28 which is entirely cytoplasmic, suggesting that GP63_31 might have a more prominent role in host invasion. Preliminary docking analyses showed that Ld_GP63_28 and Ld_GP63_31 share host substrates with Lm_GP63. However, GP63_28 preferentially interacts with substrates involved in immune modulation, while GP63_31 targets cytoskeletal remodelling factors. These findings emphasize the complementary roles of GP63 isoforms in Ld pathogenesis, offering insights for targeted therapeutic interventions.

Fig. 1



SE02.02

The impact of the menstrual cycle on human skin volatile profiles and mosquito preferences

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Historically, women have been underrepresented in biological and medical research, resulting in significant knowledge gaps with adverse implications for women's health. This exclusion often aims to avoid potential temporal variations linked to the female reproductive cycle. In studies on human odors and disease vector attraction, such gaps can critically impact human health. Yet, sex-specific differences in odor cues influencing mosquito attraction remain largely unexplored.

To address this gap, we conducted a longitudinal study investigating variations in female body odors throughout the menstrual cycle. We collected skin volatile samples from female subjects during the menstrual, fertile, and luteal phases, as well as from male controls. These samples were analyzed using two-dimensional gas chromatography-mass spectrometry (GCxGC-MS), providing crucial insights into sex differences in body odor and the potential effects of the menstrual cycle. Additionally, we tested the relative attraction of the primary malaria vector, *Anopheles gambiae*, to odors from the same individuals over time.

Our findings may inform targeted strategies for improved health interventions and disease prevention and control, ultimately contributing to better public health outcomes.

SE02.03

Mosquito-borne parasite surveillance in Perth, Western Australia

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Introduction: Avian Haemosporidia are highly diverse, with 250 morphospecies described and 5,131 unique lineages within the *Plasmodium*, *Haemoproteus*, and *Leucocytozoon* genera. They have a global distribution, except for Antarctica, and there are fewer detections in oceanic regions These genera have a complex heteroxenous lifecycle, with sexual reproduction occurring in a haematophagous vector (e.g. mosquito) and asexual reproduction within its primary and intermediate hosts.

Xenomonitoring has provided a convenient and cost-effective surveillance method for Mosquito-borne pathogens. In Western Australia, routine xenomonitoring screens for human mosquito-borne viruses, therefore, the prevalence of mosquito-borne parasites (MBP) has not been described.

Objectives: This study aims to a) determine the prevalence of MBP, b) compare the geographical distribution of identified MBP and c) Provide further evidence for suspected vectors of detected MBP.

Material and Methods: Mosquitoes were collected using CO2-baited CDC mosquito traps at 10 locations, 14 times over 2 years, around Perth"s metropolitan areas. Mosquitoes were morphologically identified to species level and pooled according to species, location, and date. Mosquito pools were screened using conventional and nested PCR for species within the *Dirofilaria*, *Haemoproteus*, and *Plasmodium* genera. Sequences were edited using Geneious Prime (Version 2021.0.3), and lineages were identified using BLAST-NCBI and MalAvi databases.

Results: 3,379 mosquitoes in 469 pools were screened for MBP. Haemosporidia parasites were detected in 21 pools, with 13 (61.9%) identified in *Culex* spp. pools. Phylogenetic analysis revealed three previously identified MBP (*Plasmodium* spp. BELL01 & MYNA02, and *Haemoproteus zosteropis*). A novel Avian *Plasmodium* lineage was detected, with a 5% sequence divergence from previously identified lineages. *Dirofilaria* was not detected.

Conclusion: This study identified a diverse range of avian haemosporidia. The novel Avian *Plasmodium* lineage suggests that Perth may harbour a regionally specific lineage. Detected lineages only occur within the oceanic region; however, the pathogenicity of these strains to native birds remains unknown. The results agree with *Culex* spp. as the primary vectors. However, detection in other genera may suggest that vector competence may expand outside of the *Culex* genus.

SE02.04

Use of the salivary peptide gSG6-P1 as a biomarker for assessing human exposure to *Anopheles* spp. bites in a context of LLINs use in two localities of the East region of Cameroon

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[4]University Joseph Ki-Zerbo,19 Ouagadougou, Laboratory of Fundamental and Applied Entomology, Ouagadougou, Burkina Faso
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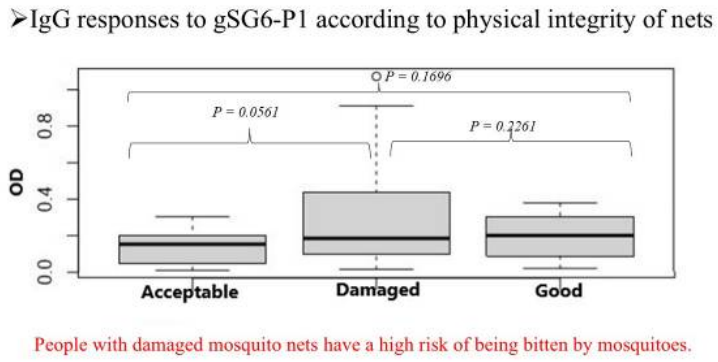
Introduction: Human IgG antibody response to *Anopheles gambiae* gSG6-P1 salivary peptide was reported to be a pertinent indicator for assessing human exposure to mosquito bites and evaluating the risk of malaria transmission. However, in the East region of Cameroon, no study using this tool to highlight how anthropological factors can affect human vector contact are available. In this study, we aimed to determine the anthropological factors favoring human-vector contact in a context of LLINs use, using the gSG6-P1 salivary peptide.

Methodology: In October 2022 during the main rainy season, blood samples were collected from people living in Belabo and Ouami. A questionnaire was administered to people who had agreed to take part in the study, and their mosquito nets were collected. Malaria infection was determined using Rapid Diagnostic Test. The level of IgG Anti-gSG6-P1 response as a biomarker of human exposure to *Anopheles* bite, was assessed using enzyme-linked immunosorbent assay.

Results: Around 85% of the nets collected in the field had at least one hole, with a high percentage of damaged nets in both sites. Despite the fact that the difference was not significant between the level of antibody response and the status of the net, people with a damaged had a higher IgG response than other. Comparison of the levels of IgG Ab response between *Plasmodium*-infected and uninfected individuals showed that this response was significantly ((p=0.0206) higher in the group of infected individuals than in uninfected ones. A significant difference (p=0.0103) in IgG response to gSG6-P1 was observed between people who had vegetation around their house compared to those who did not have.

Conclusion: Parameters such as the presence of vegetation around houses, as well as the condition of the mosquito net, influence the level of exposure to mosquito bites and consequently the persistence of malaria.

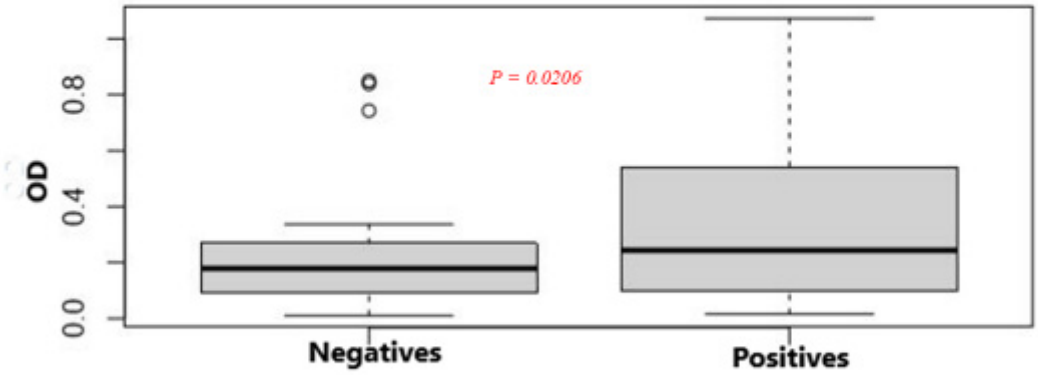
Fig. 1



People with damaged mosquito nets have a high risk of being bitten by mosquitoes.

Fig. 2

➢ IgG response between *Plasmodium*-infected and uninfected individuals



People with the highest Ab response to gSG6-P1 were more likely to be infected by *P. falciparum* than people with a low response.

SE02.05

Interactions of *Trypanosoma cruzi*, triatomines and the microbiota – A review

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Trypanosoma cruzi is the causative agent of Chagas disease, a neglected disease endemic to Latin America. It develops in the intestinal tract of the blood-sucking vectors, the triatomines. These intestinal regions are also colonized by the intestinal microbiota, which includes many different fungi and bacteria as well as the mutualistic bacterial symbionts. *T. cruzi* strains show great biological heterogeneity. The effects of the flagellate on the triatomines depend on the *T. cruzi* strain. The effects of the triatomines on the flagellate are reflected in differences in the ability to establish in the vector and in developmental peculiarities. *T. cruzi* multiplies mainly in the small intestine and rectum, and it is in the latter that the metacyclic trypomastigotes develop. The rate of metacyclogenesis is increased after blood ingestion, whereas starvation of the vector leads to more spheromastigotes. After feeding of long-term starved nymphs, stages of multiple divisions of *T. cruzi* develop. The mutualistic symbionts multiply after blood ingestion, but only in the anterior midgut. These regions have a high antibacterial activity against *Micrococcus luteus*. The symbionts are lysed in the posterior midgut, where the antibacterial activity is low. The function of the symbionts is unknown, but does not appear to be the supply of B vitamins. Since the two verified mutualistic bacteria are Actinomycetales, their unique compounds, mycolic acids, may be of importance. The surface coat of blood trypomastigotes induces the synthesis of antibacterial compounds in the intestine in a *T. cruzi* strain-dependent manner. However, this has no effect on the symbionts *Rhodococcus rhodnii* and *R. triatomae* in the triatomines *Rhodnius prolixus* and *Triatoma infestans*, respectively. After a knockout of the antibacterial compounds, more non-symbiotic bacteria and less *T. cruzi* develop. The growth of non-symbiotic microorganisms is also promoted during long-term infections of nymphs with *T. cruzi*, possibly indicating a suppression of intestinal immunity. These results point to the complex interactions between *T. cruzi,* the triatomine vectors and the non-symbiotic and symbiotic bacteria.

SE02.06

Malaria vector bionomics and transmission patterns in Olama, an equatorial forest region of South Cameroon

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Introduction and Objective: Malaria remains a major public health problem in Cameroon. In rural areas of the equatorial forest region, the wide range of available breeding sites generally favour vector diversity and high densities. Malaria is perennial and is transmitted by local vectors such as *Anopheles moucheti* and *An. gambiae s.l.,* which are responsible for high malaria transmission. However, recent environmental changes resulting from intensified human activities, including deforestation, urbanization, agriculture, promoted the proliferation of these highly anthropophilic vector species with serious consequences on malaria epidemiology. The current study aimed to investigate vector bionomics and malaria transmission patterns in Olama.

Methods: Adult mosquitoes were captured by human landing catches and CDC light traps in Olama in January-december 2023. Malaria vectors were morphologically identified and analyzed for *Plasmodium falciparum* circumsporozoite protein detection using ELISA method and molecular identification of *Anopheles gambiae* species using PCR.

Results: A total of 8240 mosquitoes were collected. Overall seven anopheles species were identified. A predominance of *An. moucheti* 98.32% (4757/4838 Anopheles mosquitoes). Of the 107 *An. gambiae* s.l. processed by PCR, 50.46% (54/107) were *An. coluzzii* and 24.29% (26/107) *An. gambiae s.s*. The infection rate by *P. falciparum* was 0.52% (11/2122 mosquitoes processed). The entomological inoculation rate was 2.21% infective bites/man/night. The human biting rate was 117.54 bites per man per night. Malaria transmission risk wasn"t significantly high indoor (122.42 ib/m/n) compared to outdoor (112.67 ib/m/n); (X=0.427; P=0.51).

Conclusion: *Anopheles moucheti* remained the main malaria vector species. Although a decrease in malaria transmission intensity has been reported, this level is still far from the target of the national malaria control programme. The changes in vector bionomics, population non-adherence to control measures, deforestation, construction of dams, affect the effectiveness of vector control measures in the forest region and could have effects on local malaria transmission. A call for immediate actions to improve control strategies.

Key words: Malaria, Transmission, Equatorial forest region, Bionomic, *Anopheles moucheti*.

Fig. 1

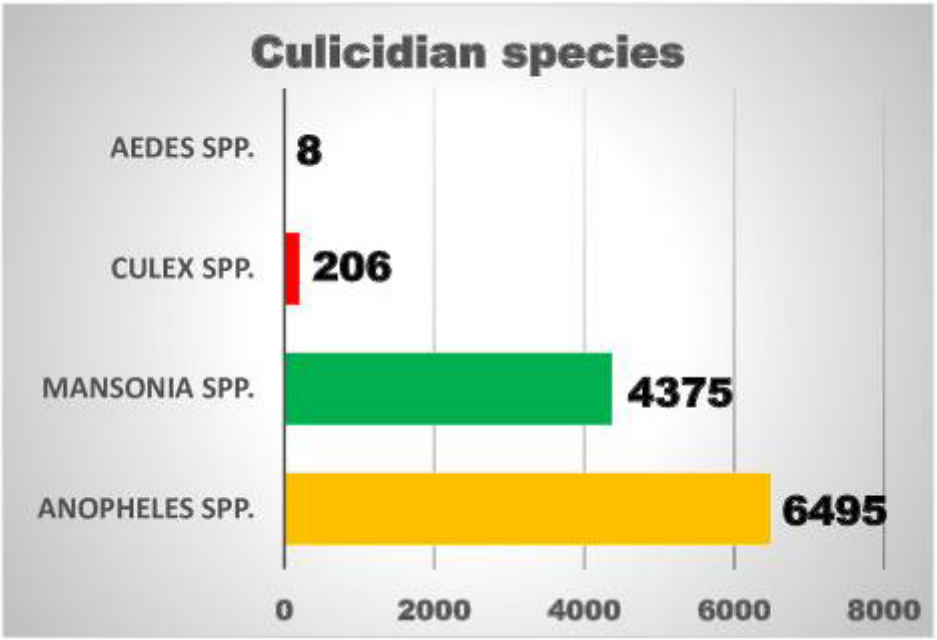

Fig. 2

Anopheline species	N	%
An. moucheti	5315	82,24
An. gambiae s.l.	659	10,2
An. funestus	38	0,59
An. marshallii	354	5,48
An. ziemanni	92	1,42
An. paludis	5	0,07
Total	6463	100
Taxa_S	6	

SE02.07

Revealing complex mosquito behaviour in bioassays to improve vector control

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For many mosquito-borne diseases, such as malaria and dengue, vector control remains the most effective strategy and requires the availability of affordable, safe and effective vector control products. To develop and test these products, standardised bioassays are used that typically measure simple endpoints, such as mortality or biting rates, but fail to capture how mosquitoes interact with these products. Recently, automated video tracking of mosquito flight paths has been adopted as a comprehensive method to observe mosquito behaviour. We use this strategy to evaluate the impact of insecticide-treated nets and to understand how topical repellents prevent mosquitoes from biting. For example, we found that while permethrin is often referred to as being repellent, permethrin-treated nets actually increase the chance of mosquitoes passing through the holes of such a net due to heightened excitation. In testing topical repellents in an arm-in-cage set-up, we observed that – although repellents reduced bites – they did not prevent skin contact. Both studies underscore the complex effects of chemical interventions on mosquito behaviour. We will discuss the importance of understanding mosquito behaviour beyond simple endpoints for the development of new vector control tools and provide an overview of available automated video tracking systems to record mosquito flight paths in the laboratory and the field setting.

SE02.08

Studies on the co-localization of TBE-Virus and *Borrelia* species at selected TBEV-foci in Baden-Wuerttemberg

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In Central Europe, *Ixodes ricinus* ticks are vectors for a wide range of pathogens, including the Tick-Borne Encephalitis Virus (TBEV) and *Borrelia burgdorferi* s.l.. Both pathogens are of public health importance. In Germany, the TBEV is primarily represented by a genetically diverse subgroup of the European subtype, while *Borrelia* while Borrelia has an even greater genetic diversity and comprises at least eight species that can infect humans. Co-infections of ticks with multiple pathogens, such as TBEV and various *Borrelia* species, have been documented, but the ecological and molecular interactions between these pathogens remain unclear.

This study investigates the potential influence of *Borrelia* on the prevalence and spatial distribution of TBEV within tick populations. Specifically, we examine the co-infection dynamics between various TBEV strains and *Borrelia* species within TBEV microfoci in the district of Ravensburg, a region experiencing a sharp increase in human TBE cases since 2017. By analyzing TBEV foci and nearby TBEV-free sites, both local and in the metropolitan area of Stuttgart as controls, we aimed to address whether specific *Borrelia* species are associated with TBEV foci and if co-infections with *Borrelia* may influence the restricted distribution patterns observed for TBEV in the region.

A total of 4801 *Ixodes ricinus* ticks were collected from the different sites and subsequently screened for the presence of TBEV and *Borrelia*. Co-infections within individual ticks carrying both pathogens were identified, offering important insights into the potential interactions between these pathogens within tick populations. The preliminary results indicate that TBEV and *Borrelia* infections can occur simultaneously in individual ticks and are a factor of considerable importance for epidemiological modeling and the development of targeted control strategies.

SE03.03

Interrogation of entamoeba histolytica in a human intestinal tissue microphysiological system

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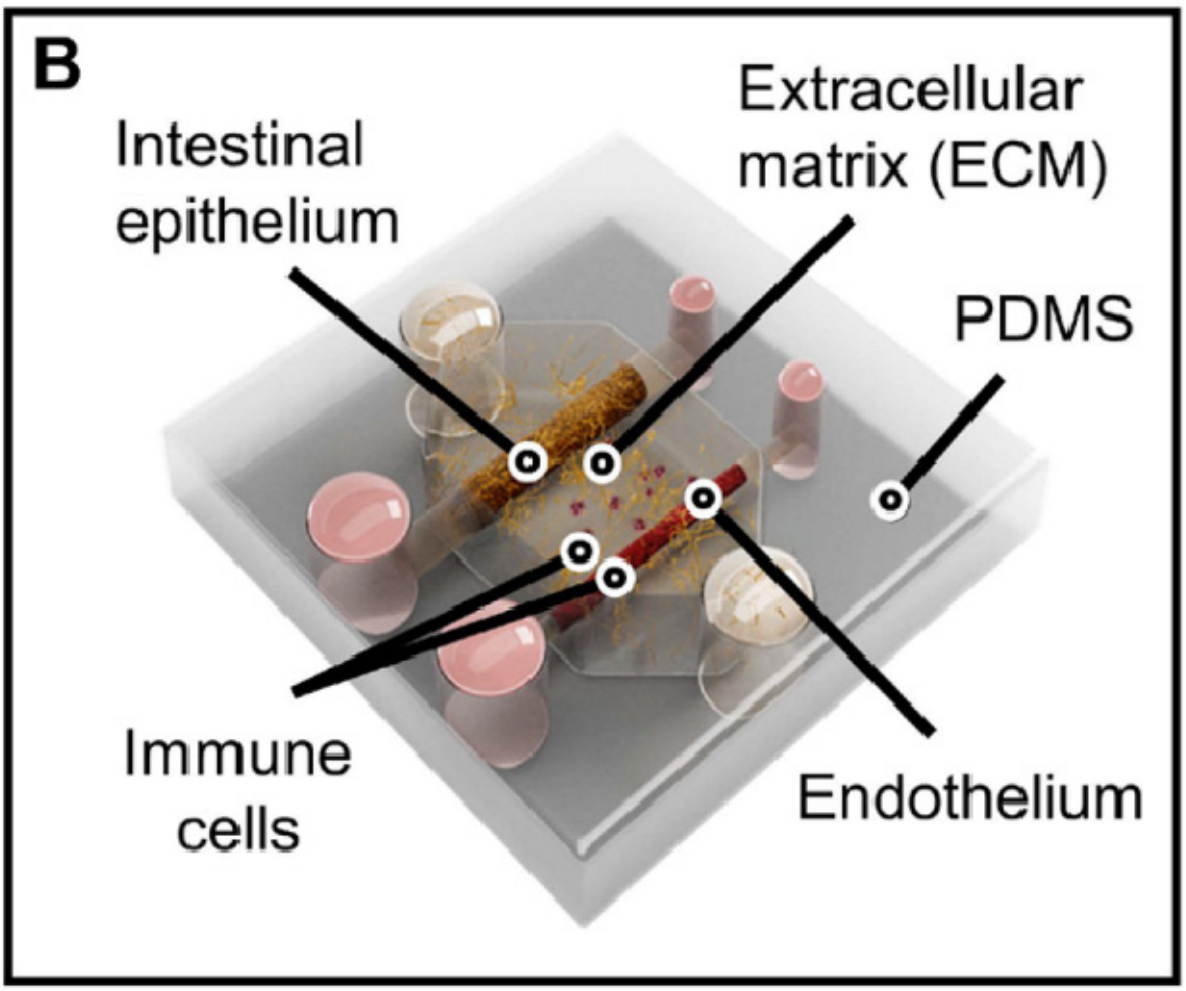
The infectious burden of protozoan parasites is extremely significant worldwide and some parasitic species, such as *Entamoeba histolytica (E. histolytica)*, are vastly understudied. Despite its high worldwide burden, little is known about the dynamics of *E. histolytica* pathogenesis in the gut. This gap in research knowledge is largely due to the difficulties associated with analyzing host-pathogen interactions and parasite dissemination in applicable models for human disease. In recent years, great strides have been made in the field of microfluidics, allowing for the integration of human organ systems which can facilitate tissue-tissue crosstalk and effectively model these complex microenvironments. We have developed a human microphysiological system of intestinal tissue and a vascular compartment to investigate *E. histolytica* pathogenesis. This system not only models human architecture but integrates the microbiotic environment necessary to accurately represent the microbial interactions that underly *E. histolytica* non-pathogenic and pathogenic infection.

Fig. 1



SE03.04

Cellular responses to insecticides: Insights using new cell lines

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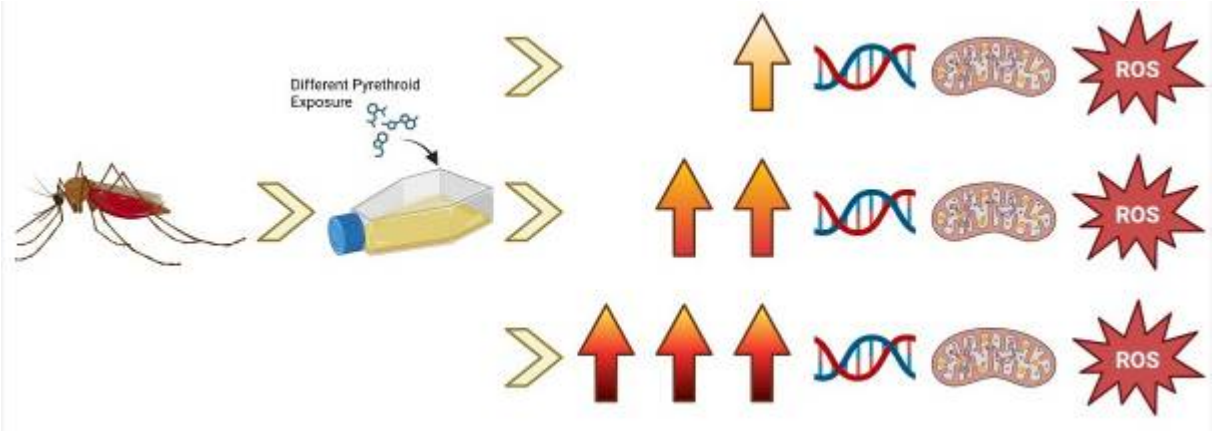
Insecticide treated bed nets (ITNs) are the most important tool in malaria prevention. Traditional ITNs have relied on a single class of insecticides, pyrethroids, which target the mosquito nervous system. Next generation ITNs have been introduced, which incorporate an additional chemistry, such as chlorfenapyr, a pyrrole insecticide. Chlorfenapyr works by inhibiting oxidative phosphorylation and thereby killing the cell.

It has been shown that pyrethroid exposure can affect mosquito metabolism, with mosquitoes resistant to pyrethroids having a higher respiration rate, which is depressed upon exposure. Recent work in the lab has further shown that exposure to pyrethroid insecticides lead to a reactive oxygen and nitrogen species response, which may impact mitochondrial function.

Despite now being distributed across Africa, the interactions between pyrethroids and chlorfenapyr are not well understood, with some evidence that chlorfenapyr alone is a more effective insecticide in some settings. Untangling the complex relationship between these two chemistries using whole mosquitoes is difficult due to multiple factors, including conversion of chlorfenapyr, metabolic activity and differences in sizes. To overcome this, we have generated novel primary cell lines from lab reared mosquitoes under different pyrethroid susceptibility backgrounds. Using these cell lines, we can explore the secondary effects of pyrethroid exposure to mosquito tissues. We show that at the cellular level, pyrethroid exposure has different outcomes between susceptible and resistant mosquitoes. Strikingly, we find that even in the same chemical classification, different pyrethroids elicit different responses.

By utilising novel primary cell lines, we demonstrate that pyrethroid exposure leads to distinct metabolic responses depending on the mosquito's susceptibility. These results underscore the need for further investigation into how different pyrethroids affect mosquito physiology and their potential interactions with chlorfenapyr. Understanding these dynamics will be crucial for improving future malaria control strategies.

Fig. 1



SE03.05

Custom microfluidic solutions for parasitology: Towards the host-on-a-chip

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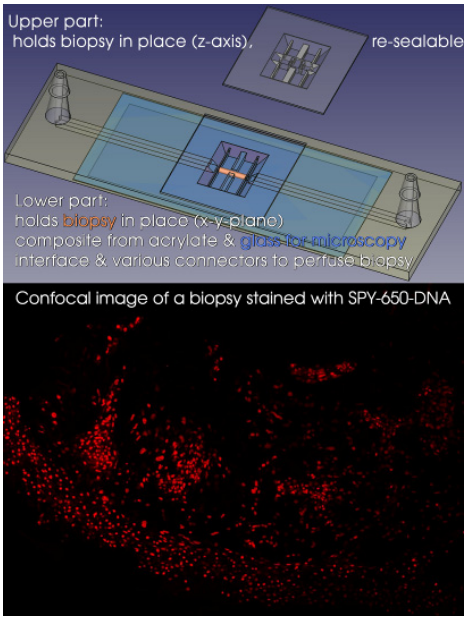
Replicating the intricate host-parasite interactions using organ-on-Chips has the potential to unlock new insights, from diagnostics to therapeutic testing. Organ-on-Chips are based on microfluidic devices. While off-the-shelf devices exist, they are often inadequate for addressing the specific needs for each host-parasite interaction (e.g. membranes, channels and special geometries). Tailored designing and 3D-printing of dedicated microfluidic devices unlocks many parasitology studies.

Using our recenltly developed "biopsy holder" (see Figure) as example, we present a workflow to create devices, crafting them in a variety of materials such as acrylate, nylon, or composites with glass or ceramics. Optional coatings ensure compatibility with cell cultures, organoids, and other experimental systems. This system maintains tissue viability for extended observation and analysis, showcasing the ability to integrate biological relevance with experimental practicality.

For more complex investigations, our microfluidic tools support the cultivation of organ-on-chip systems, providing physiologically relevant environments to study host-parasite interactions. This includes mimicking human organs affected by parasitic infections, such as the gut or liver, or testing the systemic side effects of antiparasitic drugs on interconnected organ systems within a single chip. By tailoring devices to fit each research question, we ensure that researchers can explore these critical areas with precision and efficiency.

By offering rapid prototyping and a service-oriented approach, we eliminate the constraints of standard solutions, enabling parasitology researchers to unlock new insights into diagnostics, host simulations, and therapeutic development.

Fig. 1



SE04.01

A qualitative insight into barriers and facilitators to C*utaneous leishmaniasis* prevention and management in Tigray, northern Ethiopia

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Introduction: Cutaneous leishmaniasis (CL) is a skin disease caused by a group of parasite species transmitted by a bite of sand fly. It is a neglected tropical disease that frequently affects rural and vulnerable communities with little access to health care services and is frequently associated with long-term disability and stigma. Ethiopia, on the Horn of Africa is among the countries with a high burden of cutaneous leishmaniasis. There are approximately 20,000 to 30,000 cases each year and the disease is endemic in highland areas at an altitude of 1400-3175 meters above sea level. Despite the high burden of cases, it is still difficult to provide appropriate care, only in tertiary care facilities are provide the service.

Objective: The aim of this study was to explore barriers and facilitators to prevent and manage cutaneous leishmaniasis in Tigray, northern Ethiopia

Methods: The study was conducted in CL endemic area found in Tigray, Northern Ethiopia. A six-month ethnographic study which was carried out in Tigray's CL-endemic districts. Thematic content analysis was used to generate the main findings. A total of 33 interviews with CL patients, caregivers, medical professionals, traditional healers, and leaders in the community were and three focused group discussions interviews were conducted. Eight field work observation report were also developed through participant observation while the fieldwork was going on.

Result: Individual barriers like lack of knowledge, financial constraints, and poor seeking care behavior. At the communal level, impediments like Poverty, stigma, social constructed and culturally practiced beliefs and perception towards the disease found to be aggravating factors not to seek the timely treatment at the right place. Lack of access, lack of knowledge and skill of health providers, unavailability of drugs and supplies, poor data management, and a lack of program support, inadequate surveillance in endemic areas and information gaps at health facilities level. Delayed diagnosis and treatment also another challenges

Conclusion: Although there are several factors that affect CL prevention and management, the most significant one is that people found in CL endemic area cannot easily receive CL diagnosis and treatment, and both the general public and healthcare professionals lack adequate knowledge of the disease.

Fig. 1

Fig. 2

SE04.02

Self-sufficiency with consequences – A case report of human ascariosis

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Ascaris lumbricoides is seen as the causative agent of human roundworm infection, whereas a zoonotic infection with *A. suum* is often not considered. Nevertheless, most human cases of roundworm infection in European countries are caused by *A. suum*. Some researchers even debated that both are the same species with variants having different host preferences. This can result in an unclear epidemiological situation and ineffective control, as the case report presented here shows.

A family living in northern Germany contacted the Institute of Parasitology of the University of Veterinary Medicine Hannover to investigate the undetermined cause of a recurring human roundworm infestation. From 2005, first the children and then all members of the family suffered from reoccurring roundworm infection and were therefore treated with mebendazole at alternating intervals (in 2011, 2012 and 2015). In 2016/2017, one of the family members exhibited respiratory symptoms and coughed up worms. Over the years, the family consulted several physicians, each of whom assumed an *A. lumbricoides* infection and considered the reinfections to be a hygiene issue within the family. An infestation with *A. suum* was excluded or was not considered. During the anamnesis, it became apparent that the family regularly fattened pigs for self-sufficiency and fertilized their vegetable garden with manure from these pigs. The family sent in roundworm specimens isolated from their and the pigs" faeces, faecal samples and soil samples for examination.

The adult worms measured 10 to 15 cm and were morphologically identified as *Ascaris* species. *Cox1* gene sequences of these adults clustered with reference sequences from adults associated with pig infection. Therefore, the family most likely suffered from zoonotic infection. While no eggs were found in the faecal samples of the family, the pigs had an average EpG of 1040. A total of 67 *Ascaris* eggs were found in 250 g of soil from the vegetable garden, 42 of which were embryonated and thus potentially infective. Following these results, the family discontinued their pig housing in 2017 and relocated their vegetable garden. No further roundworm infection was recorded in the following years.

In 2022, a follow-up examination of soil samples from the former vegetable garden and pig farming area was conducted. Even after 5 years, the former vegetable garden was still highly contaminated with 24 eggs per 250 g soil, of which 16 were embryonated. Two pigs were experimentally infected with 85 of these embryonated eggs each. One pig, that harboured three females and one male had an EpG of up to 866, whereas the other pig harboured only one male and did not excrete eggs.

It can be assumed that the roundworm infection of the family was caused by the consumption of insufficiently washed vegetables that were contaminated with roundworm eggs from the pig manure and even after 5 years, these eggs still are a potential source of zoonotic infection.

SE04.03

Evaluation of the PAPRIKA method for predicting *Cryptosporidium* contamination of karst water resources

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Introduction: In 2019-2020, the largest cryptosporidiosis outbreak never reported in France occurred in south-east of France. Several thousands of patients were concerned. An intense rainfall caused an episode of *Cryptosporidium parvum* contamination in the karstic springs used to supply drinking water network to several municipalities. Population was supplied with bottled water for several months. Economic and health consequences were strong for health authorities.

Objectives: In this context, a study was carried out to evaluate a prediction model for *Cryptosporidium* oocysts dissemination in karst aquifer.

Materials & Methods: The PaPRIKA vulnerability assessment method was evaluated. The PaPRIKa method is based on four criteria: protection of the groundwater, rock type of the reservoir (saturated zone), infiltration and karstification. Based on the PaPRIKA vulnerability mapping, several sampling points were defined. From January to July 2021, eight surface waters were sampled monthly according to a methodology adapted from the ISO EN NF T 90-455 standard method. Infectivity of isolated *Cryptosporidium* oocysts was evaluated and strains were genotyped. Potential correlation with microbiological potability indicators of tapwater was evaluated.

Results: The global vulnerability map of the studied region was obtained using the PaPRIKA vulnerability assessment method. Sampling sites contamination varied according to seasonality and most vulnerable suspected sites using the PaPRIKA vulnerability method were actually the most contaminated. Up to 7/8 sampling sites were contaminated by oocysts in January and no oocyst were detected in both February and March. No significant correlation was observed with other investigated microbial parameters (total flora, coliforms, *E. coli*, anaerobic sulphite-reducing spores, intestinal enterococci) neither with water features (temperature, conductivity and turbidity). Regarding the potential correlation between *Cryptosporidium* contamination and precipitation: oocysts were mainly detected when it rained during sampling or when it rained for several days before sampling or both. Results on subtyping showed that contamination varies over time even for a same sampling site and that a wide diversity of subtypes are circulating. Subtypes IIa were dominating and the IIaA15G2R1 and IIaA20G1R1 ones were the most represented. 86% of detected isolates were still infective.

Conclusion: The PaPRIKA vulnerability assessment method appeared effective to predict *Cryptosporidium* oocysts circulation in karst aquifer. Water resources contamination appeared frequent, influenced by rainfall and surrounding livestock areas. The PaPRIKA vulnerability assessment method could be proposed to health authorities for the prediction (or even prevention) of cryptosporidiosis in a context where the parasitic contamination research is not mandatory on water potability quality assessment.

SE04.04

Impact of using *Dihydroartemisinin piperaquine* for malaria mass drug administration in an endemic area of Ghana

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Ghana ranks 12th among countries that contributed 70% of the global malaria burden. Targeting asymptomatic carriage in endemic countries in Africa using mass drug administration (MDA) could lead to malaria pre-elimination in endemic areas. The need for data to inform policy on MDA implementation is urgent.

We hypothesize that implementing MDA will interrupt transmission paving the way to pre-elimination in these endemic communities.

A population of 8,000 (6000 in the intervention arm and 2,000 in the control arm) were targeted through bimonthly rounds of MDA between December 2023 and August 2024. All test were perfomed using RDTs.

Community health workers (CHW) went from door-to-door testing all participants using RDTs and treating with DHAP. Data was analysed using SPSS Statistics 26.

MDA using DHAP resulted in a more than 95% reduction in RDT positivity (RDT+) in the intervention communities compare to bassline. RDT+ December 2023 was 27.8% (456/1642) [95% CI 25.6, 29.9] and 0.7% in August 2024 (p-value < 0.001, x^2=4798.521) following 5 rounds of MDA. This significant decline in asymptomatic RDT+ was observed across all ages. The overall incidence was post-MDA was <10/1000 population compared to 278/1000 population at baseline. The slight fluctuation observed at this very low level of prevalence during intervention 4 and 5 shows that there need to extern MDA to eliminate reservoirs or implement vaccine for the under post. Severe anaemia reduced from 24.4% to 2% in children <15 year while moderate anaemia fluctuated over time. At baseline the proportion of non-falciparum were low but as the prevalence decreased over time their proportions increased very significantly.

These findings suggest that implementing MDA using DHAP in a moderate-to-high transmission setting using DHAP is feasible with high impact. Community engagement and ownership is critical for sustainability of results. Work is ongoing to assess the impact on MDA on markers of resistance in the study site. Reductionof the paraside load in moderate-to-high transmission settings could pave the way for usccessful vaccine implementation. Above all there is need for a institution of a strong social component that encourages community ownership of such interventions. The non-falciparum infections to be increase a Pf prevalence decrease to very low levels in the population. There is need to investigated in to this. Molecular analysis are ongoing.

SE04.05

Enrichment of helminth mitochondrial genomes from faecal samples using hybridisation capture

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Introduction: Analysing soil-transmitted helminth populations and tracking their transmission remains challenging due to the technical and logistical difficulties of obtaining adult worm material. Whole genome sequencing (WGS) methods have been applied directly to faecal samples for phylogenetic and diagnostic marker recovery and have shown variable and inconsistent results, particularly favouring *Ascaris lumbricoides*-positive samples with extremely high egg load.

Materials and Methods: Our study leveraged a hybrid-capture approach with ~1000 probe/bait targets to enrich mitochondrial genome sequences of *A. lumbricoides* and *Trichuris trichiura* directly on faecal (n=23) and worm (n=1) specimens. These data were subsequently compared to results from WGS.

Results: The hybrid-capture approach achieved >1,000 fold-enrichment for *Ascaris* and *Trichuris* with up to 50% of the total reads being attributed to parasite targets. The minimum eggs per gram (epg) concentration required for each species to achieve > 50% mitochondrial genome coverage was also identified to be 336 epg for *Ascaris* and 48 epg for *Trichuris*. Variant calling analysis showed that 90% of the SNPs are shared between WGS and hybrid capture, with their shared frequencies achieving a very strong correlation ($R > 0.99$, $p < 0.001$).

Conclusion: The hybrid capture method described here provides a reliable and scalable way of generating Ascaris and Trichuris mitochondrial genome data directly from faecal samples for downstream population genetic and phylogenetic analysis. This technique overcomes a limiting factor of STH sequencing from faecal samples and paves the way for broader hybrid-capture-based genome-wide applications and future molecular epidemiology.

SE04.06

Incidence of intestinal parasitic infections and their associated risk factors among toddlers in rural and urban areas of Lahore, Pakistan

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Background: Intestinal Parasitic Infections (IPIs) are major health problems in many developing countries due to their poor socio-economic status. They are more severe in young children due to their weak immune system and malnutrition especially among toddlers. The aim of study was to determine the prevalence of Intestinal Parasitic Infections among toddlers.

Methodology: 150 stool samples were collected and diagnostic procedures were performed. Two techniques were applied to the stool samples i.e. Direct Wet Mount by use of Saline or Iodine and Formalin Ether Sedimentation technique to observe larvae, cysts, eggs, trophozoites and adults of different Intestinal Parasites.

Results: Overall prevalence of IPIs among toddlers was found 52.7%. The results had revealed that helminth infections (44.7%) were more common than protozoan infections (8%). *Ascaris lumbricoides* was most common intestinal parasite (18%) followed by *Entamoeba histolytica* (11.3 %), *Hook-worm* (6%), *Trichuris trichiura* (4.7%), *Hymenolepsis nana* (4.7%), *Giardia lamblia* (3.3%), *Taenia saginata* (2.7%), and *Enterobius vermicularis* (2%). Statistical analysis of data was done by applying Chi-sq. test and *P*- value < 0.05 was considered statistically significant.

Conclusion: Various factors like nail trimming, habit of eating washed fruits and vegetables, residential area, family income, hygienic conditions, habit of shoe-wearing, availability of safe drinking water and deworming were found statistically significant. The findings of the study suggest that these factors should be considered in control and prevention of IPIs. Also, It is vital to know the incidence and effects of infection based on the parasites in order to apply therapeutic mediations and control measurements.

Key words: Intestinal parasitic infections, toddlers, helminths, protozoa, deworming.

SE04.07

Treatment of cerebral malaria

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Cerebral malaria (CM) is a lethal immunopathological disease caused by *Plasmodium falciparum*. It is associated with mainly activation of immune responses that results in inflammation, endothelial damage, blood brain barrier (BBB) destruction, cerebral edema, seizure and coma.

Several methods were examined for eliminating the parasites or ameliorating CM immunopathology in a CM model, *P. berghei* ANKA infected mice: **a**. Injection of liposome-encapsulated glucocorticoid, β-methasone hemisuccinate decreased BBB destruction and CM related immunopathology. It was accumulated in the brain of mice and reduced CM pathology; cerebral inflammation, hemorrhages and edema. **b**. Rivoceranib, an anti-VEGFReceptor-2 that blocks vascular endothelial growth factor (VEGF) cascade prevented BBB damage and reduced CM as well. **c**. Artemisone (ART), an artemisinin derivative prevented severe disease. Few successful methods of its delivery were examined to overcome the need for repetitive injections: **c1**. release from subcutaneously injected gel, **c2**. use of a lipid-based ART microemulsion applied by gavage, intranasal or transdermal delivery. **c3**. Release from fibrous polymer nanocarriers (NFN). This goal was achieved by preparation of ART-loaded hydrophobic NFN and application in infusion system which unlocks the usage of hydrophobic drug eluting nanocarriers, that is a simple programmable system with reduced compliance complications.

Overall, combined immunomodulatory and anti-plasmodial therapy is suggested. The above-mentioned methods could be extended for treatment of other diseases.

We acknowledge the GIP-DFG for the financial support, Cipla for the donation of ART and Elevar Therapeutics for Rivoceranib.

SE05.02

Fishing for answers: Assessing temporal trends in freshwater fish parasite diversity in Ireland

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Parasites are often associated with the spread of disease and considered a danger to conservation efforts. However, while parasites do exert pressure on the fitness of their hosts in ecological and evolutionary terms, parasite-host dynamics are comparable to more accepted predator-prey relationships and require more attention. Parasites contribute hugely to the biodiversity and biomass of ecosystems they inhabit and can also be useful indicators of ecosystem health.

However, while parasites are clearly important and starting to get more recognition in conservation objectives, many are still undiscovered or undescribed. With growing concern for species extinctions, it is inevitable that parasites are also going extinct, many possibly without ever having been discovered. An ecosystem's richness, species dominance, prey availability, and even the existence of a food source for organisms that eat free-living parasite stages could all be negatively impacted by the loss of parasites.

Here we examine parasite diversity in freshwater fish in Lough Corrib in the west of Ireland. To date 138 fish comprising of eight species have been dissected. Eels appear to have the highest parasite intensity and species richness, followed by perch, brown trout, roach and pike. This work will develop an updated reference point for parasites of freshwater fish in Lough Corrib that can be compared to available historical data and international studies to monitor ongoing and future trends in parasite diversity and abundance.

SE05.03

Ecology of fear meets parasite ecology: How predation risk can affect parasite–host interactions and wildlife diseases

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Landscapes of fear can shape the dynamics of entire ecosystems. Prey species can exhibit physiological, behavioural, or morphological changes in response to a perceived risk of predation to avoid being preyed upon. These changes can indirectly influence other species by altering interactions (e.g., changes in feeding behaviour), leading to knock-on effects on the ecosystem, such as trophic cascades. While these indirect effects of fear have been well-studied for herbivore-plant and predator-prey interactions, much less is known about their impact on parasite-host interactions and wildlife diseases. In this talk, I introduce a conceptual framework to explain how the perceived risk of predation by host organisms can influence parasite-host interactions, affecting disease dynamics. I outline various pathways through which parasite-host interactions can be impacted, and provide examples from recent experimental research involving a marine predator-prey/host-parasite system (including crabs, molluscs, and trematodes). Finally, I discuss the broader implications of predation risk for parasite and host populations and identify gaps in current knowledge.

SE05.04

Three decades of anisakid nematode infections in harbour porpoises of the North- and Baltic Sea: Trends and health effects

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Harbour porpoises are infected by anisakid nematodes, which become mature in the stomach of marine mammals after completing a multi-stage life cycle including free-living larvae and crustacean intermediate and fish paratenic hosts. They cause gastritis and ulcerations in their definite hosts and anisakidosis in humans when accidentally ingested with undercooked fish. A stranding network collected harbour porpoises along the coasts of Germany since 1990 and investigated their health status. Necropsy reports and samples from ~1500 harbour porpoises were analysed for prevalence, intensity (none, mild, moderate, severe) and lesions associated with anisakids. A GLM with logistic regression analysed sex and age class differences as well as time trends from 2004-2023.

Anisakis simplex s.l. is the most common gastric nematode in porpoises in the North- and Baltic Sea and anisakid nematodes were prevalent from 1990 onwards. Prevalence of stomach nematodes increased slightly in the period from 2004-2023 in porpoises from the North- and Baltic Sea. Prevalence was significantly lower in the Baltic Sea (11%) compared to the North Sea (19%). No differences in infection patterns between male and female porpoises were found. In adult's prevalence (23%) was significantly higher than in juveniles (11%) while neonates were almost never infected. Intensity of infection did not change over time nor showed differences between North- and Baltic Sea, age and sex of porpoises. Pathological changes associated with stomach nematode infections were correlated to intensity of infection and included chronic ulcerative gastritis of varying severity and characteristics. The striking differences in infection patterns observed between North- and Baltic Sea and between age classes reflect ecosystem characteristics, diet as well as life history of porpoises. Environmental conditions may drive prey species composition and the viability of larval stages and intermediate hosts. The epidemiology of this generalist, trophically transmitted nematode with zoonotic implications needs to be monitored closely.

SE05.05

Having IMPACT on monitoring parasite diversity – First experimental results evaluating eDNA as an integrative tool for studying fish parasites

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Global parasite biodiversity is facing an extinction crisis, with up to 30% of species in each major parasite group predicted to be extinct by 2070. Although parasites can serve as integrative biological indicators of ecosystem responses to global change, parasites remain the most neglected components of biodiversity monitoring and have only recently begun to be considered in conservation discussions. Monitoring of parasite biodiversity is time-consuming, labor-intensive, require specialised expertise and remains ethically debated, as it involves the sacrifice of many hosts. 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 allowing them to be detected in environmental matrices without sampling the hosts. Given that eDNA is increasingly used in aquatic biodiversity monitoring worldwide, it offers a great opportunity for obtaining and integrating parasite diversity data from existing monitoring programs. Recent literature reviews show that eDNA has proven useful in the detection of single parasite species. However, there is no consensus on the optimal sampling conditions (i.e., filter pore size and water volume) for detecting certain parasite groups. Moreover, there are very few studies targeting multiple parasite species within and across defined parasite groups at once. Here, we will present the first results from an indoor experiment conducted within the framework of the Biodiversa+ project IMPACT where multiple filter types were tested for the simultaneous detection of multiple fish parasite groups using eDNA.

SE06.01

Parasitic nematode infection factors regulate the innate immune signaling and function in Drosophila

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Entomopathogenic nematodes form mutualistic complexes with Gram-negative bacteria. These parasites are commonly used for the biological control of insect pests and disease vectors, and they have also emerged as excellent research tools for studying the molecular basis of nematode pathogenicity and the features that allow them to persist and multiply within the host. The goal of this work was to leverage the Drosophila infection model to identify novel parasitic nematode effector molecules and explore their impact on the regulation of host anti-nematode innate immune signaling and function. For this, we generated axenic nematodes devoid of their symbiotic bacteria and used a combination of gene expression analyses, expression of candidate virulence factor genes in insect cells, injection of *Drosophila*adults with recombinant proteins, and phenotypic characterization of the injected flies. We have found that the excreted-secreted (ES) products of the parasitic nematode Heterorhabditis bacteriophora can suppress the activity of the Immune deficiency (Imd) pathway in Drosophila. Also, we have shown that a nematode serine carboxypeptidase can activate the Activin branch of the TGF-beta signaling pathway in Drosophila wild-type adults and the Imd pathway in Activin-deficient flies. In the same manner, we have further identified a putative uridine diphosphate - glycosyltransferase, an invertebrate-type lysozyme, and a serine carboxypeptidase and determined theirimmunomodulatory capacities in *Drosophila*. These findings indicate the array of infection factors that entomopathogenic nematodes (in the absence of their symbiotic bacteria) can utilize to undermine the insect defense and eliminate their host. Understanding the molecular mechanisms of nematode infection and host anti-nematode immune responses in Drosophila will be used not only for the development of novel means for parasitic nematode control, but also to make inferences about the emergence of parasitism among Clade V nematodes.

SE06.02

Bovine PMN responses to extracellular vesicles released by *Besnoitia besnoiti* tachyzoites and *B. besnoiti*-infected host cells

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Introduction: Bovine besnoitiosis is a re-emerging cattle disease caused by the apicomplexan parasite *Besnoitia besnoiti,* which severely affects individual animal welfare and profitability in cattle industry. We recently showed that *B. besnoiti* tachyzoite exposure to bovine polymorphonuclear neutrophils (PMN) effectively triggers neutrophil extracellular trap (NET) formation, leading to parasite immobilization hampering host cell infection. So far, the triggers of this defense mechanism remain unclear. Emerging evidence indicates that extracellular vesicles (EVs) modulate PMN effector functions, such as ROS production or NET formation.

Objetive: Study whether exposure of bovine PMN to EVs from different cellular sources affects classical PMN effector functions and cytokine/chemokine secretion.

Material and methods: EVs were isolated from *B. besnoiti*-infected and non-infected host cells (bovine umbilical vein endothelial cells, BUVEC), from tachyzoite-exposed bovine PMN and from *B. besnoiti* tachyzoites. EV concentration and size was determined by Nano-Flow cytometry and EV nature was confirmed by both classical EV markers (CD9 and CD81) and transmission electron microscopy (TEM).

Results: PMN stimulation with both BUVEC- and tachyzoite-derived EVs significantly induced extracellular DNA release whilst EVs from PMN failed to affect NET formation. BUVEC and tachyzoite EV-driven NET formation was confirmed microscopically by the presence of DNA decorated with neutrophil elastase (NE) and histones in typical NET structures. Moreover, confocal microscopy revealed EVs to be internalized by bovine PMN. Referring to PMN activation, EVs from the different cellular sources all failed to affect glycolytic or oxidative responses of bovine PMN as detected by Seahorse®-based analytics and luminol-based chemoluminescence, thereby denying any role of NADPH oxidase (NOX) activity in EV-driven NET formation. Finally, exposure to *B. besnoiti*-infected BUVEC-derived EVs induced IL-1β and IL-6 release, but failed to drive CXCL8 release of bovine PMN.

Conclusion: We overall demonstrated that EVs of selected cellular origin owned the capacity to trigger NOX-independent NET formation, were incorporated by PMN and selectively fostered IL-1β and IL-6 release.

SE06.03

The C-type lectin receptor MINCLE interferes with eosinophil function and protective intestinal immunity in *Strongyloides ratti*-infected mice

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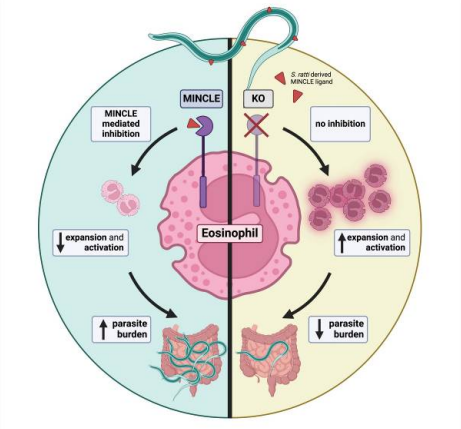
Strongyloides ratti is a helminth parasite that displays tissue-migrating and intestinal life stages. Myeloid C-type lectin receptors (CLRs) are pattern recognition receptors that recognize pathogen-derived ligands and initiate immune responses. To date, the role of CLRs in *S. ratti* infection has not been investigated. Here, we show that *S. ratti*-derived ligands are recognized by the CLR Macrophage inducible Ca2+-dependent lectin receptor (MINCLE). While MINCLE-deficiency did not affect initiation of a protective anti-*S. ratti* type 2 immunity, MINCLE-deficient mice had a transient advantage in intestinal immunity. Unravelling the underlying mechanism, we show that next to macrophages, dendritic cells and neutrophils a fraction of eosinophils express MINCLE and expand during *S. ratti* infection. MINCLE-deficient eosinophils exhibited a more active phenotype and prolonged expansion *in vivo* and displayed increased capacity to reduce *S. ratti* motility and produce reactive oxygen species *in vitro*, compared to wild-type (WT) eosinophils. Depletion of eosinophils in *S. ratti*-infected mice after the tissue-migration phase elevated intestinal worm burden in MINCLE-deficient mice to the WT level. Thus, our findings establish a central contribution of eosinophils to parasite ejection from the intestine and suggest that *S. ratti*-triggered signalling via MINCLE interferes with eosinophil mediated ejection of *S. ratti* from the intestine.

Fig. 1



Graphic summary On the left: wild type situation: *S. ratti*-derived ligands engage MINCLE on eosinophils. Expansion and activation of eosinophils is muted and establishment of intestinal *S. ratti* infection promoted. On the right: MINCLE knock out (KO) mice display increased expansion and activation of eosinophils, ejection of *S. ratti* parasites from the intestine is accelerated

SE06.04

Activation of natural killer cells and the development of a memory-like phenotype after helminth infection

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Helminths are the most common infectious agents worldwide, with more than 400 million people suffering from lymphatic filariasis or schistosomiasis. Type 2 inflammation is a hallmark of nematode tissue infection and is implicated both in immunopathology and eosinophil-dependent immunity. Type-2 innate lymphoid cells (ILC2) are usually associated with the initiation of type 2 inflammation in helminth infection.

However, we noticed that they surprisingly failed to expand following *Brugia malayi* experimental peritoneal filarial infections. Conversely natural killer (NK) cells, usually associated with a Type 1 immune response, rapidly expanded, and represented over 90% of the ILC population in the first week of infection.

Interestingly, specific ablation or depletion of the NK cell compartment in RAG2 or RAG2gc immunodeficient mice using anti-NKp46 or asialo GM1 antibody injections led to increased susceptibility to chronic adult *B. malayi* infection and impaired granulocyte recruitment to the site of infection.

We have also demonstrated that in RAG2 deficient mice, drug clearance of a primary *B. malayi* infection followed by challenge infection led to resistance against early larval *B. malayi* establishment. This innate resistance was associated with bolstered NK and eosinophils whereby NK cells expressed markers of memory-like/enhanced activation (increased expression of IFNg and Ly6C, see Figure 1). Our data thus promotes a novel functional role for NK cells in immunoprotection against experimental primary and secondary filarial infection which can proceed in the absence of adaptive immune regulation.

Interestingly, we also observed similar NK cell expansions following murine *Schistosoma mansoni* helminth infections over a 15 weeks" time-course longitudinal experiment. NK cell activation data is currently being analysed and will inform on potential wider scope of NK cell functional fates than initially thought and on their role to play in innate-memory protection from *S. mansoni* infection.

Fig. 1

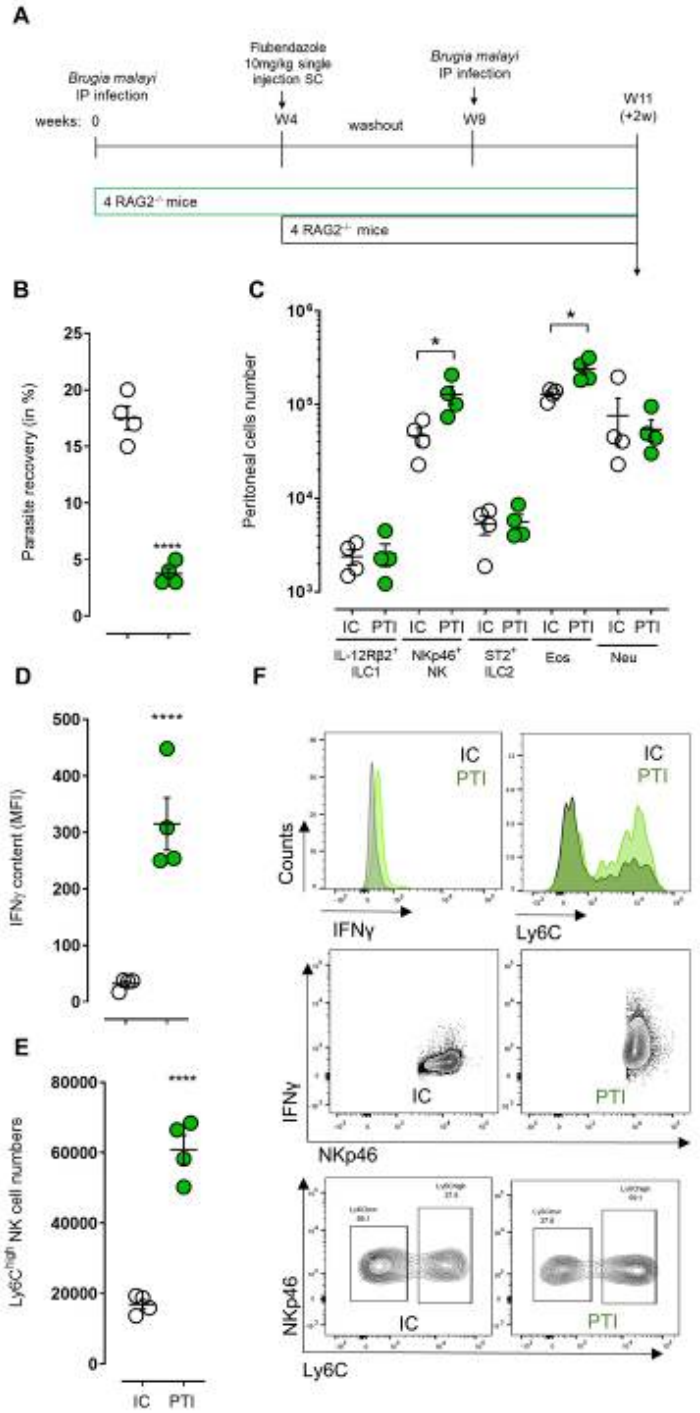

Figure 1: NKp46⁺ NK cells display signs of enhanced/memory-like activation and boost anti-filarial immune response in challenge after immune-priming. (A) Schematic representation of the study design where *B. malayi* infected RAG2$^{-/-}$ mice were treated with flubendazole at 10mg/kg 4 weeks post-infection to clear them out of remaining worms before being challenged again at week 9 with *B. malayi* parasites and a readout 2 weeks post-challenge. (B) Parasite recovery in infected control mice (IC, clear) or primed treated infected (PTI, green) mice 2 weeks post-challenge. (C) Innate lymphoid cells (IL-12Rβ2⁺ ILC1, NKp46⁺ NK, ST2⁺ ILC2) and granulocytes (eosinophils – Eos and neutrophils – Neu) numbers in the peritoneal cavity of infected control (IC, clear) or primed treated infected (PTI, green) mice at 2 weeks post-challenge. (D) IFNγ content expressed as MFI in peritoneal NKp46⁺ NK cells from IC (clear) or PTI (green) mice 2 weeks post-challenge. (E) Ly6C^{high} NKp46⁺ NK cell numbers in the peritoneal cavity of IC (clear) or PTI (green) mice at readout. (F) Representative histograms and flow plots for IFN and Ly6C expression or cell counts on either pre-gated NK cells or depending on NKp46 expression in IC and PTI mice at readout. Unpaired T-tests, n=4, single experiment. Significance is given as *: p<0.05 and ****: p<0.0001.

SE06.05

Redundant and nonredundant functions of group 2 innate lymphoid cells during S*trongyloides ratti* infection in mice

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Introduction: *Strongyloides ratti* is a rodent-specific parasitic nematode that displays tissue-migrating and intestinal life stages. Infections are controlled in the context of a type 2 immune response. While innate effector cells play important roles in reducing worm burden during the first week post-infection (p.i.), infection termination depends on the adaptive immune system. After a cleared infection, mice become semi-resistant to reinfections. Previous studies suggest that group 2 innate lymphoid cells (ILC2) contribute to expelling *S. ratti* from the intestine via the secretion of IL-9, which triggers mucosal mast cell activation.

Objective: This study aims to decipher the function of ILC2 during the infection with *S. ratti*.

Material & Methods: We use a novel mouse model lacking ILC2 in otherwise immunocompetent mice (Nmur1iCre-eGFP Id2fl/fl) to analyze the role of ILC2 in the anti-*S. ratti* immune response.

Results: ILC2 deficiency increased tissue-migrating larvae day 2 p.i., intestinal parasite burden day 6 p.i., and larval shedding in feces until 3 weeks p.i. Along this line, ILC2-deficient mice exhibited reduced goblet and tuft cell hyperplasia, lower eosinophil levels, and decreased mast cell activation. However, infection clearance occurred with wild-type (WT) kinetics within one month. Notably, no defect in type 2 cytokine production, Th2 polarization, or antibody production was detected in ILC2 KO mice. Experiments testing the role of ILC2 in establishing protective memory responses showed a comparable reduction of intestinal parasite burden in ILC2-deficient and WT mice during a second infection compared to a first infection. However, killing of tissue-migrating larvae day 2 p.i. infection was less efficient in ILC2 KO mice compared to WT littermates.

Conclusions: In summary, these results suggest that ILC2 plays a non-redundant role in controlling the initial parasite burden but are redundant for Th2 cell polarization and clearance of the infection. The role of additional ILC2-derived effector cytokines is under current investigation.

SE07.03

Digitalization of clinical specimens by microscopic scanners in combination with Artificial Intelligence (AI) provides new tools to improve diagnostics in high-income countries and for virtual training in low-income countries

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Introduction: Parasitic diseases pose major global health challenges, especially in tropical regions. The primary method for diagnosing parasitic infections is microscopic examination. Although alternatives like nucleic acid amplification methods (e.g., real-time PCR) exist, microscopy remains widely used due to its versatility and global accessibility. However, this method is labour-intensive, requires highly trained technicians, and is prone to errors with false-negative rates exceeding 10%. Hence, improvement of microscopic detection of parasites in routine patient care is needed.

Objectives: To improve the accuracy and efficiency of parasite detection Erasmus MC and Leven Vision, a Dutch start-up software company, joined forces to develop an AI-driven automated microscopic detection system. By a commercially available microscope scanner digital images of clinical specimens can be produced, that can subsequently be interpreted by machine learning algorithms. These algorithms facilitate parasite detection by selecting those specimen areas suspected of parasite presence with parasite species prediction and probability score. In high-income countries, this system can be used for automated examination of clinical specimens in patient care. In low-income countries, the obtained digital images can be used for educational purposes by training technicians with the virtual microscopy tools developed by the EQALM working group for virtual microscopy.

Materials & methods: The developed automated microscopic detection system was trained for 15 gastro-intestinal parasites and its performance was compared to manual microscopic examination by expert technicians of 4 distinct clinical expertise centres in the Netherlands. For educational purposes, virtual microscopy software has been developed, such that feedback can be given on observed morphological characteristics and evaluated in an external quality assessment set-up. The added value of the virtual training tool will be evaluated in low-income countries in the eWHORM programme.

Results: Based on the examination of 45 blinded stool samples the automated system at a confidence threshold of 0.8, had a high sensitivity (99%) and a specificity of 82% that could be increased to 100% by expert confirmation of the selected areas. The results of the comparison with expert manual examination will be presented at the conference, as well as the results for reporting morphological characteristics in clinical specimens by virtual microscopy.

Conclusion: A robust automated AI-driven microscopic detection system has been developed for the detection of gastro-intestinal parasites and virtual microscopy is a valuable tool for training technicians in low-income countries.

SE07.04

Development and validation of an AI based platform for the identification of novel chemical compounds as anthelmintic starting points

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Schistosoma mansoni is a major contributing species to the infectious human disease schistosomiasis, which affects more than 240 million people worldwide. A single chemotherapeutic agent, praziquantel (PZQ), is currently used for the control of this disease. However, PZQ is ineffective against juvenile worms, necessitating repeated treatment and raising concerns around the development of resistance. New drugs are urgently needed for the sustainable control of this disease and strategies to objectively assess compound suitability utilising medium- and high-throughput platforms are essential to identify new chemical starting points.

We have developed and trained a machine-learning-based pipeline using data from 12,126 whole-organism, phenotypic screens (11,167 in-house, 959 extracted from PubChem) to predict compounds with plausible anti-schistosomal properties. This pipeline was used to virtually screen a compound library (8513 small molecules) consisting of phase I, phase II and phase III drugs curated from ChEMBL for predicted activity. A consensus prediction from four separate voting classifiers yielded 745 potentially active anti-schistosomal compounds from this collection. Similarity analysis of compound structures using SkelSpheres descriptors, removal of previously screened anthelmintic compounds and sourcing compounds available for purchase led to us pursuing 19 hits for experimental testing.

These 19 compounds were tested experimentally in our whole-organism phenotypic assays; 12 compounds were found to be active against schistosomula stage parasites (at 10 uM) and were subsequently tested against adult worms. Out of these 12 compounds, 8 were also active against adult worms at 20 uM (42% hit rate overall) and several were found to have EC_{50}s in the high nanomolar/low micromolar range. Target validation is currently underway and further characterisation of these molecules is on-going. When combined with existing high-throughput phenotyping assays and medicinal chemistry support, artificial intelligence (AI) bridges critical gaps in funding and resource availability by offering cost-effective, scalable and rapid solutions to advance drug discovery for schistosomiasis, ultimately contributing to improved global health outcomes for underserved populations.

SE08.02

Occurrence of *Klossiella equi* in European equids: A neglected parasite of horses?

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Introduction: *Klossiella equi* [1] is a protozoan parasite, infecting equid kidneys via oral ingestion. Within the epithelial cells of Henle's loop, this monoxenous parasite undergoes gamete formation, producing fully sporulated, polysporocystic oocysts that are eventually excreted in urine. Although infections are often asymptomatic, renal complications such as haematuria and nephrosis may occur in immunocompromised animals [2,3].

Klossiella equi has been reported in Iran [4], Afghanistan [5] or Canada [2], but its presence in Europe remains underexplored. This study builds on the findings of Léveillé et al. (2019) by examining the occurrence of *K. equi* in selected European regions, focusing on its detection in kidney tissues and urine.

Materials & methods: In 2023–2024, kidney and urine samples were collected via partnerships with institutions in Spain, Italy, Romania, Netherlands and the Czech Republic, with 55 paired kidney and urine samples obtained from the same animals. 181 kidney and 85 urine samples have been analyzed, with Dutch samples still in progress.

DNA was extracted using the DNeasy Blood & Tissue Kit (QIAGEN). The presence of *K. equi* was tested by PCR targeting 575 bp of mitochondrial DNA outside gene coding regions using primers Api LSUG_F and Haem_RNA_14_R [2].

Results: *Klossiella equi* was detected in 30 % of kidney samples. Regional prevalence was highest in Spain (61 %), followed by Italy (43 %) and Romania (13 %). Urine positivity rates were 12 % in the Czech Republic and 57 % in Spain. Among 55 paired samples, the positivity rate was 76 %.

Conclusion: This study highlights *K. equi*'s presence across all examined European regions, suggesting widespread distribution throughout the continent. These findings underscore the need for further research to assess its pathogenic potential, genetic diversity and role in equine health and epidemiology.

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SE08.03

Unravelling the epidemiology of ticks and tick-borne infections in Benue state, Nigeria

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Introduction and Objective: Ticks infesting cattle pose a persistent challenge to Nigeria's livestock sector, primarily as vectors for pathogens and parasites including bacteria such as *Anaplasma* and *Rickettsia* species and protozoa such as *Babesia* and *Theileria* species. The epidemiology of these infections is complex, being influenced by transhumance-based farming practices, extensive trade and movement of cattle, often between countries and climate change. This project aims to quantify the epidemiology of tick-borne infections in Benue State and identify determinants of this epidemiology as a basis for evidence-based control interventions.

Materials and methods: Cattle present in livestock markets across Benue State were surveyed for ticks in early 2022 and summer 2023. Cattle handlers/owners completed a questionnaire to ascertain the provenance of their animals and details of recent movements of them. Ticks were identified by reference to taxonomic keys and DNA extracts prepared from them were used as templates in diagnostic PCR-based assays for *Anaplasma*, *Rickettsia, Babesia* and *Theileria* species.

Results and Discussion: a total of 1316 ticks were collected from 366 cattle. Ticks were identified as *Amblyomma variegatum* (the most prevalent), *Hyalomma impeltatum*, *R. (Boophilus) microplus*, and *R. (B) geigyi*. These ticks were found to harbour *Thieleria mutans, Theileria velifera, Babesia caballi, Anaplasma marginale*, *Ehrlichia ruminantium*, *Coxiella burnetti, Rickettsia africae* and *Rickettsia aeschlimannii*. Collation of these data with the information gained from questionnaires revealed some correlation between the provenance of cattle and their associated tick fauna and thus the identity and prevalence of pathogen/parasite infections.

Conclusion: Benue State is a hub for livestock trade rather than a centre of cattle rearing, so most of the cattle in the State are introduced from elsewhere. Our surveys show the diversity of places from where cattle are introduced, reflecting extensive national and transboundary trade. The provenance of cattle appears to influence their tick fauna and the diversity of tick-borne pathogens/parasites, and thus we propose that the livestock trade in Nigeria and beyond is an important determinant of tick and tick-borne pathogen/parasite epidemiology in the country. Of specific concern is the abundance of *Hyalomma* species encountered as climate change is likely facilitating their establishment further south which may lead to diseases previously restricted to northern Nigeria becoming more widespread across the country.

SE08.04

Comparative proteomic analysis of metronidazole-sensitive and resistant *Trichomonas vaginalis* suggests a novel mode of metronidazole action and resistance

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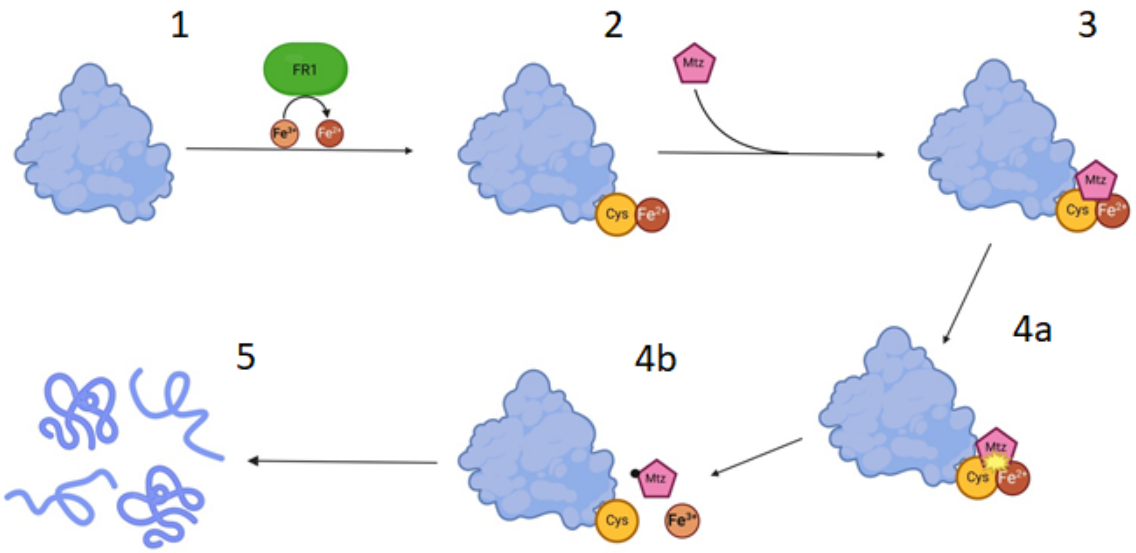
The single cell parasite Trichomonas vaginalis occurs worldwide and infects the urogenital tract of humans, especially in women. Because no vaccine is available the management of the disease is limited to administration of metronidazole and related drugs (5-nitroimidazoles). 5-nitroimidazoles are special because they are only toxic to anaerobic and microaerophilic microbes such as T. vaginalis rendering them safe for use in humans and animals. Resistance to metronidazole has remained comparably rare, but it occurs much more often in some parts of the world complicating treatment considerably.

In this study we compared the total protein expression profiles of metronidazole-susceptible and –resistant strains to identify proteins specifically associated with resistance. Surprisingly, the number of proteins found was very low, and when comparing the protein expression profiles of resistant clinical isolates and strains with resistance induced in the laboratory we only found one single protein to be downregulated in all data sets. We also found that this protein, flavin reductase 1 (FR1), reduces iron in the cell. Reduced iron, in turn, binds to proteins in the cytoplasm forming complexes which can react with metronidazole and render it toxic. Consequently, we found that in resistant T. vaginalis, FR1 is not expressed thereby keeping proteins safe from metronidazole.

This project was supported by the Austrian Science Fund [Project P35545]

Fig. 1



SE08.05

Development of tools to assess the risk of *Leishmania* transmission – I. selection of recombinant antigens for standardised ELISA assay

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Leishmaniases are a group of medically and veterinary important diseases caused by protozoan parasites of the genus *Leishmania* (Kinetoplastida) and transmitted by blood-feeding sand flies (Diptera: Phlebotominae). To assess the risk of *Leishmania* transmission, host exposure to sand flies can be measured through the detection of host antibodies to vector salivary proteins. Anti-sand fly saliva antibodies are elicited by repeated exposure of the mammalian host to sand fly salivary proteins deposited into the host skin during blood feeding. These antibodies are species-specific and correlate with the intensity of host exposure to sand fly bites, hence serving as a marker of exposure and consequently as a risk marker for *Leishmania* transmission. Anti-sand fly saliva antibodies are typically measured using sand fly salivary gland homogenate (SGH) prepared from glands dissected from sand fly females. However, the development of recombinant salivary proteins offers more standardised approach, independent of having colonised sand flies. The aim of our study is to develop a standardized ELISA assay based on recombinant sand fly salivary antigens. We focus on three Old World sand fly vectors: *Phlebotomus perniciosus* and *P. tobbi* as vectors of *Leishmania infantum*, and *P. papatasi* as a vector of *L. major*. In previous studies, we developed an ELISA assay based on *P. perniciosus* recombinant salivary protein 03B (rSP03B, a yellow-related protein). For *P. papatasi* and *P. tobbi,* we are developing new recombinant antigens using sera from dogs bitten by these species collected in endemic areas in Turkey. Sera were screened using SGH-ELISA and positive sera were analysed via immunoblot and used to identify antigenic proteins in immunoprecipitation assay followed by proteomic analysis. Four candidate salivary proteins from each species were expressed in *Escherichia coli* and tested as risk markers of sand fly exposure. Among these, *P. tobbi* rSP38 (a yellow-related protein) and *P. papatasi* rSP36 (an apyrase) were selected as the most reliable antigens to replace SGH in serological assays. The three recombinant antigen-based ELISA assays have been optimised and are now available to measure exposure to vector bites in dogs, complementing other surveillance and leishmaniasis control tools. Within the CLIMOS project (https://climos-project.eu/), they will be used to monitor exposure in sentinel dog populations in Portugal, Spain, and Italy, serving as an early warning surveillance tool for circulating *Leishmania* infection and to evaluate the efficacy of protective measures.

Funding: The CLIMOS consortium is co-funded by the European Commission grant 101057690 and UKRI grants 10038150 and 10039289. The six Horizon Europe projects, BlueAdapt, CATALYSE, CLIMOS, HIGH Horizons, IDAlert, and TRIGGER, form the Climate Change and Health Cluster.

SE09.01

Treatment of Malaria: It is never too late

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Malaria is spread exclusively through bites of infected Anopheles mosquitoes. The mosquito bite introduces the parasites from the mosquito's saliva into a person's blood where they invade the red blood cells. Most deaths are caused by P. falciparum, whereas P. vivax, P. ovale, and P. malariae, generally cause a milder form of malaria. Lack of reliable vaccination with 40 to 70 % protection and drug resistance against therapeutics such as Artemisinin, Mefloquine, Lumefantrine, Quinine or Chloroquine necessitates new therapeutic approaches such as monoclonal antibodies which prevent the penetration of Plasmodium species to red blood cells before they travel to the liver where they mature and reproduce.

Ketantin binds to the CD147 receptor to the epitope 5H8 on the red blood cell and prevent binding of RAP 2 Ligand of Plasmodium to CD147 epitope 5H8 of red blood cells and thereby preventing penetration of Plasmodium into red blood cells. In January 2020 FDA has issued a Fast Track certification, IND 143872 and Phase I is completed in USA, now Phase II started in April 2024 in Africa in Kenya, Rwanda, Gabon and Ghana in more than 8 centers. The anticipated TPP is ≥90% currative efficavy after 1month, with 1 month treatment and 6 months follow up. Treatment application is s.c. every 1.5 months. The dosage form for children is 5 mg/0,5 ml and 30mg/0,5 ml for adults in prefilled syringes. Approval is expected in 2029. We are looking for strategic partners for marketing of Ketantin for treatment of Malaria in Africa.

Ketantin is a IgG2 humanized monoclonal antibody, expressed in CHO DG44 cells and produced in suspensión culture, purification is performed by Protein A affinity chromatography, followed by AIEX and CIEX chromatography. The manufacturing process is robust. The DSP is validated for adventitious viral contaminant removal. The purity is 99,99%. Ketantin is a safe, specific and potent biologic of high quality.

Ketantin is available for global licensing.

Keyword: Monoclonal IgG2 Antibodies, Malaria, High quality Antibody Therapeutic, Licensing Opportunity.

SE09.02

Exploration of emodepside combination chemotherapy for the treatment of soil-transmitted helminth infections

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Soil-transmitted helminthiasis affects around one quarter of the worldwide population, primarily in tropical and subtropical regions with unsatisfactory sanitary conditions. Control relies on mass drug administration, where single doses of anthelmintics are administered at regular intervals to an entire population. Resistance development, caused by high drug pressure, is an ongoing concern. Hence, new treatments are needed and combination therapies might be the way forward. We explored emodepside as a partner in combination therapy and characterized the drug interaction effect with key anthelmintics *in vitro* and *in vivo* in different rodent models of soil-transmitted helminths. On the larval stage of different hookworms, the combination of emodepside and ivermectin revealed synergistic effects as measured by its fraction inhibition concentration (FIC <0.7) across all tested ratios. On adult *H. polygyrus* worms, combining emodepside with low concentrations of macrocyclic lactones and benzimidazoles, we observed synergistic effects which decreased the EC50 of emodepside to <0.04 µM, corresponding to a 2.5-fold decrease. Similar results were observed against adult *Trichuris muris* worms, with both the combination between emodepside and albendazole (FIC <0.7) and the combination of emodepside and ivermectin (FIC <0.9) revealing synergistic effects across all tested ratios. The combinations of emodepside with ivermectin or albendazole were chosen for *in vivo* testing in the *H. polygyrus* and *T. muris* mice models and results are forthcoming. These results will contribute towards the optimization and improvement of new treatment options for soil-transmitted helminthiasis.

SE09.03

Slo-1 K and TRP-2 are synergistic drug targets of emodepside and diethylcarbamazine in filaria

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Objectives: Emodepside has been found to have a selective anthelmintic effect on soil-transmitted helminths as well as filaria without undue toxic effects on their mammalian hosts. We are seeking to find drug combinations that enhance the activity of emodepside.

Materials and Methods: We have used patch-clamp techniques on dissected *Brugia malayi* muscle*, Xenopus* oocytes expression with voltage-clamp and patch-clamp on channels expressed in HEK293 cells to study the effects of emodepside, diethylcarbamazine and other compounds on muscle ion-channels.

Results: Slo-1K channels are calcium-activated big conductance potassium channels that are found in different tissues of vertebrates and invertebrates including filarial parasites. We have found that emodepside activates filarial Slo-1K channels and that the classic anthelmintic diethylcarbamazine has a synergistic effect on the action of emodepside by activating TRP-2 channels and increasing cytosolic calcium. We have modeled the binding of emodepside to Slo-1 K channels using molecular docking. Recently our studies have focused on BK channel activators to increase the effects of emodepside and have found that GoSlo-SR-5-69 is a potent positive allosteric modulator of emodepside on *Onchocerca volvulus* and *Brugia malayi* Slo-1 K expressed channels. We have also studied the single-channel effects of emodepside on *Onchocerca volvulus* Slo-1 K channels expressed in HEK293 cells. We find that emodepside increases the opening frequency, the mean single channel conductance and the probability of the channel being open and this can explain the positive allosteric effects of emodepside. TRP-2 channels in *Brugia* muscle cells and expressed in HEK293 cells are activated by diethylcarbamazine and arachidonic acid and calcium imaging reveals that their opening leads to an increase in cytosolic calcium.

Conclusions: The effect of diethylcarbamazine on TRP-2 channels and the increase in cytosolic calcium can explain the synergistic effect on emodepside, Fig. 1. The action of selective Slo-1 K channel activators may be another approach to increase the spectrum of activity of emodepside and delay the onset of resistance. Modeling the docking of emodepside in the Slo-1 K channel found favorable docking in the S6 site of the channel. Our studies are continuing as we are seeking to characterize the drug targets of emodepside and diethylcarbamazine to find synergistic drug combinations.

Acknowledgment: NIH National Institute of Allergy and Infectious Diseases grants R01AI047194 and R01AI155413 and the EA Benbrook Endowment to RJM for financial support.

Fig. 1

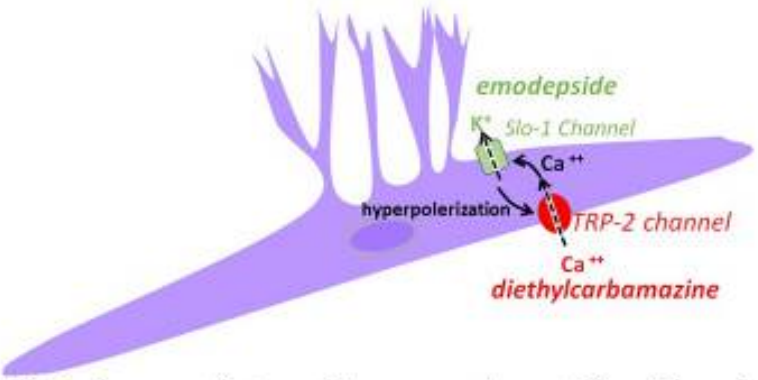

Fig. 1. Summary diagram of the proposed synergistic actions of diethylcarbamazine (DEC) and emodepside. DEC activates TRP-2 channels and increases cytosolic Ca^{2+} that activates the Ca^{2+}-activated Slo-1 K channels. Emodepside also activates Slo-1 K channels and hyperpolarizes the muscle cell that in-turn increases the driving force and entry of Ca^{2+}. Emodepside and DEC have a synergistic interaction.

SE09.04

Bridging *Leishmania* cell biology and drug discovery: Establishment of *in situ* assays for intracellular trafficking as druggable target

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Antileishmanial drug discovery has witnessed significant progress over the last two decades empowered by advanced functional genomic technologies. However, the high attrition rate of compounds in clinical development emphasizes the need to explore new target-validated antileishmanial leads. To meet the " Know your Target" principle of drug discovery, we engineered *Leishmania* lines to investigate the mode-of-action and activity of new antileishmanial compounds. In *Leishmania*, like any eukaryotic cell, spatiotemporal molecule trafficking between subcellular organelles is maintained by endosomes, while active shuffling of molecules between the cytoplasm and nucleus relies on dynamic nucleocytoplasmic transport.

First, by elaborate mode-of-action studies of antileishmanial aminopyrazoles, we discovered that disrupting the assembly of endosomes is an exploitable and druggable pathway. MUT-SEQ and CRISPR-Cas9 gene editing has independently confirmed an association between 10-30-fold aminopyrazole resistance and multiple independent heterozygous mutations in *LINF_180011100*, encoding an FYVE domain-containing protein. Next, genetic fusion with an N-terminal green fluorescent protein (GFP) tag demonstrated that the protein primarily localizes in cytoplasmic/endocytic vesicles and revealed an impact of treatment with aminopyrazoles. Proteomic analysis of co-immunoprecipitates with the GFP-tagged LINF_180011100, confirmed the interaction with recycling endosomes that are associated with the ribosomal translation machinery and mitochondria.

Our second initiative was to visualize and target Nuclear Protein Import (NPI). NPI relies on interactions between cargo molecules carrying a nuclear localization signal (NLS) and their specific transport receptors, regulated in turn by a Ran GTPase cycle. Using genetic engineering, the mCherry fluorescent protein with a C-terminal NLS, was introduced into the *L. infantum* genome. Confocal fluorescence microscopy demonstrated parasites with a nuclear localisation of mCherry. As a specific inhibitor of importin-β, importazole exhibited the anticipated dose-dependent inhibition of NPI as well as a broad antiprotozoal activity. Next, assay specificity was demonstrated by evaluating the effect of lead compounds of the Drugs for Neglected Diseases initiative with different modes-of-action, revealing antileishmanial activity independent of NPI impairment.

Collectively, our transgenic *Leishmania* lines can provide an *in situ* read-out of endosomal assembly or nuclear protein transport. In addition to offering opportunities for mechanistically informed drug discovery, this also provides an innovative research tool for understanding the *Leishmania* cell biology.

SE09.05

Pathway towards chemical validation of compounds targeting genetically validated *myo*-inositol metabolism in *T. cruzi*

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myo-Inositol is one of the nine naturally occurring inositol stereoisomers. It is ubiquitous amongst eukaryotes and acts as an essential metabolite with roles in signal transduction and membrane formation. In the protozoan parasite *Trypanosoma cruzi*—the causative agent of Chagas" disease—*myo*-inositol acts as a precursor to phosphatidylinositol (PI), an essential membrane lipid component. PI in turn is then required for formation of inositol phosphorylceramide (IPC), various phosphoinositides, and glycosylphosphatidylinositol (GPI)-anchored mucin-type molecules and glycoproteins. Both of these GPI-anchored molecules coat the parasite"s cell-surface allowing the parasite to participate in multiple essential steps in parasite-host interactions.

In *T. cruzi, myo*-inositol is proposed to be both *de novo* synthesised and scavenged from the environment. *myo*-Inositol *de novo* synthesis begins with the isomerisation of D-glucose-6-phosphate to D-*myo*-inositol-3-phosphate in a NAD^+ dependent manner, which is catalysed by inositol-3-phosphate synthase (*Tc*INO1). *TcINO1* has been genetically validated as an essential *T. cruzi* gene. Biochemical *Tc*INO1 studies have shown *Tc*INO1 demonstrates Michaelis Menten kinetics with its natural substrate and differential scanning fluorimetry (DSF) studies show *Tc*INO1 requires both its natural substrate and cofactor to be stabilised.

DSF was used to screen heterocyclic fragment libraries, such as the Maybridge Rule of Three and Otava Chemicals libraries, for compounds that interacted with *Tc*INO1. The top stabilising compounds that interacted with *Tc*INO1 were from the Otava Chemicals library and these were tested *in vivo* in wild type *T. cruzi*, with several demonstrating $EC_{50} \leq 50$ µM. The best compound had an $EC_{50} = 6.11 \pm 0.31$ µM, which is on par with Nifurtimox—one of the two currently approved anti-Chagas" disease therapies. The unoptimised lead compound also demonstrated a selectivity index of 81.83*,* which is better than Nifurtimox. Importantly the most potent three compounds also showed altered EC_{50}s in *TcINO1* genetically altered *T. cruzi* cell lines. These compounds also affected enzyme activity of recombinantly purified *Tc*INO1 enzyme and initially suggest these compounds might be possible positive homotropic cooperative compounds with *Tc*INO1.

SE09.06

Searching for novel anti-malarials using target based drug discovery

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There is a pressing need for new, affordable and safe anti-malarial drugs, with novel mode-of-actions. Current treatments, such as artemisinin-based combination therapies (ACTs), have been effective against the *Plasmodium* parasite. However, resistance to these established treatments is increasingly prevalent. To address this, the development of anti-malarials with novel mode-of-actions is crucial.

A crucial step in designing new antimalarial drugs is to uncover its target within the *Plasmodium* parasite. In the last decades, discovery of an anti-malarial targets was typically performed retroactively, where compounds were first phenotypically screened for anti-malarial activity, followed by mechanism-of-action studies to identify the target. While this approach has led to many promising candidates, the process can be highly challenging, particularly for novel *Plasmodium* targets for which no orthologue is present. Furthermore, this workflow may overlook potential druggable targets, as it is restricted by the initial selection of compounds in the screening library.

At TropIQ, we utilize a Target Based Drug Discovery (TBDD) approach to discover novel anti-malarials. Promising druggable targets are identified, based on genetic inhibition studies to determine their essentiality within the *Plasmodium* life cycle. This approach is followed by focused screening of compound libraries against specific targets. Knowning the target beforehand facilitates the rational design of more potent and selective compounds. TBDD is further aided by the growing availability of structural data on *Plasmodium* proteins and their orthologs as well as machine learning-based structure prediction of protein-ligand binding sites.

Promising *Plasmodium* drug targets are recombinantly expressed and purified in our lab. Next, we establish target-specific biochemical assays to define target activity and/or function. These assays allow us to screen for compounds that modulate target function, potentially leading to the development of new antimalarials. Our current focus includes multiple *Plasmodium falciparum* enzymatic targets, which are all critical for parasite survival, such as *Pf*.ACb, *Pf*.Gyrase, *Pf*.SHMT, and proteins of the MEP pathway.

In case of *Pf*.ACb we have set up and enzymatic assay to measure the readout of cyclic AMP from ATP by the recombinantly expressed protein. This assay is highly optimized and automated to make it suitable for high throughput screening of large compound libraries. Various screens, including ~50.000 compounds, were performed leading to several hit compounds that are currently begin explored in both biochemical as whole cell assays during hit validation studies. This project exemplifies our general workflow in the early stages of Target Based Drug Discovery in which we aim to identify novel chemical matter that can kill the *Plasmodium* parasite.

SE09.07

Chemical strategy to eliminate a long-neglected parasitic disease: Development of a potent trypanosome alternative oxidase inhibitor active against the causative agents of animal trypanosomiasis

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Animal African Trypanosomiasis (AAT) is a wasting disease that is caused by a blood-dwelling protozoan parasite called trypanosome and transmitted through the bite of an infected tsetse fly vector. The disease affects wildlife, and domestic animals like sheep, goats, cattle, horses, and donkeys across mainly poor rural communities in sub-Saharan Africa where it is a major constraint to livestock production and draught power for farmers. AAT is estimated to cause $2.75 to $4.5 billion in annual losses. Isometamedium and diminazene are used to treat AAT however, these drugs are sometimes ineffective due to increasing drug resistance and the presence of a cheaper counterfeit versions. Hence, new effective and affordable drugs are needed to control the disease. Targeting parasite specific metabolism with chemical compounds that are not cross resistant with existing drugs is a useful strategy to curb AAT. The mitochondrion-based Trypanosome Alternative Oxidase (TAO) has been validated as essential for respiration and survival of bloodstream forms of trypanosomes. Since TAO is absent in mammals and it is conserved among *Trypanosoma* species, it offers a promising target for chemotherapy. But previous TAO inhibitors were unsuccessful *in vivo* due to their inability to cross the parasite"s membranes. We present here, a novel approach that involves boosting the trypanocidal efficacy of a mitochondria targeted 2,4-dihydroxybenzoate by conjugating the inhibitor with lipophilic cation (LC) that crosses lipid bilayers by non-carrier mediated transport, and thus accumulate specifically in the parasite"s mitochondrion, driven by the transmembrane potentials of trypanosomes. This design provided an LC–TAO inhibitor conjugate that is active in the low nanomolar range against wild-type and resistant strains of trypanosomes (*T. b. brucei, T. evansi, T. equiperdum*, and *T. congolense*), with promising selectivity over human cells. *In vitro* biochemical assessments confirm TAO inhibition. Kinetic assay of the inhibitor against recombinant TAO revealed a noncompetitive inhibition mode, while X-ray diffraction analysis of the crystal structure of rTAO-inhibitor complex gave molecular insights into the mode of inhibition and shows that the inhibitor occupies an allosteric binding site distant from the active site. Thus, the LC–inhibitor conjugate strategy provides a useful strategy for developing new potent class of trypanocides.

SE10.02

Exploring parasite miRNAs in *Wolbachia*-parasite-human interactions

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Parasitic filarial nematodes *Wucheria bancrofti*, *Brugia malayi*, and *Brugia timori* are the causative agents of the debilitating, neglected, infectious lymphatic filariasis. Adult worms reside in the human lymphatic system where they damage the lymphatic valves and vessels and cause lymphedema. Parasites have also evolved a mutualistic association with the intracellular bacteria, *Wolbachia,* essential for worm development, reproduction, and survival. The mechanism for such dependency is being studied to explore the unique potential of using *Wolbachia* as a novel chemotherapeutic target against human filarial infections. Here we examined the role of *B. malayi* regulatory RNAs (bma-microRNAs) in Wolbachia-parasite and parasite-host interactions. First, we compared the expression of bma-microRNAs in worms treated with doxycycline (anti-*Wolbachia* agent) *vs* control to identify which parasite microRNA are modulated by the presence of *Wolbachia*. We identified 13 upregulated and 20 downregulated miRNAs in worms treated with doxycycline, as compared to controls**,** four of which are unique to *Brugia* with no homologs in other filariae. We used miRNA-mediated interference to silence two miRNAs (bma-mir-86 and bma-mir-5864) highly expressed in adult female worms to study their role in *Wolbachia* and worm biology. The treatment with miRNA inhibitors significantly reduced *Wolbachia* numbers and induced apoptosis in treated worms, as compared to control worms treated with scrambled miRNA inhibitors. Filarial worms have also been shown to secrete microRNAs that can be detected in the biofluids of infected animals and humans. Selected 2 bma-microRNAs were found in extracellular vesicles secreted from adult and microfilaria worms. We hypothesized that *B. malayi* microRNAs are involved in the damage to lymphatic cells, which could lead to lymphedema. Lymphatic endothelial cells (LECs) were treated with miRNA-mimics, which mimic selected parasite miRNAs, and the expression of the human proteins was analyzed. Bma-mir-86 and bma-mir-5864 reduce cell-to-cell connection and adhesion of the cells, as well as increase the permeability of the endothelial monolayer. Therefore, parasite miRNAs are secreted by the worms in the lymphatics and participate in the pathology. Defining the role filarial miRNAs play in worm biology and pathogenesis could help establish mechanisms that could be targeted therapeutically.

SE10.03

An SRP-independent targeting (SND) pathway plays a key role in modifying human red cells by malaria parasites

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The virulence of *Plasmodium falciparum*, the causative agent of severe malaria, relies on its capacity to remodel human red blood cells (RBCs). This remodeling involves the export of hundreds of parasite proteins to the host cell, enabling immune evasion and cytoadherence, both critical for pathogenesis. While proteins with N-terminal signal peptides utilize canonical secretory pathways for export, mechanisms driving the endoplasmic reticulum (ER) entry of proteins lacking signal peptides, including the major virulence factor PfEMP1, remain poorly characterized. Here, we identify a Signal Recognition Particle (SRP)-independent targeting (SND) pathway in P. falciparum and establish its role in trafficking parasite proteins. We show that PfSnd2, a central component of this pathway, localizes to the ER membrane and mediates the trafficking of a subset of parasite proteins lacking N-terminal signal peptides. Disruption of PfSnd2 through conditional knockdown impairs the export and surface localization of PfEMP1 and other surface-exposed proteins. Furthermore, we delineate key structural elements required for ER targeting via this pathway, providing new insights into its molecular mechanism. These findings establish the SND pathway as a critical contributor to *P. falciparum* virulence and underscore PfSnd2 as a potential therapeutic target for combating malaria.

SE10.04

To die or not to die – Exploring a p1/s1 3' nucleotidase/nuclease as novel antileishmanial drug target

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Introduction: The absence of effective therapeutics for Leishmaniasis highlights the need for novel antileishmanial treatments. To expand the scope of potential drug candidates, we are characterizing a p1/s1 3' nucleotidase/nuclease (3'nt/nu) in *L. major* as drug target and screen for inhibitors of this enzyme. As all protozoans, *Leishmania* are purine auxotroph and depend on metabolite salvage from their host environment. Thus, they possess several ecto-enzymes to convert nucleic acids and nucleotides into nucleosides, enabling their transport into the parasites to be utilized in purine metabolism.

Objectives: Enzymatic activities of p1/s1, its localization and involvement in host-parasite-interaction were studied using *L. major* and human primary immune cells to characterize it as novel drug target.

Results: Employing quantitative proteomics, we identified a class I nuclease p1/s1 cluster in *L. major*, comprising ecto-enzymes with 3' nucleotidase and nuclease activities. Once we confirmed secretion of p1/s1 protein, dual ecto-3' nucleotidase and endonuclease activity on intact *L. major* and in the culture supernatant was demonstrated. This activity is reduced in *p1/s1*$^{-/-}$ parasites. The mechanisms by which *L. major* compensated for the loss of p1/s1 are currently investigated by genomic and proteomic approaches.

In addition, these functions revealed two mechanisms of host-pathogen interactions that facilitate infection establishment: Utilizing their 3' nucleotidase activity, *Leishmania* generate extracellular adenosine to suppress inflammatory cytokine secretion from macrophages and reduce T-cell proliferation in human primary cells. The presence of ecto-endonucleases further allows these parasites to degrade and escape neutrophil extracellular traps, another potent first-line innate immune mechanism in pathogen defense.

Conclusion: Generating a null mutant deficient for the p1/s1 genomic cluster allows studying its potential as antileishmanial drug target. So far, p1/s1 exhibits multiple beneficial characteristics of a potent drugable protein. In addition to its manifold roles in parasite metabolism and immune evasion, this enzyme class lacks mammalian homologues, yet it is expressed in the infective stages of all *Leishmania* species. Consequently, we are now testing and identifying compounds derived from a virtual screening as specific 3' nucleotidase/nuclease inhibitors, with the aim of developing them further as novel antileishmanial compounds.

SE10.05

The role of non-coding RNAs in regulating gametocyte development in malaria parasites

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To analyze the role of non-coding RNAs (ncRNAs) during sexual differentiation of *P. falciparum*, we used direct RNA sequencing by Oxford Nanopore Technology (ONT) to identify true non-coding RNAs on a genome-wide level. Previous studies have mainly annotated ncRNAs based on Illumina sequencing data, which uses a reverse transcriptase that can lead to potential artefacts in identifying ncRNAs. Further, ONT allows us, due to the long read technology, to get full-length ncRNAs, including their splicing patterns and easily differentiate overlapping mRNAs and ncRNAs transcribed from the same strand. We sequenced 500 ng of total RNA from asexual stages and stage V gametocytes from *Pf.* NF54 parasites and, in addition, a tightly synchronized time course of gametocyte development from *Pf.* Dd2 parasites. We generated between 441,000 and 1,409,000 reads aligning to the reference genome 3D7. Out of these reads, between 3 - 13% aligned outside of annotated features, e.g., protein-coding genes, rRNA, annotated ncRNAs, and pseudogenes, providing a high number of potential new ncRNAs. Further bioinformatic analyses and predictions of potential open reading frames (or lack thereof) confirmed identification of more than 1000 well-supported ncRNAs. Non-coding RNAs can have different modes of action. They can directly influence the transcription of a gene by being transcribed in antisense, they can inhibit transcription of a neighboring gene on the same strand, or they can act in *trans* and influence translation, mRNA stability, recruitment of epigenetic factors, and interact with RNAs/proteins involved in different pathways. All of these different ncRNA types were identified within our ONT data set, and further analysis revealed ncRNAs specifically regulate genes during gametocytogenesis by being transcribed antisense or close to a gene. To find binding partners for *trans*-acting ncRNAs, we are currently combining bioinformatic analyses with RNA/RNA-Duplex sequencing to directly identify interactions between ncRNAs and mRNAs. Overall, this study will provide novel insights into the role and importance of ncRNAs in the regulation *Plasmodium* gene expression.

SE11.01

Endangered parasites: No one else cares but parasitologists do

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It is now well established that biodiversity is globally being lost at an accelerating pace. Since virtually every macroscopic organism has its own parasites, every extinct or endangered host species means the same fate for its parasites, in what is called "co-extinction". Several prominent cases of such a disappearance have been documented for ticks from rhinos and giant turtles, lice from weasels, phthirapterans from vultures, warble flies from elephants, and so on. Some parasites, such as pubic louse, disappeared because of the altered lifestyle of their human host, while others are being eliminated by disrupted life cycles and ever improving hygienic conditions. While none of this applies to the ecto- and endoparasites of freshwater and marine fish, they are progressively disappearing as well. I will describe the case of insect trypanosomatids from tropical countries, and one possible way of retaining their diversity. The general aim is clear – the quest to save parasites is as important as the quest to save their hosts.

Fig. 1

Fig. 2

SE11.03

Long-term trends in parasite diversity and abundance: Approaches and patterns

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Parasites exist in every ecosystem, affecting nearly all organisms and playing a complex role in human societies. On one hand, they contribute significantly to biodiversity and support ecosystem stability by performing essential ecological functions. On the other, they can impose health burdens on their hosts, causing diseases in both animals and humans. Despite their significance, how parasitic organisms are affected by anthropogenic environmental change remains poorly understood. In other well-studied groups such as free-living birds, mammals and insects, long-term ecological datasets have been instrumental in elucidating temporal trends in abundance or diversity and linking them to anthropogenic drivers. For parasites however, overarching long-term trends in abundance or diversity have yet to be identified, perhaps because such long-term datasets are hard to obtain. Here we provide an overview of the research approaches developed to study long-term changes in parasite systems and the trends highlighted by these studies. Our aims were to help researchers make informed methodological decisions when designing their research, and to provide recommendations for future long-term research on parasite ecology. To this end, we performed a systematic literature search on long-term analyses of eukaryotic parasites of wild animals and identified four types of approaches deployed to gather long-term data: 1) long-term monitoring, 2) snapshot resampling, 3) literature-based research and 4) natural history collection-based studies. Our results revealed striking differences in the temporal scope, geographical scale of sampling, sample sizes and taxonomic resolution of parasite identification among these approaches. However, no overarching trends in parasite abundance or diversity were identified. When detected, temporal changes were often linked to anthropogenic disturbances, but these claims were rarely supported by inferential analyses. Overall, our results show that our understanding of long-term trends in parasite systems remains hampered by data scarcity and research biases. To address these issues, we advocate for the establishment of large-scale parasite monitoring programs combined with existing ecological monitoring projects, as well as the development of new scalable biomonitoring tools. We also highlight the importance of valorising historical data and preserved biological material in museum collections to obtain baseline information on parasite systems.

SE11.04

Host whole genome sequence data represent an untapped resource for inferring parasite biogeography

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Organisms may be exposed to vastly different biotas during island colonisation resulting in novel interactions, including between hosts and parasites. Parasites are diverse and ubiquitous, imposing varied pressures on their hosts. Yet, most studies of island biogeography tend to overlook parasites or focus on single groups of parasites. This bias reflects a long-term focus on one-host one-parasite systems and may be due to the challenges posed by studying parasites such as costs of sampling, the specialist skills required for identification and their generally cryptic nature. However, ignoring parasite diversity neglects the influence of varied life histories (e.g. transmission routes) on patterns of biogeography. With the advent of high-throughput sequencing, an increasing amount of genomic data is becoming freely available on public repositories. Whole genome sequence (WGS) data generated from the blood or tissues of free-living organisms could inadvertently capture information about endogenous parasites infecting the host. In this study, we analyse WGS data generated from blood samples of the silvereye, *Zosterops lateralis*, aiming to identify endogenous parasites in birds from different island populations in the southwest Pacific. We examine how data from unintentionally sequenced parasites provide insights on how patterns of island biogeography may be explained by parasite life histories. In particular, our analyses provide evidence that abiotic factors, such as rainfall, differentially impact parasite prevalence in island populations depending on mode of parasite transmission. Our study highlights how parasite life histories can influence parasite biogeography and demonstrates how WGS data can be repurposed to unravel complex host-parasite interactions.

SE12.01

Integrative transcriptomic analysis of *Plasmodium falciparum* clinical samples across severe disease phenotypes

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Background: Emerging drug resistance in the malaria parasite *Plasmodium falciparum* necessitates novel therapeutic approaches. A detailed understanding of the parasite's molecular mechanisms within the host is crucial for developing effective treatments.

Patients & Methods: We employed multi-probe, custom-designed microarrays, Illumina-based pooled RNA sequencing, and Oxford Nanopore direct RNA sequencing to analyze the transcriptomes of *Plasmodium falciparum* clinical isolates. These isolates were obtained from different patients across three severe malaria manifestations: Hepatic Dysfunction (HD), Cerebral Malaria (CM), and Thrombocytopenia (THR).

Results: Our study provides direct evidence for natural antisense transcripts (NATs) originating from the mitochondrial and apicoplast genomes. Although mitochondrial activity is diminished in blood-stage malaria, a key finding is the correlated upregulation of sense-antisense transcript pairs encoded by both nuclear and mitochondrial genomes in the CM cluster. In THR, mitochondrial electron transport chain (mETC)-associated antisense transcripts are significantly upregulated, suggesting their involvement in severe pathogenesis through potential disruption of mitochondrial bioenergetics. RNA methylation profiles in HD highlight insights into N6-methyladenosine (m6A) and 5-methylcytosine (m5C) modifications in both sense and antisense transcripts, revealing a striking overlap of differential methylation of isoforms with either of the two modifications.

Conclusion: This study provides a comprehensive view of *P. falciparum* splicing events, transcript modifications, and antisense-mediated regulation across nuclear, mitochondrial, and apicoplast genomes. The integration of long- and short-read sequencing with microarray data offers a robust, *ex-vivo* transcriptomic dataset, unveiling the parasite's complex post-transcriptional landscape in severe malaria phenotypes.

SE12.02

Advancing parasite diagnostics: A rapid protocol for isolation of *Schistosoma* cell-free DNA designed for use at the point-of-care

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Parasitic infections, particularly those responsible for neglected tropical diseases (NTDs), pose a significant global health burden. These diseases have profound impacts in endemic regions and an increasing presence in non-endemic areas due to globalization [1]. Early and accurate diagnosis is crucial for effective control and elimination strategies, especially as many parasitic infections manifest with nonspecific clinical symptoms. Recent advances in the detection of cell-free DNA (cfDNA) - short fragments of extracellular DNA secreted by host and pathogen cells in bodily fluids such as blood and urine - have shown promise as a diagnostic biomarker for a range of pathogens, including parasites [2].

Current cfDNA isolation methods often require costly and complex procedures that are inaccessible in resource-limited settings. We have developed a rapid, cost-efficient, and user-friendly cfDNA isolation protocol designed specifically for point-of-care use. The novel method utilizes magnetic particles, requires minimal equipment, and is completed in as little as 10 minutes. Unlike many commercial kits, this protocol is free of hazardous substances, environmentally friendly, and generates minimal plastic waste.

We validated the performance of our protocol using clinical specimens from patients infected with Schistosoma mansoni. Benchmark tests against conventional commercial kits demonstrated comparable or superior sensitivity and specificity for cfDNA detection by this new protocol. The protocol"s robustness and accessibility make it a promising tool for parasite diagnostics, particularly in resource-limited regions where the burden of disease is the greatest.

By making available such a convenient non-invasive diagnostic tool, that is easy to implement at not only the point-of-care and clinical settings, but also for large-scale community screening and surveying, we are closely supporting the WHO roadmap for NTD elimination in these impacted localities. It also enables rapid case detection in nonendemic areas, offering a powerful means to address the growing challenges posed by parasitic infections in a globalized world.

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SE12.03

Global genetic diversity of soil-transmitted helminths reveals population-biased variation that impacts diagnostic targets

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Introduction: Soil-transmitted helminths (STHs) impact a significant portion of the global population, perpetuating poverty and disease in some of the most neglected communities worldwide. STH control relies on microscopy-based diagnostics to monitor parasite prevalence and enable post-treatment surveillance. However, molecular diagnostics are rapidly being developed due to their increased sensitivity, particularly in low-STH-prevalence settings. In large-scale epidemiological and clinical trials, qPCR-based diagnostics targeting repetitive sequences have been used to confirm elimination or monitor programmatic progress. Understanding helminth genetic diversity and its potential impact on molecular diagnostics is vital for STH epidemiology and elucidating their natural transmission patterns within and between populations.

Methods: Using low-coverage genome sequencing data, we assessed the presence of STHs in n=1000 worm, faecal, and purified egg samples from 27 countries. We characterised (i) the genetic diversity and connectivity of parasite populations and (ii) the impact of genetic variation between STH populations on the performance and efficiency of conventional molecular diagnostics such as qPCR.

Results: We identified contrasting patterns of genetic variation and connectivity between globally distributed STH species. The genetic diversity of *Trichuris trichiura* follows anticipated patterns of geographical radiation (e.g., neighbouring countries share genetic variation compared to more distant populations), however, the population analysis of *Ascaris* spp. revealed that using multiple reference sequences from different geographical isolates can influence the interpretations of the genetic mixing within and between *Ascaris* spp. We also defined substantial copy number and sequence variants in current diagnostic targets in *A. lumbricoides* and *Trichuris trichiura*. Validation of these single nucleotide genetic variants showed that they can significantly influence the ability of qPCR to detect diagnostic targets through in vitro assays.

Discussion: Our study provides insights into the diversity and genomic epidemiology of STHs. Further work to define the genetic connectivity of STH populations may enable the identification of transmission zones and networks, offering a more precise approach to prioritising control efforts. Our analyses present a clear rationale for the comprehensive evaluation and validation of repetitive sequences currently utilised as targets for molecular diagnostics. Collectively, such data and subsequent studies will form the foundation for the genomic epidemiology of all STHs and their sustainable control as a public health concern.

SE12.04

Understanding infection versus transmission dynamics of *Schistosoma mansoni* pre- and post-treatment, and the relationship between egg, antigen and DNA based diagnostics – A latant class analysis

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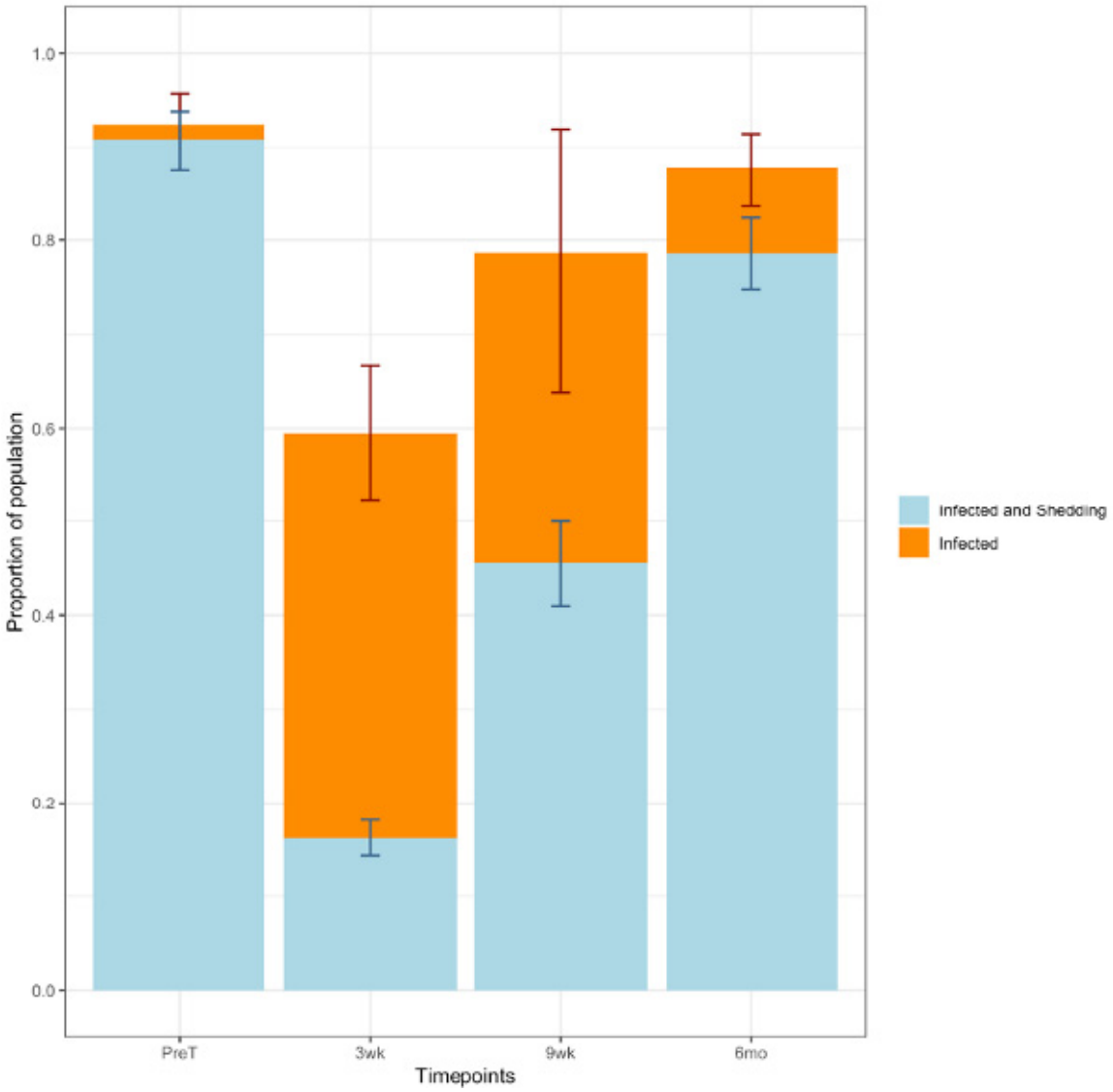
Over 240 million people are infected with *Schistosoma*. The World Health Organization has set goals for schistosomiasis elimination as a public health problem by 2030, defined as <1% prevalence of heavy-infections measured by Kato-Katz (KK) in school-aged children. However, KK lack sensitivity, and commonly overestimate drug efficacy. The point-of-care circulating cathodic antigen test (POC-CCA) improves on sensitivity, especially for low intensities and post treatment, but specificity is not 100%. In addition, the relationship between antigens and eggs changes post treatment, resulting in different drug efficacy measures and many individuals who are POC-CCA positive but KK negative. Understanding what proportion of KK negative, POC-CCA positive individuals are true infections, and if they are contributing to transmission, will help inform and guide control programmes. We aimed to estimate the true proportion of school-aged children who are infected with *Schistosoma mansoni* and at risk of morbidity, and what proportion are shedding eggs and contributing to transmission in a high-endemicity Ugandan community. A Bayesian Latent Class Model was developed and fit to data from three days of duplicate KK, miracidia hatching, qPCR of stool and blood spots, and POC-CCA G-scores at pre-treatment, and 3, 9, and 22 weeks post-treatment. Incorporating miracidia hatching data and stool qPCR greatly improved predictions of those shedding eggs, as well as resulting in improved, higher, specificity estimates for POC-CCA. Baseline egg and antigen-based diagnostics were comparable, but at 3 weeks post treatment, egg-based diagnostics lack sensitivity and vastly overestimate clearance, with only a quarter of estimated infections shedding eggs. Miracidia hatching data were the most comparable to model estimates of individuals shedding eggs. In conclusion, after treatment, most infected individuals are not shedding eggs, and therefore not contributing to transmission at that stage. However, egg-based diagnostics overestimate the efficacy of treatment, and more robust diagnostics are needed in order to monitor elimination goal attainment.

Fig. 1


1.0
0.8
0.6
0.4
0.2
0.0
Proportion of population
PreT
3wk
9wk
6mo
Timepoints
Infected and Shedding
Infected

SE12.05

Automated image-based Flow Cytometry for High-throughput coproscopical parasite detection and quantification using Machine Learning algorithms

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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.

A novel strategy to reduce anthelmintic drug usage and decelerate resistance development is accurate detection and quantification of parasitic helminth eggs via image-based flow cytometry and subsequent analysis via machine learning (ML) algorithms.

In this study, an automated image-based flow cytometry indicates the state-of-the-art performance of parasitic egg detection and classification, outperforming manual microscopic examination in time, costs, and detection accuracy. 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 achieveing an accuracy of over 95 % and high reproducibility, but significant different computing time. The approach was sucessfully 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 catogarisation).

An optimised diagnostic workflow will reduce the level of specialization and time needed for coproscopical examination and subsequently reduce the associated costs for diagnostics.

SE12.06

New tools for detecting amoebic liver abscess: Evaluation of two serological assays

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Introduction: Amoebiasis caused by *Entamoeba histolytica* is a prevalent parasitic disease in low-income countries but occurs less frequently in European countries. The extraintestinal form, typically hepatic, can be fatal if untreated, underscoring the importance of timely and accurate diagnosis. Diagnosis relies on a combination of clinical, radiological, and serological findings. In a previous study (Pretot et al., ESCMID Global 2024), we evaluated the four serological assays available on the European market. Since then, the antigen used in the Bordier ELISA (*E.histolytica* IgG, Bordier) has been significantly modified, and a new immunochromatographic test (ICT), *E.histolytica* IgG ICT (Bordier), has been introduced.

Objectives: The aim of this study was to assess the performance of two new serological tests for the diagnosis of amoebic liver abscess.

Materials & methods: A biobank of 424 serum samples from eight French University Hospitals was utilized. This included 81 samples from patients with confirmed amoebic liver abscess and 343 samples from healthy donors or patients with other parasitic and non-parasitic conditions (hepatic diseases, immune dysfunction). Tests were conducted following the manufacturer's instructions. Sensitivity, specificity, likelihood ratios (LR) and accuracy were calculated for each test. For the ELISA assay, optimal threshold was determined using a Receiver Operating Characteristic curve and the Youden index, implemented with the pROC package in R Software v4.3.1 (R Core Team 2021, Boston, USA).

Results: Test performance is summarized in Table 1. The ICT sensitivity was 92.6% and its specificity was 97.1%. In contrast, ELISA sensitivity and specificity, using the manufacturer's recommended threshold, were 100.0% and 89.2% respectively. The ELISA optimal Youden index was 0.93, achieved with a threshold index of 1.49. At this threshold, the sensitivity was 98.8% and the specificity was 94.5%. All positive and negative LR calculated for the ELISA assay (at both thresholds) and for the ICT indicated very strong diagnostic contributions.

Conclusion: These new tests demonstrated excellent performance. The ELISA assay with optimized threshold detected most amoebic liver abscesses while minimizing false-positive results. However, it requires a large-scale automated system, making it more suitable for high-income countries. The ICT provided slightly lower sensitivity but even greater specificity, with a very strong diagnostic contribution. It could serve as a confirmatory method for positive ELISA results in high-income countries or as a standalone test in resource-limited, endemic regions. Its balanced sensitivity and specificity, combined with its single-use format and ease of interpretation, make it well-suited for such contexts. A study conducted in an endemic area would ideally complement our findings.

Table 1: Analytical performance of two new *Entamoeba histolytica* serological tests assessed on 424 serum samples.

Fig. 1

Table 1: Analytical performance of two new *Entamoeba histolytica* serological tests assessed on 424 serum samples.

Serological test	Threshold	Sensitivity (%)	Specificity (%)	Accuracy (%)	LR^+	LR^-
***E. histolytica* IgG Bordier ELISA**	1 (manufacturer)	100.0 [95.5-100.0]	89.2 [85.5-92.1]	91.3 [88.2-93.6]	9.3 [8.8-9.8]	0.000
	1.49 (optimised)	98.8 [93.3-99.8]	94.5 [91.5-96.4]	95.3 [92.8-96.9]	17.8 [16.1-19.8]	0.013 [0.002-0.095]
***E. histolytica* IgG Bordier ICT**	NA	92.6 [84.8-96.6]	97.1 [94.7-98.4]	96.2 [94.0-97.7]	31.8 [26.1-38.8]	0.076 [0.055- 0.106]

LR^+: positive likelihood ratio; LR^-: negative likelihood ratio;

NA: not applicable; [*x-x*]: 95% confidence interval.

SE12.07

Enhancing safety of malaria diagnostics in patients at risk for viral hemorrhagic fever

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Introduction: Malaria and viral hemorrhagic fever (VHF) have overlapping symptomatology, creating clinical and diagnostic challenges in travelers returning from co-endemic areas. In those patients, rapid and accurate malaria diagnosis is critical, but is complicated by stringent safety measures required for VHF. To improve safety of sample handling, we introduced the use of blood collection tubes containing virucidal guanidine-isothiocyanate (GITC)-based Zymo DNA/RNA Shield buffer (Zymo Research, Irvine, CA, USA).

Objectives: To assess diagnostic accuracy of two rapid antigen tests (RATs) and two rapid nucleic acid amplification tests (NAATs), after mixture of patient blood with Shield buffer.

Materials & Methods: Samples had been stored at -80℃ before use for this study. One volume of whole blood was mixed with two volumes of buffer to mimic concentrations that would ideally be present in a full collection tube after a clinical venipuncture. The mixtures were kept at room temperature for at least 30 minutes before further use. Further handling of the samples was done in a laminar flow cabinet in a BSL-2 facility. After 30 minutes, the BinaxNOW™ (Abbott, IL, USA) and Biozek (Inzek BV, Apeldoorn, The Netherlands) RAT and the Alethia Malaria LAMP (Meridian Bioscience, OH, USA) and EasyNAT Malaria Cross Primer Assay (CPA) (UStar Biotechnologies, Hangzhou, China) were performed with the mixtures according to the manufacturer"s instructions.

Results: In 26 *P. falciparum*-positive samples, histidine-rich protein (HRP)-2 detection was excellent for the Biozek RAT (25/26), but poor for the BinaxNOW RAT (9/26). Both RATs failed to detect pan-*Plasmodium* aldolase in any of the mixtures. In contrast, both the LAMP and CPA performed excellent and detected DNA of all 47 *Plasmodium*-positive mixtures.

Conclusion: These data show that rapid and accurate malaria diagnostics remains possible on whole blood mixed with this buffer. We propose the use of this buffer in patients with high suspicion of malaria and in need of rapid and accurate malaria diagnostics, but in whom VHF cannot be completely excluded.

SE14.01

Toxoplasma gondii micropore is required for regulating the membrane reservoir during parasite replication

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During intracellular development, apicomplexan parasites demonstrate the ability to uptake proteins from the host cell through endocytosis. In *Toxoplasma gondii*, recent studies have identified the micropore as an important endocytic structure and elucidated its composition (1, 2). The maintenance of the micropore depends on the kelch-domain protein K13, a key factor in malarial drug resistance to artemisinin (3). Depletion of K13 leads to a striking accumulation of plasma membrane, resulting in disorganised parasites and indicating that the micropore is critical for maintaining plasma membrane homeostasis.

We investigated plasma membrane dynamics and recycling in *Toxoplasma gondii*. Intracellular parasites share a plasma membrane (PM), which undergoes a continuous cycle of endocytosis and exocytosis, mediated by Rab5b and MyoF. Interestingly, we discovered that the parasites form an extracellular plasma membrane reservoir (PMR) before daughter cell formation. This PMR is reabsorbed at the end of replication, ensuring membrane homeostasis. However, when K13 is deleted, the parasites lose the ability to reabsorb the PMR, resulting in its accumulation, disruption of membrane homeostasis, and eventual parasite death. These findings directly link endocytosis to the PMR reabsorption process.

Keywords:

Toxoplasma gondii/ Micropore/ Endocytosis/ Plasma membrane/ Plasma membrane reservoir

1. Koreny L, Mercado-Saavedra BN, Klinger CM, Barylyuk K, Butterworth S, Hirst J, et al. Stable endocytic structures navigate the complex pellicle of apicomplexan parasites. Nature Communications. 2023;14(1):2167.
2. Wan W, Dong H, Lai D-H, Yang J, He K, Tang X, et al. The Toxoplasma micropore mediates endocytosis for selective nutrient salvage from host cell compartments. Nature Communications. 2023;14(1):977.
3. Birnbaum J, Scharf S, Schmidt S, Jonscher E, Hoeijmakers WAM, Flemming S, et al. A Kelch13-defined endocytosis pathway mediates artemisinin resistance in malaria parasites. Science. 2020;367(6473):51.

Figure 1: Differentiation between the mother PM and the de-novo PM using SAG1-Halo. A combination of two membrane-permeable dyes was used at different time points, one before invasion, and one after replication. Scale bar 2µm

Figure 2: Formation of the Plasma Membrane Reservoir (PMR) in RH parasites using α-SAG1. Ultra-expansion microscopie. Scale bar 5µm.

Fig. 1

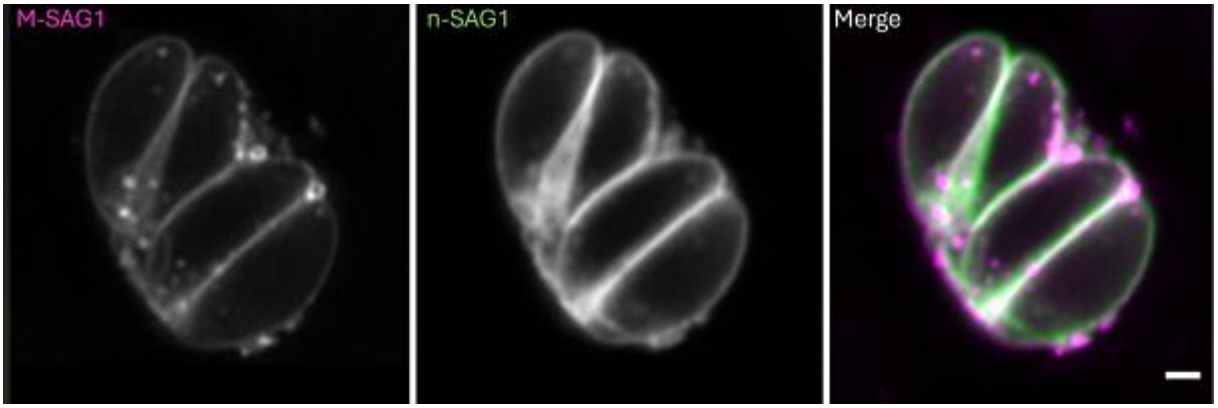
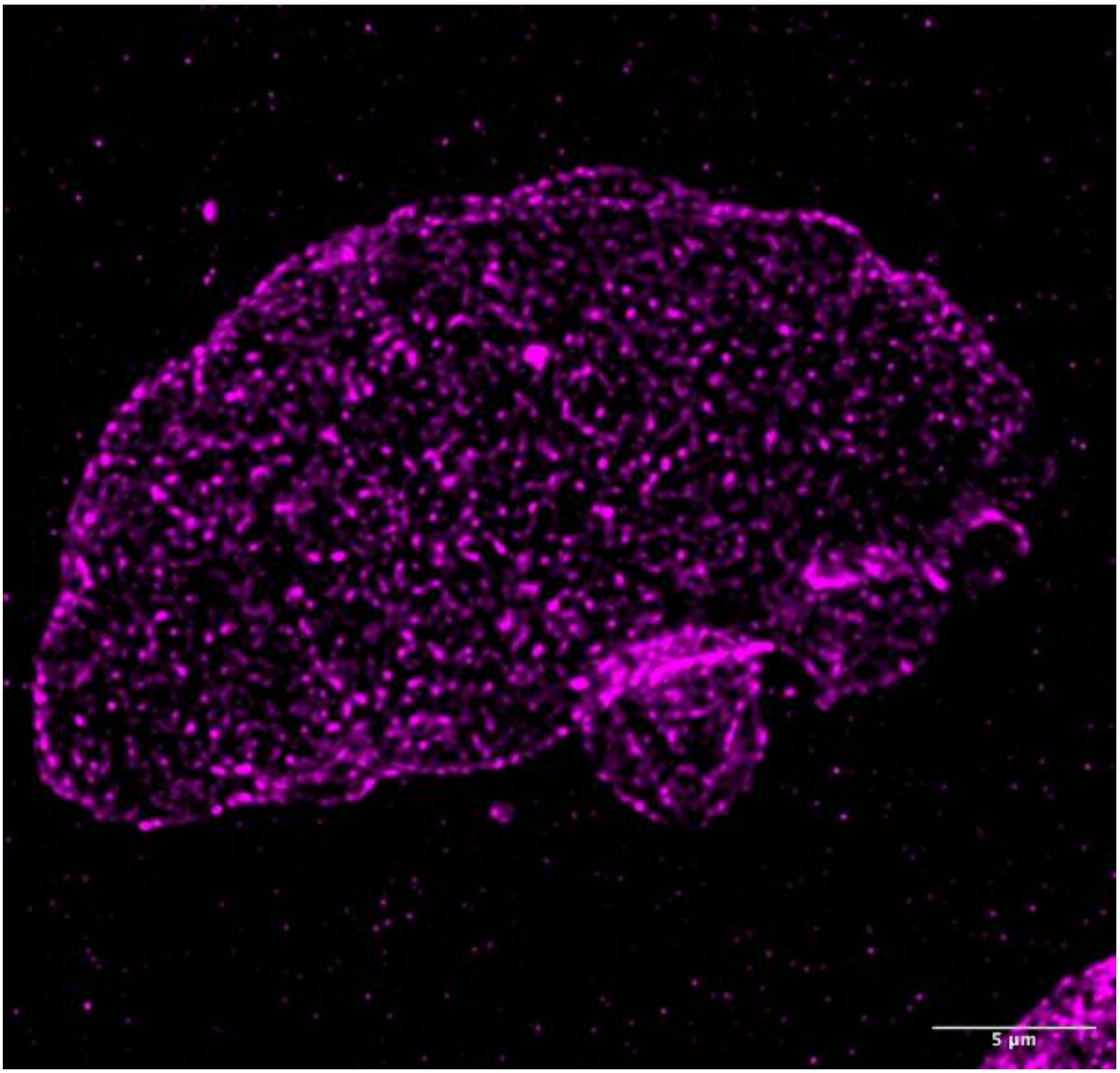


Fig. 2



SE14.02

A PROPPIN regulates endocytic membrane dynamics and food vacuole integrity in malaria parasites

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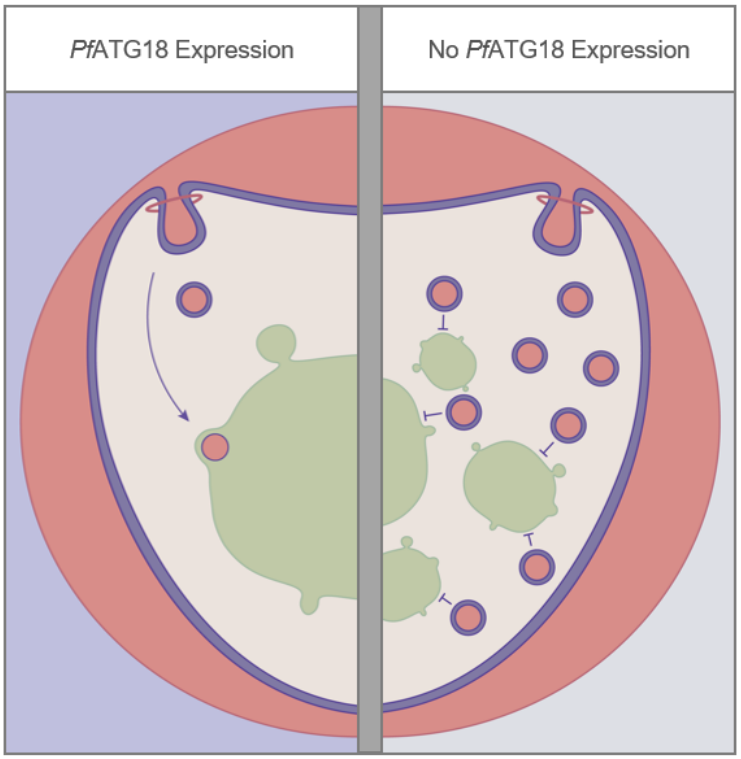
The *Plasmodium* food vacuole is a unique parasite compartment whose functions in hemoglobin catabolism and heme detoxification are indispensable for the survival of malaria parasite blood stages. Throughout intraerythrocytic development, the vacuole undergoes various fusion and fission events that contribute to vacuole homeostasis, maintenance and function. Transport vesicles filled with host cell cytoplasm as well as ER/Golgi-derived vesicles fuse with the food vacuole membrane to deliver hemoglobin and parasite proteins, respectively. To date, the mechanisms and molecular machinery underlying these vacuolar membrane dynamics remain poorly delineated. Here, we combine conditional reverse genetics in the human malaria pathogen *Plasmodium falciparum* with quantitative live cell imaging and volume electron microscopy to characterize the functions of a putative membrane scission protein: the PROPPIN family member autophagy-related protein 18 (ATG18), homologs of which are believed to participate in fragmentation of yeast vacuoles as well as formation and removal of endosomal transport carriers in mammalian cells. In malaria parasites, point mutations in the ATG18 coding sequence have been associated with partial resistance to artemisinin-based antimalarials. Here, we demonstrate that *Pf*ATG18 localizes to the membrane of the food vacuole and to transient endosomal compartments. We find that conditional inactivation of *Pf*ATG18 is lethal to the parasites and causes fragmentation of the food vacuole. Resulting vacuolar fragments progressively lose their ability to fuse with incoming endosomes, which in turn prevents the delivery and processing of hemoglobin. Our studies highlight *Pf*ATG18 as a central regulator of vacuolar membrane dynamics with essential functions during asexual parasite proliferation in the human bloodstream.

Fig. 1



SE14.03

Actin-like proteins 3 and 5a are essential for *Plasmodium* transmission

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The malaria-causing parasite *Plasmodium* relies on an adaptable cytoskeleton and specialized molecules to transmit to *Anopheles* mosquitos. *Plasmodium* expresses a set of actin-related proteins unique to apicomplexans, termed actin-like proteins (Alps). However, the importance and specific roles of these Alps in *Plasmodium* progression are not yet understood.

In-silico analysis of the predicted structures of Alp3 and Alp5a revealed that despite an amino acid sequence similarity of only 19% and 21% to actin, respectively, the actin-fold core was conserved. In order to determine their functional relevance, Alp3 and Alp5a knockout (KO) lines were generated in the rodent model *Plasmodium berghei* and subsequently characterised across different life cycle stages. Deletion of either Alp did not affect blood stage growth and ookinete gliding motility. However, the Alp3KO line had highly reduced oocyst loads compared to wild type, while sporozoite formation in these oocysts was unaffected. Deletion of Alp5a led to smaller and fewer oocysts as well as severely impaired sporozoite formation. Complementation lines confirmed that oocyst phenotypes were specifically caused by Alp knockouts.

These findings suggest that both Alp3 and Alp5a are indispensable for *Plasmodium* transmission at different steps of initial mosquito infection. Alp3 is essential for oocyst formation in mosquitos, while Alp5a is required for oocyst maturation and sporozoite development. By understanding the molecular mechanisms of both Alps will provide important information about oocyst biology as well as the role of specific actin-related proteins during parasite transmission.

SE14.04

Shedding new light on the male-female interaction of schistosomes: The first scRNA-seq analysis of a platyhelminth organ uncovered a RAR-family nuclear receptor in *Schistosoma mansoni* ovaries essential for meiosis progression

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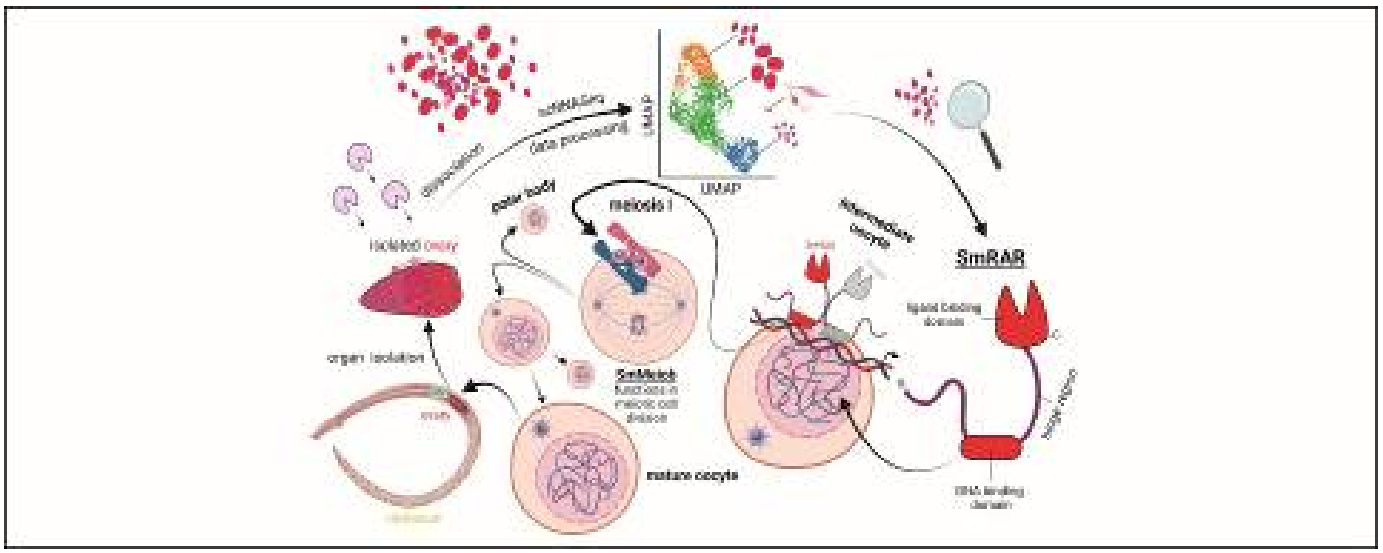
Studies on transcription regulation in platyhelminth development are scarce, especially for parasitic flatworms. For the first time in platyhelminth research, we employed organ-specific single-cell transcriptomics to identify genes that may control reproductive development in *S. mansoni*. Unlike other trematodes, schistosomes exhibit distinct sexes, with egg production reliant on the pairing-dependent maturation of female reproductive organs. Despite this significance, the molecular mechanisms underlying ovary development and oocyte differentiation remain largely unexplored.

Utilizing an organ isolation approach for *S. mansoni*, we extracted ovaries of paired females for single-cell RNA-seq with disassociated oocytes. A total of 1,967 oocytes expressing 7,872 genes passed QC filtering. Unsupervised clustering revealed four distinct cell clusters: somatic cells, germ cells and progeny, intermediate-stage, and late germ cells. Among others, we identified a hitherto uncharacterized transcription factor of the retinoic acid (RA) nuclear receptor (RAR) family, SmRAR. This gene appeared to be most abundantly transcribed in intermediate-stage oocytes, and it is strongly regulated being expressed: (i) stage-preferentially in adults, (ii) sex-preferentially in females, (iii) high abundantly in the ovary and low abundantly in testes, and (iv) pairing-dependently only in females and their ovaries. RNAi-based functional analyses of Sm*rar* and STRING-predicted, functionally associated genes like Sm*meiob* (meiosis-specific, OB domain-containing; also pairing-dependently and ovary-preferentially expressed) demonstrated their decisive roles in oocyte differentiation and meiosis progression in females after pairing. Upon Sm*rar* (as well as Sm*meiob*) RNAi, oocyte differentiation stopped after stem cell (oogonia) division. The identity of SmRAR indicated a contribution of RA provided by the host. Indeed, pilot experiments with 9-cis RA showed increased egg production of *S. mansoni* couples *in vitro*, while RA signaling inhibition caused the opposite. These result and the proven role of SmRAR for oocyte differentiation strongly suggest an additive influence of RA, and thus the host environment, for oocyte differentiation.

From our findings, we conclude that the schistosome male fulfills the role of "biological-technical assistance" for the female, which depends on pairing to (i) drive the expression of SmRAR and many other pairing-dependently expressed gene as a prerequisite to (ii) "correctly interpret" the host environment for her own development. This is a novel aspect for schistosome research and may be stimulating for young researchers to delve into the largely unexplored subject of transcription factors/NRs in platyhelminth development.

Moescheid et al. (2025) The retinoic acid family-like nuclear receptor SmRAR identified by single-cell transcriptomics of ovarian cells controls oocyte differentiation in *Schistosoma mansoni*. Nucleic Acids Research (in press)

Fig. 1


UMAP
UMAP
isolated ovary
organ isolation
polar body
meiosis I
mature oocyte
SmRAB

SE14.05

Unique actin-related protein Alp1 governs ookinete motility and malaria transmission

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Plasmodium motility is essential for transmission to and from mosquitoes, with the turnover of actin filaments being a central feature of productive cell movement. Ookinetes employ motility to penetrate the mosquito midgut epithelium to colonize their new host. The molecular basis of this movement is only poorly understood and many highly specialized molecules that are critical for motility and mosquito transmission remain unknown. Actin-related proteins (Arps) are known to play critical roles in motility, trafficking and chromatin remodelling. The *Plasmodium* genome encodes actin-like proteins (Alps): apicomplexan-unique Arps that contain striking insertions around a common actin fold. However, the importance, functions and stage-specific contributions of Alps in *Plasmodium* progression remain unknown. Here, we characterized the role of Alp1 using the *P. berghei* infection model.

Gene ablation approaches indicated that *Alp1* plays an essential role in transmission to mosquitoes, whereby Alp1 knockout ookinetes were immotile and unable to infect midguts. Complementation with *P. falciparum Alp1* rescued ookinete motility, indicating a high degree of cross-species functional conservation. Selected unique regions were mutated to corresponding actin equivalents and had only modest effects on ookinetes, suggesting the role of other residues or multiple regions in Alp1 specialised function. Alp1 is located in speckle structures throughout the cell. We also present the actin chromobody probe in ookinetes and show novel structures in ookinetes that are different from another motile parasite stage, revealing the similarities and differences in actin dynamics at different parasite stages. Finally, we show that deletion of *Alp1* renders actin filaments more prone to stabilization, pointing to a potential role of Alp1 in promoting actin filament turnover in ookinetes. We have thus identified a novel Arp that has evolved a specialist function to govern ookinete motility and facilitate malaria transmission.

SE14.06

Hot and greasy: Characterization of the surface lipidome of *Trypanosoma brucei*

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The bloodstream form of *Trypanosoma brucei* inhabits both blood and diverse tissues of an infected mammal, where the parasitic cells feature a dense coat of a variant surface glycoprotein (VSG). The tightly controlled VSG coat shields invariant proteins and the plasma membrane from the mammalian immune system.

We want to deepen our understanding of surface processes at the biophysical limit by artificially recreating the surface of the trypanosome and explore the lifecycle dependant changes in and on the surface of the parasite. Synthetic membranes with variable compositions give us the option to control all aspects of the system to investigate the mechanisms behind the VSG coat and its relationship with the plasma membrane.

We developed a versatile artificial membrane system. Phospholipids and trypanosome-derived VSG proteins are reconstituted into protein-dense proteoliposomes. These proteoliposomes match the lipid-to-protein ratio of the VSG coat of trypanosomes and can be composed into supported lipid bilayers. The diffusion behaviour of both lipid molecules and VSG is analysed with single-molecule fluorescence microscopy.

To extend our control of the system beyond the protein composition, we currently solve the replacement of the primitive lipid bilayer with a chemically diverse and structurally functional membrane. Therefore, we purify and identify the surface lipidome of *T. brucei* during key lifecycle stages of the parasite and use the trypanosome-derived lipid composition to create a fully cell-mimicking membrane system. We developed a novel cyclodextrin-based method to siphon lipids directly from the outer leaflet of the parasite plasma membrane. The technique has proven to avoid harmful lysis and is scalable for dynamic yield. We use the method for our identification of the surface lipidome of slender, stumpy and procyclic stages by mass spectrometry, where we found distinct changes in the lipid composition between stages, and in lipid preparation for direct incorporation into our membrane systems.

SE15.01

3D Tokuyasu: Expanding immuno electron microscopy into three dimensions

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The Tokuyasu method has significantly advanced electron microscopy by enabling high-resolution immunolabeling of ultrathin cryosections while preserving antigenicity and cellular architecture. Key features of the method include gelatine embedding, sucrose infiltration, rapid freezing in liquid nitrogen, and sectioning at cryogenic temperatures (-90°C to -120°C). This approach combines the spatial resolution of electron microscopy with immunolabeling techniques to reveal the ultrastructure of biological specimens.

Although the method has been used since the 1970s, no detailed protocol focusing on trypanosomatids existed, and published studies were limited to two-dimensional visualizations. To address this gap, we developed a comprehensive workflow for precise localization of specific proteins and molecular complexes within parasite cells. Our approach combines semithin cryosectioning, immunolabeling and electron tomography to generate high-resolution 3D reconstructions. Using the endosomal apparatus of *Trypanosoma brucei* as a model, we will discuss the preparation process, troubleshooting strategies, and recent advancements that broaden the method's applications and potential insights into cellular architecture and molecular interactions.

SE15.02

Pooled image-based genetic screens in *Trypanosoma brucei*

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Pooled genetic screens can be used to uncover novel cell biology through massively parallel perturbation of thousands of genes in an organism. Pooled genetic screens using RNAi have been used extensively in *Trypanosoma brucei* to uncover mechanisms of drug resistance, gene expression control and cell signalling. Although extremely powerful, these screens have been limited by readouts such as cell fitness, differentiation and one-dimensional fluorescence. Applying more complex readouts such as fluorescent imaging would allow genetic screens that could identify genes which control protein localisation or assembly of biological structures. In just the last few years, several new technologies have demonstrated application for pooled image-based genetic screens. Intelligent Image Activated Cell Sorting (iIACS) is one such technology. iIACS combines machine learning and imaging cytometry to sort cells based on specific imaging phenotypes at a throughput of 1 x 10^6 cells per hour.

Here, we investigated the potential application of iIACS for pooled image-based genetic screens in *T. brucei*. To test the ability of iIACS to image *T. brucei* cells with different localisations, we endogenously tagged several proteins in different cellular compartments at high efficiency without drug selection markers using recombinant Cas9. We successfully imaged *T. brucei* proteins in several biological compartments using iIACS. Furthermore, we developed a convolutional neural network (CNN) trained on images of a fluorescently tagged nucleolar protein in *T. brucei.* Using this CNN, we were able to effectively isolate nucleolar tagged cells from flagellar tagged cells based on localisation alone using iIACS at high efficiency and specificity. As a proof-of-concept for combining iIACS with genetic screens, we present preliminary data of a small-scale RNAi library screen for proteins which alter morphology of the endoplasmic reticulum when knocked down. Overall, we anticipate that iIACS will be a powerful technology allowing researchers to perform pooled image-based genetic screens in *T. brucei* and other single celled parasitic organisms.

SE15.03

Biophysical methods to quantify mechanics, shapes and forces

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[1]MPZPM, Erlangen, Germany

Advancements in physical methods, optics, and microscopy have been pivotal in medicine and biology, particularly in the mechanical phenotyping of cells and tissues. However, the role of mechanical properties in parasitology - specifically regarding host interaction, parasite locomotion, attachment, and infection - remains underexplored. In this presentation, I will discuss the quantification of parasite biophysical properties using atomic force microscopy (AFM), Brillouin microscopy (BM), and optical diffraction tomography (ODT). I will address challenges in sample preparation, including the use of vibratomes and cryotomes for sectioning larger samples, and how varying resolution and penetration depths can be utilized for specific applications. I will present preliminary studies of *Toxoplasma* and *Giardia*. For *Toxoplasma*, we investigated the mechanical properties of the parasitophorous vacuole (PV) within dendritic cells using BM and AFM. Our findings show that parasites are significantly stiffer than the cell nucleus and are surrounded by a softer, more viscous matrix within the PV, creating a unique mechanical environment that facilitates parasite survival and dissemination. In the case of *Giardia*, we examined the ventral disc (VD), a specialized structure used for host tissue attachment. Employing BM and ODT, we characterized the mechanical properties of the VD and surrounding surfaces, giving rise to a better understanding of the attachment mechanisms of this parasite. This study provides the first biophysical characterization of various parasites, their structural adaptations, and their interactions with the host microenvironment. These preliminary findings will be validated and expanded to additional model systems in future research.

SE15.04

Modeling trypanosome motility in blood suspensions

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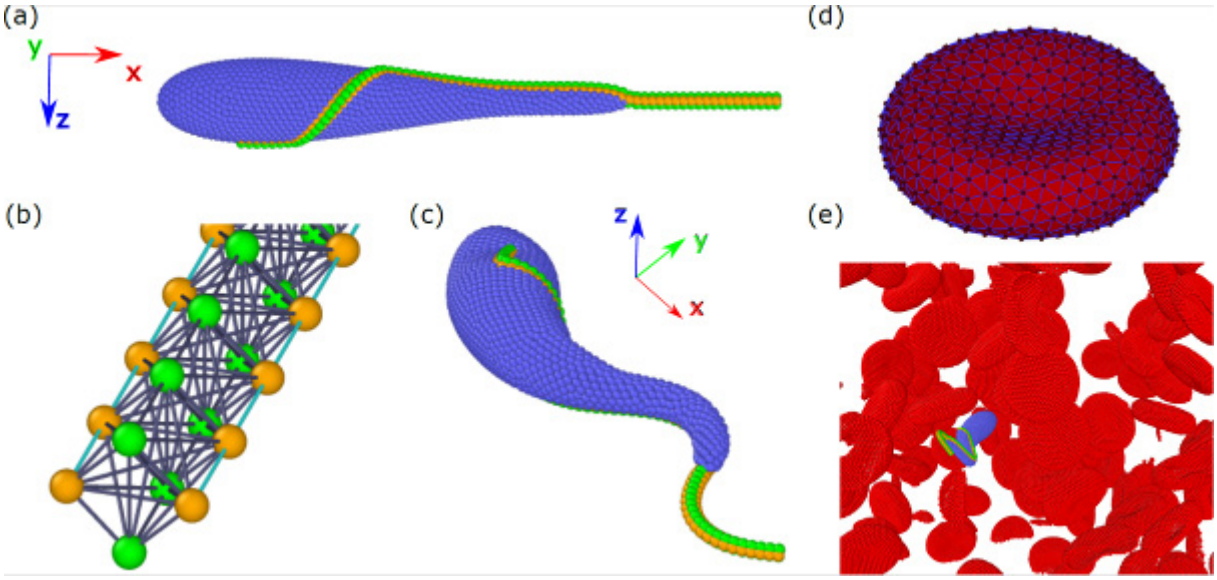
We investigate the vital motility of the protozoan *Trypanosoma brucei* [1] via numerical simulations, in which a trypanosome model is informed by experimental observations. The cell body is represented by a set of vertices distributed homogeneously on a pre-defined elongated surface, forming a triangulated elastic network of springs. This network model incorporates bending rigidity, area conservation, and volume conservation constraints [2]. For the generation of propulsion, a flagellum is attached to the cell body [3]. The flagellum consists of four parallel filaments, two of which are embedded in the body and used for generating a propagating bending wave. We examine the parasite behavior for various conditions, including different flagellum and body stiffnesses, beating frequencies, actuation wavelengths, and amplitudes. Our simulations yield swimming velocities and rotation frequencies around the swimming axis that are in a good agreement with experimental measurements. Additionally, we investigate the importance of various actuation characteristics, such as orientation of the beating plane and the stress-free conformation of the flagellum. We have also started to study parasite motility in a stationary blood suspension, which serves as a first step to understand trypanosome behavior in one of its natural environments such as blood vasculature.

[1] Heddergott et al. (2012). Trypanosome motion represents an adaptation to the crowded environment of the vertebrate bloodstream. PLoS pathogens, 8(11), e1003023.
[2] Fedosov, D. A., Noguchi, H., & Gompper, G. (2014). Multiscale modeling of blood flow: from single cells to blood rheology. Biomechanics and modeling in mechanobiology, 13, 239-258.
[3] Overberg et al. (2024) Modelling trypanosome motility, bioRxiv 2024.09.27.615450

Figure: (a) Trypanosome model [3]. Membrane particles in dark blue, flagellum particles in green (passive) and orange (active). The passive flagellum particles are part of the membrane. (b) Sketch of the flagellum model. (c) Trypanosome with actuated flagellum. (d) Sketch of a triangulated RBC, where the network vertices are connected by springs, from [2]. (e) Swimming trypanosome within a RBC suspension.

Fig. 1



SE15.05

Molecular tools for functional genetics in *Trypanosoma vivax*

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Trypanosoma vivax is the most widespread trypanosome causing animal African trypanosomiasis (AAT). Despite its huge veterinary importance, it is the least studied among the major species of trypanosomes that cause AAT (*T. brucei, T. congolense and T. vivax*). This is partly due to challenging *in vitro* cultivation but also to a great dearth of genetic tools for functional studies. The first *T. vivax*-specific DNA construct was developed over a decade ago for constitutive transgene expression, but since then, there has been little advancement in transgenesis in this parasite.

Here we report the development and application of a toolkit for functional genetics using *in vitro*-differentiated *T. vivax* trypomastigotes with bloodstream form-like biology. We developed methods to routinely modify these cells at specific loci, and used genomic and transcriptomic data to create species-specific constructs for transgenesis. We identified loci suitable for high-level and tightly regulated expression and generated a line stably expressing T7 RNA polymerase and Tet repressor. Using this, we demonstrate the ability to inducibly silence genes using species-specific RNAi constructs, and the expression of ectopic genes. We further demonstrate gene knockout using conventional knockout and efficient one-step homozygous knockout using Cas9 ribonucleoprotein, and have applied these to investigate the function of genes associated with drug resistance and of relevance to vaccine development. Finally, we developed a new transgenic cell line that produces >10,000 independent clones in a single transfection, providing a potential for generation of genome wide high-complexity mutant libraries. This work brings *T. vivax* from a point of being essentially untractable, to now being a new model for functional genetics and provides a huge opportunity to address fundamental biological questions in this important but neglected parasite.

SE15.06

Polycistron perturbation by precision-edited histone H4-tails in trypanosomes

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Trypanosomatid genomes comprise many long polycistronic transcription units that are subject to unconventional transcription controls. Bidirectional RNA polymerase II transcription initiates at sites with modified histones and with unconventional "promoters", for example, and in bloodstream form African trypanosomes, telomere-adjacent polycistrons are transcribed in a monoallelic fashion by RNA polymerase I, producing variant surface glycoproteins (VSGs). Post-translational lysine acetylation in the highly divergent trypanosome histone H4 N-terminal tail has been correlated with transcription control, but such a role has not been directly demonstrated. Here we use precision editing to directly assess histone H4 N-terminal tail lysine function. We used an inducible CRISPR/Cas9 system to delete all (>40) native copies of histone *H4*, replacing them with a single ectopic *H4* gene. Using the resulting histH4one strains, we saturation mutagenized H4 lysine-4 ($H4^{K4}$), $H4^{K10}$, and $H4^{K14}$ and used multiplex amplicon-seq to monitor relative fitness. $H4^{K10}$ mutations were not tolerated, but we were able to derive a panel of strains exclusively expressing novel $H4^{K4}$ or $H4^{K14}$ mutants. Since $H4^{K4}$ acetylation is typically diminished at RNA pol-II transcription initiation sites, we analysed H4 glutamine-4 ($H4^{K4Q}$; constitutively acetylated mimic) mutants in more detail. Transcriptomic and proteomic analyses revealed reduced expression adjacent to RNA pol-II transcription initiation sites and relaxed silencing of telomere-adjacent *VSG* expression sites. This analysis of precision edited $hist^{H4}$one trypanosomes provides direct evidence for polycistronic transcription control by histone H4 N-terminal tails.

SE16.01

From fundamental insights to malaria parasite migration to new vaccine approaches

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The extracellular stages of malaria parasites can undergo gliding motility, a form of migration during which the parasite does not change its shape and allows them to proceed extremely fast. This motility is based on an actin-myosin motor that is located underneath the plasma membrane and has been the focus of many studies including also in *Toxoplasma gondii* (Singer et al. 2023). We will present some of our molecular and biophysical approaches to study gliding motility in Plasmodium sporozoites (Figure 1), the forms of the malaria parasite transmitted by mosquitoes. Sporozoites form in oocysts at the mosquito midgut wall, need motility to exit from these and to enter the salivary glands. Within salivary glands few sporozoites move, probably to distribute in the salivary canals ready for transmission in the flow of saliva. The mosquito deposits sporozoites in the skin, where the parasites immediately proceed to glide at high speeds to enter blood or lymph vessels. Those entering the blood vessels are carried to the liver, where they exit from the circulation and penetrate the parenchyma to invade and differentiate within a hepatocyte. We have identified *Plasmodium* unique proteins or protein domains that are essential for this journey and have also determined some key divergent residues in actin and myosin. During these studies we made a serendipious discovery that lead to a new concept for experimental vaccination of animals. For this we now generate specifically slow growing blood stage parasites that undergo a limited growth within the host and are eventually eliminated by the immune system (Figure 2). These parasites develop normally within mosquitoes and can be transmitted by mosquitoes. In rodent model a single mosquito bite is sufficient for full protection (Sattler et al., 2024). We currently move this concept to human infecting parasites.

Sattler et al., (2024) Experimental vaccination by single dose sporozoite injection of blood-stage attenuated malaria parasites, EMBO Mol Med 16: 2060-79.

Singer and Frischknecht, (2023) Still running fast: *Plasmodium* ookinetes and sporozoites 125 years after their discovery

Figure 1: Scanning electron microscopy of a *Plasmodium* sporozoite. Image by Leandro Lemgruber

Figure 2: Example single shot vaccination of attenuated sporozoites. From Sattler et al., 2024.

Fig. 1

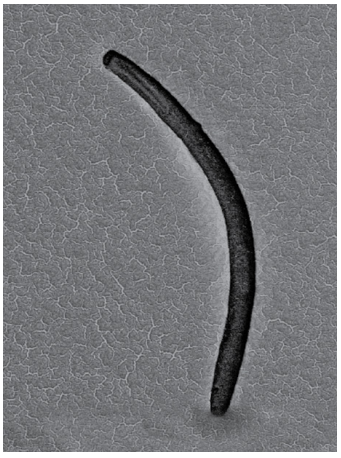
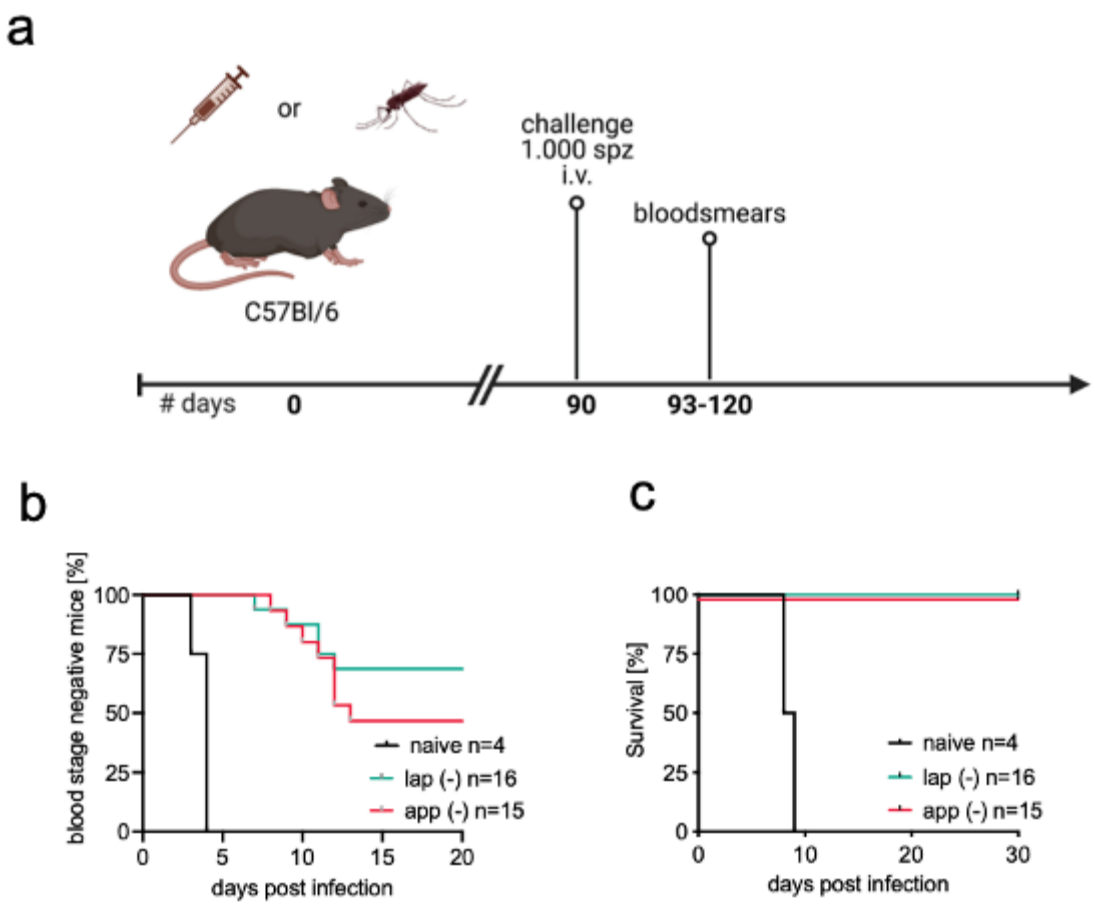


Fig. 2


a
or
challenge
1.000 spz
i.v.
bloodsmears
C57Bl/6
days
0
90
93-120
b
blood stage negative mice [%]
100
75
50
25
0
0
5
10
15
20
days post infection
naive n=4
lap (-) n=16
app (-) n=15
c
Survival [%]
100
75
50
25
0
0
10
20
30
days post infection
naive n=4
lap (-) n=16
app (-) n=15

SE16.02

The genetic basis of parasite persistence and virulence in *Cryptosporidium*

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The single celled protozoan parasite *Cryptosporidium* is a leading cause of diarrheal disease and a major driver of early childhood mortality. Studies in humans and animals have shown marked differences in the severity and duration of this infectious disease. Here we rigorously demonstrate some of these differences to be rooted in parasite genetics. We develop a powerful model for forward genetic discovery taking advantage of the single host sexual life cycle of this parasite. Using two *C. parvum* strains with dramatically different disease outcomes in mice, we mapped three specific loci associated with hypervirulence and persistence through genetic crosses. The parental strains differ by approximately 5,000 single nucleotide polymorphisms, which, combined with *Cryptosporidium's* small genome size, offers high resolution for bulk segregant analysis of the progeny. Five independent crosses demonstrate high significance quantitative trait loci and remarkable reproducibility. We validate our findings through knockout and gene replacement studies in both parents. Swapping the gene encoding the highly polymorphic glycoprotein 60 (GP60), the locus most strongly associated with virulence in our crosses, results in a 50-fold difference in parasite burden. In further experimentation we demonstrate that this is not due to gain of the virulent allele but the result of loss of the non-virulent allele, which has important mechanistic implications. However, swapping GP60 does not confer persistence, and we discover a role for additional previously unstudied secretory proteins encoded on chromosome 7 and 2. We conclude that virulence and persistence are heritable parasite traits, and that they are governed by the activity of distinct proteins secreted by the parasite.

SE16.03

Impact of the filarial infections *O. volvulus, L. loa* and *M. perstans* on the metabolic and immunological profile of lean and overweight/ obese individuals in Cameroon (FIMMIP)

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Type 2 diabetes is among the ten leading causes of death as identified by the WHO in 2019 with the prognosis that type 2 diabetes prevalence will increase the strongest in sub-Saharan Africa reaching an 134% increase by 2045. The FIMMIP study was designed to investigate the impact of filarial infections on metabolic diseases in rural Cameroon. In this open label pilot trial, 2171 participants infected with the filarial nematodes *Onchocerca volvulus* (n=378)*, Loa loa* (n=61)*, Mansonella perstans* (n=122), multiple filarial species (n=165) or filariae-free endemic participants (n=1441), being lean (BMI <25) or overweight and obese (BMI >25) were analysed for their parasitological, anthropomorphic, metabolic and immunological profile. Filariae-infected participants had significantly reduced levels of circulating liver enzymes (ALP, ALT, AST and γGT) as well as increased markers associated with kidney health (urine microalbumin, serum creatinine). Similarly, C-reactive protein, a marker associated with obesity-derived inflammation, was significantly reduced in filariasis patients. Strikingly, glycated hemoglobin (HbA1c) values were significantly reduced in filariasis patients and diabetes prevalence (HbA1c >48 mmol/mol Hb) was 2.3 times lower in the filariasis patients (31% vs. 13.4%). Furthermore, comparing the impact of *O. volvulus, L. loa* and *M. perstans* infection indicated filarial-species dependent differences with *M. perstans* infections inducing the strongest beneficial impact on liver enzymes, CRP levels, kidney markers, and lowest rate of diabetes prevalence (9.4%). Filaria-infected individuals displayed significantly increased levels of adiponectin which correlated with decreased BMI and waist circumference. Interestingly, pro-inflammatory cytokines (IL-1β, IL-6 and IL-18) were significantly altered in a species-dependent manner, with *Mansonella perstans* infected individuals displaying the lowest inflammatory profile. Taken together, our study suggests that filarial infections improve metabolic parameters and protect against the development of type 2 diabetes with *M. perstans* infected individuals showing the most striking effects.

SE16.04

Eimeria bovis-driven effects on host cell organelles during first merogony

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Background: The parasitic protozoan *Eimeria bovis* is one of the most pathogenic *Eimeria* species affecting cattle. *E. bovis*-induced typhlocolitis is a globally spread calf disease with a substantial economic impact on cattle industry. First merogony of *E. bovis* occurs intracellularly in bovine endothelial cells and leads to the formation of macromeronts up to 400 µm in size. Previous findings have shown that *E. bovis*-infected endothelial cells undergo a critical parasite-driven metabolic exploitation. Consequently, *E. bovis*-infected host cells show phenotypical changes and dysfunctions.

Objective: The current project aims to study how *E. bovis* affects the spatial arrangement of host cell organelles and if classical mitochondria-, endoplasmic reticulum- and Golgi-related signaling pathways are changed by infection.

Materials and Methods: Primary bovine umbilical vein endothelial cells (BUVEC; n = 4) were infected with *E. bovis* sporozoites and cultured up to the release of first generation merozoites. At selected time points of merogony I (at days 4, 8, 12, 17, and 22 p. i.), crude protein was extracted from both *E. bovis*-infected cells and non-infected controls. Western blotting was used to quantify the expression of organelle markers (mitochondrial marker: AIF, lysosomal marker: LAMP1, Golgi marker: GM130) and of key molecules of the following pathways: Endoplasmic reticulum protein folding pathway**:** ERO1, ERP44, ERP57, ERP72, GRP94, PDI, ER- and Golgi-associated protein pathway**:** calnexin, PDI, RCAS1, syntaxin 6. To illustrate the spatial organelle distribution, *E. bovis*-infected BUVEC were fixed and stained for ER, mitochondria and Golgi and analyzed by confocal microscopy.

Results: In *E. bovis*-infected host cells, mitochondria and Golgi structures were repositioned close to the parasitophorous vacuole. At day 4 p. i., Golgi structures were frequently found squeezed between sporozoites and the host cell nucleus. At 22 days p. i., the Golgi volume was increased in *E. bovis*-infected cells compared to control cells. Additionally, a significant expansion of the mitochondrial network was observed during macromeront development. Protein expression kinetics revealed an upregulation of most proteins of the ER protein folding pathway (ERO1, ERP57, ERP72, GRP94, PDI). In contrast, proteins of the ER- and Golgi-associated pathways like calnexin, RCAS1, and syntaxin 6 were not changed by *E. bovis* infection.

Conclusion: The intracellular development of *E. bovis* macromeronts induced a spatial reorganization of both the mitochondrial network and Golgi apparatus in host cells. Furthermore, both the overall Golgi and mitochondrial volume increased by infection. The selective upregulation of several proteins of the ER-folding pathway suggested a substantial impact of *E. bovis* infection on the host cellular protein synthesis.

SE16.05

Vascular endothelial cell involvement in the pathogenesis and host-parasite interaction of canid angiostrongylosis: A comparative *in vitro* study in red foxes (*Vulpes vulpes*) and domestic dogs

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Angiostrongylosis caused by the nematode *Angiostrongylus vasorum* is a cardiopulmonary parasitic disease that predominantly affects domestic dogs and red foxes. While the disease in dogs often manifests as respiratory and cardiovascular dysfunctions accompanied by coagulopathies, red foxes largely remain asymptomatic. This study explored the responses of primary aortic endothelial cells from both species to *A. vasorum* antigens, including excretory-secretory products, adult worm antigens, and first-stage larval antigens. The results showed that ESP elicited only mild activation, whereas adult and L1 antigens induced more robust inflammatory responses. The expression of inflammatory markers was time-dependent, reflecting dynamic endothelial activation that evolved over the course of stimulation. Canine endothelial cells displayed a delayed yet pronounced and narrowly focused pro-inflammatory response, potentially linked to tissue damage during infection. In contrast, fox endothelial cells exhibited a rapid and diverse inflammatory profile, consistent with their role as reservoir hosts and the asymptomatic nature of their infections. These findings underscore species-specific host-parasite adaptations, revealing fundamental differences in immune responses between dogs and foxes. They provide valuable insights into the mechanisms driving differential pathogenesis in canine and vulpine angiostrongylosis, highlighting the intricate evolutionary interplay between host immunity and parasite survival strategies.

SE17.02

Do all intestinal parasites affect the host's neuroendocrine system in the same way? A survey on *Anguilla anguilla* infected with different enteric helminths

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Introduction: Fish intestinal helminths induce changes in the tissue architecture and often inflammation at their attachment site. The host''s intestinal neuroendocrine system (NES) regulates the inflammatory mechanism through the release of molecular neuro-mediators. In fish parasitized with cestodes and acanthocephalans, changes in the amount of the NES components have been demonstrated with immunohistochemistry but similar information is lacking for Trematoda and Nematoda.

Objectives: No comparative study has been conducted on the effect that parasites of different taxa have on the intestinal NES in a single fish species. The extent of changes in the NES of *Anguilla anguilla* were investigated in specimens parasitized with species of 4 helminth taxa.

Materials & Methods: Twenty-five specimens of *A. anguilla* were sampled on several occasions from the Comacchio lagoons and Lake Trasimeno. Specimens harbored the following parasite taxa: 5 uninfected, 5 infected with *Deropristis inflata* (Trematoda), 5 with *Proteocephalus macrocephalus* (Cestoda), 5 with *Acanthocephalus rhinensis* (Acanthocephala) and 5 with *Contracaecum rudolphii* A larvae (Nematoda). Antibodies against serotonin and met-enkephalin were applied to histological gut sections from infected and uninfected eels. The obtained microscopic images were analyzed with the open source software ImageJ to quantify the mean number of immunoreactive neurons and endocrine cells in the myenteric plexus and epithelium of the intestine, respectively. Mean length percent of immunoreactive myenteric plexus was also measured.

Results: An increase in the neurons and nerve fibers immunoreactive to both antibodies in the myenteric plexus was observed in infected eel intestines compared to uninfected eels. In eels harboring cestodes, acanthocephalans and nematodes, the mean number of serotonin-immunoreactive neurons was significantly higher compared to uninfected eels and those with trematodes. The number of intestinal epithelial endocrine cells immunoreactive to serotonin and met-enkephalin was significantly enhanced in eels infected with trematodes, cestodes and acanthocephalans.

Conclusion: In *A. anguilla*, different parasite taxa affect the occurrence and density of serotonin and met-enkephalin intestinal components of the NES in distinct ways. Probably the difference is caused by the helminth attachment organs, site of infection and the depth of the worm penetration in the intestinal wall.

SE17.03

Parasite management in young horses

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Infections with gastrointestinal nematodes can be a major health problem in horses. Young horses are more susceptible to severe infections, potentially resulting in clinical disease. Results of 2690 faecal egg counts (FEC) of Swiss horses up to 7 years of age were analyzed to determine the pattern of egg excretion. The mean strongyle FEC measured in eggs per gram of faeces (epg) in the 8 age groups (< 1, 1, 2, 3, 4, 5, 6, 7 years) were (CI) 198 (122 - 306), 481 (386 - 601), 388 (323 - 457), 332 (278 - 395), 293 (240 - 358), 205 (169 - 248), 127 (101 - 158) and 88 (68 - 113) epg (Fig. 1). The reduction from the highest strongyle FEC measured in the yearlings until year 7 was 82%. Mean values of the age groups '< 1 - 3 years' and '4 - 7 years' were 373 and 171 epg resp. ($p<0.0001$). The foals showed the lowest mean strongyle FEC. The main reasons are most likely the relatively low roughage intake from pasture, the milk-feeding and the moderate infection pressure from herbage induced by the mares, most of which only show a low egg excretion. The substantial increase in strongyle egg excretion after weaning is reflecting the period when the majority of foals are transferred to the rearing stables. The proportion of negative samples in the McMaster analysis (sensitivity 50 epg) increased from 42 to 71% between 1 to 7 years. The results indicate that the majority of horses can significantly reduce strongyle FEC between the age of 3 and 7y. Patent infections with *Parascaris* were largely limited to the first 2 years of life, with foals showing the highest values. Our data formed the basis for a reorientation of parasite-management at the Lipizzan stud of the Spanish Riding School in Austria, where parasite control had previously been performed by strategic group treatments. Between 2016 and 2018 all 4 age categories (foals up to 3-year-olds; approx. 150 in total per year) were included in the faecal monitoring. Anthelmintic treatments were age-specific and in a variable mode depending on FEC. For the foals, blanket treatments at the age of 8 weeks with fenbendazole and 18 weeks with pyrantel were performed, mainly directed against *Parascaris*. Further treatment was given after transfer to the gender-specific rearing groups in late summer and - depending on result - in November. The yearlings underwent 4 annual block treatments with diagnostic-related anthelmintic drug selection. To enable sufficient refugia formation, a combination of group-treatment and selective therapy was used for the 2- and 3-year-old horses. Without any negative health effects, the total of anthelmintic treatments per horse during the rearing period could be reduced from 15 to an average of 10. During the study period, the average strongyle FEC in the 1-3-year old horses decreased from 522 epg to 414 epg ($p<0.05$). With *Parascaris*, the reduction in the same period was 95% (from 12.4 to 0.6 epg, $p<0.05$). The results demonstrate a clear potential for a diagnostic-based optimization of anthelmintic usage in young horses.

Fig. 1

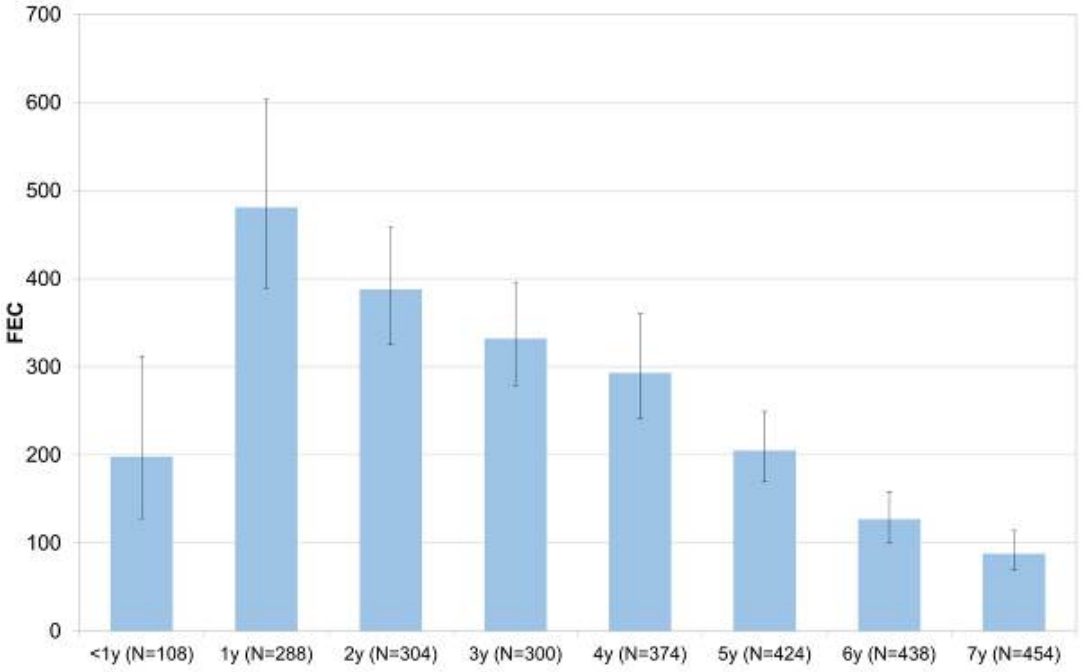

Fig. 1: Mean strongyle egg counts in horses from < 1 to 7 years of age

e

SE17.04

Detecting circulating microRNAs in a mouse model of canine dirofilariasis

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Dirofilaria immitis is a filarial nematode that causes heartworm disease in canines. Heartworm drug and vaccine discovery research is somewhat hindered by the logistical challenges that come with the need to use domestic dogs as definitive host for experimental infections, limiting in vivo heartworm research to industrial labs or institutions with specialised animal facilities. Recent studies have shown that immunodeficient NGS mice can sustain D. immitis infection for at least 15 weeks, marking them as potential surrogate hosts for heartworm clinical research. Several previous papers have reported the presence of 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. These data would support the use of NGS mice as animal model for D. immitis infection, accelerating biomarker and clinical discovery research for this important pathogen. 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 processed using the mirdeep2 algorithm. This identified miRNAs of both host and parasite origin. Forty-one parasite miRNAs were identified. Of these, 36 were present in at least one replicate from day 70 infected plasma samples, and absent from day 70 sham controls. No miRNAs were detected at earlier timepoints. This is the first report of D. immitis miRNA detection in the plasma of heartworm infected mice, with the data appearing qualitatively comparable to miRNA datasets from infected canines at infection timepoints consistent with presence of adult parasites. This work supports the use of NGS mice as a surrogate host for D. immitis infection, and could accelerate biomarker and clinical discovery research for canine heartworm.

SE17.05

Albendazole specifically disrupts the microtubule cytoskeleton and protein turnover in the tegument of the model cestode *Mesocestoides corti*

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Parasitic flatworms, such as cestodes and trematodes, are covered by a syncytial tissue known as the tegument. It consists of a superficial band of cytoplasm (the distal tegument) that is connected by cytoplasmic bridges to multiple cell bodies (cytons) that lay beneath the basal lamina and contain the nuclei. We characterized the organization of the cytoskeleton in the tegument of the model cestode *Mesocestoides corti*, and determined the effects of albendazole and albendazole sulfoxide on its organization. These anthelmintics are known to target beta tubulin in helminths, and have been extensively studied in nematodes, but the specific cells and tissues that are affected are not well understood in parasitic flatworms. We show that microtubules in the distal tegument have a unique organization, with bouquets of microtubules radiating from the cytoplasmic bridges, suggesting a role in intracellular traffic. In contrast, actin filaments were largely absent from the distal tegument. The microtubules of the tegument were specifically sensitive to low, chemotherapeutically relevant concentrations of albendazole and albendazole sulfoxide. This was correlated with the accumulation of secretory material in the cytons, and low concentrations of albendazole strongly reduced the incorporation of newly synthesized proteins in the distal tegument. Unexpectedly, albendazole also induced a global decrease in protein synthesis, which was independent of the activation of the unfolded protein response. Our work identifies the tegument as a sensitive target of benzimidazoles in cestodes, and indicates that translational inhibition may contribute to the anthelmintic effect of benzimidazoles.

SE18.01

Novel intervention strategies for schistosomiasis elimination

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Introduction: Elimination of schistosomiasis as a public health problem by 2030 is a declared goal of the WHO. Pemba Island, Tanzania, achieved this goal in 2017 and now proceeds towards transmission interruption. The existing spatial heterogeneity of *Schistosoma haematobium* infections calls for targeted interventions.

Methods: In the SchistoBreak project, implemented from 2020-2024, new adaptive intervention approaches were investigated for their contribution to elimination. In low prevalence areas, a new schistosomiasis surveillance-response approach, including test-treat-track-test-treat (5T) and snail control activities, was assessed for its sensitivity and potential to prevent recrudescence. In hotspot areas, the impact of a multidisciplinary intervention package, consisting of mass drug administration, behavior change communication, and snail control measures was investigated. Annual school- and household-based cross-sectional surveys were conducted to monitor *S. haematobium* prevalence and infection intensities, schistosomiasis-related knowledge, attitudes and practices, and economic status.

Results: Each year, more than 6000 individuals were surveyed. The 5T strategy was very useful to identify and treat infected individuals. Across the 3 years of implementation, the surveillance-response approach showed a sensitivity of 43% and the low prevalence levels were mostly maintained. In hotspots, prevalences were significantly reduced in schoolchildren and showed a decreasing trend in the community in Year 1 and 3, but slightly increased in Year 2. Hotspot areas were hallmarked by a large number of poor and rural households, and waterbodies containing *Bulinus*. Behavior change communication significantly improved knowledge and attitude scores of exposed schoolchildren. The overall prevalence remained ~1% across all years, with heavy intensities ≤0.3%.

Conclusions: The novel adaptive intervention approaches did not result in interruption of transmission within 3 years. Yet, important insights and evidence were generated that can inform control program decisions and the development of schistosomiasis elimination guidelines.

SE18.02

Investigating hybrid *Schistosoma* spp imported infections to Europe

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Introduction: Human schistosomiasis, affecting an estimated 250 million people, is classically attributed to six *Schistosoma* species, most commonly *S. mansoni* and *S. haematobium*. However, molecular methods used to genetically characterize infections over the last ~15 years revealed a more complex picture, with inter-species hybrids between human/animal *Schistosoma* species being commonly identified. Hybrids are of particular interest due to the potential development of new phenotypes influencing fecundity, hosts" and geographical range, and infectivity; different pathogenicity, drugs" susceptibility, and obstacles to control efforts are also speculated. Considering the potential of *Schistosoma* to spread across borders in suitable conditions, as also witnessed in Europe, vigilance about the types and distribution of hybrids is of interest to understand any impact on disease epidemiology and control.

Objectives: Through a network of European centres specialized in travelers and migrant health, we aimed to expand the knowledge concerning geographical origin and genetic composition of *Schistosoma* infections imported into Europe.

Materials & Methods: We analyzed 95 stool or concentrated/filtered urine samples from patients diagnosed with urinary (n=59; 62%) or intestinal (n=36; 38%) schistosomiasis. *Schistosoma* were genetically characterized using multi-locus analysis of Cox1 and ITS1+2 rDNA regions after PCR and Sanger sequencing. When ITS1+2 data was inconclusive, a partial region of the 18S rDNA was analyzed.

Results: Samples were from individuals infected in sub-Saharan African countries. In one stool, the genetic profile from suggested *S. bovis* infection. Mixed genetic profiles were obtained from 25 (26%) samples suggesting mixed species or hybrid infections. The majority 24 (96%) were associated with urinary schistosomiasis, most of which from Mali (9/24; 37%). Genetic profiles were *S.haematobium/S.bovis* (17/25; 68%), *S. haematobium/S. bovis/ S. curassoni* (6/25; 14%), and *S. haematobium/S. curassoni* (2/25; 8%).

Conclusions: With the possible inaccuracy deriving from countries of potential infection having been identified from travel history collected during routine clinical visits, to our knowledge this is the first time that

Schistosoma hybrids (*Sh/Sb/Sc*) were reported from Ghana and *Sh/Sb+/-Sc* hybrids from Burkina Faso. Deepening the knowledge about the ecology and consequences of the spread of these *Schistosom*a genetic profiles is of paramount importance.

SE18.03

Ticks in the landscape: Forest fragmentation impacts on I*xodes ricinus* density

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Tick-borne diseases constitute a considerable challenge for both human and animal health. Landscape features may influence local tick abundance by modulating abiotic conditions and tick host densities. Understanding these relationships is key to developing effective prevention strategies, particularly in light of increasing anthropogenic land transformation.

This Germany-wide study analysed the relationship of landscape features with local density of the most important tick-borne disease vector in Europe, *Ixodes ricinus*, determined at 83 sites during 2018-2020. In addition to analysing land cover data within a 500 m, 1000 m and 2500 m buffer zone around each site, satellite images were used for a more accurate assessment of habitat fragmentation, amount of edge habitat and presence of paved streets in the 500-m zone. Generalized linear mixed models were constructed, including meteorological variables to account for regional differences in climate. A higher fragmentation index, i.e. ratio of edge habitat to forest area, as well as an increasing proportion of coniferous forest within the 500 m buffer were associated with a significantly lower questing *I. ricinus* nymph density. In the subset of sites dominated by forest or shrub cover, the cumulative length of paved streets also had a significant negative impact. At the same time, increasing total forest cover within the 2500-m buffer was an additional significant predictor of lower tick density in this dataset from a densely populated country, i.e. with some human influence at all sites. These contrasting patterns detected at different spatial dimensions may indicate that *I. ricinus* thrives most in forest patches of intermediate size that form a heterogeneous landscape mosaic with agricultural or urban areas.

SE18.04

Poor efficacy of anthelmintic treatments against gastrointestinal strongyle nematodes in old world camels in Germany due to presence of resistant *Haemonchus contortus* and *Trichostrongylus colubriformis* revealed by deep amplicon sequencing

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Gastrointestinal nematodes pose a significant health risk to grazing livestock, including camelids, and cause high economic losses which are further increased by anthelmintic resistance. The study presented here examined the gastrointestinal parasite communities of Old World Camels (OWCs) in Germany and evaluated the efficacy of anthelmintic treatment. For this purpose, nine OWC-keeping farms in Germany were visited in spring 2023. Selection of the drug used for treatment was done by the farms/veterinarians independently. The FLOTAC method (multiplication factor 1) was used to determine the number of eggs per gram faeces (epg) in 107 OWCs (*Camelus bactrianus*: 86.0%; *Camelus dromedarius*: 6.5%; hybrids: 7.5%) before and 14 days after treatment (paired sample size: 100 OWCs). The software packages eggCounts and bayescount were used to calculated the faecal egg count reduction (FECR). Prevalence and relative abundance of strongyle species were analysed using deep amplicon sequencing of internal transcribed spacer 2 (ITS-2) PCR products (nemabiome analysis). Egg shedding intensities and prevalence differed widely between farms. For dosing of anthelmintics, animals were not weighed with a scale on most farms but only estimated. A significant egg reduction was found on 6/8 farms that treated with an anthelmintic ($p < 0.05$, Wilcoxon matched-pairs signed rank test). However, the FECRs were calculated for seven farms and ranged between 26.6-90.8% after treatment with albendazole, fenbendazole, ivermectin, moxidectin or doramectin indicated anthelmintic resistance. Only treatment with monepantel on a single farm indicated susceptibility (>99% FECR). Diversity of strongyle species was low. In decreasing order, *Trichostrongylus colubriformis*, *Haemonchus contortus, Camelostrongylus mentulatus*, *Cooperia oncophora* and *Trichstrongylus axei* were the most abundant strongyles found in OWCs, most of them shared with domestic ruminants in Germany. However, *C. mentulatus* has, to the knowledge of the authors, not been detected in German domestic ruminants so far. After deworming, *T. colubriformis* and *H. contortus* were strongly dominating the strongyle communities. These species are also known to be frequently resistant in domestic ruminants. In contrast, *C. mentulatus* and *C. oncophora* were consistently eliminated by treatments. The data show that routine treatments for OWCs in Germany show poor efficacy. Since some species are eliminated by treatment and others survive suggests underdosing is not the main problem but that resistant strongyle species lead to treatment failures. However, due to the limited sample size, estimation of animal weight and no drugs licensed for OWCs in Germany, the term resistance should be used with care.

SE18.05

Genomic epidemiology: A new frontier in lymphatic filariasis elimination program

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Diseases caused by filarial nematodes, onchocerciasis and lymphatic filariases, are targeted for elimination with mass drug administration (MDA) as key strategy. Despite decades of MDA, ongoing transmission in some areas remains a challenge. Acceleration of elimination can be achieved by integration of genomic and epidemiological data. However, advances in filarial genomic epidemiology have been slow compared to other parasite groups. Thus, our understanding of transmission dynamics and knowledge of genetic changes within populations over time is limited.

To overcome technical obstacles that have impeded genomic epidemiology, we have developed a WGA-based approach that enables full nuclear genome analysis of individual microfilariae (Mf) without compromising the genome-wide coverage of variants and the reliability of genotype calls. Using this approach, we sequenced geospatial and temporal samples of individual Mf of *Onchocerca volvulus* (n=305) and *Brugia malayi* (field collected n=220, lab strain n=76), and *Wuchereria bancrofti* (n=343).

Our results from these species show that i) nuclear genome diversity analysis can uncover fine-grained genetic structure in the population in geographically close countries compared to mitochondrial haplotype data, ii) the number of reproductively active adults, both female and male, can be estimated by reconstructing maternal and paternal sibship families among Mf using autosomal and sex chromosomal SNPs, iii) reconstructing and temporally tracking sibling relationships across pre- and post-treatment samples results in differentiation between new and established maternal families, suggesting reinfection in some participants and recrudescence in others, and iv) in case of *B. malayi,* while genetic structure of the parasite is determined by both geographical isolation and host species, a distinct population of *B. malayi* is shared by both animals and humans as definitive hosts.

These results provide a solid foundation for using nuclear genome information from Mf for filarial population genomic predictions. Our advances can be used to develop genetic and genomic surveillance tools that could generate essential information to guide elimination programs for lymphatic filariasis and onchocerciasis.

SE18.06

Pre-school age participation in mass drug administration: Analysing the impact on community-wide schistosomiasis control

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Historically, pre-school aged children (PSAC) have not been offered Praziquantel (PZQ), the drug used to treat schistosomiasis, as part of MDA programs. Recently there have been calls to include PSAC, due to recognition of morbidity in this age group and identification of areas with a high prevalence in PSAC [1]. WHO now recommends annual preventative chemotherapy for all age groups from two years old in moderate and high prevalence areas and access to treatment control morbidity for all ages, including PSAC younger than two [2]. To facilitate this, a new formulation suitable for PSAC, called arpraziquantel (L-PZQ) has been developed by Merck KGaA and the Pediatric Praziquantel Consortium [3]. Although the efficacy of L-PZQ has been established at an individual level, it is unknown what the impact of the inclusion of PSAC into MDA will be on a population level.

Intuitively, the effectiveness of including MDA in PSAC will depend on the demography of a population and the relative contact rates of each age group. We have used an individual-based stochastic model to demonstrate the effects of including PSAC in MDA on the prevalence of schistosome infection while varying the contact rates of the PSAC population, and the ability to meet WHO target of elimination as a public health problem (EPHP). We also assess the impact of paediatric drug administration on childhood health using a "Worm Years" metric based on the total infections during an individual"s lifetime as a proxy for morbidity.

We show that including PSAC in MDA will almost always lead to a reduction in morbidity for many scenarios of transmission intensity and drug coverage levels. However, it does not necessarily result in a substantial increase in the probability of EPHP. The proportion of all schistosome infections in each age group is a key factor in determining the effectiveness of different MDA programmes which vary the age groups to be prioritised for treatment.

These results show the importance of understanding the distribution of infections by age group and the underlying demography of the population when planning MDA. Policy makers should also be aware that including PSAC in MDA may not help to reach the WHO target of EPHP. However, a reduction in the average summed worm infection burden at the age children typically start attending school is highly desirable in increasing the long-term benefit of MDA in early childhood.

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SE18.07

A Germany-wide map of I*xodes ricinus* density

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The most common European tick species, *Ixodes ricinus*, acts as the principal vector of various *Borrelia* species, of tick-borne encephalitis virus and several other pathogens of public health relevance in Europe. Comprehensive data on tick abundance and the underlying ecological drivers are crucial for developing awareness and control strategies and assessing future changes in tick-borne disease risk. In the present study, we provide a Germany-wide map of *I. ricinus* density to aid in disease transmission risk assessment. During 2018−2020, questing tick density was assessed at 83 sites across the whole country by drag flagging, whereby 43,676 *I. ricinus* nymphs and 5,668 adults were collected. Correlations between climate, land cover, and monthly *I. ricinus* nymphal density were assessed by multivariable modelling, while accounting for seasonal and regional differences. Long-term climatic data as well as land cover were utilized to project tick density across the whole country. The highest tick hazard was observed in areas near the coast characterized by little annual temperature variability, mild winters and moist springs, and in forest-rich mid-elevation mountain ranges, while the dry eastern region and the south, characterized by high annual temperature variability, showed lower tick density. Additionally, a lower proportion of nymphs relative to adult ticks was collected on average in the east and the south compared to the north-west. The fact that the high tick density in northern Germany is contradictory to a previous estimation based on a combination of regional studies [1] illustrates the need for an extensive and coordinated sampling effort to reliably estimate tick abundance at larger spatial scales. Combined with data on tick-borne pathogens, this study enables estimating the density of infected ticks and consequently the risk of acquiring an infectious tick bite. Moreover, the observed relationships with climate and land cover can help to predict future developments of tick hazard under different climate scenarios in Central Europe.

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SE19.01

Identification of the *Trypanosoma brucei gambiense* genes sufficient for resistance to the human trypanolytic lytic factor

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Trypanosoma brucei brucei is killed by TLF, a human innate immunity complex containing Apolipoproteins A1 and L1 along with Haptoglobin Related Protein. Human infective *T. brucei gambiense* has evolved a mechanism to prevent TLF action and previous work has suggested: first, that the TgsGP gene is necessary but not sufficient for short term growth in the presence of human serum, and second, there is a polymorphism in the haptoglobin haemoglobin receptor (HpHbR) that may reduce the uptake of TLF. Here, we identify a set of genetic changes present in *T. b. gambiense* that are sufficient to confer on *T. b. brucei* Lister 427 the ability to proliferate in culture medium containing 10% human serum.

The ability to resist TLF was assayed by measuring proliferation rate in a modified HMI-11 medium containing 10% human serum (HMI-11HS) in place of the usual 10% heat inactivated foetal bovine serum. Deletion of the TgsGP gene from *T. b. gambiense* Eliane did not prevent proliferation in HMI-11HS although there was sometimes a population crash before resistant cell lines emerged. These cell lines continued to proliferate indefinitely at a rate equivalent to wild type and proliferated in HMI medium containing 50% human serum.

This finding was used to identify other factors involved in resistance to TLF. *T. b. brucei* Lister 427 cells were modified to express TgsGP and the HpHbR gene was deleted. These modifications were not sufficient for survival in HMI-11HS. The cell line was transfected with sheared *T. b. gambiense* genomic DNA and resistant clones selected using human serum. Three separate clones were obtained and RNAseq analysis showed all three had integrated a copy of Tbg.972.2.1820, a polymorphic variant of the homologous gene Tb927.2.3340. The gene encodes a protein of unknown function containing a PLAC8 domain.

To identify the minimum components sufficient for proliferation in human serum, DNA encoding Tbg.972.2.1820 was transfected into a range of cell lines derived from *T. b. brucei* Lister 427. This showed that expression of TgsGP and Tbg.972.2.1820 alone was sufficient for proliferation in both HMI-11HS and in HMI-50% human serum at rates equivalent to *T. brucei*. Thus, expression of TgsGP in conjunction with Tbg.972.2.1820 is sufficient for resistance to TLF.

Both alleles of Tbg.972.2.1820 were deleted in *T. b. gambiense* and cell lines selected in HMI-11, these grew at wild type rates and the gene is therefore not essential. Upon transfer to HMI-50% human serum, the cell lines were unable to survive with live cell numbers undetectable after 24 h. Thus, expression of Tbg.972.2.1820 and TgsGP are sufficient to confer on *T. b. brucei* the ability to proliferate in human serum.

SE19.02

Repurposing *Trypanosoma brucei* as a novel antigen presentation system: The VAST platform for antibody generation

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Panosome GmbH has developed the VAST (VSG-Immunogen Array by Sortase Tagging) platform, a novel technology for eliciting immune responses in mice (1). The VAST platform leverages the unique biology of *Trypanosoma brucei*, a unicellular extracellular parasite and the causative agent of African trypanosomiasis. The parasite's surface is covered by ~10 million copies of Variant Surface Glycoprotein (VSG), consisting of N- and C-terminal domains. The N-terminal domain, approximately 350 amino acids long, is the host-pathogen interaction site, while the C-terminal domain anchors the protein to the membrane via a GPI-moiety (2).

We have engineered the VAST platform by introducing a Sortase A (SrtA) recognition sequence into the N-terminal domain of VSG. SrtA, a bacterial enzyme, forms covalent bonds between molecules with LPSTGG- and GG- (or AA-) motifs (3). This modification enables the attachment of any molecule of interest—synthesized with an LPSTGG-tag—onto VSGs on the *T. brucei* membrane. The resulting high-density display of the molecule creates an immunodominance effect (4), focusing the immune response on the attached antigen. This focusing effect makes the VAST the ideal tool to generate therapeutic antibodies against particularly small epitopes, which are otherwise ignored by the immune system.

Using the VAST platform in mice, we generated a robust immune response against fentanyl, a small molecular synthetic opioid. We isolated eight monoclonal antibody sequences, expressed them as IgGs, and characterized their properties. Affinity studies revealed that these antibodies bind fentanyl with picomolar affinity, supported by x-ray crystallography showing fentanyl buried in the antibody"s binding pocket. *In vivo* experiments demonstrated that administering the antibodies successfully reversed fentanyl intoxication in mice.

In addition to small molecules, the VAST platform has been extended to oncological targets such as Mucin-1 (MUC-1), a glycoprotein expressed in epithelial cells. In cancer, MUC-1 becomes hypoglycosylated with distinct sialylated sugar moieties (5), making it an attractive therapeutic target. The VAST platform elicited strong immune responses against the truncated/small MUC-1 glycans, generating recombinant monoclonal antibodies that bind to cancer cell lines, including OvCar3 and SKOV3.

In summary, the VAST platform repurposes *T. brucei* as a powerful antigen presentation system, enabling robust antibody generation against challenging targets such as small molecules, glycan, and peptide antigens. This groundbreaking technology opens new therapeutic possibilities across a range of diseases.

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SE19.03

Membrane protein dynamics in *Trypanosoma brucei*

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Due to its extracellular lifestyle, *Trypanosoma brucei* is constantly exposed to the immune system of its host. While switching their predominant variant surface antigen (VSG) conveys long-term escape, they also heavily rely on the mobility of their surface coat, which enables them to remove antibodies from their surface (Engstler et al., 2007) . As the fast turnover on the surface must be mirrored in speed for degradation or recycling, we now also turn our attention to the inside of the cell.

New studies using light and electron microscopy revealed a complex, three-dimensional membrane system with overlapping subdomains of classical endosomal markers (Link et al., 2024). Its dynamics however are still unknown. Using advanced three-dimensional single molecule imaging, the HALO-tag as a labelling interface and photostable dyes, the speed and behavior of several marker molecules was measured with high spatial and temporal resolution to elucidate the functional dynamics of the endosomal system.

Since the image modalities changed, a new analysis workflow was developed. To facilitate efficient, easy and unbiased data processing, visualization and analysis of the gathered data, ThirdPeak, a software with graphical user interface was developed (Müller et al., 2024) . After validating its performance and the knowledge gain of using 3D data with VSG diffusion from the cell surface and artificial diffusion data, the dynamics from within the cell were elucidated.

Using membrane-bound markers within the endosomal system reveal slower diffusion compared to the surface, which could be necessary for efficient sorting. Comparing the dynamics of the canonical markers for early, late and recycling endosome, which are present in the same membrane subdomain, confirmed significant different behaviors and speeds between them, with the early endosome marker being highly dynamic like VSG, while the markers for late and recycling endosome remained mostly immobile, potentially enabling recruitment of further downstream effector proteins.

Imaging of lysosomal markers showed a fast, short-lived connection forming between the lysosome and the flagellar pocket, potentially documenting a novel lysosomal stress relief mechanism.

We therefore provide a new tool for the analysis of cellular dynamics. Using the newly gathered information on protein dynamics within the parasite provide further evidence to complement existing hypothesis. Introducing a significant amount of automatization to the data acquisition will allow for even more efficient and detailed insights, helping to better understand this highly efficient sorting system within the parasite.

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SE19.04

Architecture of the *Toxoplasma gondii* apical polar ring and its role in gliding motility and invasion

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In *Toxoplasma gondii*, the conoid comprises a cone with spiraling tubulin fibers, preconoidal rings, and intraconoidal microtubules. This dynamic organelle undergoes extension and retraction through the apical polar ring (APR) during egress, gliding, and invasion. The forces involved in conoid extrusion are beginning to be understood, and its role in directing F-actin flux to the pellicular space, thereby controlling parasite motility, has been proposed. However, the contribution of the APR and its interactions with the conoid remain unclear. To gain insight into the APR architecture, ultrastructure expansion microscopy was applied to pinpoint known and newly identified APR proteins (APR2 to APR7). Our results revealed that the APR is constructed as a fixed multilayered structure. Notably, conditional depletion of APR2 resulted in significant impairments in motility and invasion. Electron microscopy and cryoelectron tomography revealed that depletion of APR2 alters APR integrity, affecting conoid extrusion and causing cytosolic leakage of F-actin. These findings implicate the APR structure in directing the apico-basal flux of F-actin to regulate parasite motility and invasion.

SE19.05

Plasmodium actin-like protein Alp2b plays vital roles in male gametogenesis

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Sexual reproduction and subsequent transmission of *Plasmodium* depend on the formation of motile male gametes, which are rapidly formed through microgametogenesis, an intricate process involving simultaneous nuclear replications and axoneme formation. This results in the emergence (exflagellation) of eight gametes from a single male gametocyte. Actin-like proteins (Alps) are evolutionarily divergent apicomplexan-specific members of the actin superfamily and, given their prevalence, likely confer specialised functions in the parasite. The *Plasmodium*-specific Alp2b contains several unique insertions into the conserved actin core structure, including the enlargement of the D-loop and H plug. However, the exact function and stage-specific contribution of Alp2b in parasite progression are unknown.

The deletion of Alp2b in *P. berghei* did not affect blood stage growth or gametocyte commitment but resulted in a complete block in exflagellation and thus transmission to mosquitoes. Male gametes showed a delay in axonemal development and were unable to undergo nuclear segregation. This phenotype was partially rescued by complementation of *P. falciparum* Alp2b, indicating a degree of cross-species conservation. Replacement of the *P. berghei* Alp2b D-loop or H-plug with the respective sequences of actin abolished exflagellation. This indicates that the larger Alp2b-specific D-loop and H-plug are functionally critical. More selective mutations in each region identified critical amino acid residues within D-loop, while the conserved residues at the tip of H-plug are interchangeable with the corresponding actin sequence. Taken together, this shows that Alp2b function relies on specific conserved amino acid residues within its unique regions, while some residues may also contribute structurally.

In this study, we show that Alp2b is a critical factor for microgametogenesis and that its absence completely inhibits the production of male gametes, thus sexual reproduction and transmission. This provides the first insight into a previously unexplored member of the actin superfamily and the evolution of its members to facilitate parasite transmission.

SE21.01

SNARE-mediated hemoglobin trafficking unveils new endocytic sorting mechanisms in malaria parasites

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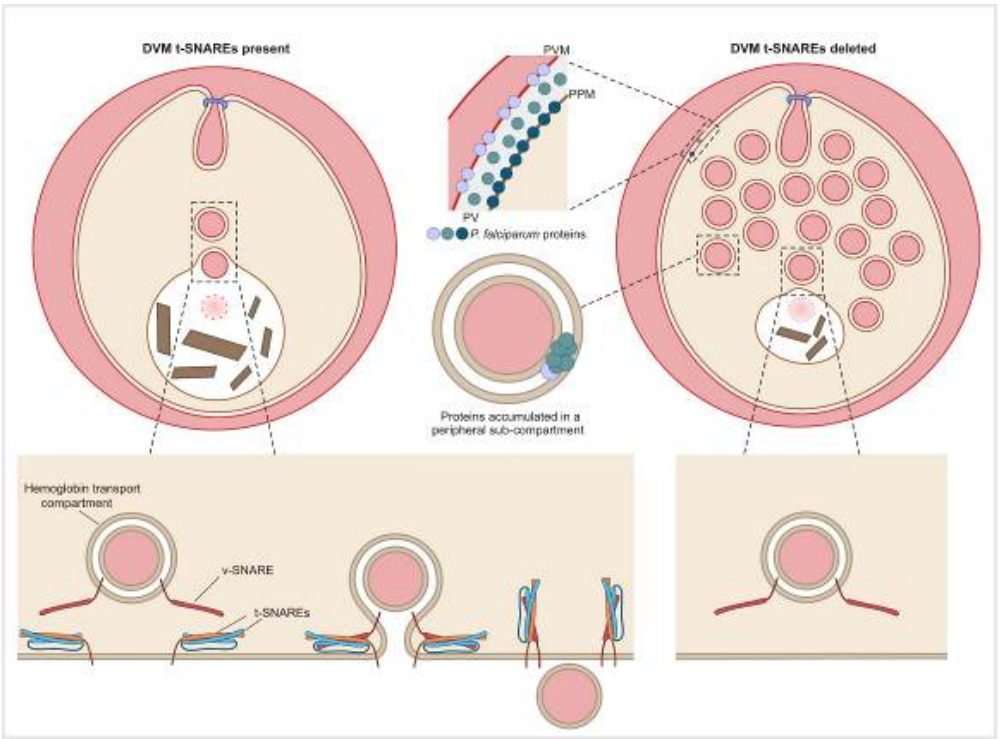
Throughout its intraerythrocytic development, the malaria parasite endocytoses up to 80% of the red blood cell´s cytoplasm which is trafficked within hemoglobin transport compartments (HTCs) to the digestive vacuole (DV) for degradation. Endocytosis and catabolism of hemoglobin are essential survival strategies of the parasite that modulate antimalarial drug efficacy. However, the mechanisms underlying endocytic delivery of hemoglobin to the DV remain poorly delineated. Here, we investigate the role of *Plasmodium* SNARE proteins in the fusion between HTCs and the DV membrane (DVM). SNARE proteins are important fusogens of vesicles (v-SNAREs) and target organelles (t-SNAREs) which interact in *trans* to mediate membrane unification. We have identified two DVM-resident SNARE proteins as key mediators of endocytic hemoglobin delivery in *Plasmodium falciparum*. Their conditional genetic inactivation resulted in the persistence of HTCs that appeared unable to fuse with the DVM. This culminated in intracellular accumulation of undigested hemoglobin and parasite death. We found that both proteins associate with the vacuolar membrane independent of endosomal delivery, indicating that they function as *bona fide* t-SNAREs. We have identified two additional SNARE proteins on the HTC surface that might engage the vacuolar t-SNAREs to form a functional *trans*-SNARE complex at the HTC – DV interface. In-depth analysis of fusion-defective parasites further revealed that proteins originating from the parasite´s two surrounding membranes are severely depleted from the HTCs. By contrast, soluble proteins of the parasitophorous vacuole appeared to be concentrated in a peripheral HTC sub-compartment. Ultrastructure expansion microscopy suggests that this redistribution of internalized parasite proteins already occurs during hemoglobin uptake. Hence, our findings identify an essential v-SNARE – t-SNARE axis in hemoglobin delivery and provide evidence for selective protein sorting during endocytosis of host cell cytosol by malaria parasites.

Fig. 1



SE21.02

A central pair assembly complex revealed by combinatorial RNAi and ultrastructure expansion microscopy

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The protozoan pathogen *Trypanosoma brucei* contains a single motile flagellum which is essential for its motility, infectivity and transmission. This flagellum is highly conserved making *T. brucei* an important model system for understanding its structural assembly. Motility of the flagellum is orchestrated by a central pair (CP) of microtubules which emerges from the "basal plate", an electron-dense structure situated at the distal end of a specialised compartment called the "transition zone" (TZ). Previously, we defined a high quality, fully validated TZ proteome. From this proteome, we identified two basal plate components, basalin and TZP103.8, and demonstrated their essential role in nucleating the CP microtubules and, therefore, motility [1,2].

Here, using a novel RNAi screen on the TZ proteome, we identify an additional two CP Assembly Proteins (CPAPs), bringing the total to four. Using three-colour ultra-expansion microscopy (UExM) co-localisation we performed a cartographical analysis to determine CPAP nanoscale organisation. We show that three of the examined CPAPs "cap" the distal, immature pro-basal body (pBB) and, at the mature TZ, form a complex arrangement of toroids that surrounds a central core containing TZP103.8. Using combinatorial epitope tagging, a novel mutagenesis and UExM analysis of the CPAPs, we show that one of these is the "master recruiter" at the pBB.

Based on our evidence and given that the pro-basal body is the progenitor of the basal body (BB) which gives rise to the flagellum, we propose that the pBB contains a "pre-basal plate". As the pBB body matures to the BB, this pro-basal plate remodels to form the basal plate on the new TZ. Once the mature basal plate forms, downstream effectors, including TZP103.8, are recruited to build a "central pair assembly complex" (CPAC) that nucleates the CP microtubules. Finally, we speculate that although the proteins described here are divergent, they serve highly conserved functions for motile cilia implying that more advanced bioinformatics methods will reveal orthologous candidates in other organisms.

1. *Dean S, Moreira-Leite F, Gull K. 2019 Basalin is an evolutionarily unconstrained protein revealed via a conserved role in flagellum basal plate function. eLife **8**, e42282. (doi:10.7554/eLife.42282)*
2. *Dean S, Moreira-Leite F, Varga V, Gull K. 2016 Cilium transition zone proteome reveals compartmentalization and differential dynamics of ciliopathy complexes. Proc Natl Acad Sci USA **113**, E5135–E5143. (doi:10.1073/pnas.1604258113)*

SE21.03

Riveted to the spot: Identification of novel *P. falciparum* suture proteins involved in the maintenance of the gametocyte subpellicular microtubule corset

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During its complex life cycle, *Plasmodium falciparum* undergoes dramatic morphological transitions, particularly during gametocytogenesis, when the parasite develops a distinct banana-shaped (falciform) form. This unique morphology is driven by the elongation of the **inner membrane complex (IMC)** and the underlying **subpellicular microtubules (SPMTs)** as the gametocyte matures. Together with the parasite plasma membrane (PPM), these components form the pellicle, a critical structure for maintaining the parasite"s shape. Despite its importance, the molecular mechanisms underlying the formation and stabilization of the pellicle remain poorly understood.

We recently identified ***Pf*SPM3**, a novel protein associated with the subpellicular microtubules (SPMTs), which is critical for the development of falciform gametocytes. To investigate the molecular function of *Pf*SPM3, we performed a BioID proximity labeling assay using *Pf*SPM3 as bait, followed by mass spectrometry to identify potential interacting proteins. Using live-cell fluorescence microscopy, we created an atlas of the subcellular localization of selected candidates.

Unexpectedly, alongside proteins co-localizing with the longitudinal SPMT structures, we identified several proteins localized to transversal structures resembling the **IMC sutures**—proteinaceous fibers that connect the IMC plates. To further explore their roles, we generated knockout lines to assess the functions of proteins associated with either microtubule or suture-like localizations.

Our findings reveal multiple new suture proteins and suggest that some of these proteins are concentrated in specific regions of the sutures, where they may mediate connections between the IMC and the underlying SPMTs. Notably, disruption of one suture-like candidate caused a dramatic loss of the falciform shape of late stage gametocytes and affected the localization of *Pf*SPM3 itself.

SE21.04

The remarkable metamorphosis of malaria parasites inside the host erythrocyte

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Malaria parasites replicate inside erythrocytes of the host. To enter an erythrocyte, the parasite binds to the erythrocyte surface and then pushes itself into the host cell, so that it ultimately resides within a membrane-bound compartment – the parasitophorous vacuole (PV) – inside the erythrocyte. As the parasite grows, the membrane of the PV (PVM) must also expand to accommodate the expanding parasite. Inside the erythrocyte, the parasite is subject to the surveillance of erythrocytes by the spleen where old, small and deformed parasites are removed. To avoid removal and successfully pass through the spleen, erythrocytes must be very flexible. Therefore, inside the infected erythrocyte the parasite must also be very flexible. However, the invasive form of the parasite is very rigid. We have now found that very soon after invasion the parasite undergoes a metamorphosis, rapidly transforming from a small, round cell to a larger, very flexible amoeboid-shaped cell with a significantly larger surface area than the merozoite. This transformation requires the parasite protein PV6; parasites lacking this protein remain small and spherical after invasion. Furthermore, erythrocytes infected with parasites lacking this protein are more likely to be removed in eh spleen. As PV6 is a phospholipid transfer protein, we speculate that this protein is responsible for the transfer of parasite lipids to the PVM. By expanding the PVM, PV6 allows the surface area of the parasite itself to increase and the parasite to assume an amoeboid shape, which in turn provides the parasite the flexibility to pass through the spleen.

In the human malaria parasite *Plasmodium falciparum*, but not in *Plasmodium knowlesi*, another human malaria parasite, PV6 is additionally required for completing the development of the parasite in the erythrocyte. *P. falciparum* parasites lacking this protein are smaller than wildtype parasites and, in many cases, their PVM becomes porous. Hence, PV6 has an essential role for the metamorphosis of the parasite after invasion and, in the case of *P. falciparum*, the complete development of the parasites. This is the first investigation of the parasite very soon after invasion and the expansion of the PVM at later stages in the intraerythrocytic cycle.

SE21.05

An accelerated *in vitro* maturation model of *Toxoplasma gondii* tissue cysts for the investigation of co-opted cyst wall components

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Mining previous transcriptomics data from all *Toxoplasma gondii* life cycle stages, we identified several genes with expression peaks exclusive to tissue cysts and oocysts, encoding secreted proteins. Here, we address the question whether these proteins are indeed incorporated in these protective wall structures in both life cycle phases. Given the absence of an *in vitro* culturing system for oocysts, we tested this first in tissue cysts and leveraged an optimized culturing pipeline to localize epitope-tagged proteins and study the effects of gene knockouts on tissue cyst formation and morphology.

Current alkaline pH-based induction of tachyzoite to bradyzoite differentiation *in vitro* inadequately captures the biology of tissue cysts. To address these limitations and to produce more mature cysts, we developed an accelerated and scalable model system using alkaline pH combined with serial passage and re-infection with bradyzoites. The resulting later generations of cysts exhibit marked morphological differences compared with those produced under standard conditions, indicating slower replication rates. RNA-Seq analysis further revealed significant expression differences between early and mature *in vitro* cysts, shedding light on the molecular pathways driving tissue cyst maturation.

This study not only advances our understanding of tissue cyst biology but also offers insights into the elusive biology of oocysts. These findings have the potential to inform future therapeutic strategies targeting chronic and transmissive stages of *T. gondii.*

SE21.06

Sarcoendoplasmic reticulum calcium ATPase is an essential and druggable *lipid-dependent* ion pump in *Toxoplasma gondii*

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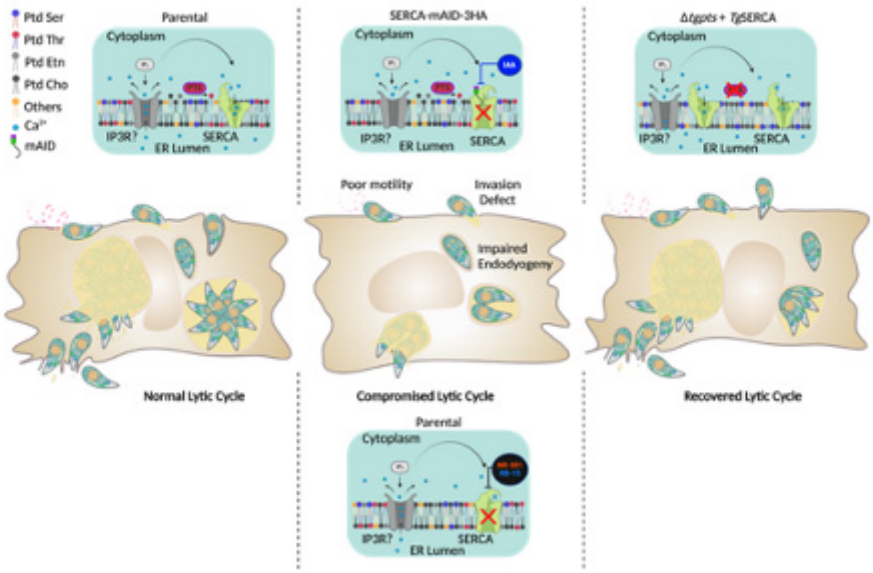
Toxoplasma gondii is a common intracellular pathogenic protist causing acute and chronic infections in many warm-blooded organisms. Calcium homeostasis is pivotal for its asexual reproduction in mammalian host cells, and sarcoendoplasmic reticulum calcium-ATPase (SERCA) is considered vital for maintaining ion homeostasis within the parasite. This work studied the physiological relevance, structure-function relationship and therapeutic value of SERCA in the acutely-infectious tachyzoite stage of *T. gondii*. A conditional knockdown of SERCA, localized in the endoplasmic reticulum, by auxin-inducible degradation is lethal due to severe defects in parasite replication, motility and invasion. The latter phenotypes are caused by dysregulated micronemal secretion and calcium homeostasis in the SERCA-depleted mutant. Structure-function modeling and ligand docking of *Tg*SERCA with a library comprising >5000 chemicals identified two ATP mimics (RB-15, NR-301) that inhibited tachyzoite growth by impairing the parasite motility, invasion, microneme secretion and calcium levels. Finally, ectopic expression of SERCA restored the lytic cycle of a phosphatidylthreonine-null and phosphatidylserine-enriched mutant with perturbed calcium homeostasis, motility, invasion and egress. Not least, these lipids are primarily expressed in the parasite ER, co-localizing with SERCA. In summary, we demonstrate SERCA as an indispensable and druggable "lipid-assisted" calcium pump in *T. gondii* and report new "drug-like" molecules with therapeutic potential against acute toxoplasmosis.

Fig. 1



SE21.07

Mitochondrial vulnerabilities in dormant *Toxoplasma gondii* bradyzoites revealed by drug screening, proteomic and metabolomic analysis

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Toxoplasma gondii is a widespread foodborne parasite that causes chronic infections, which current treatments fail to eradicate. This failure results in recurring acute disease in both humans and animals. Unlike the rapidly replicating tachyzoite stage, the persistent bradyzoite stage remains challenging to study due to its non-replicating nature and the limited availability of reverse genetic tools. Consequently, it is difficult to determine whether parasite drug tolerance is responsible for the inability of existing treatments, such as Atovaquone, to eliminate parasites and prevent recurrence.

To address this challenge, we developed a scalable *in vitro* culture system using human myotubes that enables the generation of mature tissue cysts at scale. To identify effective drugs and their targets, we screened over 650 compounds from the MMV Pathogen Box and a selection of myxobacterial compounds against both pan-resistant bradyzoites and tachyzoites. We then employed metabolomics, proteomics, and chemical mutagenesis to pinpoint essential parasite processes.

Metabolic profiling and stable isotope-labeling revealed that Buparvaquone and MMV1028806, two hit compounds, inhibit pyrimidine synthesis and disrupt the TCA cycle in tachyzoites, similar to Atovaquone, a known bc1-complex inhibitor. Interestingly, both pathways exhibit reduced activity during chronic infection. Furthermore, the metabolic profiles of treated bradyzoites resemble energy starvation responses, characterized by increased AMP levels and depletion of glycolysis intermediates and phosphocreatine, a metabolite previously unknown in this parasite. These findings suggest that the mitochondrial electron transport chain (mETC) is crucial in both parasite stages but serves distinct functions.

Argyrin D, a myxobacterial compound known to inhibit translation by binding to elongation factor G in other microbes, also effectively targeted bradyzoites and inhibited tachyzoite replication. *T. gondii* possesses three translationally active compartments. Stable-isotope and time-resolved proteomics demonstrated that Argyrin D blocks translation of two mitochondrially encoded proteins after 12 hours of treatment, while after 24 hours, a broader proteomic response is observed in non-mitochondrial compartments. This suggests that the mitochondrion is translationally active in dormant bradyzoites and highlights the need for continuous proteomic remodeling of this organelle during chronic infection.

Collectively, our data indicate that the mitochondrion may constitute a vulnerability of the parasite. These findings offer insights into the failure of Atovaquone to eradicate *T. gondii* and prevent recurring infections.

SE21.08

The impact of DNA replication on the evolutionary landscape of *Trypanosoma cruzi* genomic compartments

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Introduction *Trypanosoma cruzi* possesses a highly repetitive genome, primarily due to the presence of multigene families organized in large clusters. This genome is compartmentalized into two distinct regions: Core, containing conserved and/or hypothetical genes, and Disruptive, characterized by rapidly evolving members of multigene families (MGFs). Some MGF members, such as DGF-1 and gp63, are distributed across both compartments. In eukaryotes, DNA replication initiates at specific regions called origins, which are "licensed" when the prereplication complex binds to these sites. During the S-phase of the cell cycle, only a subset of licensed origins is activated. A previous study constructed an atlas of replication origins in the *T. cruzi* genome, identifying origins licensed by Orc1Cdc6, classified as predominant, flexible, or dormant, as well as origins independent of Orc1Cdc6 (ORC1-free). Predominant origins are associated with multigene families, while ORC1-free origins are primarily found in the Core compartment (Vitarelli et al., 2024,doi:10.1128/mbio.00319-24).

Objectives This study aimed to investigate whether different origin types, and consequently distinct DNA replication dynamics, correlate with the genetic variability observed in the genomic compartments.

Materials & Methods We used the epimastigote form of the *T. cruzi* CL Brener strain to calculate replication fork directions and identify origins, termination sites, initiation zones, and termination zones. SNPs were mapped across the genome, and their distributions were analyzed in relation to genomic regions, zones, and gene families. Origin firing efficiency was also evaluated. To localize Orc1b, a potential prereplication complex component, and Mcm7, a key subunit of the MCM helicase essential for DNA replication, we conducted ChIP-seq experiments.

Results Our analysis revealed a higher SNP density in predominant origins compared to flexible or dormant ones. Similarly, frequent termination sites exhibited an elevated number of SNPs. Interestingly, Orc1Cdc6-containing origins were fired more frequently than ORC1-free origins. Additionally, Orc1b and Mcm7 were more abundant in Orc1Cdc6-containing origins (60% and 12%, respectively) compared to ORC1-free origins (6% and 2%, respectively).

Conclusion We propose that the lower efficiency of origin firing in the Core compartment, likely due to the absence of Orc1Cdc6 and corroborated by reduced Orc1b and MCM levels, contributes to its low genetic variability. Conversely, the high efficiency of origins associated with multigene families, coupled with frequent terminations in specific regions, promotes SNP generation in these families.

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SE22.01

Antigenic variation of malaria parasites counteracts the host immune system

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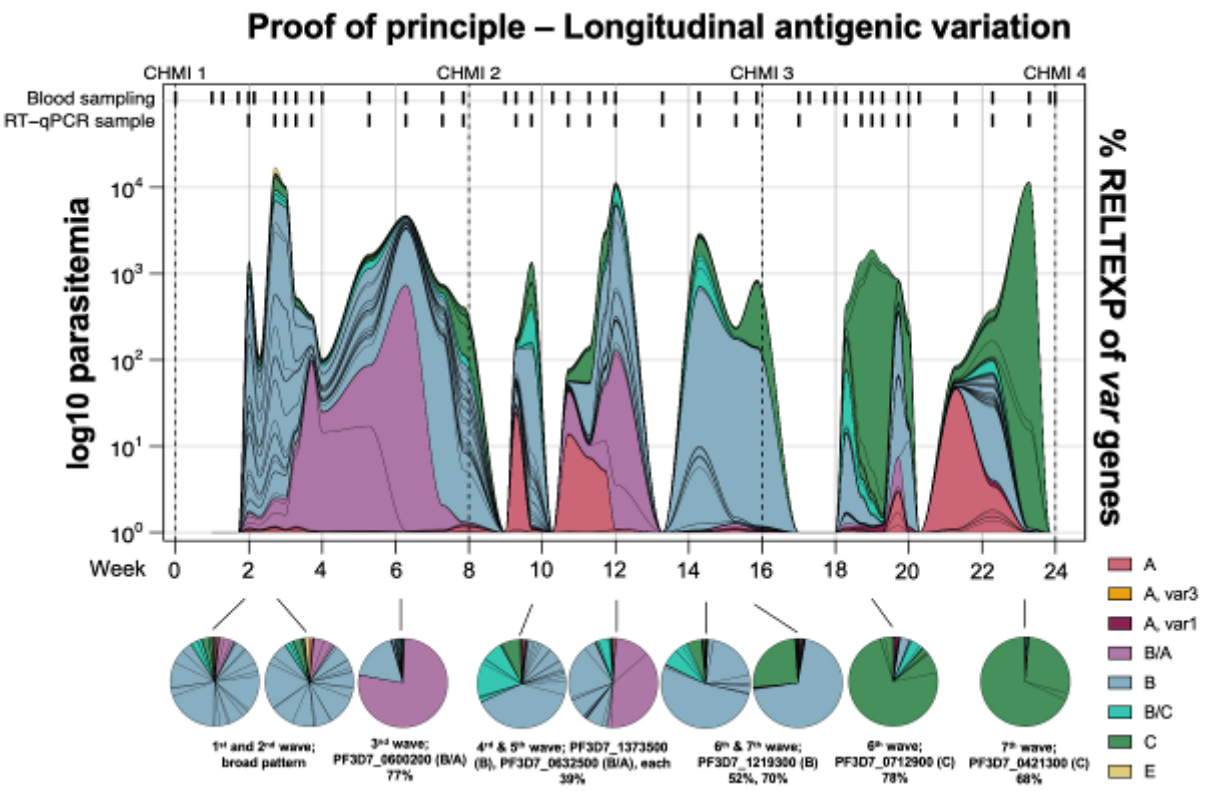
The pathogenesis of falciparum malaria is related to the expression of a highly polymorphic gene family known as *var*, which encodes the major surface antigen *Plasmodium falciparum* erythrocyte membrane protein 1 (PfEMP1). Infections with Plasmodium falciparum can be long-lasting and are characterized by periodic peaks and troughs in peripheral parasitemia, which is hypothesised to rely on antigenic variation of PfEMP1, but this long-standing antigenic variation hypothesis has never been demonstrated in human infections.

Analyzing samples from a longitudinal study of 56 controlled human malaria infections in Gabonese adults using cryopreserved sporozoites (Sanaria® PfSPZ Challenge [NF54]) we provide evidence for an association between successive peaks of parasitemia and expression of distinct *var* gene variants. Our data link absence of expression of certain *var* genes with recognition of the encoded PfEMP1 by antibodies that were either pre-existing or gained during infection. An exception to this rule is the first wave of parasitemia at the infection onset in naïve or less pre-exposed individuals, in which parasites express a highly diverse *var* gene pattern consisting of subtelomerically located group B and severity-associated group A genes. With increasing immunity and declining parasitemia during the course of infection, parasites from all longitudinally tracked volunteers show a shift towards a more homogenous expression of single variants of the *var* gene group B/C and C. Staging based on preliminary RNA-seq data suggest higher proportions of circulating schizonts in waves with parasites expressing C-type *var* genes.

With this *in vivo* study, we provide mechanistic insights into the gradual exhaustion of the *var* gene repertoire across different parasite waves, most likely driven by an increase of PfEMP1 antibodies. With increasing length of infection, peripheral parasitemia decreases and the circulating parasites express type C *var* genes. This observation indicates that the parasites withhold the entire set of group C *var* genes until later phases of infection, possibly at the expense of cytoadhesive capabilities.

Fig. 1


Proof of principle – Longitudinal antigenic variation
CHMI 1
CHMI 2
CHMI 3
CHMI 4
Blood sampling
RT–qPCR sample
log10 parasitemia
% RELTEXP of var genes
Week
A
A, var3
A, var1
B/A
B
B/C
C
E
1st and 2nd wave; broad pattern
3rd wave; PF3D7_0600200 (B/A) 77%
4th & 5th wave; PF3D7_1373500 (B), PF3D7_0632500 (B/A), each 39%
6th & 7th wave; PF3D7_1219300 (B) 52%, 70%
8th wave; PF3D7_0712900 (C) 78%
7th wave; PF3D7_0421300 (C) 68%

SE22.02

Filarial-induced vaginal eosinophils – Key players for the immunity against sexually transmitted infections

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While more than 1 million sexually transmitted infections (STIs) are acquired daily, sub-Saharan Africa bears approximately 40% of the global burden, overlapping with a high prevalence of helminth infections. One reason might be helminths' immunomodulatory capacity, which supports parasite survival but might impair immunity against bacterial and viral infection. Indeed, we and others showed that helminths are a risk factor for Human Immunodeficiency Virus (HIV) and Human Papillomavirus (HPV) infections. Since helminths do not directly colonise or pass through the female reproductive tract, the mechanisms of impaired vaginal immunity remain uncertain. To decipher parasitological, immunological, and pathological factors that might drive impaired vaginal immunity against STIs, we developed a filaria (*Litomosoides sigmodontis*) HSV-2 co-infection mouse model. We observed exacerbated vaginal inflammation in *L. sigmodontis*-HSV-2 co-infected mice during patency accompanied by increased infiltration of vaginal eosinophils and innate lymphoid cell subpopulations (e.g. ILC2) compared to HSV-2 mono-infected mice. Furthermore, pro- and anti-inflammatory cytokines and chemokines like Eotaxin-1 were increased in the vaginal lumen of these co-infected mice and eosinophil infiltration seems to depend on the CCR3 signalling pathway. Moreover, we revealed a delayed HSV-2 spread towards neurons and reduced epithelial cell lysis within the vaginal tissue of co-infected mice. HSV-2-driven vaginal pathology was not enhanced in acute and pre-patent *L. sigmodontis*-infected mice, highlighting that the immunomodulatory capacity of chronic filarial infections impairs vaginal immunity against STIs. Depletion of eosinophils (dblGATA mice) even enhanced the exacerbated vaginal pathology including increased ulceration and induced neurological symptoms. These findings suggest that *L. sigmodontis*-induced eosinophils play a key role in vaginal immunity against STIs and provide mechanistic insights into the reported clinical associations between human helminth infections and the increased risk to STIs.

SE22.03

The genetic basis of *Drosophila*-trypanosomatid interaction

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The overwhelming majority of trypanosomatid research focuses on the few species of medical relevance, however, the vast majority of trypanosomatids infect wild animal populations only. Recently, trypanosomatids have been discovered to be common insect parasites in the wild, including different species of *Drosophila*, which presents a great opportunity for insect-trypanosomatid research. *Jaenimonas drosophilae*, is a natural and virulent trypanosomatid parasite of *Drosophila*, that decreases fecundity and causes pupal mortality in *D. melanogaster.* Here, we investigate the genetics of diptera-trypanosomatid infection studied from both the host and parasite sides. To unveil the host response to the infection, we performed screening of ~150 *Drosophila* Genetic Reference Panel (DGRP) lines for susceptibility to the *J. drosophilae* which showed a wide variation in the parasite susceptibility and further performed GWAS to investigate the genetic basis of the host response. We compared the other available DGRP phenotypes with the susceptibility to *J. drosophilae* and found a correlation with traits responsible for behaviour, memory and pathogen survival, as well as with *Wolbachia* endosymbiont presence. To reveal how the parasite overcomes *Drosophila* gut defences and establishes in the host, we sequenced *J. drosophilae* genome and performed a transcriptome study to compare the parasite gene expression between the parasite during establishment in *Drosophila* gut and *in vitro*. The analysis of differentially expressed genes of parasite in the gut compared to in vitro log growth phase showed GO terms enrichment for metal ion binding pathway, and other genes involved in various processes such as ATP-binding and microtubule movement. Our study shows that the *Jaenimonas-Drosophila* system has the potential to be a powerful model for investigating the effects of trypanosomatids on insect hosts, and for further understanding insect immunity, mechanisms of host-parasite interaction and host-parasite co-evolution.

SE22.04

Impact of parasitism on fatty acid profiles of *Poecilia reticulata*

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Fatty acids, especially long-chain polyunsaturated fatty acids (LC-PUFA) are important to many physiological processes in fish, including reproduction, immunity and neural development. While fish constitute rich sources of omega-3 LC-PUFAs, mainly docosahexaenoic acid (DHA; 22:6n-3) and eicosapentaenoic acid (EPA; 20:5n-3), they have a limited enzymatic capacity to convert C18 PUFA such as 18:3n-3 to EPA and DHA. Thus, LC-PUFAs must be naturally acquired via the diet from primary producers (*e.g.* photosynthetic microalgae, heterotrophic protists and bacteria). DHA and EPA are precursors for eicosanoids, which are integral for immune function in fish, however the impact of ectoparasitic infection on these essential fatty acids remains largely unexplored. Here, utilising the guppy-*Gyrodactylus* host-parasite model system, we assessed the impact of parasitism on the fatty acid profiles of fish. Briefly, adult female ornamental guppies (*Poecilia reticulata*) were split into two treatment groups, uninfected, and those infected with the monogenean ectoparasite *G. turnbulli*. Following a 17-day infection trajectory, fish were further subdivided into three infection categories: resistant (parasite population that failed to increase with the infection being cleared or a small number of worms persisting), responder (parasite numbers initially increased but plateau or decreased over time indicating a delayed immune response) and susceptible (parasite population that kept increasing), and the liver and fillet harvested and used for analysis of total fatty acids. Given that freshwater fish face higher extinction rates than any other vertebrate group, understanding the interplay between infectious diseases and fatty acids which are fundamental for fish health and welfare is of paramount importance.

SE22.05

Host-specific platelet-activating factor acetylhydrolase selectively remodels diacylglycerophospholipids to control schistosome development

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Introduction: Schistosomiasis, a major neglected tropical disease, is currently treated with only one drug, praziquantel (PZQ), effective only against adult worms. However, high reinfection rates and potential development of drug resistance following widespread use of PZQ emphasize the need for a deeper molecular understanding of the host-parasite crosstalk as a basis for urgently needed novel drugs. Here, we identify and characterize a soluble schistosomicidal phospholipase that influences the diacylglycerophospholipid metabolism and, thereby, the survival and development of *Schistosoma mansoni*.

Methods and results: We found that MsPAFAH, the mouse-specific isoform of the platelet-activating factor acetylhydrolase (PAFAH), was upregulated during schistosome infection in mice. Using ultra-performance tandem mass spectrometry and electron microscopy imaging, we demonstrated the lethal effects of recombinant mouse, but not human, PAFAH on all juvenile and adult parasite stages *ex-vivo*, strongly impairing fecundity, pairing stability, and reproductive organ integrity in worms, in addition to altering stem cell development. This activity was associated with substantial sex-dependent changes in ether-phospholipid composition and distribution within the schistosome tegument. MsPAFAH specifically decreased the availability of phospholipid species containing unsaturated fatty acids, namely the eicosenoic and docosatetraenoic acids 16:0_20:1 and 18:0_22:4, while increasing levels of respective hydrolysis lyso-products diacyl- and ether-phospholipids (20:1 and 18:0), predominantly in males. Supplementation of metabolized fatty acids C20:1/eicosenoic acid and 22:4/adrenic acid rescued the viability of female worms, confirming the essential role of the metabolism of these diacylglycerophospholipids in schistosome survival.

Conclusion: These findings unravel how a host phospholipase interferes with schistosome biology and development by regulating diacylglycerophospholipid availability and can thus open new avenues for schistosomiasis drug development and control.

SE22.06

Bridging physics and health: Surface swimming as a key mechanism in schistosome transmission

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Objectives: While infectious diseases are traditionally tackled through molecular and drug-based approaches, pathogens must overcome fundamental physical challenges to maintain transmission. Imagine searching for someone in a vast skyscraper without knowing which floor they're on—this mirrors the challenge faced by schistosome cercariae, microscopic parasites affecting 240 million people globally, as they search for human hosts in large water bodies. We investigate how these parasites achieve remarkably efficient transmission despite seemingly insurmountable physical constraints.

Materials & Methods: We pioneer an interdisciplinary approach combining biomechanical modeling, fluid dynamics, and field studies in Senegal. Using novel microscopy techniques, we analyze cercarial swimming behavior in both laboratory and natural settings, developing a mathematical framework that treats these parasites as weight-asymmetric self-propelled swimmers interacting with the air-water interface. This approach bridges the gap between physics and parasitology to reveal previously hidden transmission mechanisms.

Results: Our biomechanical analysis reveals a sophisticated physical adaptation: cercariae achieve stable surface swimming at a precise angle through a balance of gravitational torque and surface forces. Just as finding someone becomes dramatically easier when you know they're on the top floor, this previously unknown mechanism concentrates over 80% of parasites in the top millimeter of water, transforming a daunting three-dimensional search into a manageable two-dimensional one. The efficiency gain is remarkable: surface-swimming parasites can explore a square meter of water surface in under 10 minutes, compared to the month-long timeframe required to search the same volume of water. Field studies demonstrate how cercariae exploit the physics of fluid interfaces, swimming in a slow-moving viscous sublayer at the surface where water velocities are reduced by up to two orders of magnitude. This allows parasites to persist in human-contact zones rather than being swept away by typical currents that move 100 times faster than they can swim, explaining how remarkably low parasite concentrations in water (0-3 per 100L) can maintain high transmission rates in endemic areas.

Conclusion: By reframing disease transmission through the lens of physics, we uncover a critical mechanism underlying schistosomiasis persistence in endemic regions. This finding demonstrates how physical principles can complement molecular approaches in understanding and controlling infectious diseases. More importantly, it reveals an unprecedented opportunity for disease control through interventions targeting surface-swimming parasites, potentially offering more sustainable solutions than drug treatment alone. Our work establishes a new paradigm for addressing global health challenges through interdisciplinary science and community-engaged intervention design.

Fig. 1

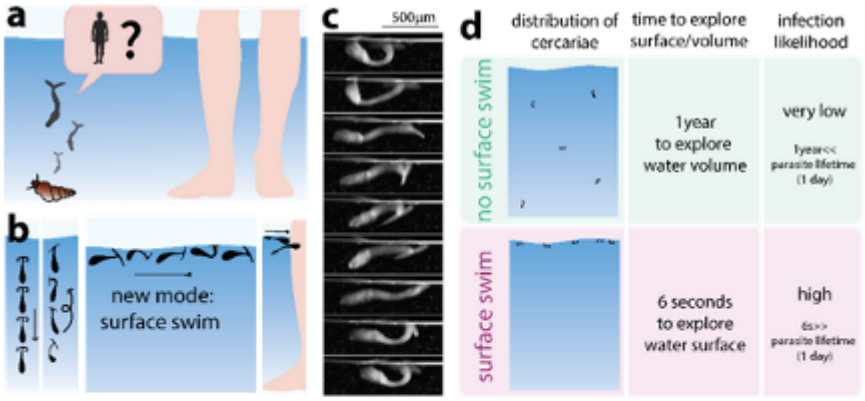

Figure 1| Surface swim is essential for efficient infection by schistosome parasites

(a) Schistosome cercariae need to explore the water body to find a human, within the span of the parasite lifetime (10-20 hours). (b) In addition to known swimming modes of upward swim and passive sink, I have uncovered a mechanism through which cercariae can swim at the air-water interface. Time-lapse of a surface swimming cercaria, observed at 200 frames per second with SALP. (c). (d) Comparison between the infection likelihood in a scenario without surface swim (very unlikely infection), compared to a scenario with surface swim (infection is likely).

Fig. 2

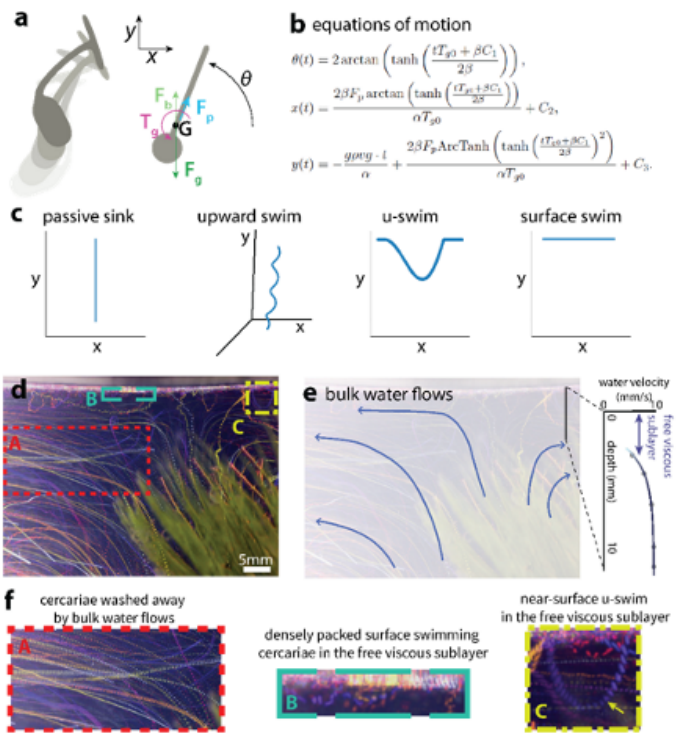

Figure 2| Surface accumulation of cercariae emerges from biomechanics and water flows

A biomechanical model of cercariae incorporating gravity and self-propulsion (a) yields analytical solutions (b) that predict four distinct swimming modes. Field measurements in Senegal using SALP and time-lapse imaging (d) reveal that while bulk water flows exceed cercarial swim speed (e), a slow-moving viscous sublayer at the surface(e) enables accumulation of the surface swimming parasites while cercariae in the bulk are washed away (f).

SE22.07

Heat shock protein 90 as a vital signalling hub in *Schistosoma mansoni*: Towards a "systems biology" approach to understand aspects of schistosome growth and survival

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Heat shock proteins (HSPs) are molecular chaperones often produced by cells in response to hostile conditions. Among the HSP superfamily, the HSP90s are unique as they are not only involved in *de novo* folding of proteins, but they facilitate final maturation, stabilisation, and activation of a diverse protein clientele. Of the 55 HSPs present in the parasite *Schistosoma mansoni*, three are HSP90-like. Here we aimed to characterise in detail the importance of the cytosolic HSP90 alpha-isoform 2-like protein (Smp_072330) to growth, development, and survival of *S. mansoni* with an emphasis on the developmental stages that parasitise the human host, their stem cell proliferation, and cellular signalling. Laser scanning confocal microscopy revealed that HSP90 protein was prominently expressed in the sub-tegument, tegument and head-tail junction of skin stage schistosomula, the testicular lobes of male worms, and the ovary and sub-tegument of female worms. Western analyses revealed striking HSP90 upregulation in developing schistosomula over 21 days *in vitro* culture. Furthermore, phenotyping assays revealed that the HSP90 inhibitors gedunin, 17-AAG, and EC-144 profoundly reduced the viability of skin schistosomula as early as two days in culture and restricted their development to the lung/liver schistosomulum stages. Liver stage schistosomula and adult male and female worms were also killed by 17-AAG/EC-144 *in vitro*, with egg laying supressed in surviving adult worms; pre-isolated eggs were also killed by the drugs. Strikingly, 17-AAG/EC-144 also blocked somatic and germinal Edu+ stem cell proliferation in the skin, lung and liver schistosomula, the testicular lobes of males, and ovary of females. Knockdown of HSP90 (Smp_072330) by RNAi resulted in ~50% reduced protein expression, attenuated stem cell proliferation and restricted schistosomulum growth. The involvement of HSP90 in regulating selected protein kinase signalling pathways was also demonstrated by the decreased levels of phosphorylated Akt, ERK1/2 and p38 MAPK in 24 h schistosomula following treatment with 17-AAG or EC-144. Finally, protein interactome analyses highlighted the complex interplay of *S. mansoni* HSP90 with client proteins, including protein kinases, with several mediating HSP90 phosphorylation. Collectively these findings demonstrate the crucial importance of HSP90 to intra-mammalian schistosome life stages and highlight HSP90 as a potential drug target to control human schistosomiasis.

SE22.08

The impact of *Blastocystis* sp. on 5-fluorouracil efficacy in colorectal cancer treatment

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Blastocystis sp. (Blastocystis), an enteric protozoan with significant genetic diversity, is often linked to gastrointestinal issues such as diarrhea, nausea, and abdominal pain, particularly in immunocompromised patients. This study evaluates the influence of Blastocystis on the chemotherapeutic efficacy of 5-fluorouracil (5-FU) in colorectal cancer (CRC). Using both in vitro and in vivo models, we examined how solubilized antigens from Blastocystis impact the activity of 5-FU. Experiments with HCT116 cancer cells showed a marked reduction in 5-FU's inhibitory potency on proliferation when co-incubated with Blastocystis antigens, while effects on normal colon fibroblast cells were negligible. In vivo results demonstrated an increase in tumor multiplicity and severity of intestinal histopathology in rats treated with 5-FU in the presence of Blastocystis infection. Molecular analyses revealed upregulation of pro-inflammatory cytokines and stress-related genes, alongside suppression of apoptotic pathways. These findings suggest that Blastocystis infection may compromise the therapeutic outcomes of 5-FU in CRC patients, emphasizing the need for improved infection management during chemotherapy.

SE23.02

An investigation into the mechanisms of action and resistance of quinapyramine in African trypanosomes

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Quinapyramine is a veterinary trypanocide, originally introduced in the 1940s, that was taken out of use in the 1970s because it appeared to induce cross-resistance with isometamidium, ethidium, diminazene. It was, however, reintroduced in 1984, but only outside of sub-Sharan Africa, particularly for the treatment of surra in camels and horses (and off-label against dourine), and is still in use today. The mechanism of action was the subject of early studies but never satisfactorily resolved and the mechanism of (cross)-resistance is completely unknown. We induced resistance in T. equiperdum and T. evansi, which only in T. evansi was associated with substantially reduced growth rates even at modest resistance levels. Higher levels of resistance in T. equiperdum were associate with loss of the kinetoplast, as well as ~10-fold cross-resistance with ethidium and lower cross-resistance with diminazene. Quin-Res T. evansi were cross-resistant with diminazene and isometamidium. T. brucei strains AT1-KO (diminazene, arsenical resistant) and ISMR1 (isometamidium resistant) also displayed resistance to quinapyramine as did diminazene-resistant T. congolense. Quinapyramine uptake was investigated using its innate fluorescence, and found to be inhibited by adenosine. Likewise, adenosine uptake through the TbAT1/P2 transporter was inhibited by quinapyramine, with moderate affinity. Treatment of T. brucei with quinapyramine did not appear to impact DNA synthesis and either kinetoplast or nuclear division, but did greatly affect cytokinesis, leading to multi-nucleated cells. Investigations of the mode of action and of resistance are ongoing and the results obtained to date will be presented.

SE23.03

Genomic and transcriptomic characterisation of eprinomectin resistance in *Haemonchus contortus* collected from dairy ewe farms

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Anthelmintic resistance significantly impacts animal welfare and productivity worldwide. We have investigated the genetic basis of eprinomectin (EPR) resistance in *Haemonchus contortus*, a highly pathogenic gastrointestinal nematode of small ruminants, using samples collected from dairy sheep farms in a small geographic area of southwest France. In this area, EPR is routinely used as a deworming treatment, and several cases have shown alarming clinical failures. Worm isolates from six farms were characterised for their susceptibility or resistance to macrocyclic lactones (ML) using a motility assay on third-stage larvae (see abstract A. Lespine). Whole genome sequencing, followed by genome-wide analyses, was used to compare phenotypically differentiated isolates. In EPR-resistant worms from different farms, a discrete genomic region on chromosome 5 exhibited significant genetic differentiation, previously identified as a signature of ivermectin resistance in *H. contortus*. We further analysed the transcriptomes of adult male and female worms from infested animals, revealing variation in the expression of genes located on chromosome 5 that could be associated with the resistance phenotype. These data provide a unique opportunity to link farm treatment histories and drug phenotype data with genomic and gene expression variation, paving the way for identifying novel molecular targets, improved diagnostics, and treatments for helminth infections.

SE23.04

Glycoengineering of nematode antigens using insect cells: A promising approach for producing bioactive vaccine antigens of the barber's pole worm *Haemonchus contortus*

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The H11 glycoproteins, located on the intestinal microvilli of the barber''s pole worm *Haemonchus contortus*, comprises a group of homologous aminopeptidases essential for the parasite's digestion of blood meals. Native H11s are highly effective vaccine antigens, capable of eliciting a robust protective immunity in sheep and goats against *H. contortus* infection. However, the recombinant production of H11 antigens using conventional expression systems and transgenic *Caenorhabditis elegans*, failed to replicate the protective efficacy of the native form, likely due to inappropriate glycosylation and suboptimal protein folding. To address these limitations, we developed a novel strategy to produce recombinant *Haemonchus* antigens in glycoengineered insect cells. By introducing *C. elegans* genes encoding key nematode glycoenzymes, we altered the native N-glycosylation pathways of *Trichoplusia ni*-derived Hi5 cells. This enabled the recombinantly expressed antigens to better mimic the glycosylation patterns of native H11s. We successfully expressed soluble H11 antigens featuring nematode-specific glycan epitopes, including the tri-fucosylated core and the Galβ1,4Fuc motif, with structural verification performed via HPLC and MALDI-TOF-MS/MS. The glycoengineered antigens retained their aminopeptidase activity and could stimulate cytokine secretion from ovine peripheral blood mononuclear cells (PBMCs) *in vitro*. A preliminary vaccine trial in sheep further indicated their potential to induce protective immunity. These findings demonstrate the feasibility of producing bioactive *H. contortus* antigens as promising vaccine candidates to combat this parasitic nematode.

Keywords: glycoprotein, glycoengineering, N-glycan, insect cell, *Haemonchus contortus,* recombinant vaccine

SE23.05

Beyond Suspicion: Confirming the prevalence and occurrence of drug resistant *Fasciola hepatica* in sheep, goats and cattle in the Southern Tablelands of NSW, Australia

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Fasciola hepatica is a zoonotic parasite of international significance. In Australia, it is the 13th most important cause of losses to the sheep industry due to its impacts on body condition score (BCS), fibre quality and yield. Resistance to the frontline drug, Triclabendazole (TBZ), was first detected in Australia in 1995 and has since spread throughout the world. Recently, livestock producers from the NSW Southern Tablelands have raised concerns over suspected increases in drug-resistant parasites. In response, this project set out to evaluate the prevalence of *F. hepatica* on naturally infected sheep, cattle, and goat properties in the region, and confirm or deny suspicions of drug resistance. To do so, eight farms encompassing nine mobs (seven sheep, one goat, one cattle) of 45 animals were split into three treatment groups of 15 animals per group and were administered either TBZ, AVOMEC DUEL/Albendazole (ABZ) (positive control for sheep and goats, respectively), or water (negative control). Treatments were administered according to individual animal weights on Day 0. Faecal samples, animal weight, and BCS were collected and recorded on Day 0 then again 21 days later. A traditional sedimentation and faecal egg count (FEC) and a commercially available coprological ELISA (cELISA) were used to determine within-herd prevalence. Faecal egg count and coprological reduction tests (FECRT and CRT, respectively) were used to determine drug efficacy and therefore resistance or susceptibility on two farms (one sheep and one goat) that met the criteria for drug efficacy determination. Of the eight farms tested, four had a within-herd true prevalence of >25% and are therefore likely to experience production losses due to *F. hepatica* infection. Our results showed evidence of TBZ resistance (89%-92% efficacy) on one sheep property, TBZ susceptibility (97%-98% efficacy) on the Goat Property and, interestingly, ABZ resistance (77%-79% efficacy) on the Goat Property. This is the first study to investigate drug resistant *F. hepatica* in goats in Australia. The results underscore the continual threat of resistance and highlights the need for ongoing surveillance and development of alternative parasite control options, to safeguard the remaining efficacy of TBZ as long as possible.

SE23.06

Validation and characterisation of a transporter that mediates Isometamidium resistance in the livestock pathogen *Trypanosoma congolense*

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The unicellular protozoan parasite *Trypanosoma congolense* is the primary cause of African animal trypanosomosis (AAT), a debilitating livestock disease that is also caused by *T. vivax* and to a lesser extent, *T. brucei*. This disease poses a significant threat to livestock health and agricultural productivity across sub-Saharan Africa, with an estimated 3 million cattle deaths annually. Isometamidium remains the only drug available with both prophylactic and curative properties. Despite reports of resistance since the 1970s, a definitive molecular mechanism of resistance remains unresolved, especially in the clinically relevant species, *T. congolense*. In this study, the role of a previously identified protein, TcoDMT was validated via the analysis of *in vitro*-derived mutants. We show that expression levels of this protein correlate strongly with isometamidium sensitivity - overexpression and knock-out result in hypersensitivity and resistance, respectively. Functional analyses reveal that the protein is a phenanthridine transporter localised to the trypanosome mitochondrion, and is closely related to nucleotide sugar transporters. Interestingly, ablation of expression of the DMT orthologue in *T. brucei* has no effect on isometamidium sensitivity, suggesting that the role of this protein may be species-specific. This study validates, for the first time, a transporter with a defined role in isometamidium action and resistance, advancing our understanding of drug resistance mechanisms in parasitic protists, and informing strategies to combat trypanosomosis in endemic regions.

SE23.07

Translating the protective effects of an invariant *T. vivax* antigen from mouse to cattle

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African trypanosomes constrain animal agriculture in Sub-Saharan Africa by causing African Animal trypanosomiasis, a disease described to lies at the heart of Africa"s struggle against poverty. Effective vaccines against African trypanosomes were considered unachievable due to the sophisticated system of antigenic variation employed by these parasites to elude the host immune response.

Using systematic genome-led vaccinology screens in murine models, we found that vaccination with a single recombinant protein comprising the extracellular region of a conserved cell surface protein localised to the flagellum membrane termed "invariant flagellum antigen from *Trypanosoma vivax*" (IFX) induced long-lasting protection. Immunity was passively transferred with immune serum, and recombinant monoclonal antibodies to IFX could induce sterile protection and revealed multiple mechanisms of antibody-mediated immunity, including a major role for complement.

To translate our findings from mice to cattle, we have established a *Trypanosoma vivax* cattle infection model suitable for vaccine efficacy studies. Preliminary vaccination experiments using IFX formulated as recombinant protein-in-adjuvants have shown that antibody levels obtained in cattle were low compared to mice. Other vaccine platforms such as SpyCatcher-VLPs are investigated to improve the IFX antibody response in cattle.

SE24.03

The impact of soil and habitat factors on the presence of *Galba truncatula*, the main snail intermediate host of liver and rumen fluke in Europe

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Introduction: *Galba truncatula* is the main intermediate host of the livestock parasites *Fasciola hepatica* and *Calicophoron daubneyi* in Europe. Fascioliasis is estimated to cause over €1 billion to the global agricultural industry [1] and *C. daubneyi* has spread to several European countries in recent decades [2]. Due to limited available anthelminthics, and growing resistance in *F. hepatica* [3], understanding the environmental preferences of the intermediate snail host is vital to reduce livestock infection rates.

Objectives: To find and investigate current gaps in the environmental preferences of the snail Galba truncatula.

Materials and Methods: An initial systematic literature review of 198 papers showed that the impact of soil properties on *G. truncatula* were poorly understood. Therefore, 91 habitats across 8 Welsh farms were surveyed for G. truncatula, and soil samples taken from each habitat and analysed. Multivariable analysis was conducted in R using the glmmTMB package where binomial mixed models were created using a stepwise methodology to identify factors associated with *G. truncatula* presence in habitats.

Results: A significant negative association between log soil organic matter content and *G. truncatula* presence was found (OR = 0.15, p = 0.028) whilst the odds of detecting *G. truncatula* snails was x3.3 (1.2 – 9) greater in habitats with a soil OM content above 8.3% (p = 0.012). The odds of detecting the snails were also x8.7 (95% C.I 1.6 – 47.5) greater in habitats with a soil pH above 5.27 (p = 0.018).

Conclusions: The negative association between soil organic matter and snail presence may be tied to their long-noted aversion to peatland habitats. As such, peatland restoration and increasing the organic content of soils may be beneficial to livestock farmers, and should be investigated further in future research.

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SE24.04

Heavy metal concert in the ocean: Patterns of accumulation in seals and their parasites

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The rising intensity of anthropogenic activities has led to a continuous increase in metal pollution in marine ecosystems. Many of these elements might be accumulated in marine organisms, exerting detrimental effects from single individuals to populations. This emphasises the crucial requirement for effective monitoring techniques, including the utilisation of bioindicators to evaluate pollutant levels and bioavailability in natural systems. While various free-living organisms (e.g. mussels) have long been employed as sentinels for pollution, recent studies suggest that parasitic helminths offer a remarkable potential as bioindicators for metal pollution. For example, studies on freshwater fish-parasite systems have demonstrated that parasites such as acanthocephalans and cestodes can accumulate different elements (e.g. lead and cadmium) at concentrations up to 2,700 times higher than those in host tissues. This high accumulation capacity enables certain parasites to act as contaminant sinks, with infected hosts often exhibiting reduced contaminant burdens compared to their non-infected counterparts. However, most of the studies focused on (freshwater) fish-parasite systems, leaving a significant knowledge gap regarding marine mammals and their associated parasites. The study of marine mammals and their parasites is of particular interest due to their exposure to a high number of trace elements in the marine environment. When considered in combination with their long lifespan, this can provide valuable monitoring data on pollution dynamics in marine ecosystems.

Fresh carcasses of grey seals (n=21), harbour seals (n=22) and harbour porpoises (n=24) that had stranded in the North and the Baltic Seas were necropsied. The concentrations of 15 elements (Ag, As, Cd, Co, Cu, Cr, Fe, Mn, Mo, Pb, Se, Sn, Sr, V and Zn) were determined in seven distinct host tissues (liver, kidney, lung, muscle, stomach, large and small intestine) and helminths from the gastrointestinal tract and lung using inductively coupled plasma mass spectrometry (ICP-MS). The initial analyses reveal differences in element enrichment among the various species, with grey seals and harbour seals displaying comparable concentrations. Within species, variations in element enrichment were observed based on factors such as geographic origin (North Sea vs. Baltic Sea), sex, and age group. Regarding parasites, the study found that they accumulate elements like cobalt, manganese, and strontium at significantly higher concentrations than their hosts. Additionally, notable differences were identified among parasite groups: cestodes, for example, exhibited substantially higher concentrations of cobalt, copper, manganese, lead, and strontium compared to acanthocephalans and nematodes.

SE24.05

Understanding the ecological implications of aquatic parasites in changing environments

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Despite significant advancements in recognizing the ecological importance and implications of aquatic parasites, our understanding of how environmental conditions influence parasite occurrence and impact remains incomplete. In the context of global ecological change, an integrative approach is essential to elucidate the role of aquatic parasites in ecosystems. Parasites respond variably to environmental stressors, with their abundance either increasing or decreasing depending on the intensity and nature of the stressors, as well as their life cycle complexity. For instance, heteroxenic parasites often decline under elevated environmental stressor impavt, whereas monoxenic parasites may thrive. Moreover, growing evidence highlights the complex interactions between parasitism and pollution, revealing both synergistic and antagonistic effects on host organisms. While parasitism can exacerbate the negative impacts of pollutants by impairing host defenses, it may also mitigate these effects by reducing pollutant concentrations in infected hosts, potentially offering a paradoxical advantage. However, this protective effect is counterbalanced by potential pathological impacts of the parasites themselves.

This presentation will explore key insights into the field of environmental parasitology, focusing on the dual role of aquatic parasites as both stress mitigators and amplifiers within food webs. Selected examples from aquatic ecosystems will illustrate these dynamics, providing a deeper understanding of the multifaceted interactions between parasites, hosts, and environmental stressors.

SE24.06

Bridging knowledge gaps in trematode life cycles: Insights from South African freshwater ecosystems

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Freshwater ecosystems in South Africa harbour a considerable diversity of trematodes, yet the intermediate stages of these parasites and their interactions especially with invertebrate hosts remain poorly understood. This study fills critical gaps by assessing the diversity, prevalence and host associations of trematode infections in freshwater snails and linking cercariae and metacercariae stages to elucidate life cycles. A total of 1,332 freshwater snails from six genera (*Radix, Pseudosuccinea, Bulinus, Biomphalaria, Physa and Tarebia*) were sampled between March 2023 and October 2024 at eight sites stretching from the border of the Kruger National Park to the Tsitsikamma National Park. Trematode infections were detected in 64 snails, with 17 taxa identified through morphological and molecular analysis. Notable species include *Schistosoma mattheei* and *Fasciola gigantica*, both important pathogens affecting humans, livestock and wildlife. Continued molecular analysis is expected to advance resolution and refine identifications. Cercariae shedding was assessed using a three-day screening protocol, followed by dissection to identify sporocysts and rediae. By integrating snail host data with findings on metacercarial stages in vertebrate hosts from a complementary study, this research elucidates partial life cycles for three trematode genera: *Petasiger, Tylodelphys,* and *Uvulifer*. Additional matches are anticipated following the completion of molecular analyses. This is one of the first studies in South Africa connecting trematode life stages across multiple hosts, which offers novel insights into the complexity of trematode-snail interactions and parasitic biodiversity. By shedding light on transmission dynamics, this work represents a significant step toward a comprehensive understanding of African freshwater trematode life cycles.

SE25.01

Infection dynamics of avian *Plasmodium* parasites in zoo-kept birds and wild passerines

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Introduction: Avian malaria is caused by protozoa of the Genus *Plasmodium*.These parasites utilize various bird species as intermediate hosts and mosquitos for transmission and sporogony. In birds, infection causes both blood and tissue pathology, the latter resulting from infection of reticuloendothelial cells in various organs. Interestingly, the clinical outcome appears to vary depending on parasite, host species and immunity.

Aims and objectives: In this study, we are performing a comparative investigation of the biology and pathologic lesions associated with *Plasmodium* parasites infecting zoo-kept penguins and wild birds. Our approach utilises a combination of host and parasite genotyping, blood analyses, as well as histological and immunohistochemical examinations of blood and tissue samples collected opportunistically from penguins at the Zoo Zürich and from dead wild birds in Switzerland.

Results: In penguins, we identified *P. relictum* and *P. matutinum* infections causing severe mononuclear inflammation due to the abundance of intracellular meronts in the spleen, lung, and liver. Infection in erythrocytes is rare and often characterized by low parasitemia, potentially resulting in underdiagnosis through blood analyses. Interestingly, positive cases are also detected in some asymptomatic older penguins, while most fatal cases are in young penguins. *P. matutinum-infected* blackbirds examined have a lower parasitic load with milder inflammatory response seen in tissues compared to penguins. Erythrocytic merogony is apparent in *P. relictum-infected* sparrows, contrary to the situation in penguins.

Conclusions: Serosurveillance coupled with diagnostic assays is needed to confirm if immunity correlates to infection outcomes in penguins. Our data also demonstrate that severe tissue merogony contributes to the fatality of young unexposed penguins while passerines tolerate infection. More studies are needed in experimental hosts to investigate dynamics of infection and tissue tropism. Our studies will close major knowledge gaps in the understanding of the biology of avian *Plasmodium* and provide a rationale for diagnostics and treatment.

SE25.02

Individual host factors and parasite co-infections affect the probability and intensity of endoparasite infections in German dairy herds

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Endoparasite infections can cause health issues and economic losses in dairy farming. Individual host factors (e.g., parity number, lactation stage) and co-infections with different endoparasite taxa may affect infection probability and intensity, but have not yet been sufficiently researched. Hence, this study aimed to evaluate the influence of host factors and co-infections on the most prevalent endoparasite taxa in dairy cows.

Faecal samples from 1,126 dairy cows from 24 herds were examined with the Baermann technique, FLOTAC, and a classical sedimentation method to determine lungworm larvae, gastrointestinal nematode eggs, trematode eggs and coccidian oocysts per gram of faeces [EPG/OPG]). The effect of parity number, lactation stage (days in milk) and the presence of co-infections with other endoparasite taxa on both egg/oocyst excretion probability and intensity was analysed in two-part hurdle models for the most prevalent taxa. The herd, sampling month, and breed were included as random effects.

The most frequently detected endoparasite taxa were strongyles (mean in-herd prevalence: 46.2%, mean EPG: 6.5), *Fasciola hepatica* (mean in-herd prevalence: 13.0%, mean EPG: 0.3), rumen flukes (mean in-herd prevalence: 43.8%, mean EPG: 19.0) and coccidia (mean in-herd prevalence: 21.0%, mean OPG: 7.0). Other parasites, i.e. *Moniezia* spp., *Capillaria* spp., and *Trichuris* spp., were less abundant with mean in-herd prevalences of 5.1%, 4.2% and 2.1%, respectively. *Dictyocaulus viviparus* larvae were absent in all sampled herds. The hurdle models revealed that the parity number had a significant effect on all four most prevalent taxa. The probability of strongyle egg excretion decreased after the first parity, likely due to developing immunity. However, in cows from the fourth parity onwards, the probability of strongyle egg excretion increased again. The probability of *F. hepatica* egg excretion and the intensity of rumen fluke egg excretion both increased with increasing parity number. Coccidian oocysts were excreted most frequently and intensely in first-parity cows. The lactation stage only affected strongyle egg excretion, with higher probabilities in early lactation, when cows are potentially more susceptible to diseases due to a negative energy balance. A co-infection with strongyles increased the probability of coccidian oocyst excretion (P=0.008), while a co-infection with coccidia was associated with both higher probability and intensity of strongyle egg excretion (P=0.011 and P=0.007, respectively). Additionally, a co-infection with coccidia resulted in a decreased intensity of rumen fluke egg excretion (P=0.022).

In conclusion, parity number, lactation stage, and parasitic co-infections significantly influenced parasite egg and oocyst excretion in dairy cows, highlighting the importance of considering these factors in parasite control and management strategies on dairy farms.

SE25.03

Development of "Tremabiome" technology for fluke species differentiation using deep amplicon sequencing and qPCR assay to detect *Fasciola* infection in livestock in the United Kingdom

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Introduction: Fasciolosis, a parasitic liver fluke disease, poses a significant economic challenge to ruminant production worldwide. Traditional diagnostic methods for detecting liver fluke infections in ruminant faecal samples, such as sedimentation and microscopy, are often labour-intensive and may lack sensitivity and specificity.

Methods: This study introduces "Tremabiome", a deep amplicon high-throughput sequencing technique targeting the rDNA ITS-2 region, for the accurate identification and differentiation of fluke species (n=21), with a particular focus on *Fasciola hepatica* and *Calicophoron daubneyi* and qPCR to identify *Fasciola*. Correction factors derived from mock mixtures of different fluke egg and adult worm DNA addressed sequence representation biases, enabling reliable quantification of sequence reads. Both methods were validated using fluke egg-positive faecal samples (n=127) out of total screened faecal samples (n=402) and adult *F. hepatica* worm DNA (n=10) collected from cattle (n=154), sheep (n=233) and others (n=15) in the UK.

Results: The assay exhibited a detection threshold as low as five eggs of *F. hepatica* or *C. daubneyi*. The results revealed the presence of *F. hepatica* (3.2%) and *C. daubneyi* (12.6%) eggs in faecal samples with co-infections (14.4%) being more common than single-species infections. In total, 29 and 32 amplicon sequence variants (ASVs) were identified for *F. hepatica* and *C. daubneyi*, respectively, with phylogenetic analysis confirming their alignment to species-specific clades. Compared to newly developed qPCR, the "Tremabiome" approach demonstrated a significant relationship ($p<0.0000000002629$), although "Tremabiome" detecting *F. hepatica* in samples (n=20) that qPCR could not identify.

Conclusions: "Tremabiome" analysis offers significant potential for epidemiological surveillance, particularly in light of the economic impact of *F. hepatica* on livestock and the potential for co-infection with *C. daubneyi*. By uncovering species diversity, co-infection patterns, and genetic variation, this technique enhances diagnostic accuracy and provides a robust framework for managing and controlling liver fluke infections in ruminant populations.

Keywords: Fasciolosis, *Fasciola hepatica*, *Calicophoron daubneyi*, "Tremabiome", qPCR, flukes diagnostics

SE25.04

Taeniidae in wild African carnivores – New insights into species diversity, with implications for host ranges and distribution patterns

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Introduction: The Taeniidae, including the four recognised genera *Taenia*, *Hydatigera*, *Echinococcus*, and *Versteria*, is a tapeworm family with a two-host life cycle, requiring both definitive and intermediate hosts to be mammals. While research focuses predominantly on taeniid species of medical and economic importance, limited information is available regarding sylvatic Taeniidae. Species descriptions of cestodes in African wildlife hosts are mostly based on morphology and have not yet been genetically verified due to the restricted access to fresh material. Therefore, the true biodiversity and distribution of Taeniidae in African wild mammals remain poorly understood.

Materials and Methods: The present study analysed taeniid samples (eggs, adults, cysts) collected from 13 species of wild carnivore definitive hosts and three species of intermediate hosts from Ethiopia, Namibia, and South Africa. Molecular and morphological methods were employed for the identification of species. A morphological classification key was developed for this study to narrow down candidate species. Genetic data obtained through PCR and sequencing of various gene regions (*nad1*, *cox1*, *cob*, 12S rRNA, 18S rRNA), were utilised to construct phylogenetic trees and haplotype networks.

Results: A total of 22 distinct taeniid species (two *Echinococcu*s spp., four *Hydatigera* spp., and sixteen *Taenia* spp.) were detected in the examined hosts. Twelve of the genetic lineages (54.5%) could not be identified at the species level through alignment with previously published sequence data. The novel lineages were detected in cheetahs, caracals, lions, leopards, servals, spotted hyaenas, African wild dogs, and one *Taenia* species with zoonotic potential was found in black-backed jackals. In some cases, morphological comparisons with reference material facilitated species assignment and rediscovery, while genetic network analyses revealed geographical distinctions and host specificity.

Conclusion: The new data highlights the rich diversity of Taeniidae in African terrestrial wildlife and provides further insights into host range, speciation and the dispersal of certain taeniid species.

SE25.05

Multiresistance in a marine ectoparasite, *Lepeophtheirus salmonis*

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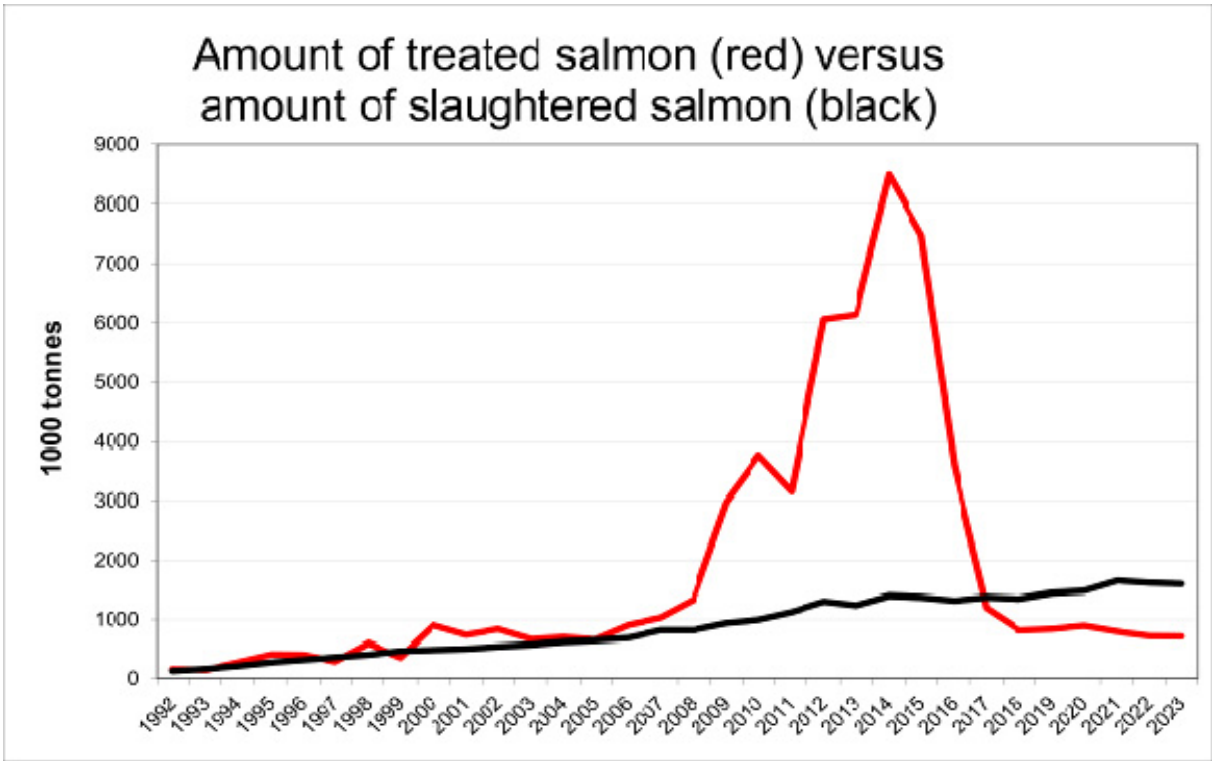
Production of farmed Atlantic salmon is the most important animal industry in Norway. The annual production is five times larger than the combined production of all other meat-producing animals.

The marine copepode *Lepeophtheirus salmonis*, the salmon louse, is the most pathogenic ectoparasite on salmon. Strict regulations on the maximum number of parasites allowed per fish have over the years triggered an overuse of antiparasitic agents to control the parasite in the farms as well as to protect wild salmonids from being infected with lethal numbers. The overly reliance on chemotherapeutants for salmon lice control has rendered this parasite multiresistant and forced introduction of several non-medicinal treatment options for its control.

To date, resistance towards azamethiphos (organophosphate), deltamethrin (pyrethroid), emamectin benzoate (macrocyclic lactone) and hydrogen peroxide (topical disinfectant) is widespread and has been demonstrated from field efficacy data, bioassays and molecular methods. This presentation will focus on the faults made in the past, the surveillance of the problem and possible ways forward including vaccine development.

Fig. 1



SE26.02

The interaction of schistosomes with the maternal microbiota and their respective influences on the offspring immune system

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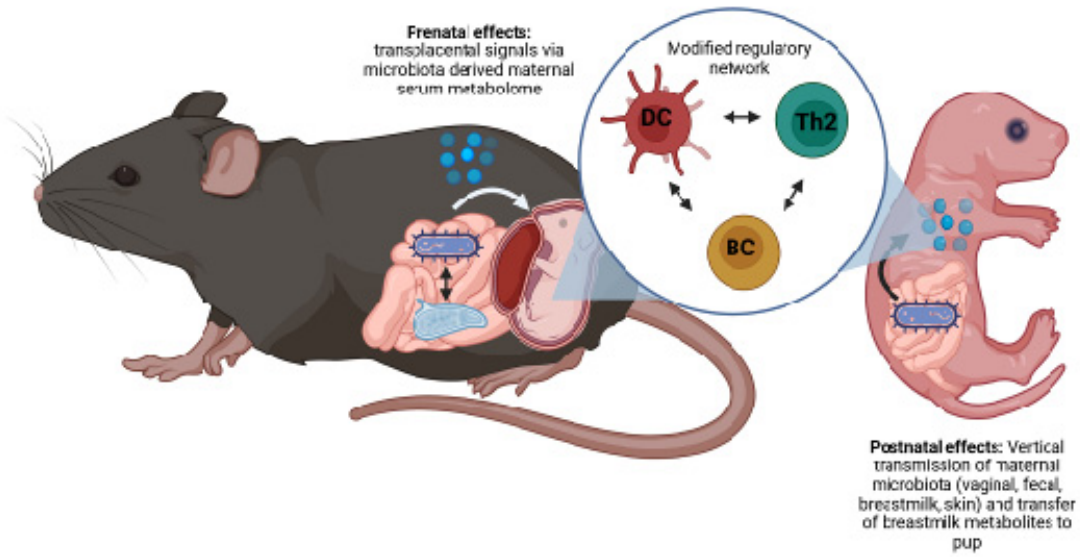
Schistosomes are parasitic worms which manipulate the host immune system to ensure their prolonged survival, with bystander effects on the infected host and their progeny including reduced allergic sensitivities and impaired vaccine responses. We propose that these immune alterations in the offspring stem from specific maternal signals that are modified during chronic infection. Given that 40 million women of child-bearing age are infected in Sub-Saharan Africa, it is imperative to delve deeper into these signals. To better understand whether the offspring immune system is impacted pre- or postnatally, we carry out a cross-foster experiment in mice from regulatory and Th2 phases of infection and analyse antigen-presenting and costimulatory molecule expression on B cells and dendritic cells in the spleen, mesenteric lymph node and bone marrow as well as frequencies of their stem cell precursors. We show that pre- vs postnatal exposure to an infected mother have opposing effects on the expression of these antigen-presenting and costimulatory molecules on B cells and that this effect is stronger in the pre-weaning phase compared to in adult offspring. We therefore analyze immunomodulatory metabolites in stool, serum, and breastmilk using both untargeted and targeted metabolomics. Our findings reveal that schistosomiasis alters the levels of microbial metabolites, including short-chain fatty acids, tryptophan metabolites, and bile acids. We linked this to changes in the microbiota using 16s rRNA sequencing of the maternal and offspring stool. In the future, we will further investigate immunomodulatory components of serum and breastmilk including cytokines and corroborate this data with serum samples from our human schistosomiasis mother-child cohort (Helmvit).

Fig. 1



SE26.04

Do microbiomes of filarial nematodes contribute to disease pathogenesis?

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Recently we have discovered that 70% of parasitic nematodes host a large and diverse RNA virome (Quek et al. 2024, Nature Microbiology 9(10):2488-2505. doi: 10.1038/s41564-024-01796-6). Previous work in our laboratory has highlighted the contribution of *Wolbachia* bacterial endosymbionts of filarial nematodes as drivers of inflammatory disease pathogenesis. Does the newly discovered RNA virome also contribute to disease pathogenesis? We have focussed on a rhabdovirus, OVRV1, which is ubiquitous based on transcriptome mining, PCR and immunohistochemistry in *Onchocerca volvulus* parasites. Interestingly, we detected antibody responses to OVRV1 glycoprotein (gp) with seropositivity of 93-100% in infected or exposed communities in Africa, suggesting that these individual's immune system is exposed to the virus and are most likely infected by this nematode-associated virus. OVRV1 is phylogenetically related to lyssaviruses, including rabies virus, and as such may contribute to the disease pathogenesis of onchocerciasis-associated epilepsy, a recently recognised clinical presentation in children and adolescents exposed to high levels of *O. volvulus* transmission. To determine the fusogenicity and the resulting cell tropism of OVRV1 gp we have created lentiviral pseudotypes decorated with OVRV1 gps to define human cell susceptibility to infection. Pseudotyped lentiviruses provide an opportunity for rapid throughput to determine the functionality of putative viral glycoproteins, as well as provide mechanistic and tropism information in the absence of isolated infectious virus. To probe for cell susceptibility to OVRV1 gp-mediated entry, we exposed human cell lines of different origins (IRF3 KO lung epithelial A549 cells, embryonic kidney HEK293T cells and TZM-bl cells, a derivate of human cervix carcinoma HeLa cells) to GFP-encoding lentiviral particles decorated with OVRV1 gp. Subsequently, we quantified reporter expression two days post-transduction, using an established protocol for production of lyssavirus-gp pseudotypes. Vesicular stomatitis virus (VSV)-gp pseudotypes served as positive control and rhabdoviral reference. Addition of OVRV1 gp lentiviral pseudotypes to cells resulted in dose-dependent GFP expression, providing robust evidence for the ability of OVRV1 gp to mediate entry into human cells and strengthening our hypothesis of OVRV1 infection-induced pathogenesis in humans. Current experiments are testing the susceptibility of advanced human induced pluripotent stem cells (iPSC)-derived bi/tri-partite neurospheroid culture systems to determine OVRV1 infection of neural cells and tissues.

SE27.02

Household environmental contamination with soil-transmitted helminths and associated factors among residents of peri-urban areas in Jimma city, Southwest, Ethiopia

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Introduction: Soil-transmitted helminth (STH) infections are among the most common and widespread neglected tropical diseases (NTDs) in Ethiopia and globally. They are transmitted by eggs present in human faeces, which contaminate the soil in areas where sanitation is poor. This study aimed to determine soil contamination rate, and associated risk factors among residents of Peri-urban kebeles in Jimma city, Oromia, Ethiopia, 2021

Methods: A community-based cross-sectional study was conducted in Peri-urban kebeles of Jimma city from May to June 2021. A total of 459 soil samples were collected from 153 household compounds (children"s playground, toilet area, and Refused dumps site) using a systematic random sampling. The collected soil samples were then examined microscopically using optimized soil straining flotation method. Data on Socio-demographic and predisposing factors were collected using a semi-structured questionnaire and checklist. The data were entered into Epidata and exported to SPSS for further analysis. Descriptive statistics were used to summarize household characteristics. Logistic regression was performed to determine the risk factors associated with STH contamination. The level of statistical significance was set at $P < 0.05$.

Results: The overall soil contamination rate in at least one location within a household was 39.2% with Ascaris was being the predominant species (35.9%). The most contaminated site with any of the Soil-transmitted helminth eggs was the refuse damp site (21.6%) followed by the Toilet area (18.3%), and the children"s playground (7.8%). Multivariate analysis confirmed that unimproved toilet facilities, having a domestic animal, and self-reported history of STH infection were a significant predictors of soil contamination with Soil-transmitted helminths.

Conclusion and recommendation: the current study finding indicated that STH eggs were prevalent in the household"s environments of a peri-urban community of Jimma city. This wide range of soil contamination rates suggested that the community was at a high risk of acquiring STH infection. This suggests a need to strengthening the existing comprehensive approaches aimed to prevent and control STH infection and STH environmental contamination in these community. These approaches should focus on large scale deworming to reduce the infection burden, improving WASH to reduce environmental contamination and promoting health education aimed to alter behavior to reduce environmental contamination and risk of infectionKeywords: Soil contamination rate, STHs, Risk Factors, Peri-urban, Southwest Ethiopia.

Fig. 1

Table1: Household characteristics of Peri-urban community of Jimma city, Ethiopia, 2021

Variables	Categories	Frequency	Percentage
Types of houses	Mud plastered	146	95.4
	Stone walls	7	4.6
House floor types	Earthen	123	80.4
	Cement	30	19.6
Toilet facilities	Improved	91	59.5
	Unimproved	62	40.5
Drinking water sources	Protected	14 6	95.4
	unprotected	7	4.6
Sources of water for domestic use	Protected	136	88.9
	unprotected	17	11.1
Solid waste disposal system	Proper	16	10.5
	improper	137	89.5
Liquid waste disposal system	Proper	6	3.9
	improper	147	96.1
Types of Toilet Floors	Earthen/sand	134	87.6
	cement	19	12.4
Toilet has lids/cover	Yes	12	7.8
	No	141	92.2
Family size	<5	67	43.8
	>5	86	56.2
Under 5-year children	Yes	86	56.2
	No	67	43.8
Child defecation sites	Safe[a]	57	66.3
	unsafe	29	33.7
Child feces disposal site	Safe[b]	63	73.3
	Unsafe	23	26.7
Presence of domestic animals in the compounds	Yes	46	31.4
	No	105	68.6

Safe child defecation[a] :-toilet facility and Use child potty
Safe disposal[b]:- Put/rinsed in toilet or latrine

Fig. 2

Table 3: Bivariate and multiple logistic regression analysis results of factors associated with the soil-contamination rate among Households Peri-urban kebeles of Jimma city, Southwest, Ethiopia, 2021.

Variables	Categories	Soil-transmitted helminths		COR (95% CI)	P-value	AOR (95% CI)	P-value
		Positive No (%)	Negative No (%)				
Kebeles	Bore	13(35.1)	24(64.9)	0.84(0.34–2.06)	0.709	0.80(0.20–3.20	0.760
	Kofe	6(35.3)	11(64.7)	0.84(0.26–2.70)	0.781	0.55(0.06–4.69	0.592
	Hora gibe	7(70.0)	3(30)	3.63(0.82–15.88)	0.087	4.90(0.42–57.0	0.204
	Jiren	16(37.2)	27(62.8)	0.92(0.39–2.17)	0.852	0.27(0.05–1.34	0.110
	Ifabula	18(39.1)	28(60.9)	**1**		**1**	
Age of SAC	5–10 years	36(33.0)	73(67.0)	1		**1**	
	11-15 years	24(54.5)	20(45.5)	2.43(1.19–4.97)	0.015*	1.58(0.39–6.2	0.515
Family size	≤ 5	20(29.9)	47(70.1)	1		**1**	
	>5	40(46.5)	46(53.5)	2.04(1.04 -4.01)	**0.037***	1.73(0.51–5.80	0.374
Toilet facilities	Improved	29(31.9)	62(68.1)	1		1	
	Unimproved	31(50.0)	31(50.0)	2.13(1.09–4.15)	**0.025***	3.35(1.17–9.56	**0.024****
History of STHs at SAC	Yes	39(53.4)	34(46.6)	3.22(1.63–6.34)	**0.001***	3.19(1.11–9.12	**0.03****
	No	21(26.2)	59(73.8)	1		1	
Children < 5 years in the	Yes	36(41.9)	50(58.1)	1.29(0.66–2.49)	0.448	-	-
	No	24(35.8)	43(64.2)	1		1	-
Place for defecation	Safe	19(33.3)	38(66.7)	1		1	
	Unsafe	17(58.6)	12(41.4)	2.83(1.12–7.12)	0.027*	2.63(0.90–7.71	0.077
Stool disposal site	Safe	21(33.3)	42(66.7)	1		1	
	Unsafe	15(65.2)	8(34.8)	3.75(1.37–10.24)	0.010*	2.88(0.95–8.71	0.060
Toilet has lid/cover	Yes	2(16.7)	10(83.3)	1		1	
	No	58(41.1)	83(58.9)	3.49(0.73–16.54)	0.115*	0.35(0.03–3.38	0.371
Toilet floor	Earthen/sand	56(41.8)	78(58.2)	2.69(0.84–8.54)	0.093*	2.75(0.27–28.1	0.392
	Cement	4(21.1)	15(78.9)	1		1	
Visible faeces in compou	Yes	14(60.9)	9(39.1)	2.84(1.14–7.06	0.026*	2.19(0.49–9.72	0.299
	No	46(35.4)	84(64.6)	1		1	
Presence of domestic animals	Yes	25(52.1)	23(47.9)	2.17(1.08–4.36	0.029*	3.95(1.25–12.4	**0.019****
	No	35(33.3)	70(66.7)	1		1	

SE27.03

Leveraging one health approaches for the elimination of neglected tropical diseases in Nigeria

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Introduction: Neglected Tropical Diseases (NTDs) represent a significant public health challenge in Nigeria, affecting vulnerable populations, particularly in rural and underserved areas. These diseases perpetuate cycles of poverty, poor health, and reduced productivity. The One Health framework, which emphasizes the interconnectedness of human, animal, and environmental health, provides a promising interdisciplinary approach to addressing NTDs holistically. This study focuses on the application of One Health strategies to mitigate the burden of NTDs such as lymphatic filariasis, schistosomiasis, and onchocerciasis in Nigeria.

Materials and Methods: This study employed a mixed-methods approach, integrating data from public health initiatives, veterinary services, and environmental interventions. It reviewed the implementation of mass drug administration (MDA), integrated vector management, and environmental sanitation programs. Cross-sectoral collaboration among public health officials, veterinarians, environmental scientists, and local community stakeholders was also analyzed. Community engagement and capacity-building efforts were evaluated using case studies and program reports, with a focus on identifying best practices and barriers to implementation.

Results: Preliminary findings indicate that integrating One Health principles into NTD control efforts significantly reduces transmission rates. The combination of veterinary and environmental health measures with traditional public health interventions demonstrated measurable progress in reducing disease burden. However, persistent challenges were noted, including inadequate funding, weak institutional frameworks, and limited awareness among affected populations. Community participation and improved surveillance systems emerged as critical factors for sustained success.

Conclusion: The study highlights the effectiveness of the One Health approach in combating NTDs in Nigeria. Scaling up such strategies, supported by strong institutional frameworks and community involvement, could accelerate progress towards the elimination of NTDs. The findings underscore the need for multisectoral collaboration to address complex health challenges, offering valuable insights for endemic regions worldwide.

SE27.04

Application of *in silico* and genomics approaches to improve control of the giant roundworm *Ascaris*

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Introduction: *Ascaris* is a major health burden in humans globally and causes considerable production losses in pigs. Periodic treatment with benzimidazole anthelmintics is the mainstay of control of *Ascaris* infections, leading to concerns that anthelmintic resistance will arise, jeopardising sustainable control and achievement of WHO 2030 elimination targets. We have been using in silico and genomic approaches to explore resistance mechanisms in *Ascaris* and alternative control options.

Materials & methods: Beta-tubulins are major benzimidazole targets. Sequence data in repositories was searched to identify *Ascaris* β-tubulin isotypes and expression in different *Ascaris* tissues was investigated using RT-qPCR and transcriptomic data analysis. In silico docking and molecular dynamics simulations were conducted to investigate binding of benzimidazoles to *Ascaris* ß-tubulins. An amplicon deep sequencing approach was used to screen an archive of *Ascaris* specimens for mutations in two β-tubulins which might be involved in anthelmintic resistance.

In separate work, *Ascaris* proteomes predicted from whole-genome sequencing data were analysed for potential vaccination targets using bioinformatic tools (including Vacceed, VaxiJen, Bepipred 2.0) which tested for different characteristics such as sub-cellular location, T-cell and B-cell molecular binding, antigenicity, allergenicity and phylogenetic relationship with other nematode proteins.

Results: Seven *Ascaris* β-tubulin isotypes were identified, with isotypes A (BtA) and B (BtB) the most widely expressed in adult worms. In silico analyses revealed that seven isotypes were capable of binding benzimidazoles and identified key residues involved in binding. Amplicon sequencing yielded 187 results from BtA and 164 from BtB. No SNPs previously associated with benzimidazole resistance in nematodes were identified in *Ascaris* BtA or BtB.

Out of 100,000 protein sequences analysed, four transmembrane proteins were predicted to be potential vaccine candidates: a Piezo protein, two voltage-dependent calcium channels and a protocadherin-like protein. These were expressed in the muscle or ovaries of *Ascaris* and all contained high affinity epitopes for human T-cells and B-cells.

Conclusion: With decreasing sequencing costs and expanding "omic datasets, *in silico* and "omic approaches are increasingly useful to explore parasite transmission dynamics, investigate drug resistance mechanisms and identify new vaccine and drug targets.

SE27.05

In vitro and _In vivo_ characterization of niclosamide ethanolamine against the fox tapeworm, _Echinococcus multilocularis_

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The fox tapeworm, *Echinococcus multilocularis*, is the most important foodborne parasite in Europe. It causes the severe and neglected disease alveolar echinococcosis (AE) in humans and other animals. The tumor-like growth of this parasite is caused by the larval metacestode stage mainly in the liver of patients. Drug treatment options are based on benzimidazoles, which are not always efficacious and can induce adverse effects. The discovery of new and better drug treatment options against AE is urgently needed.

Niclosamide is an anthelmintic drug, but it does not reach the tissue-dwelling parasites like the AE metacestode in the liver, as it is poorly absorbed from the intestine. We here repurposed niclosamide ethanolamine (NEN) from the field of cancer research, as it shows an improved systemic exposure profile with higher drug levels also in the liver. The activities of niclosamide and NEN *in vitro* on *E. multilocularis* metacestode vesicles (EC50<0.2 µM) and primary parasite cells (EC50<0.3µM) are well in the range of NEN levels reaching the liver. Using the Cell Mito Stress Test in a Seahorse machine we found that NEN acts as a mitochondrial uncoupler in *E. multilocularis* primary cells, which can, at least partially, explain its mode of action on the parasite.

We also performed an *in vivo* study in a secondary AE mouse model. In this model, metacestode material from *in vitro* cultures was directly injected into the peritoneal cavity of BALB/c mice, resulting in rapid growth mimicking the advanced stage of the disease. The treatments were performed by semi-voluntary micropipette drug administration using condensed milk, twice per day, 5 times per week, for 9 weeks. However, contrary to control treatment with albendazole (200 mg/kg), oral treatment with NEN (40 mg/kg) was ineffective against AE in mice. A possible explanation for this outcome could be the drug's metabolization and thus limited bioavailability in the parasite. This is currently further investigated based on mouse plasma and parasite tissue samples. Future investigations should explore new formulations and derivatives of niclosamide to enhance its efficacy against AE *in vivo*.

SE27.06

Screening of the MMV pandemic response box reveals a promising novel compound for the treatment of alveolar echinococcosis

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The metacestode stage of *Echinococcus multilocularis* causes the emerging zoonotic disease alveolar echinococcosis (AE). Today"s chemotherapeutic treatment options rely solely on benzimidazoles, which only exhibit a parasitostatic effect, inhibiting metacestode growth without achieving parasite eradication. Thus, there is an urgent demand for novel treatment options against AE.

In this study, we employed whole organism-based drug screening to test the 400 compounds of the Medicines for Malaria Venture (MMV) Pandemic Response Box on *E. multilocularis in vitro.* Using damage-marker release assay in combination with metacestode viability assays five compounds were found to be active. These compounds, namely, alexidine, carbendazim, 2,4-diiodoemodine, ESI-09 and oxfendazole were further characterized by assessing parasiticidal potential on *E. multilocularis* primary cells and cytotoxicity on mammalian cells. With an IC50 of 2.45 ± 0.86 µM against *E. multilocularis* primary cells and an IC50 of 37.33 ± 0.73 µM and 9.02 ± 1.30 µM against confluent and pre-confluent RH- cells respectively, ESI-09 demonstrated the broadest therapeutic window.

ESI-09 was first described as an exchange protein activated by cAMP (EPAC) inhibitor and more recently also as a mitochondrial uncoupler in mammalian cancer cells. To investigate the effect of ESI-09 on *E. multilocularis* mitochondria, we employed Seahorse assays, TMRE assays and transmission electron microscopy. We have found that indeed also in *E. multilocularis* ESI-09 can act as a mitochondrial uncoupler. Concluding, screening of the MMV Pandemic Response Box has revealed ESI-09 as the compound with the broadest therapeutic window against *E. multilocularis*. Additionally, our experiments have shown that ESI-09 acts on the mitochondria of the parasite. If ESI-09 is also efficacious *in vivo* is to be tested.

SE27.07

Emerging pathogenic strongylid infections in the Virunga massif mountain gorillas

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Emerging health challenges threaten the conservation of mountain gorillas (*Gorilla beringei beringei*), particularly with increasing reports of strongylid nematode-associated gastrointestinal disease in the two extant populations. Using metabarcoding, we evaluated strongylid diversity in fecal samples from the entire Virunga Massif gorilla population in 2018, analyzing factors such as spatial distribution, habitat, and environmental gradients that influence parasite diversity and abundance. Additionally, archived biobank samples from 2001 were analyzed to assess temporal variation. We identified *Hyostrongylus* nematodes—strongylid pathogens typically associated with suids—as dominant contributors to gastric lesions observed in necropsies, particularly in high-altitude regions. Spatial analyses linked strongylid community composition to environmental gradients, underscoring the role of climate and vegetation in shaping parasitic dynamics. Temporal variation between 2001 and 2018 revealed an increasing dominance of *Hyostrongylus* in areas with elevated disease occurrence, coinciding with population growth, limited spatial expansion, and changes in social structure since 2007. Our findings highlight the critical need for continued monitoring of parasitic diseases in isolated populations like mountain gorillas. The emergence of *Hyostrongylus* demonstrates the importance of long-term biobanking, ecological monitoring, and One Health approaches to mitigate health threats. These results offer insights into managing island populations and emphasize the broader conservation relevance of understanding host-parasite-environment interactions to prevent biodiversity loss.

SE29.01

A genome-wide CRISPR base editing screen reveals novel drug resistance biomarkers in *Leishmania*

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Leishmania parasites cause a spectrum of diseases in their mammalian host, ranging from cutaneous lesions to lethal visceral infections. With no human vaccine available, anti-leishmanial drugs remain the primary treatment option for the foreseeable future. Understanding the mechanisms of action of these drugs, identifying their molecular targets, and uncovering potential resistance pathways are critical for effective treatment strategies.

Here we utilize a CRISPR/Cas9 cytosine base editor (CBE) for a genome-wide reverse genetic screen to identify drug resistance biomarkers in *Leishmania*. By introducing premature stop codons into 94% of protein-coding genes, we systematically disrupt gene function and identify both known and novel drug resistance-associated genes. Validation experiments by CRISPR mediated gene replacement confirm the identification of six previously unknown proteins required for miltefosine and/or amphotericin B sensitivity, as well as several ceramide biosynthesis-related genes linked to pentamidine resistance. Notably, we also find that the loss of genes encoding intraflagellar transporters and BBS9, but not other BBSome components, confers resistance to antimony. In addition, we identified novel resistance markers for the experimental anti-leishmanial compound 1c, including two linked MAP kinases associated with flagellar length phenotypes. Starting to gain further mechanistic insight into these mutants, mass spectrometry analysis of two hypothetical proteins with transmembrane domains, previously unlinked to amphotericin B resistance, revealed sterol deficiencies similar to those observed in sterol C-5 desaturase mutants.

Overall, our study identifies unique and largely non-overlapping drug resistance biomarkers for multiple anti-leishmanial drugs. Additionally, fitness screening with the same libraries generates a comprehensive genome-wide map of essential and fitness-associated genes in *Leishmania*, demonstrating the effectiveness of base editing for reverse genetic screening in *Leishmania* research.

SE29.02

The nonrandom tridimensional nuclear organization of *Trypanosoma cruzi*

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The protozoan *Trypanosoma cruzi*, the causative agent of Chagas disease, exhibits polycistronic transcription, with approximately 50% of its genome composed of repetitive sequences, including both coding (primarily multigenic families) and non-coding regions (such as ribosomal DNA, spliced-leader (SL), and retroelements, etc). Genomic sequences, including repeats, motifs of architectural proteins, and nonprotein-coding RNA loci are crucial for genome folding. Here, we evaluated the genomic features associated with higher-order chromatin organization in *T. cruzi* (Brazil A4 strain) through extensive computational processing of high-throughput chromosome conformation capture (Hi-C) using the mHi-C pipeline, designed to handle multimapping reads. We demonstrated that applying canonical Hi-C processing, which overlooks repetitive DNA sequences, results in a loss of DNA-DNA contacts, misidentifying them as CF boundaries. Our analysis revealed that loci encoding multigenic families of virulence factors are enriched in chromatin loops and form shorter and more compact chromatin folding structures, in contrast to loci encoding core genes. We uncovered a non-random 3D genomic organization in which nonprotein-coding RNA loci (tRNAs tRNAs, snRNAs and snoRNAs) and transcription termination sites are preferentially located at the boundaries of the chromatin-folding domains, while pseudogenes and multigenic family genes located in unstructured genomic regions. Our data indicate 3D clustering of tRNA loci, likely optimizing transcription by RNA polymerase III, and a complex interaction between spliced-leader RNA and 18S rRNA loci, suggesting a link between RNA polymerase I and II machineries. Finally, we highlighted a group of genes encoding virulence factors that interact with SL-RNA loci, suggesting a potential regulatory role. Our findings provide insights into 3D genome organization in *T. cruzi*, contributing to the understanding of supranucleosome-level chromatin organization and suggesting possible links between 3D architecture and gene expression.

SE29.03

Investigating the role of some compounds in targeting RNA-binding proteins and mRNA polyadenylation in trypanosomes

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Trypanosoma brucei, the parasite responsible for African trypanosomiasis, exists in two forms: bloodstream forms in mammals and procyclic forms in tsetse flies. In Kinetoplastids, most protein-coding genes are organized into polycistronic transcription units, with mRNAs processed via 5" *trans*-splicing and 3"-polyadenylation. The regulation of mRNA stability and translation primarily occurs post-transcriptionally, with RNA-binding proteins (RBPs) playing a crucial role, especially in the 3"-untranslated regions (3"-UTRs) of mRNAs (Clayton 2019).

In our previous study (Bishola Tshitenge and Christine Clayton, 2022), we identified the RNA-binding protein DRBD18 as essential for the survival of bloodstream-form *T. brucei*. RNAi-mediated depletion of DRBD18 led to growth arrest and affected the processing of over 200 mRNAs, including RBP10, which has an unusually long 3"-UTR (7.3 kb). DRBD18 depletion caused the accumulation of mRNA isoforms with shortened 3"-UTRs, which were often more abundant than their longer counterparts in control cells. These findings suggest that DRBD18 plays a pivotal role in regulating 3"-UTR length and mRNA processing.

The RNA-immunoprecipitation demonstrated that DRBD18 associates with mRNAs containing long 3"-UTRs, particularly those with polypyrimidine tracts. Mass spectrometry analysis revealed that DRBD18 interacts with the Nup76 complex and the nuclear export protein MEX67, suggesting that it helps export mRNAs with long 3"-UTRs to the cytosol. Single-molecule fluorescence in situ hybridization revealed that DRBD18 binding prevents splicing factors from recognizing internal polypyrimidine tracts within 3"-UTRs, ensuring correct 3"-UTR length. Additionally, DRBD18 binding promotes the export of these mRNAs, which is crucial for proper gene expression. This is the first time a trypanosome RNA-binding protein has been linked to determining *trans*-splicing and polyadenylation patterns of specific mRNAs. Our results show that DRBD18 depletion leads to alternative polyadenylation and accumulation of incorrectly processed mRNAs in the nucleus, which are unable to be properly exported to the cytosol. This discovery opens new avenues for targeting mRNA processing to combat trypanosomiasis, especially given the challenges posed by antigenic variation in variant surface glycoproteins, which complicates vaccine development.

Our current project, funded by TWAS-UNESCO, aims to evaluate novel compounds—including phenolic acids, natural compounds, and benzoxaboroles—for their ability to target DRBD18 and/or mRNA polyadenylation. In our first study, currently under review in *Nature Scientific Reports*, we explored the antioxidant potential of three medicinal plants, which contain alkaloids, saponins, flavonoids, iridoids, and anthraquinones. Moving forward, we plan to assess the effects of these compounds on *T. brucei*, with the goal of identifying new strategies to eliminate trypanosomiasis and potentially other diseases.

Fig. 1

SE29.04

Modeling interactomes of key *Leishmania trans*-regulators controlling parasite surveillance, infectivity and virulence

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Leishmania protozoan parasites present peculiar gene expression fundamentally dependent upon post-transcriptional control. This elevates the importance of RNA binding proteins (RBPs) for gene regulation in these parasites. Building upon the comprehensive RBPome we isolated previously (Pablos, Ferreira et al., MCP, 2019), a BarSeq CRISPR-derived trans-regulator knockout (RBP KO) screen reveals essential roles of RBPs for cellular differentiation and capacity to infect macrophages and mice. Individual RBP KO cell lines for critical trans-regulators isolated from the screen show normal growth dynamics during sandfly-infective stages while macrophage infection is inhibited or ablated. This validates essential roles for specific RBPs in Leishmania viability, transmission and virulence. Fifteen proteins were endogenously tagged, immunoprecipitated (IP) and submitted to transcriptomic and proteomic analysis to identify associated RNA and protein RNP components. Importantly, strong correlations exist between specific RBP protein complexes; interactome modeling groups correlative networks of the RNPs into 2 dominant modules implicit in parasite infectivity. Furthermore, gene ontology classifications of RNAs associated with these RBPs highlight select mechanisms. Overlapping associated transcript interactions will reveal common RNA targets. Examining regulation of associated RNAs may reveal gene regulatory pathways inherent to parasite survival and mammalian infection. These regulatory RNPs represent novel virulence complexes.

SE29.05

In vitro development of schistosomes – Old questions, new approaches

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Schistosomes are blood flukes that cause Schistosomiasis, a major Neglected Tropical Disease (NTD) that affects >250 million people worldwide and ~600 million at risk of infection in tropical endemic areas. With genetically-determined male and female individuals, schistosomes are the exception among flatworms, which are largely hermaphrodites. However, the parasites only become sexually dimorphic during development within the mammalian host. Cellular and molecular mechanisms underlying this process are poorly understood, partly due to intrinsic challenges in assessing the *in vivo* parasite development. Therefore, robust and reproducible approaches for maintaining and developing parasites *in vitro* may overcome these difficulties. Scarce studies to date have focused on culture protocols aiming at tackling the sexual dimorphism establishment in schistosomes. Here, we refined a protocol for long-term culture of newly transformed cercariae (human infective larvae) that developed *in vitro* into sexually dimorphic forms. We assessed the effect of two different sera, Foetal Bovine Serum (FBS) and Human Serum (HS), added to the culture medium supplemented with human red blood cells (hRBCs). The development of the parasites was followed over time and analysed by bright field microscopy and confocal imaging. Striking differences between the two conditions were observed; firstly, hRBCs were digested and the resulting hemozoin became apparent within the intestine from day 5 post-transformation in HS- but not in FBS- cultured worms. Second, while the majority of the latter did not progress beyond an early liver stage, sexual dimorphism was definitely established in the HS-cultured worms, albeit delayed compared to the *in vivo* development. EdU pulse: chase experiments revealed a continuous proliferation of parasite cells over time only in HS-cultured parasites. Moreover, we employed single cell RNA-seq to reveal cell types, genes, and molecular pathways underlying the observed phenotypes. These developments pave the way to study parasite development *in vitro*, positively impacting the 3Rs principles (Replacement, Reduction and Refinement) for animal research. Additionally, outcomes from this research may unveil targets for novel control strategies aiming at interrupting life cycle progression.

SE29.06

In vitro characterization of the unique *T. brucei* mrna deccaping enzyme alph1

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An important step in the control of eukaryotic gene expression is the regulation of mRNA degradation, which commonly starts with deadenylation, followed by the removal of the 5´ m7G-cap, and ends with the 5´-3´ exoribonucleolytic decay of the mRNA. Like in other eukaryotes, mRNA decay in Kinetoplastida follows these steps, with conserved enzymes for the deadenylation and 5´-3´ exoribonucleolytic decay. However, Kinetoplastida lacks homologs of the canonical enzyme responsible for 5´ cap removal, the nudix hydrolase DCP2, and its major partners. Instead, Kinetoplastida relies on a different and unique enzyme, the ApaH-like phosphatase ALPH1, to remove the cap. ALPH1 consists of a central catalytic domain, an unstructured and non-essential N-terminus, and a structured and more conserved C-terminus. It is important to point out that ALPH1 is essential for parasite survival, and ApaH-like phosphatases are absent in mammals, which brings special attention to this protein as a potential drug target for the treatment of parasitic diseases caused by kinetoplastids. With this motivation, we investigated and characterized the activity of *Trypanosoma brucei* ALPH1 *in vitro*.

First, we used caped RNA substrates and monitored decapping via a band-shift on a gel. To our surprise, we found that both the trypanosome-specific, heavily methylated cap 4 and the non-methylated cap 0 are cleaved by ALPH1, and this cleavage was also independent of the RNA sequence. Moreover, ALPH1 was able to cleave cap analogues and thus is not dependent on RNA. When directly comparing methylated and non-methylated cap analogues, we discovered that non-methylated cap analogues are much better substrates for ALPH1. This preference is surprising but may be related to the enzyme"s origin from the bacterial ApaH protein, known to cleave (non-methylated) Np4A nucleotides. Most interestingly, the m7G methyl group shifts the cleavage site preference from the pyrophosphate bound near the m7G to the pyrophosphate bound farther from the m7G. When transferred to an RNA substrate, this means that the m7G methyl group ensures the production of a monophosphate RNA, which is required for downstream degradation by the exoribonuclease, though at the cost of cleavage efficiency.

Our detailed analysis of the substrate preferences of *T. brucei* ALPH1 has already contributed to the optimization of an assay for a high throughput screen to find ALPH1 inhibitors. These are potentially exploitable as drugs to treat Kinetoplastida-caused diseases and we will present results from the first screens.

SE29.07

Investigation of the malate dismutation pathway as a potential drug target in *Echinococcus multilocularis*

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The zoonosis alveolar echinococcosis (AE) is caused by the metacestode of the fox tapeworm *Echinococcus multilocularis*. AE is characterized by tumor-like growth of metacestodes, primarily in the liver, and is fatal if untreated. Current treatments rely on benzimidazoles, which act parasitostatically but do not achieve a cure. Thus, novel therapeutic strategies are urgently needed.

Previous studies revealed significant release of succinate and acetate from *in vitro* cultured metacestode vesicles, suggesting an active malate dismutation (MD), a mitochondrial pathway that facilitates oxygen-independent ATP generation. MD utilizes an alternative electron transport chain and the unusual electron carrier rhodoquinone, absent in mammals, making it a promising drug target.

We demonstrated that *E. multilocularis* metacestodes and primary cells survive prolonged anoxia. Using targeted metabolomics and transcriptomics, we observed increased succinate production and elevated expression of transcripts involved in rhodoquinone biosynthesis under low oxygen conditions. Among these transcripts, *coq-2* exhibited the highest increase in expression. Notably, *E. multilocularis* exclusively expresses the rhodoquinone-specific isoform of *coq-2*, underscoring the importance of this pathway for the parasite.

Future experiments will explore the essentiality of MD for parasite viability and its potential for therapeutic intervention. These findings provide a strong foundation for developing novel treatments targeting parasite-specific metabolic pathways.

SE29.08

The *Plasmodium falciparum* histone methyltransferase SET2 regulates gene expression linked to cytoskeletal organization in gametocytes

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The intracellular and extracellular survival of the malaria parasite *Plasmodium falciparum* in the human and mosquito hosts depends on rapid morphological and physiological changes coordinated by various gene regulatory mechanisms. Growing evidence emphasizes the contribution of epigenetic control mechanisms, particularly histone post-translational modifications, during intraerythrocytic replication and immune evasion of the asexual blood stage parasites. The role of histone post-translational modifications during the sexual development of the parasite, however, is not well studied. Previous chemical loss-of-function studies in gametocytes using the histone methyltransferase inhibitor BIX-01294 resulted in the dysregulation of gene expression and, consequently, impairment in gametocyte development and gametogenesis.

In this study, we aimed to phenotypically investigate the role of the histone methyltransferase *Pf*SET2, one of ten known SET proteins of *P. falciparum*, in gametocyte development. Localization and expression of *Pf*SET2 in the *P. falciparum* blood stages were investigated by immunofluorescence assay and Western blotting. To functionally characterize *Pf*SET2, *Pf*SET2-KO parasite lines were generated via the selection-linked integration-mediated targeted gene disruption method and subjected to cell-based assays. Morphologic changes in the *Pf*SET2-KO lines were further investigated via high-resolution and electron microscopy. Additionally, comparative transcriptomics were performed.

We demonstrate that *Pf*SET2 is expressed in the nuclei of the asexual and sexual blood stages. While *Pf*SET2-KO does not affect intraerythrocytic replication, it significantly impairs gametocyte formation and particularly, male gametogenesis. Late stage *Pf*SET2-KO gametocytes exhibit abnormal morphology due to defects in cytoskeletal organization. Additionally, the development of flagellated male gametes was disrupted, probably stemming from cytoskeletal abnormalities during flagella formation. In accord with these findings, *Pf*SET2-KO parasite exhibit deregulated genes encoding for axoneme regulation and transcription.

Our findings demonstrate that *Pf*SET2 plays a crucial role for cytoskeletal organization during the maturation of transmissible gametocytes.

SE30.01

Trypanosoma brucei brucei-induced aggregated NETs (*agg*NETs) are dependent on purinergic P2X1 and P2Y6 receptors

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Introduction: *Trypanosoma brucei brucei* is an important parasite able to infect a wide range of hosts, including humans, domestic animals and wildlife. In cattle, the disease is known as Nagana, which severely impacts the herd health status, leading to significant economic consequences. In the mammal host, *T. b. brucei* lives extracellularly in the bloodstream, where it multiplies as trypomastigote forms. Here, the parasite can directly interact with leukocytes of the innate immune system, such as polymorphonuclear neutrophils (PMN). Former studies showed that *T. b. brucei* stages effectively induce neutrophil extracellular trap (NET) formation in PMN. While purinergic signalling is known to play a pivotal role in PMN activation and immune function, the role of this signalling pathway in *T. b. brucei*-activated bovine PMN and aggregated NET release (*agg*NETs) remains poorly understood.

Objective: The aim of the current study was to investigate the formation of *agg*NETs induced by *T. b. brucei*-bloodstream trypomastigotes and to assess the role of different purinergic receptor-dependent signaling pathways and oxygen concentration in *T. b. brucei*-induced *agg*NET formation.

Material and Methods: NET release was illustrated by scanning electron microscopy (SEM). NET formation in presence or absence of the purinergic inhibitors NF449 (P2X1 inhibitor) and MRS2578 (P2Y6 inhibitor) and under different oxygen concentrations (5% O2, hipoxia and 21% O2, normoxia) was assessed via the detection of citrullinated histones, neutrophil elastase (NE) and DNA in NET-like structures by immunofluorescence microscopy. Parasite-driven PMN activation (in the presence or absence of the inhibitors) was measured on the level of oxygen consumption (OCR) and extracellular acidification (ECAR) rates via Seahorse® analyses. 3D holotomographic illustration was performed via 3D holotomographic microscopy (Nanolive®).

Results: Exposure of bovine PMN to *T. b. brucei* resulted in a significant activation of PMN since oxidative (OCR) and glycolytic (ECAR) PMN responses both revealed increased early after co-culture. Pre-treatments of PMN with the purinergic inhibitors NF449 and MRS2578 diminished OCR and ECAR values, thereby confirming a P2X1- and P2Y6-dependent parasite-driven PMN activation, respectively. As expected, SEM and immunofluorescence analyses illustrated that parasite encounter induced the formation of *agg*NETs at 4 and 18 hours of exposure, with no differences observed regardinO2 concentrations. The role of purinergic signaling in aggNET formation was confirmed by PMN treatments with NF449 and MRS2578, leading to a decreased release of *T. b. brucei*-induced *agg*NETs. Live-cell 3D-holotomographic analyses was successfully applied to illustrate and calculate related volumes of *agg*NETs.

Conclusion: Alltogether, the current data highlight the key role of purinergic signaling in *T. b. brucei* trypomastigote-driven PMN activation and NET formation.

SE30.02

CD160, a crucial regulator for ILC2 function during helminth infection

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Introduction: *Strongyloides ratti* is a rodent-specific parasitic nematode that displays tissue-migrating and intestinal life stages. Infection resolves within 2-4 weeks in the context of an adaptive type II immune response. Infection termination depends entirely on adaptive immunity, as RAG-/- mice lacking T and B cells remain infected for up to one year. Nevertheless, innate immunity is highly effective in reducing the number of intestinal parasites, from approximately 80 parasites per mouse on day 6 post-infection (p.i.) to only 2-5 parasites on day 10 p.i. and later time points. We have shown previously that ejection of parasites depends on mucosal mast cells that are activated by ILC2 in an IL-9-dependent manner before adaptive immunity is established. CD160, a regulatory receptor expressed on T cells as well as innate immune cells like NK cells and ILC1, emerges as a key factor in this context.

Material & methods: The parasitic nematode *Strongyloides ratti* is a suitable model to analyse the immune response and immunoregulation during infection with a helminth that displays tissue-migrating and intestinal life stages in the mouse mode. $RAG^{-/-}CD160^{-/-}$ and $RAG^{-/-}$ mice were compared to investigate the impact of CD160 during helminth infection. Receptor expression analysis of the CD160 was performed by flow cytometry. The allergen *Alternaria alternata* was used to investigate ILC2 function *in vivo*. Cytokines were quantified via ELISA or intracellular flow cytometry staining.

Results: Here we report for the first time that CD160 is expressed on intestinal ILC2 and upregulated during *S. ratti* infection *in vivo*. Intestinal ILC2 expand in *S. ratti* infected WT mice. By contrast, $RAG^{-/-}$ $CD160^{-/-}$ mice did not show intestinal ILC2 expansion during infection. CD160-deficient ILC2 produced fewer type 2 cytokines compared to WT ILC2 in response to alarmin cytokine (IL-33) activation *in vitro* and allergen (*Alternaria alternata*) stimulation in *vivo*. Comparing *S. ratti* infection kinetics in $RAG^{-/-}$ and $RAG^{-/-}CD160^{-/-}$ mice, a consistent intestinal parasite burden of approximately 50 parasites per mouse was observed from day 6 to day 97 p.i. in $RAG^{-/-}CD160^{-/-}$ mice . Notably, the absence of CD160 correlated with a lack of intestinal mucosal mast cell expansion and activation. Finally, adoptive transfer of intestinal CD160-competent but not of CD160-deficient ILC2 reduced the worm burden in *S. ratti* infected $RAG^{-/-}CD160^{-/-}$ mice and restored mucosal mast cell activation.

Conclusion: In summary, our findings suggest that CD160 is a novel positive regulator of ILC2 during intestinal helminth infection.

SE30.03

Elucidating helminth-mediated suppression of anti-influenza vaccination efficacy

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Introduction: Pre-existing helminth infection, afflicting more than a quarter of the human population, hampers the efficacy of vaccination, pivotal against life-threatening diseases like emerging viruses. We have shown before that infection with *Litomosoides sigmodontis*, a murine model for human filariasis leads to a reduced influenza vaccine-specific humoral responses and increased viral load and weight loss upon challenge infection. Specific immune signatures to predict such subpar vaccine response in helminth-infected individuals are lacking. This study aims to better understand the influence of the murine helminth infections on vaccine responses and efficacy in detail.

Methods: To compare the suppressive influence of two helminths during both active infection and following clearance, we infected C57BL/6 mice with *L. sigmodontis* or *Heligmosomoides polygyrus*. Mice were vaccinated at day 30, 60 and 90 post infection using the commercially available adjuvanted vaccine Fluad. We analyzed the vaccine response by measuring the different antibody (Ab) isotypes in blood by ELISA. Spectral flow cytometry was used to verify the presence of regulatory B and T cells, and their expression of checkpoint molecules in different organs. Additionally, we aim to analyze the cytokine profile in the bronchoalveolar lavage fluid and serum, and compare the influenza-specific germinal center reaction in helminth-infected and non-infected mice.

Results: Vaccination of non-helminth infected mice resulted in high influenza-specific IgG1, IgG2c, IgG2b and IgG3 titres. Comparison of the influenza vaccine specific Ab titres revealed a more robust suppression of all isotypes by *L. sigmodontis* in contrast to *H. polygyrus*. In line with our previous results using a non-adjuvanted vaccine, infection with *L. sigmodontis* results in an increase of regulatory T cell subsets at the site of infection, the thoracic cavity. Surprisingly, the cellular compartment in the lung as well as the bronchoalveolar lavage was altered by *L. sigmodontis* infection, even after clearance of the infection. By contrast, changes at the cellular level were less pronounced in *H. polygyrus* infected mice.

Conclusion: Preliminary data points towards an impaired vaccine response in both helminth mouse models, with *L. sigmodontis* being more potent. In the long run, identifying immune signatures will aid in establishing the molecular pathway associated with the diminished response.

SE30.04

The metabolic program of inflammatory skin-imprinted eosinophils accounts for the chronicity of *Leishmania mexicana* infections

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Eosinophils are myeloid cells that are primarily known for their role in type 2 immune disorders and in the control of helminth parasites. Recent research has identified novel functions of eosinophils in immune homeostasis, metabolic regulation and tumour defence. However, the contribution of eosinophils to the pathogenesis of microbial skin diseases remains poorly understood. Here, we used a mouse model of chronic cutaneous leishmaniasis (CL) caused by the protozoan parasite *Leishmania (L.) mexicana* to investigate the function and transcriptomic signature of eosinophils in the skin.

In C57BL/6 wild-type mice, *L. mexicana* led to local and systemic eosinophilia, which was dependent on type 2 innate lymphoid cells and interleukin (IL)-5 and paralleled by the development of chronic CL. Disruption of initial eosinophil infiltration by anti-IL-5 treatment or genetic depletion of all eosinophils (dblGATA-1 mice) resulted in a more pronounced Th1 and M1-like macrophage response in the skin and clinical resolution of disease. Notably, the Th2 response was not affected by the lack of eosinophils. Single cell RNAseq analysis of the transcriptome of skin-infiltrating eosinophils revealed a hitherto uncharacterized inflammatory trajectory characterized by the elevated expression of *Cd274*, *Il1rn*, *Fcgr3*, *Hif1a*, and *Nfkb1*. Furthermore, eosinophils recruited into the dermis exhibited a marked upregulation of the high-affinity glucose transporter 3 (*Slc2a3*), exceeding the expression level found in *Ifng*+ Th1 cells. In accordance with the increased *Slc2a3* expression, skin lesion-derived eosinophils demonstrated significantly higher uptake of the fluorescent glucose analogue 2-NBDG compared to CD4+ T cells. Finally, sorted skin lesion-derived eosinophils, which were cultured together with in vitro generated Th1 cells, suppressed the IFNγ response of the T cells. We propose that eosinophils deprive Th1 cells of glucose and thereby impede their IFNγ production and the resolution of *L. mexicana* infections.

Collectively, our data expands the understanding of the transcriptional landscape and functional heterogeneity of distinct eosinophil subsets. Highly inflammatory eosinophil subsets and their metabolic competition with Th1 cells were identified as crucial drivers of chronic inflammation in *L. mexicana* infection.

SE30.05

Role of IL-33 in alveolar echinococcosis

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Alveolar echinococcosis (AE) is a zoonotic disease caused by the fox tapeworm *Echinococcus multilocularis*. In AE, the immune response is directly linked to parasite growth and disease progression, with high levels of inflammation resulting in parasite remission and misdirected immunity driving parasite growth. IL-33 is an alarmin, commonly expressed in mucosal barrier surfaces, which has a role in many conditions including allergy and parasitic infection. Previous research indicates IL-33 plays both a protective and exacerbatory role in parasitic infections, but its contribution to *E. multilocularis*-induced liver pathology and disease progression remains poorly understood.

Here we found that in mice, at 16 weeks post infection with *E. multilocularis*, there is elevated transcript levels of *Il33* and *Il1rl1* (encoding for ST2, the IL-33-specific receptor moiety) in the liver, but not in the spleen. Liver structural cells, including liver endothelial cells, hepatocyte progenitor cells and cholangiocytes, are the major cellular sources of IL-33. Despite an increase in the abundance of multiple immune cell types, such as Kupffer cells, peritoneal macrophages and CD4+ T cells post infection, FOXP3+ T-regs were the only cell subtype to express elevated levels of ST2. These data suggest that IL-33 is likely to influence ST2+ FOXP3+ Tregs post infection, which potentially contributes to the anergic immune response seen in late-stage AE.

E. multilocularis derived products have been shown to modulate activating/suppressing immune mechanisms in AE at various points during infection. Applying an *in vitro* approach using *E. multilocularis* vesicle fluid (VF), we found that VF induces IL-33 expression and secretion from NIH/3T3 fibroblasts. Subsequent fractionation experiments indicated that proteins, such as the highly abundant antigen B (AgB), are unlikely to be responsible for inducing IL-33. Further investigations are being carried out to determine specific compounds that induce IL-33, and how IL-33/ST2 signalling affects host/pathogen interactions during AE.

SE30.06

Naturalizing immunity to hookworm

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Hookworm infection afflicts around 500 million people worldwide and additional tools are needed to achieve the WHO NTD roadmap, including the development of a vaccine to reduce morbidity. Current laboratory models of human infection fail to replicate a key characteristic of human hookworm infections, namely that no protective immunity is established. The "translational hurdle" of generating clinically relevant insights from laboratory mouse models is widely observed in biomedical research and "naturalization" has been proposed as a solution. Naturalized mice are exposed to microbes, pathogens or even the natural environment to better induce immune system maturation. Here we implement the Wildling mouse model to study helminth infection and immunity in mice with a complex microbiome and exposome. While SPF laboratory mice display near full protection towards *Nippostrongylus brasiliensis* reinfection, we observed that Wildlings are susceptible to reinfection, in line with human observations. This is associated with a dampening of type 2 immunity in the T cell and ILC2 compartments throughout infection, as observed by scRNAseq and flow cytometry. During primary infection, morphological characteristics of *N. brasiliensis* from Wildling mice and SPF mice were similar. Interestingly, we observed a delay in expulsion of the parasite. In the small intestine, we found that the prototypical anti-helminth "weep and sweep" response was delayed in Wildling mice, with both tuft cell numbers and goblet cell hyperplasia reduced as compared to SPF mice. Naturalizing helminth research could thus bridge the current translational gap and offer a unique understanding of the host-helminth interaction.

SE30.07

Assessment of Foxp3, T-bet, and Gata3 gene expression in splenocytes of Experimental Autoimmune Encephalomyelitis (EAE) induced mice following challenge with *Toxocara canis* recombinant C-type lectin

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Toxocara canis is a zoonotic parasite causing chronic toxocariasis, with its excretory-secretory (ES) products, particularly C-type lectins, driving Th2 and regulatory immune responses.

Objectives: This study explored the effects of *T. canis* recombinant C-type lectin (rCTL) on Experimental Autoimmune Encephalomyelitis (EAE), assessing brain cell infiltration and regulatory T cell populations (CD4+ CD25+ Foxp3+) in BALB/c mice splenocytes to understand its immunomodulatory potential. In a first experience, six-week-old female BALB/c mice (n=8) received rCTL protein intravenously and intraperitoneally in six doses. After 28 days, brain and spleen tissues were analyzed. Histopathology showed no cerebral artery cell infiltration, while flow cytometry revealed higher FOXP3+ regulatory T cell percentages (2.59%, 1.64%, 1.78%) in treated mice versus controls (1.14%, 1.13%, 1.15%). The protein enhanced regulatory T cells, suggesting immunomodulatory and anti-inflammatory potential. In a second experience, female C57BL/6 mice (n=20) were divided into four groups. EAE was induced in groups 1 (MOG + TcES Ag) and 2 (MOG). After 28 days, TcES Ag decreased Number of transcribed copies of T-bet and GATA-3 gene expressions, and increased Foxp3 copies, as well as, reduced disability scores, highlighting its therapeutic potential. In a parallel study, female C57BL/6 mice (n=40) were injected subcutaneously and intraperitoneally with 30 µg rCTL three times weekly. EAE was induced using MOG35-55 peptide, with weight and clinical scores monitored. Splenocyte analysis via real-time PCR and ELISA assessed T-bet, Gata3, Foxp3 expression, and cytokine levels (IL-4, IFN-γ, TGF-β). Results showed rCTL reduced clinical disability, delayed EAE onset, and modulated immune responses by upregulating Foxp3 mRNA and increasing TGF-β production, highlighting its potential therapeutic role in EAE.

Keywords: Experimental Autoimmune Encephalomyelitis, Histopathology, Real-Time PCR.

SE31.03

Kichochofilm – The story of Shukuru: A film about schistosomiasis as an intervention in Pemba Island, Tanzania

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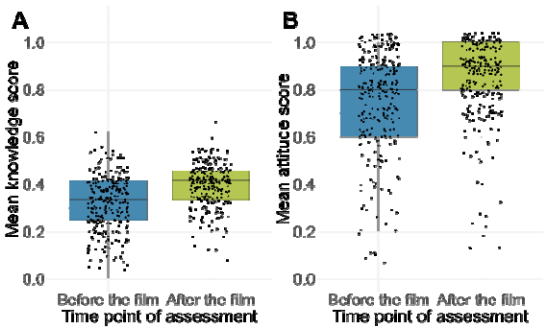
Schistosomiasis is a neglected tropical disease (NTD) that currently puts people from 78 countries at risk of infection and serious health consequences, primarily in sub-Saharan Africa. The World Health Organization recommends the implementation of treatment, snail control, and behavior change interventions to decrease the prevalence. However, behavior change interventions fitted to the culture and primarily aimed at adults are rare. Here we aim to determine the impact of the film on the knowledge, attitudes, and practices of schoolchildren and community members in Pemba, Tanzania.

From November 2023 to February 2024, a survey was conducted in 20 administrative areas in Pemba to assess the perceptions of community members on a film about schistosomiasis. In March 2024, the film was created based on the community"s perceptions. In July 2024, a study followed a pretest-posttest design to assess the impact of the film on knowledge, attitudes, and willingness to change behavior in children and community members in ten administrative areas. Linear mixed-effect models were used to assess the change in knowledge and attitudes before and after watching the film.

A total of 195 children and 195 adults were included in the impact study. Among all participants, the median knowledge score significantly increased by 0.08 units (SE = 0.01, t = 9.3, $p < 0.001$) and the median attitude score significantly increased by 0.08 units after watching the film (SE = 0.12, t = 6.9, $p < 0.001$). Before the film, 71.9% (200/278) of the participants indicated that they utilize water bodies for rice farming, swimming, washing clothes, or washing dishes. Among those, 72.5% (145/200) indicated that they never utilize protective clothing in water bodies or rice fields. After the film, 88.2% (240/272) of individuals indicated they were likely to avoid using water bodies, and 54.4% (148/272) said they would likely use protective clothing in the future.

Engaging behavior change tools, such as a film, particularly if created based on the community"s perception, can increase the knowledge and attitudes of schoolchildren and adults. Furthermore, individuals are willing to change their behavior into something that is self-protective and may protect them from being part of the transmission cycle. However, willingness to change behavior alone is not sufficient to achieve the elimination of schistosomiasis if the means to actually behave differently are not available. Hence, investments in infrastructure are needed to support the elimination of schistosomiasis.

Fig. 1



SE31.04

Designing effective public engagement in parasitology

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Public engagement and science communication are vital for building trust and fostering understanding between researchers and the general public. This is especially true in parasitology, where community involvement is critical for collecting patient samples and advancing treatment campaigns in endemic areas.

Community engagement is not a one-size-fits-all approach—it must be tailored to the unique needs and contexts of each community. This flexibility can present challenges but also offers opportunities for creative strategies. For example:

- In northern Malawi, street theatre was used to engage communities affected by African sleeping sickness.
- In northeast Thailand, a carnival at a rural food market drew attention to Opisthorchis viverrini, a parasitic infection impacting local populations.
- In southwestern Nepal, a science festival brought health and science education to isolated communities.

Central to effective engagement is prioritizing the community itself. All three initiatives employed a methodology developed at the University of Glasgow during the Wellcome Centre for Integrative Parasitology, emphasizing local leadership and co-creation at every stage, particularly when working outside the UK.

These projects were highly successful, meeting the goals set during their planning and delivering lasting benefits to the communities involved. Beyond their immediate impact, they serve as exemplary models of thorough and meaningful engagement, offering valuable insights for parasitology researchers worldwide.

SE31.05

Citizen science approach to detection of emerging ticks and tick-borne diseases

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Background: Vectors and vector borne diseases are rapidly emerging against a back drop of rapid climate and landscape change. The province of Saskatchewan represents 2 interface zones in Canada: the historical interface of the distributions of the Rocky Mountain Wood Tick (*Dermacentor andersoni*) in the west and the American dog tick (*D. variabilis*) in the east, and the western distributional limit of established populations of *Ixodes scapularis* (black-legged tick), the main vector of Lyme disease in North America. Our primary motivation is to detect establishment of this tick in Saskatchewan, as well as to determine shifts in geographic distribution of endemic ticks and detect adventitious, introduced ticks from movements of migratory wildlife, pets, and people.

Methods: We conducted passive surveillance for ticks recovered from people, animals, and the environment as part of a citizen science-based, national initiative called eTick, where digital photos of ticks are identified to genus level, and physical submissions can be requested for more definitive identification and molecular characterization. We also conduct regular systematic dragging in the environment as part of the Canadian Lyme Disease Sentinel Network. All *I. scapularis* are tested via PCR for DNA of *Borrelia* spp., *Babesia* spp., and *Anaplasma phagocytophilum*. Finally, we conduct sentinel surveillance in animals to determine exposure to Lyme disease and other tick borne zoonoses as an early warning signal for public health.

Results: Implementation of eTick has led to an increase in the number of *Ixodes* spp. detected over 5 years in a non-endemic region, and has also enabled reactive surveillance by dragging in the environment where the tick was acquired. eTick has also enabled detection of shifts in the geographic and seasonal distribution of endemic ticks. Despite regular annual detection of *I. scapularis* through passive surveillance, active surveillance indicated that endemic populations do not yet occur in Saskatchewan. However, for the first time, *I. scapularis* were detected in the province by ground dragging in 2023 and 2024, suggesting establishment may be imminent. Tick PCR and serology in coyotes and dogs suggests local transmission of *Borrelia burgdorferi*.

Conclusions: We conclude that animals make excellent sentinels for invasive ticks and tick-borne diseases, adventitial ticks regularly introduce *Borrelia* spp. (and other pathogens) into non-endemic regions, and citizen science and environmental surveillance combined reveal invasion of a new tick vector important for animal and human health. This approach is key to detecting and mitigating risks of emerging ticks and tick-borne diseases in a rapidly changing environment.

SE31.06

Co-developing a contextualised participatory health education program to address persistent schistosomiasis transmission in the endemic rural fishing community of Bugoto landing site Mayuge district Uganda

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Introduction: Schistosomiasis is a Neglected Tropical Disease transmitted by poor sanitation and lack of access to safe water (WHO, 2022). In Uganda, schistosomiasis is included in the national treatment program only through annual Mass-Drug-Administration (MDA), with no accompanying national health-education program (Lowenberg, 2014). The mainstream radio announcements for schistosomiasis sensitisation that were used, were reported to be confusing as they were locally calling schistosomiasis *ekidada* (swelling of the stomach) yet to the community *ekidada* was caused by witchcraft (Mujumbusi et al., 2023). This leaves affected communities with poor knowledge, attitudes and misperceptions about schistosomiasis prevention, transmission and treatment, negatively affecting control. Interventions targeting community sensitization and awareness have the potential to impact behavioural change through addressing contextual needs and involving end-users. This study aimed to co-develop a contextualised health education program to increase awareness and dispel myth and misconceptions about schistosomiasis transmission to improve schistosomiasis control in the endemic communities around Lake Victoria in Uganda.

Methods: Between September 2023&April 2024, we employed rural participatory appraisal at Bugoto landing site in Uganda. This involved surveys, and community meetings to identify communication challenges, knowledge gaps and schistosomiasis education priorities. Based on this, we co-developed education messages using community radios, drama and plays, posters, community meetings, training more Village Health Team members (VHTs), and showing videos about schistosomiasis lifecycle. To evaluate the outcome, we employed surveys, In-depth interviews, and focus groups discussions. Preliminary qualitative analysis was thematically conducted.

Results: The co-developed education programs led to increased knowledge about schistosomiasis, reduced the myths and misconceptions, resulted into behavioural change such as water processing, and latrine construction. Interestingly, the radio announcements increased demand for praziquantel which was earlier disliked because of perceived side-effects. Amongst the education programs, the use of community radios where the trained community VHTs sensitised the community was the most preferred.

Conclusion: These findings indicate that engaging the community to co-develop education messages addresses community needs and leads to improved knowledge, behavioral change and prevention practices. Thus, scaling up the intervention and integrating in the national MDA programs could offer a more sustainable impact.

SP02.01

Exploring methylation in apicomplexan parasites: A key regulator of cytoskeletal dynamics and motility

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Apicomplexan parasites, including *Toxoplasma gondii* and *Plasmodium spp.*, are responsible for devastating diseases such as toxoplasmosis and malaria. Their survival and pathogenicity heavily rely on intricate cytoskeletal dynamics and motility processes that drive host cell invasion, intracellular replication, and egress. Emerging evidence highlights the pivotal role of post-translational modifications (PTMs), particularly lysine methylation, in regulating these processes. In this project, we investigate the function of methylation in the recruitment and activity of key cytoskeletal proteins. Specifically, we focus on the PreConoidal Lysine MethylTransferase (PCKMT), a novel methyltransferase critical for motility initiation and cytoskeletal organization.

Using conditional knockout strains, immunofluorescence microscopy, mass spectrometry, and live-cell imaging, we aim to uncover the mechanistic roles of PCKMT and its interaction with pre-conoidal proteins such as the structural Conoid Gliding Protein (CGP) and Formin 1 (FRM1). Preliminary findings reveal that methylation is essential for the assembly and function of the parasite"s motility initiation machinery. Disruption of PCKMT results in severe motility defects, impaired invasion and egress, underscoring its importance in the parasite's lytic cycle.

Our work provides critical insights into how methylation governs parasite cytoskeletal dynamics, offering new avenues for therapeutic interventions targeting methyltransferases in Apicomplexan parasites.

SP02.02

From genome to function, from invasion to evasion – Deciphering the roles of venom allergen-like proteins in *Schistosoma mansoni*

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Introduction: Venom allergen-like (VAL) proteins are defined by the SCP/TAPS domain, which adopts a highly conserved α-β-α sandwich conformation. This strong conservation, together with their ubiquitous presence in helminth secretomes suggests a shared biological activity potentially tied to the parasitic lifestyle. In *Schistosoma mansoni*, 29 SmVAL genes had been identified to date, but functional data is scarce. However, the advances in the quality of the *S. mansoni* genome present new opportunities to explore the SmVAL family in greater depth.

Objectives: This study investigated the diversity, expression, and functions of SmVAL proteins across parasite life stages, focusing on their roles in host-parasite interactions.

Materials & Methods: Using the updated version of the *S. mansoni* genome, the SmVAL family was revisited. Meta-analysis of publicly available RNASeq data was performed to uncover the expression profiles of SmVALs across the life cycle. Functional analyses were carried out through protein expression in HEK293 cells, localization studies, immunological assays, protein-protein interaction screens, and cell culture-based functional assays.

Results: The family revision uncovered six novel SmVAL genes, bringing the total number of SmVALs to 35. RNASeq meta-analysis of all SmVAL genes revealed distinct expression profiles of SmVALs, with the highest expression observed in eggs and miracidia. Localization studies together with lack of interactions with human cell receptors indicated that egg-associated SmVALs are probably utilized by miracidia to penetrate the intermediate host. Additionally, cercarial SmVAL10 was found to alter the definitive host's immune response, promote keratinocytes proliferation, ECM remodeling, and stimulate angiogenesis.

Conclusion: This study expands the known repertoire of SmVAL genes, updates on the family relationships and expression patterns and provides a detailed characterization of candidate larval SmVALs functional roles. While egg-associated SmVALs are likely implicated in molluscan host invasion, cercarial SmVAL10 seems to be important for the mammalian one. This study expands the understanding of host manipulation through SmVAL proteins and encourages reevaluating orthologous proteins in other helminths as genomic datasets improve.

Fig. 1

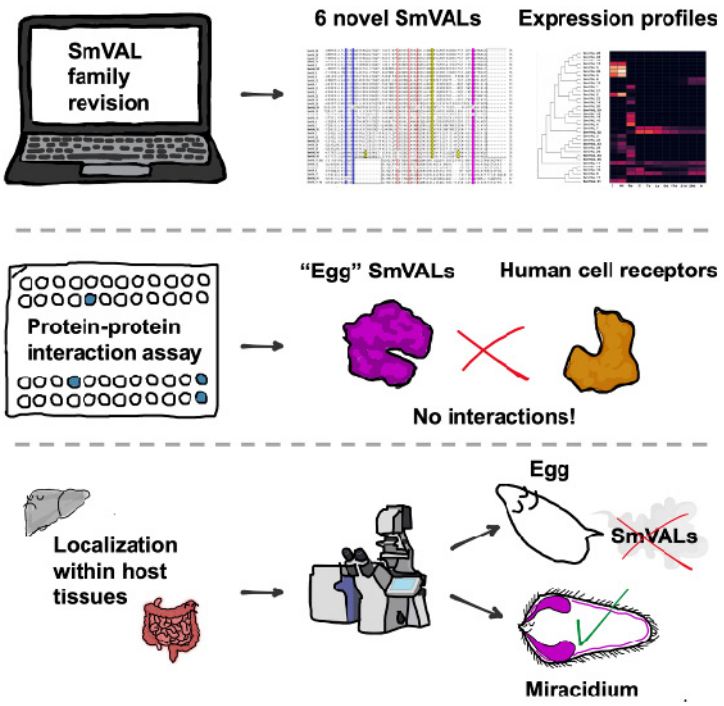
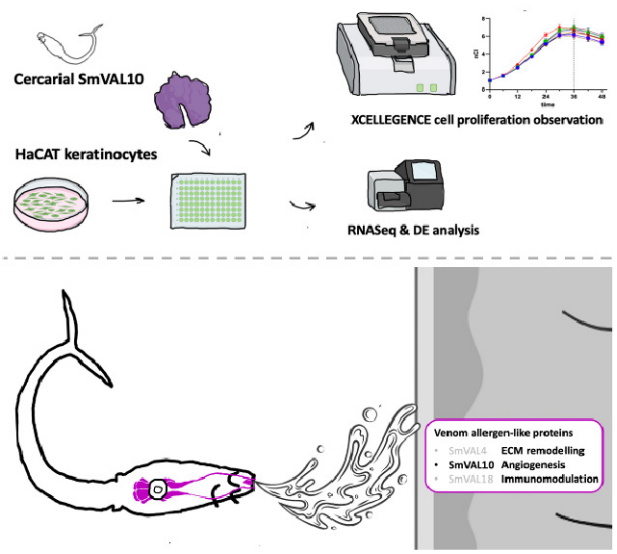

SmVAL family revision
6 novel SmVALs
Expression profiles
Protein-protein interaction assay
"Egg" SmVALs
Human cell receptors
No interactions!
Localization within host tissues
Egg
SmVALs
Miracidium

Fig. 2


Cercarial SmVAL10
HaCAT keratinocytes
XCELLEGENCE cell proliferation observation
RNASeq & DE analysis
Venom allergen-like proteins
SmVAL4 ECM remodelling
SmVAL10 Angiogenesis
SmVAL18 Immunomodulation

SP02.03

Molecular and cellular characterization of cell death pathways involved in *Leishmania* host cell exit and cell-to-cell transfer

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Introduction, Objectives: Although *Leishmania* parasites have been extensively studied, the mechanism by which the parasites exit from infected phagocytes in order to undergo cell-to-cell transfer remains unclear. Characterizing the role of cell death in the early steps of the parasite's life cycle will contribute to identifying new treatment options for leishmaniasis.

Previous infection experiments in mice and isolated human monocyte-derived macrophages demonstrated that apoptosis is elevated in infected cells. Additionally, studies showed that *Leishmania major* (*L. major)* proliferation increases cell death in infected isolated human phagocytes (Baars et al., 2023). Besides apoptosis, host cell pyroptosis can also participate in *Leishmania* spreading. By using BLaER1 monocytes as an infection model for *L. major* in a co-incubation assay, it was revealed that BLaER1 GSDMD$^{-/-}$ cells, which lack the pore-forming protein gasdermin D, are more resistant to pyroptosis. Moreover, it was discovered that infection of BLaER1 GSDMD$^{-/-}$ cells leads to a decreased parasite spread to new host cells compared to BLaER1 wild-type cells (Volkmar et al., 2023).

Results: The involvement of apoptosis in host cell exit and cell-to-cell transfer of *L. major* was characterized by using CRISPR/Cas9-generated BLaER1 knockout cell lines lacking the apoptosis executioner caspases 3 and 7 in co-incubation assays analyzed via flow cytometry. Preliminary results demonstrated a reduced secondary infection rate in BLaER1 CASP3x7$^{-/-}$ recipient cells after they were co-incubated with infected BLaER1 CASP3$^{-/-}$ or CASP3x7$^{-/-}$ donor cells compared to those that were co-incubated with BLaER1 WT or BLaER1 CASP7$^{-/-}$, respectively. Likewise, the percentage of dead donor cells was correlated with the secondary infection rate.

Conclusion: These first results indicate a role of caspase-3, but not caspase-7, in *Leishmania* host cell exit. The contribution of apoptosis in the exit mechanism will be further investigated by performing Annexin V/PI or TUNEL stainings followed by live cell imaging. Furthermore, caspase-8/10 and caspase-9-deficient BLaER1 will be generated to examine whether the intrinsic or extrinsic apoptosis pathway is engaged in *Leishmania* host cell exit.

SP02.04

Characterization of gametocyte egress vesicle proteins in *P. falciparum*

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The malaria parasite *Plasmodium falciparum* undergoes a complex life cycle involving both human hosts and female *Anopheles* mosquitoes. During a mosquito"s blood meal, intraerythrocytic sexual precursor cells of the parasite, the gametocytes, are ingested and undergo gametogenesis to form male and female gametes. Gametogenesis is triggered by specific environmental cues (T, pH, xanthurenic acid) and involves the sequential destruction of the two surrounding membranes, the parasitophorous vacuole membrane (PVM) and the red blood cell membrane (RBCM). Gametocyte egress follows an inside-out mode, during which the PVM ruptures prior to the RBCM. Two specialized types of secretory vesicles, the osmiophilic bodies (OBs) and PPLP2-positive egress vesicles (P-EVs) are key players of egress by facilitating the rupture of PVM and RBCM, respectively. A previous proximity-labelling analysis, using G377 (an OB protein), MDV1 (a male OB protein), and PPLP2 (a P-EV protein) as bait, demonstrated distinct proteomes of both vesicles and revealed 32 major gametocyte egress vesicle proteins (GEVPs).

These GEVPs included the GPI-anchored micronemal antigen (GAMA), the putative *Plasmodium* secreted ookinete proteins 1 and 12 (PSOP1 & PSOP12), as well as further proteins (GEXP12, GEVP1, GEVP3, GEST, MiGS). We here evaluate protein expression and localization of these GEVPs using high-resolution fluorescence imaging and western blot analysis. Mutant parasite lines were generated employing overexpression, inducible knockdown, and knockout vector systems. Additionally, gametocyte activation with xanthurenic acid allowed real-time analysis of the migration and secretion of the GEVPs during egress. Our initial data show that the selected GEVPs like PSOP1, PSOP12, and GAMA, display vesicular localization patterns and partial colocalization with egress vesicle markers (G377 and PPLP2). Upon gametocyte activation, GEVPs migrate to the parasite periphery and are secreted at specific time points, mimicking the migration dynamics of the established vesicle markers. These findings suggest that the identified GEVPs are likely involved in the egress process and may contribute to the regulated release of gametocytes. Further characterization of these proteins could deepen our understanding of vesicle-mediated gametocyte egress.

SP02.05

Uncovering the cellular diversity of *Fasciola hepatica* utilizing single-cell transcriptomics

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Introduction: The liver fluke *Fasciola hepatica* is a globally prevalent parasite that causes the zoonotic disease fascioliasis and resides in the bile duct of its final host where is sexually reproduces. To date, basic knowledge of the parasite"s cell types and cell-specific gene expression repertoire is missing. These kinds of data could be an invaluable resource for research on drug target genes as well as developmentally important genes. Past advances in transcriptomic technologies allow the analysis of transcripts from thousands of individual cells (scRNA-seq).

Objectives: Here, we report the first whole-organism cell atlas of the adult stage of F. hepatica, which serves as a basis for expression analysis of genes at the single-cell level.

Materials & Methods: Utilizing the Chromium kit from 10x Genomics, we developed a workflow featuring a new dissociation protocol that produced high-quality single-cell preparations from adult F. hepatica worms and enrichment of viable cells through flow cytometry. Bioinformatics analyses were performed with the Seurat software package in R, and the predicted clusters were confirmed using RNA in situ hybridization. RNA velocity was applied to predict cell differentiation lineages and parasite in vitro culture for functional gene characterization.

Results: Utilizing this workflow, we successfully annotated 15 distinct cell clusters, including gastrodermal cells, stem cells, and muscle cells. Additionally, we detected various cell clusters related to the reproductive tissue of the parasite, including the testes, ovary, and vitellarium. The resolution of this dataset allowed the discovery of dynamics along the vitelline differentiation lineages via RNA velocity. We uncovered two lineages stemming from different cell fate decisions: proliferating S1 cells and cells entering differentiation into mature vitelline cells. Further analysis revealed novel driver genes along the lineage of these cells. Finally, we could leverage this dataset for drug target research, as we identified several tissue-specifically expressed protein kinase genes, a gene family described as druggable in other helminths. The p21-activated kinase PAK4 was expressed in a functional network associated with cell adhesion and cytoskeleton organization. A commercially available inhibitor of human PAK4 showed in vitro activity against all intra-mammalian stages of the parasite.

Conclusions: With the *Fasciola* cell atlas at hand, we can gain valuable new insights into the biology of this parasite, predict gene function, and cell differentiation. Furthermore, the dataset proved its value as a resource for future drug target discovery.

SP03.01

High-dimensional spatial biology to understand host-parasite interactions – Murine acute pulmonary *Schistosoma mansoni* infection

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Schistosomiasis is a major Neglected Tropical Disease (NTDs) caused by the infection with parasitic flatworms in the genus *Schistosoma spp.*, affecting >200 million people world-wide. The life cycle involves two hosts where the parasite is exposed to multiple environments and tissue micro-niches promoting multi-organ morbidities. Thus, the worm expresses life-stage specific transcriptomic and morphologic profiles to survive in diverse landscapes. The parasite lung stage remains understudied, and no current knowledge is available on its interaction with the lung tissue. In hosts such as rats, we observe clearance of parasites in the lung, however in humans and mice these mechanisms are absent allowing the worm to traverse the lung without causing evident pathology. To date there is limited characterisation of the spatio-transcriptomic landscape of both host and parasite cells interacting with each other.

Our main objective is to delineate the immune landscape of the murine lung during acute pulmonary infection and investigate novel host-parasite interactions at the transcriptomic level. In addition to this we aim to reconstruct putative morphological compartments observed across other life stages of the worm, identify juvenile-specific morphology and mechanisms through leveraging not only single cell transcriptomic data but also observing the worm in its native spatial context.

Here we present single cell transcriptomics of lung-migrating juveniles of *S. mansoni* in a murine infection model (n=4) identifying cell populations underlying body organisation and developmental axis of the worm. We also explore tentative transcriptome perturbation and host-parasite interactions that may point to how the parasite can migrate in the absence of inflammation. Additionally, to interrogate the host-parasite interface we are currently generating a single cell spatial atlas of pulmonary *S. mansoni* infection utilising the new 10X 5K-plex Xenium Prime platform with a bespoke *S. mansoni* gene panel.

Our data shine light not only into the host-parasite interface within the lung, but also the biology of the poorly understood lung-stage of *S. mansoni*. Most importantly, we establish the feasibility of using high-dimensional spatial approaches to provide insight into the pathology of a major NTD.

SP03.02

Describing *Leishmania major* cell cycle dynamics using single-cell transcriptomics

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Dynamic gene expression across the cell cycle of the African trypanosome has been examined through population-level RNAseq and proteomics after synchronisation via elutriation, as well as using single-cell transcriptomics on unsynchronised cell populations. No such cell cycle data is available for *Leishmania*, and so we have obtained a high-resolution cell cycle regulated (CCR) transcriptome of promastigote *Leishmania major* cells without prior cell cycle synchronisation using single cell transcriptomics. Computational reconstruction of the cell cycle using periodic pseudotime inference revealed a wide range of RNAs that display dynamic expression across the cell cycle stages. A comparison of CCR genes between *L. major* and *T. brucei* reveals core and genera-specific factors. Additionally, the available data appears to suggest limited separation between *L. major* S- and G2/M cell cycle stages, a feature comparable to cell cycle stage transitions in *T. brucei* bloodstream form cells and unlike the more discrete cell cycle stages in *T. brucei* procyclic cells. Finally, we have used single cell transcriptomics to examine gene expression in *L. major* promastigotes that have entered stationary phase, which we have shown to display much reduced DNA replication, allowing us to map how genome duplication occurs. Single cell analysis will reveal how stationary phase promastigotes arise from replicating cells and what cell functions are expressed and supressed in these conditions.

SP03.03

High-resolution scRNA-seq reveals genomic determinants of VSG expression hierarchy in *Trypanosoma brucei*

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The ability of *T. brucei* to evade the host immune response is based on its capacity to switch the expression of its major surface antigen, the variant surface glycoprotein (VSG), in a non-random manner. The mechanisms driving the observed hierarchy in VSG expression remain elusive. A major challenge in unraveling this process has been the difficulty to track transcriptome changes and potential genomic rearrangements in single cells during VSG switching events. In this study, we present the establishment of a highly sensitive single-cell RNA-seq (scRNA-seq) approach tailored for trypanosomes. This approach has revealed genomic rearrangements that occur in individual cells during a switch event. Our data show that following a double-strand break in the active VSG – an important trigger for antigen switching – the type of repair mechanism and the resulting newly active VSG depend on the availability of a homologous repair template in the genome. When such a template was available, repair proceeded through segmental gene conversion, creating novel mosaic VSGs. Conversely, in the absence of a suitable template, exclusively telomere-adjacent VSGs were activated, which were recombined by break-induced replication. Moreover, in the latter scenario, a high proportion of cells switched VSG expression by activating a different telomeric expression site, but this population of cells disappeared over time. Collectively, our results reveal the critical role of repair template sequence availability in the VSG switching and selection mechanism. Additionally, our study demonstrates the power of highly sensitive scRNA-seq methods in detecting genomic rearrangements that drive transcriptional changes at the single-cell level.

SP03.04

Spatial transcriptomics – Mapping gene expression of a parasitic flatworm

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Introduction: Recent technological innovations in transcriptomics open up new avenues to gain insights into the transcriptional landscape of complex multicellular parasites, such as plathyhelminths. Spatial transcriptomics (ST) enables the visualization of entire transcriptomes in 2D within tissue sections, which helps to reveal genes important for tissue function, host-parasite interaction, and even drug target discovery.

Objectives: We obtained for the first time spatial transcriptomes for a parasitic flatworm, the liver fluke *Fasciola hepatica*, which causes zoonotic disease affecting human and animal health worldwide. We demonstrate the value of ST data for both basic research and drug discovery.

Materials & Methods: By using Curio Seeker and 10X Visium technologies, we generated ST datasets for the migratory immature stage of *F. hepatica* and for the sexually reproducing adult stage that resides in the hepatic bile duct of its host. ST involves capturing and spatial barcoding of transcripts from cryosections using oligonucleotide-coated glass slides or beads.

Results: For adult parasites, we obtained a spatial gene expression map at 55 µm resolution comprising eight tissue types, including intestine, tegument, and different reproductive organs, covering in total over 9,000 genes (Gramberg et al., Nat Commun 2024, PMID: 39414795). Differential gene expression analysis identified marker genes, and gene ontology (GO) enrichment analysis revealed characteristic biological processes and molecular functions associated with each tissue cluster. By integrating our recently obtained single-cell transcriptomics data into the ST dataset, we could improve resolution of gene expression in gonadal tissues and spatially resolve different cell differentiation stages. The gene expression map enabled us to reveal a tissue-specific expression of several known and new putative drug target genes (ß-tubulins and protein kinases), drug resistance genes (ABC transporters, GSTs), and transcription factors. By prioritizing genes expressed in tissues critical for parasite survival and by functional gene characterization using RNAi and small-molecule inhibitors, we identified an intestinal transcription factor and a tegumental protein kinase with importance for parasite survival. Using Curio Seeker, we achieved a 2D gene expression map of immature parasites with 14 clusters at nearly single-cell resolution. By a neighborhood analysis, we identified a spatial relationship of different parenchymal cell clusters. Furthermore, distinct spatial patterns of gene families related to tissue migration were obtained.

Conclusions: This work provides the first transcriptome of a trematode at spatial resolution and demonstrates how spatial transcriptomics improves our understanding of multicellular parasites. Through the identification of tissue-specific transcripts that are essential for the parasite's survival within the host, ST data can guide drug target discovery.

SP03.05

Optimisation of single-nuclei isolation and RNA sequencing of parasitic nematodes

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Single-cell/nuclei transcriptomics (sc/snRNA-seq) has revolutionised our understanding of cell, tissue, and organismal biology in humans and model organisms during health and disease. High-throughput commercial sc/snRNA-seq platforms are increasingly being applied to non-model organisms. However, established cell and nuclei isolation methods were developed primarily for model organisms like humans and mice and proved inadequate when we applied them to non-model species like nematodes. Therefore, reassessment and refinement of conventional methods are needed to address novel technical and biological challenges for non-model organisms.

We tested and compared the strengths and weaknesses of three widely-used single nuclei isolation protocols for use in snRNA-seq of parasitic nematodes. We assessed the success of the three protocols based on nuclei counts recovered, nuclei quality, and the integrity of RNA isolated from the nuclei. Using lessons learned from these protocols, we developed and optimised a novel method for nuclei isolation for parasitic nematodes (SNIP). SNIP is optimised for use with phylogenetically and morphologically distinct stages and species of parasitic nematodes, including the mouse whipworm *Trichuris muris* and the small ruminant-infecting worm *Haemonchus contortus*.

The nuclei recovered from our method are superior in quantity and quality compared to existing protocols. We test the versatility of SNIP across diverse species and life stages, validating it with snRNA-seq to reveal sex, stage, and tissue-specific gene expression. From these data, we could identify distinct stage and sex-specific nuclei clusters and clusters from each species that are biologically meaningful.

Our method will be of broad interest to parasitologists and researchers working in non-model systems interested in applying single nuclei sequencing for the first time. To support this effort, we highlight specific steps that are easily modified to adapt our method to other species of interest, including non-nematode species.

SP03.06

A single cell atlas of the *Schistosoma mansoni* cercaria reveals transcriptomic and spatial architecture of the excretory system

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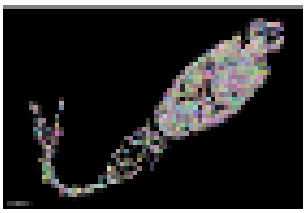
Schistosoma mansoni is a parasitic flatworm that causes schistosomiasis, a Neglected Tropical Disease affecting over 250 million people worldwide. Despite being a significant threat to public health, we lack key knowledge on the parasite's cellular composition and molecular mechanisms which limits our ability to develop novel control strategies. Schistosomes have a complex life cycle which includes a human-infective larva, the cercaria. During infection, cercariae drop their tails and transform into schistosomula, the first intra-mammalian life cycle stage. To better understand the cercaria at the molecular, cellular and tissue levels, we are developing a whole-body single-cell atlas using droplet-based single-cell RNA-sequencing (scRNAseq), multiplexed *in situ* hybridization (ISH), immunofluorescence, and image analysis. High resolution nuclei quantification via machine learning shows that the cercaria is composed of ~1429 nuclei, ~370 of which make up the tail. Using clustering analysis of the transcriptomes of 24,708 single cells, we classified 24 molecularly distinct cell type clusters. Most clusters are recognisable to the tissue level based on marker genes identified in atlases of other life cycle stages. However, the identities of three clusters in this data set are unknown. Here, we use ISH validate two of these clusters and show that one makes up the excretory tube cells in the body and the second makes up the excretory tube cells in the tail. The excretory system, also known as the protonephridial system, of flatworms is made up of flame cells and excretory tubes. While flame cells have been transcriptionally identified in existing *S. mansoni* single cell atlases, this is the first time excretory tube cells have been captured. We show that there are transcriptional differences between excretory tube cells of the body and the tail, again, highlighting that the tail is a temporary larval structure. Tail cell transcriptomes are enriched in metabolic genes, whereas the body tube cells are enriched in genes associated with epithelial formation and cell organization. Combining scRNAseq data from the cercaria with new data from the two day old schistosomula, we have identified genes that are dynamically regulated in the excretory system as the parasite moves from freshwater to the mammalian host environment and undergoes major morphological changes. Our findings demonstrate the transcriptional characterization of a novel excretory cell type.

Fig. 1



SP04.01

The physics of parasitism

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Traditionally, parasitology was concerned mainly with whole-organism studies, while modern parasitology focuses on medically relevant cellular and molecular mechanisms, in ever-increasing depth. Here, we introduce a new frontier in this field, namely the physics of parasite-host interaction. This interaction is controlled by the anatomy of the parasites, the physics of their locomotion, and the mechanics of their attachment to host structures. Parasitism as a strategy has evolved many times and hence, there are numerous convergent solutions to the challenge of how to physically hijack a host. These long periods of co-evolution have optimised and refined the biology of parasites to a high degree. Examples are parasitic tools such as suckers and shields and refined locomotive devices that allow attachment to and also navigation in various body fluids, crowded and confined spaces, and highly viscous environments - often at surprisingly high speeds. Our understanding of the physical constraints and mechanical forces acting at these dynamic parasite-host interfaces remains rudimentary. There is an urgent need for measuring the material properties and mechanics of parasites in their niches, uncovering the physical basis of their locomotion, and determining the mechanical and physical basis for their attachment. We envisage that the results obtained might yield novel ways of combating parasitic diseases based on mechanobiology, against which resistance is unlikely to evolve.

Fig. 1

SP04.02

Dynamic ventral disc contraction forces enable *Giardia* attachment and contribute to host epithelial barrier breakdown

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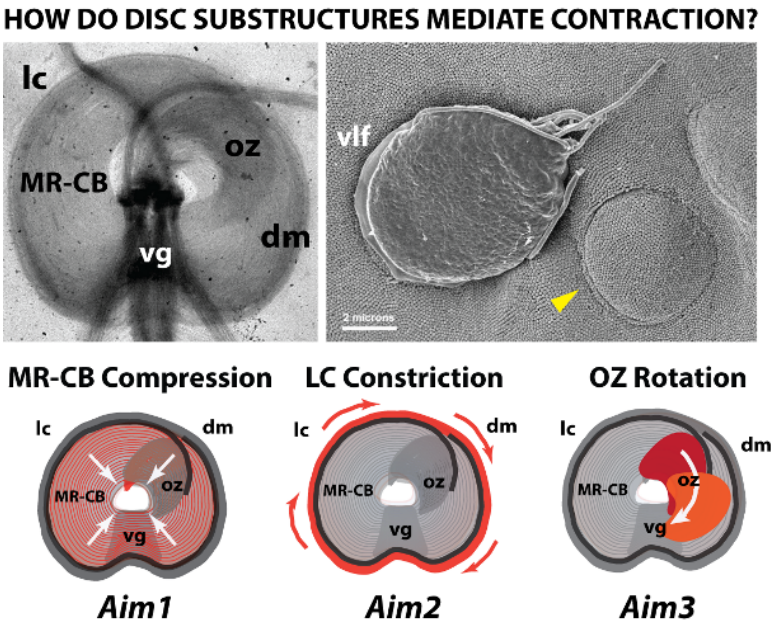
*Giardia lamblia***,** a common parasitic protist, infects the small intestine, causing acute and chronic diarrheal disease defined by weight loss and malabsorption. Infection also leads to intestinal epithelial damage defined by microvilli loss, tight junction and barrier disruption, and apoptosis. Motile Giardia trophozoites attach to duodenal microvilli via the ventral disc, a unique microtubule-based organelle. While disc-mediated attachment has been linked to ring-shaped depressions in the microvilli, the biophysical forces and molecular mechanisms of attachment as well as a direct role for attachment forces in causing epithelial damage remains unclear. Here we capitalize on advancements in both molecular genetic manipulation of *Giardia* and the use of a human *in vitro* organoid monolayer (ODM) model of infection to define disc-mediated mechanisms of attachment and reveal impacts of attachment on the host. Using high-resolution imaging we identified three types of disc contractile movements reducing overall disc diameter and volume. Analysis of a new disc-associated protein (DAP7268KO, or "Baumkuchen") deletion mutant confirmed a role for disc contraction during attachment, resolving a long-standing debate. Baumkuchen mutants exhibit defective disc contraction, and an inability to attach under fluid flow likely due to the aberrant disc structure in mutants that includes multiple overlap zone layers and multiple lateral crests that define the "Baumkuchen" disc phenotype. Infections of human organoid monolayers (ODMs) with DAP7268KO mutants revealed significantly reduced epithelial barrier breakdown compared to wild-type parasites. Overall, this work establishes disc contractility as a critical force for parasite "grasping" of host epithelium, a mechanism that enables extracellular attachment and ultimately contributes to host damage through epithelial barrier disruption. This work also highlights potential therapeutic targets to mitigate the pathogenicity of this widespread intestinal parasite.

Fig. 1



SP04.03

Adhesion forces and cellular mechanotransduction in parasitic flatworms

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Introduction: Schistosomes (blood flukes) and liver flukes infect mammalian hosts to sexually reproduce. *Schistosoma mansoni* and *Fasciola hepatica*, the two species in focus, have migratory juvenile stages in the final host and reside in a variety of host niches (vessels or tissues). As typical for trematodes, they are equipped with head and ventral suckers that aid in locomotion and attachment. We hypothesise that the regulation of biomechanical forces at an organ scale (suckers) and cellular scale (mechanotransduction) is crucial for the parasites' survival and success of infection. However, these forces are largely unknown, and biomechanic processes at the parasite-host interface are an unexplored field of research.

Objectives: The project aims to determine whether sucker-mediated adhesion forces differ between fluke species, sexes, and developmental stages, and assess the influence of the host environment, which includes factors like substrate stiffness and flow of surrounding body fluids. Furthermore, we aim to identify and functionally characterise genes related to the parasites" mechanobiology.

Results: As a prerequisite for traction-force microscopy (TFM) to determine attachment forces of parasites *in vitro,* polyacrylamide hydrogels with different stiffness (1.0 kPa-16.0 kPa) have been established according to the parasite sizes. Traction forces for *S. mansoni* were found to be in the range of 0.07-0.40 kPa for couples, 0.04-0.30 kPa for males, and 0.012-0.050 kPa for females. In the case of *F. hepatica,* traction forces were 1.0-5.0 kPa for adults and 0.5-3.0 kPa for immature worms. Furthermore, a dependency of traction forces on hydrogel stiffness was found: the stiffer the surface, the higher the traction force. Pull-off force measurements to quantify maximum sucker forces revealed higher forces for *S. mansoni* on a rigid glass surface compared to a softer hydrogel. In the case of adult *F. hepatica*, maximum and average pull-off forces were determined to be 2.0-8.0 mN and 1.0-4.0 mN, respectively. To shed light on the cellular mechanobiology, we identified the mechanosensitive receptor Piezo and the transcription factor YAP in the genome of *S. mansoni.* Pharmacological agonists (Yoda-1) or antagonists (verteporfin, Lats-IN-1) affected attachment and egg production in *S. mansoni*.

Conclusion/Outlook: Traction forces depend on the fluke species (*F. hepatica* is stronger than *S. mansoni*), life stages (immature *F. hepatica* are weaker compared to adults), sexes (*S. mansoni* males are stronger than females), and substrate stiffness. Quantification of attachment strengths and mechanosignaling, when the parasites are exposed to flow stress, is currently in progress. Piezo and YAP will be functionally characterised by RNA interference.

SP04.04

Evolution of micro-swimmer designs in distinct microenvironments

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Trypanosoma brucei is a flagellated parasite transmitted by tsetse flies that causes sleeping sickness in humans and infects various vertebrate hosts. Throughout its life cycle, *T. brucei* must adapt to different environments within its hosts, such as the bloodstream and skin, each presenting unique mechanical challenges like varying viscosity and spatial constraints [1]. Structurally, *T. brucei* has an elastic, spindle-shaped body with a flagellum attached in a helical manner [2]. Its movement begins with a planar bending wave that starts at the anterior of the cell, followed by a longitudinal rotation due to the helical flagellum attachment [3]. However, the detailed, quantitative analysis of how individual *T. brucei* cells move and how their motion behavior adapts to different microenvironments remains limited.

This study investigated the motility of *T. brucei* in environments of varying viscosity and within collagen gels using high-resolution and digital holographic microscopy. This approach enabled the capture of both 2D and 3D data on single-cell movement. The findings reveal that the parasite adjusts its rotational motion to maintain swimming speed in environments with different viscosities. In more viscous and dense collagen conditions, the cells demonstrated changes in their movement patterns, such as swimming in circular paths. This suggests that the physical properties of the surrounding environment play a significant role in shaping *T. brucei*"s movement behavior.

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SP04.05

Understanding gliding motility through the dynamics of trail formation of *Plasmodium* sporozoites

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Following deposition into the skin by a malaria infected *Anopheles* mosquito, *Plasmodium* sporozoites actively traverse the dermis to invade a blood vessel to continue their life cycle. To achieve this feat, sporozoites utilize an unusual form of substrate-dependent movement, termed gliding motility, reaching impressively high speeds of one to two µm/s. This is approximately ten-times faster than the fastest crawling cells in the human body.

It is known that gliding motility consists of apical secretion of substrate-binding adhesion proteins, their rearwards translocation through an actin-myosin motor and the subsequent decoupling of these adhesion sites through cleavage by a rhomboid protease. The dynamic interplay of apical secretion, formation and turnover of adhesion sites that result in such high speeds is poorly understood.

During gliding motility, sporozoites leave behind trails containing the main adhesin thrombospondin-related anonymous protein (TRAP) and the major surface protein circumsporozoite protein (CSP). The mechanism of deposition remains enigmatic, and trails were believed to result from shedding of these proteins directly onto the surface.

Here we attempt to visualize this highly dynamic and fast process of sporozoite gliding motility by live-cell microscopy of trail formation. For this purpose, we further optimized fluorescent-tagging of CSP without impacting parasite progression through the mosquito [1]. Using total internal reflection fluorescence (TIRF) microscopy, we could visualize CSP within the membrane trails during live gliding. We observed a highly dynamic formation and disruption of trails. Membrane trails are not deposited individually as previously thought. Instead, they protrude as connected nanotubes that stretch from the moving sporozoite and collapse into smaller fragments that form the trails. Furthermore, we could adapt this method to visualize the dynamic turnover of substrate-bound adhesins, improving our understanding of how sporozoites reach such high speeds.

Live imaging of fluorescently tagged circumsporozoite protein in moving sporozoites enables us to visualize the dynamics of their motility and furthermore may provide a potential tool to investigate CSP-antibody interactions.

[1] Thieleke-Matos C, Walz K, Frischknecht F, Singer M. Overcoming the egress block of *Plasmodium* sporozoites expressing fluorescently tagged circumsporozoite protein. Molecular microbiology. 2024;121: 565–577.

SP05.01

Thermosensory behaviors of the free-living life stages of *Strongyloides* species support parasitism in tropical environments

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Soil-transmitted parasitic nematodes infect over 1 billion people worldwide and are a common source of neglected disease. *Strongyloides stercoralis* is a potentially fatal skin-penetrating human parasite that is endemic to tropical and subtropical regions around the world. For most parasitic nematode species, reproductive adults exclusively reside within host animals. Species in the genus *Strongyloides* have a unique step in their life cycle that features soil-dwelling, non-parasitic adults; the progeny of these "free-living" adults all develop into infective larvae. The sensory behaviors that enable free-living *Strongyloides* adults to navigate and survive soil environments are unknown. *S. stercoralis* infective larvae display parasite-specific sensory-driven behaviors, including robust attraction to mammalian body heat. In contrast, the free-living model nematode *Caenorhabditis elegans* displays thermosensory behaviors that guide adult worms to stay within a physiologically permissive range of environmental temperatures. Do *S. stercoralis* and *C. elegans* free-living adults, which experience similar environmental stressors, display common thermal preferences? Here, we characterize the thermosensory behaviors of the free-living adults of *S. stercoralis* as well as those of the closely related rat parasite, *Strongyloides ratti*. We find that *Strongyloides* free-living adults are exclusively attracted to near-tropical temperatures, despite their inability to infect mammalian hosts. We further show that lifespan is shorter at higher temperatures for free-living *Strongyloides* adults, similar to the effect of temperature on *C. elegans* lifespan. However, we also find that the reproductive potential of the *Strongyloides* free-living life stage is enhanced at warmer temperatures, particularly for *S. stercoralis*. Together, our results reveal a novel role for thermotaxis to maximize the infectious capacity of obligate parasites and provide insight into the biological adaptations that may contribute to their endemicity in tropical climates.

SP05.02

Genomic and functional diversity of parasitism island genes in the parasitic nematode *Strongyloides ratti*

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Strongyloides ratti is a common parasite of rats. In its *c.*13,000 gene genome, some 900 genes are associated with parasitism, which include genes encoding astacin-like metalloproteases, acetylcholinesterases, CAP-domain, and Transthyretin-like proteins. Many of these are arranged adjacently in gene clusters referred to as "parasitism islands". These islands exhibit higher genetic diversity than other genomic regions, suggesting functional divergence among gene products.

To explore this phenomenon, we have analysed genomes and characterised the parasitism islands from several wild UK *S. ratti* genotypes. We find that parasitism island structure was diverse among different genotypes, both in the number of islands in each genome (ranging from 38 to 50), their genomic positions, and gene compositions. Focusing on astacin-like metalloproteases, structural modelling and alignment with experimental structures revealed that over 85% of astacins encoded in parasitism islands lack a zinc-binding motif that is essential for protease activity. This loss contrasts with astacins encoded outside of parasitism islands, where more than two thirds retain the motif and, presumably, protease activity. Because genes encoding these motif-deficient proteins are highly expressed we hypothesize that they may have alternative, non-protease functions. Ongoing work aims to computationally predict how diverse parasitism island CAP, astacin-like metalloprotease and acetylcholinesterase coding genes collectively confer particular functional characteristics to different *S. ratti* genotypes. These findings will advance our understanding of how genetic diversity within parasitism islands contributes to the adaptive success of *S. ratti* in diverse environments and host populations.

SP05.03

Exploring differences in gene expression of *Strongyloides ratti* and *Caenorhabditis* spp. exposed to the nematophagous *Arthrobotrys oligospora*

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Understanding the intricate relationship between nematodes and their fungal predators offers insights into ecological dynamics and potential avenues for biological control strategies of parasitic nematodes. In low nutrient conditions, some nematode-trapping fungi such as *Arthrobotrys oligospora,* form loop-like structures to trap and feed of nematodes. In this study, we investigate the interaction between *A. oligospora* and three nematode species representing diverse ecological niches: the free-living model nematode *Caenorhabditis elegans*, a fig-bearing free-living relative *C. inopinata*, and the free-living life cycle stage of the gastrointestinal parasite *Strongyloides ratti*. We observed that *A. oligospora* displayed a higher efficiency in trapping *S. ratti* nematodes compared to *Caenorhabditis*. Approximately 70% of *S. ratti* were entrapped using ~60 traps, whereas *C. elegans* exhibited ~300 traps with only 25% of nematodes trapped. We investigate molecular responses in both the fungi and the nematodes during fungal-nematode interactions and find changes in fungal gene expression depended on the nematode species it was trapping. We find an increase in reproduction and sporulation associated genes upregulated in *A. oligospora* in response to *S. ratti,* in addition to morphological differences in trap formation and fungal growth compared to the *A. oligospora* response to *Caenorhabditis* spp. We also found that genes differentially expressed in the nematode exposed to *A. oligospora* varied depending on the nematode species. *S. ratti* regulated genes relating to astacins, cuticle development and myosin-associated proteins in response to fungal stresses. Together these results suggest that different mechanisms are at play in nematode-fungi interactions depending on the species of nematode.

SP05.04

To what extent is strongyloidiasis a zoonosis?

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More than 600 million people are infected with the nematode *Strongyloides stercoralis*. Also non-human primates, dogs and cats were described as natural hosts for this parasite. FSince more than 100 years there is an ongoing controverse discussion whether or not the *S. stercoralis* in these animals is really the same species as the one in humans and if these animals serve as a reservoir for zoonotic human strongyloidiasis. The main arguments for zoonotic transmission are the fact that in many cases dogs could be experimentally infected with human derived *S. stercoralis* and that molecular/genomic investigations of wild populations of *S. stercoralis* by us and others suggested that dogs can carry *S. stercoralis* indistinguishable from the ones in humans, in addition to genotypes not found in humans. On the other hand, the scarcity of confirmed zoonotic cases of human strongyloidiasis and the fact that dogs tend not to appeare as risk factors in epidemiological studies argue against strongyloidiasis being a zoonotic disease. I will discuss the zoonotic potential and the possible epidemiological importance of zoonotic transmission of *S. stercoralis*. I will argue that, while zoonotic infection may be epidemiologically relevant in certain settings, it appears unlikely that strongyloidiasis is normally a zoonosis.

Even within human derived isolates of *S. stercoralis* there exist considerable genomic and other biological differences. This raises the question if all *S. stercoralis* in humans really belong to the same species or if they form a complex of multiple closely related species or subspecies with possibly different host ranges and pathogenetic potential. In order to further investigate this and to study biological differences, controlled experiments are desirable. This requires that different isolates are in laboratory culture. We are in the process of establishing a collection of *S. stercoralis* isolates. We culture *S. stercoralis* isolates from the wild in gerbils and we determine a set of parameters such as life cycle preference and the genome sequence and we cryopreserve the isolates in order to keep them available for later studies by us and others.

SP05.05

Neural mechanisms of skin penetration in the human-infective nematode *Strongyloides stercoralis*

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The skin-penetrating nematode *Strongyloides stercoralis* is a gastrointestinal parasite that infects over 600 million people worldwide, primarily in low-resource settings with poor sanitation infrastructure. Infections can cause gastrointestinal distress, stunted growth and cognitive impairment in children, and death in immunocompromised individuals. The infective larvae of skin-penetrating nematodes invade hosts by penetrating directly through the skin, making skin penetration a promising step for anthelmintic intervention. However, virtually nothing was known about the behavioral and neural mechanisms that drive skin penetration. We investigated skin penetration at the level of genes, neurons, and behavior. We first designed an *ex vivo* skin penetration assay that allowed us to visualize the behavior of infective larvae on either rat or human skin. We then used this assay to show that infective larvae engage in repeated cycles of pushing, puncturing, and crawling on the skin surface before ultimately penetrating the skin. Exposure to odorants found in human skin and sweat, as well as human foot odor, enhances skin penetration by *S. stercoralis*. Conversely, human-emitted odorants inhibit skin penetration by the closely related rat parasite *Strongyloides ratti*, suggesting that chemosensation contributes to host selectivity. Pharmacological disruption of dopamine signaling inhibits skin-penetration behavior in both *S. stercoralis* and the human-parasitic hookworm *Ancylostoma ceylanicum*. Moreover, CRISPR-mediated disruption of dopamine biosynthesis, chemogenetic silencing of dopaminergic neurons, or CRISPR-mediated disruption of a mechanoreceptor gene expressed in dopaminergic neurons severely impairs skin penetration by inhibiting skin-penetration behaviors. Together, our results identify an essential and conserved role for dopamine signaling in driving skin penetration across distantly related species of skin-penetrating nematodes. Finally, we identify FDA-approved topical compounds that block skin penetration when applied to the skin surface. Together, our results provide insight into the neural basis of skin-penetration behavior and pave the way for the development of topical prophylactics that prevent nematode infections by blocking skin penetration.

SP05.06

Kinetic analysis of IL-9 expressing cells during murine infection with the parasitic nematode *Strongyloides ratti*

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Objectives: During their migration helminths induce the release of tissue-derived alarmins such as IL-33 which promote the initiation of a protective type 2 immune response. We have previously shown that mucosal mast cells are central for the timely ejection of *Strongyloides ratti* from the small intestine (SI) of infected mice. Application of recombinant IL-33 accelerated the degranulation of mucosal mast cell in an IL-9-dependent manner, resulting in rapid expulsion of *S. ratti from the intestine*.

Material and Methods: We use BALB/c IL-9 GFP reporter mice to identify IL-9 producing immune cells during *S. ratti infection* and in response to IL-33.

Results: Murine *S. ratti* infection induced IL-9 expression by innate lymphoid cells (ILCs) but not CD4+ T cells in the lungs on day 6 p.i. In the small intestine, IL-9 expression by ILCs and CD4+ T cells was significantly increased at day 10 p.i. compared to naïve mice. We observed, thereby an expansion of CD4+ T cells only late in infection, while the frequencies of ILCs were not altered in *S. ratti* infected mice compared to naïve mice. By contrast, IL-33 treatment alone induced an early and pronounced upregulation of IL-9 expressing ILCs already at day 2 in the lungs. Surprisingly, intraperitoneal application of IL-33 at day 0 and 1 diminished the viability of SI cells which was counterbalanced by the simultaneous infection of the mice with *S. ratti*. Flow cytometric analysis revealed a similar kinetic of IL-9 expression by IL-33 treated and *S. ratti* infected mice. However, the expansion of ILCs in the lung and mesenteric lymph nodes (MLN) induced by IL-33 was less pronounced in mice that were additionally infected with *S. ratti*. In line with the reduced expansion of ILCs, serum IL-5 levels were lower in *S. ratti* and IL-33 treated mice compared to uninfected IL-33 treated mice. *S. ratti*-derived excretory secretory products reduced IL-9 production by Bone marrow-derived ILC2 *in vitro*.

Conclusion: In summary, artificial activation of innate cells by application of the alarmin cytokine IL-33 induced a rapid and pronounced expression of IL-9 by ILCs. *S. ratti* infection alone resulted in a late upregulation of IL-9 by ILCs and CD4+ T cells in MLN and SI. Interestingly, infection with *S. ratti* in addition to IL-33 treatment reduced the expansion of ILCs and improved the viability of SI cells suggesting counter regulation of IL-33-driven anti-helminth immune response by *S. ratti*.

SP07.01

Vaccine-link chemotherapies to support the elimination goals for onchocerciasis

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The present annual or biannual MDA of the microfilaricidal drug ivermectin (IVM) aims to interrupt transmission of onchocerciasis. However, by 2013, only a 31% reduction in microfilariae prevalence was achieved by MDA with IVM given for >20 years, clearly indicating that the WHO goal of elimination of transmission (EOT) by 2030 using IVM alone might not be met. The current lack of vaccines and macrofilaricides highlights the urgent need for developing new drugs and alternative treatment regimens. Transmission model simulations indicate that the combined use of a hypothetical macrofilaricide (with ~60% efficacy) or a vaccine with IVM would substantially increase the probability of elimination compared with the independent use of each, highlighting a need for alternative integrated treatment regimens. We present data that could support complementary treatment regimens as part of a more comprehensive strategy to eliminate onchocerciasis. We posit that addition to the toolbox of the present microfilaricidal drugs and/or future macrofilaricidal treatment regimens of a prophylactic vaccine as well as the use of repurposed drugs prophylactically can prevent the establishment of new infections and will not only improve the chances of meeting EOT goals but may support achieving a sustained elimination of onchocerciasis. Our *in vivo* studies in small animal models have shown that immunization with two vaccine candidate antigens (Ov-103 and Ov-RAL-2), co-administered as two individual adjuvanted vaccines or as an adjuvanted fusion protein (Ov-103/Ov-RAL-2), can reduce significantly the establishment of infection. Protection antibody, neutrophil and complement dependent. Furthermore, we demonstrated that Emodepside (repurposed macrofilaricidal drug under clinical development) and Moxidectin (a microfilaricidal drug) inhibit *in vitro* molting and viability of *O. volvulus* l3s and L4s, respectively as well as the motility *B. pahangi* early stages (day 42 post infection) with IC50s in the nanomolar range. Importantly, both drugs have a superior half-life and their PK profiles in humans cover the experimental IC50s for both filarial young worms. Notably, when each drug was used to treat prophylactically gerbils infected with *Brugia pahangi*, both drugs significantly inhibit the development of adult worms and fecundity of adult female worms. The repositioning of vaccines and prophylactic drugs to complement chemotherapy, would be a novel revitalizing concept to the present disease control activities that have remained focused on transmission reduction. Our findings indicate that a major programmatic shift that incorporates integrated control strategies, aimed at reducing both the overall adult worm burden and transmission, is needed to achieve the 2030 WHO elimination of transmission goals for onchocerciasis.

SP07.02

Success and failures in drug development for filarial diseases

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Twenty-one diseases are recognized as neglected tropical diseases (NTDs) by World Health Assembly resolutions, including human filarial diseases. Filarial diseases still affect an estimated 200 million people worldwide, but global efforts in recent decades have made significant progress toward eliminating filariasis as a public health problem. It is acknowledged in the WHO Roadmap that new drugs or drug regimens that can kill or permanently sterilize adult filarial worms would greatly accelerate the timelines for elimination, improve individual treatment and help achieve the goal of disease eradication.

However, this area remains underfunded, and since there is no high return on investment, there is no dedicated drug development pipeline for human filariasis. Drug development faces high attrition rates, with promising molecules failing in preclinical development or subsequent toxicological, safety, and efficacy testing, leading to high research and development (R&D) costs.

Product development partnerships between the private sector, academic institutions, and NGOs are model to facilitate drug development for NTDs and while the de novo development of anthelmintics for human use is not commercially attractive, drug repurposing from veterinary medicine is a viable approach to fill the drug development pipeline for NTDs affecting the poorest population of the world. It also has a higher chance of success with already proven drug targets in nematodes of veterinary importance. Some impressive examples of successful repurposing of veterinary drugs for human use include benzimidazoles, ivermectin (IVM), praziquantel, moxidectin, and triclabendazole. This approach has also been adopted by the DNDi, which is currently investigating emodepside (in collaboration with Bayer AG) and ABBV-4083 (a tylosin derivative, jointly developed in collaboration with the Anti-Wolbachia (AWOL) consortium and the pharma partner AbbVie). The third lead compound is the off-patent veterinary product oxfendazole for potential human use. Here we present a project update, discuss recent failures and considerations to enable patient`s access to new medicines.

SP07.03

Efficacy of oxfendazole against infective filarial larval stages

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Introduction: Tens of millions of people are affected by lymphatic filariasis and onchocerciasis worldwide. As of 2025, we still lack drugs with a safe and adult worm killing activity that fit the desired target product profile (per os, max. 7-14 days). Oxfendazole, a benzimidazole compound, has demonstrated such a target profile in pre-clinical models and is currently tested in phase II clinical trials in onchocerciasis, loiasis, mansonellosis and trichuriasis patients.

However, activity against infective larvae (L3) has not been investigated yet. Thus, we analyzed the activity of oxfendazole against this stage and the role of eosinophils using the *Litomosoides sigmodontis* model. Clearance of L3 larvae in addition to the known adult worm killing properties would indicate that oxfendazole allows curative treatments independent of the time of infection.

Methods: BALB/c WT and eosinophil-deficient BALB/c Δ*dblGata1* mice were naturally infected with the rodent filaria *L. sigmodontis*. Mice were treated with different doses of oxfendazole for 3 to 5 days starting one day after the infection. Mice were sacrificed 35 days after the infection and assessed for changes in parasitological and immunological parameters ex vivo.

Results: Treatment with oxfendazole for 5 days led to a significant reduction of recovered worms. The mean worm burden was reduced by 97% in the WT and 87% in the Δ*dblGata1* mice. Shorter treatments of three days still led to a significant albeit slightly weaker reduction in both strains. In addition to the lower treatment efficacy in the BALB/c ΔdblGata1 mice, we also observed a number of differences in the immune response in both strains including, e.g. for CD4 T cells, macrophages and *L. sigmodontis* specific-IgG1 levels.

Conclusions: Oxfendazole is one of only two novel macrofilaricidal candidates in clinical development for human filarial infections. Previously we have demonstrated that oxfendazole has a strong macrofilaricidal activity and no activity against the filarial progeny, the microfilariae. In the present study, we demonstrated that oxfendazole also has a strong activity against the infective L3 larvae in the *L. sigmodontis* model. These results indicate that treatment with oxfendazole might not only be curative of the adult stage, but also the infective larval stage, thus allowing curative treatments independent of the time of infection. This offers the opportunity to prevent or at least treat reinfections.

SP07.04

Respiratory multiple infections by bacteria, viruses, fungi, and parasites in a COPD patient

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SARS-CoV-2 is the causative agent of COVID-19, an infection that can manifest with mild to severe respiratory symptoms. A 70-year-old man with COVID-19 and COPD presented to a hospital complaining of breathing difficulties. A sample was taken, leading to the finding of the Acanthamoeba parasite. Stenotrophomonas maltophilia, a bacterium known for its resistance to most antibiotics and its significance as a nosocomial pathogen, was identified. Furthermore, for the first time, the Gloeotinia fungus was discovered as an endosymbiont of Acanthamoeba. The patient underwent successful treatment and was discharged from the hospital. Immunocompromised people should be concerned about the increasing incidence of nosocomial infections. The presence of Acanthamoeba should not be overlooked in respiratory disorders, as it has the potential to carry numerous pathogenic microorganisms as endosymbionts.

SP07.05

Investigating the role of the mTORC-related kinase KIN during translational regulation in the *Plasmodium falciparum* blood stages

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Malaria is a global health crisis, causing over 247 million cases and 619,000 deaths each year. The disease results from infection with *Plasmodium* parasites, with *P. falciparum* causing the most severe cases. The parasite"s complex life cycle alternates between human and mosquito hosts, requiring adaptation to vastly different environments and multiple stage transitions. These transitions depend on tight post-transcriptional regulation, including translational repression and storage of transcripts in RNP granules. In mammals, the mTORC pathway regulates translation, cell growth, and stress responses. Although *P. falciparum* lacks key mTORC components like the mTOR kinase, it retains downstream elements such as the S6 kinase PKB and the translational repressor DOZI, a putative 4E-BP. It also expresses homologs of upstream regulators, including PI3K, LanCL2 (termed 7-Helix-1), and AMPK (termed KIN). AMPK, a cellular energy sensor in eukaryotes, modulates global translation by inhibiting mTORC1 and promoting stress granule formation, and similarly, the plasmodial counterpart, KIN, senses nutrient availability during blood-stage replication, influencing parasite growth and survival. We here investigate the role of KIN in the *P. falciparum* blood stages using chemical approaches. Inhibition of KIN using Dorsomorphin disrupts parasite replication by causing an arrest at the schizont stage. Activation of KIN with A-769662 reduces merozoite production per schizont without altering blood stage development, leading to reduced parasite proliferation. Real-time qPCR shows increased *kin* transcript levels in mature and activated gametocytes compared to asexual blood stages. Furthermore, Dorsomorphin treatment does not affect gametocyte formation but blocks maturation at stage IV, underscoring KIN"s critical role in reaching stage V gametocytes. Additionally, KIN inhibition disrupts the eIF2α-mediated stress response, suggesting its involvement in cellular stress regulation. Our combined data hint at a key role of KIN during sexual-stage development.

SP07.06

Immune reset by L-arginine leads to the cure of chronic *Cutaneous leishmaniasis*

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Effective control of cutaneous leishmaniasis (CL) relies on IFNγ-induced nitric oxide synthase 2 (NOS2), which metabolizes L-arginine into citrulline and leishmanicidal nitric oxide (NO). In contrast, Th2-driven arginase 1 (ARG1) competes with NOS2 by diverting L-arginine metabolism towards the synthesis of urea and polyamines, which promote both immune cell and parasite cell proliferation. Whether and how ARG1 is involved in sustaining chronicity of *Leishmania* (*L.) mexicana* infections, however, remains unclear.

We investigated ARG1 contribution to chronic *Leishmania (L.) mexicana* infections. Unlike wild-type (WT) mice, Arg1ΔCx3cr1 mice lacking ARG1 in CX3CR1+ cells developed mild, self-resolving disease. Single-cell RNA sequencing (scRNA-seq) revealed that ARG1, in conjunction with elevated IFNγ, drives Ly6Chigh monocytes toward differentiation into ARG1+NOS2+chemokine+ inflammatory macrophages. This initiates a self-perpetuating inflammatory cycle, supporting parasite replication by impairing NO-mediated killing through L-arginine competition.

Metabolomics analysis demonstrated that ARG1 deletion restores L-arginine levels in infected tissues, thereby resolving inflammation. Prophylactic L-arginine supplementation prevented chronic CL development, while therapeutic L-arginine treatment in established infections induced clinical cure, reduced parasite load, and conferred protection against reinfection. Mechanistic studies revealed that L-arginine supplementation enhances immune infiltrates, Th1 activation and IFNγ production, as evidenced by flow cytometry and intracellular cytokine staining.

These findings establish ARG1 as a key driver of chronic *L. mexicana* infections through L-arginine depletion and highlight the therapeutic promise of dietary L-arginine supplementation. Restoring metabolic balance with L-arginine represents a novel strategy to resolve chronic inflammation and to achieve long-term clinical cure in CL patients.

PS001.I

Malaria unveiled: Exploring the epidemiology and population dynamics in Venezuela

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Introduction: Venezuela has been a critical hotspot for malaria, accounting for nearly 60% of cases and three-quarters of deaths in the Americas during the second decade of the 21st century. Mérida State contributes an unknown percentage of *Plasmodium vivax* and *Plasmodium falciparum* cases in the country.

Objectives: This study aims to describe the clinical and epidemiological characteristics of patients with malaria in an area of unknown transmission in Mérida State.

Materials and Methods: A retrospective study was conducted on patients seeking medical care at the main center in Mérida State. Malaria diagnosis was performed by microscopy, following national standards. Positive patients were assessed for clinical symptoms, and hematological tests were conducted at the time of diagnosis. Telephone follow-up was conducted to evaluate malaria recurrences from 2012 to 2018.

Results: Out of 200 patients, 142 (71%) tested positive for *P. vivax*, 44 (22%) for *P. falciparum*, and 14 (7%) had mixed infections. The median age of patients was 37 years, with 71% being male, of whom 82% were engaged in agriculture. The most common symptoms were fever (97%), chills (93%), and headaches (88%). There were 14 deaths due to cerebral malaria. Hemoglobin levels were significantly lower in patients infected with *P. falciparum* compared to those with *P. vivax*. Regardless of the infecting parasite, patients exhibited elevated liver function tests and lactate dehydrogenase (LDH) levels.

Conclusions: The high prevalence of malaria among young adult males engaged in agricultural activities in rural areas suggests that this occupation is a significant risk factor. The unexpectedly high number of *P. vivax* patients presenting at least one criterion of severe clinical disease raises concerns about a lack of timely diagnosis and effective treatment.

PS004.I

Seasonal dynamics of bacterial inoculum-mediated composting: A strategy for parasite and coliform-free slaughterhouse sludge compost

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This study investigates the effectiveness of bacterial inoculum in converting sludge from slaughterhouses into compost that is coliform- and parasite-free. The goal of the experiment was to monitor temperature, humidity, and pH during the composting process and evaluate the presence of coliform bacteria and parasite in the compost at different intervals. It was carried out in both the summer (June & July) and winter (December & January). The findings showed that there are notable ($P < 0.05$) seasonal differences in temperature, with summer seeing a greater and quicker rise in temperature (39°C-59°C) than winter (24°C-48°C). In contrast to lower levels in summer, humidity levels were significantly higher ($P < 0.05$) in winter. There was no discernible change in the compost's pH between the two seasons ($P > 0.05$). Moreover, a seasonal influence was seen in the decline in coliform and parasite counts, with a considerably ($P < 0.05$) larger decline in summer than in winter. It's interesting to note that, in contrast to the slower winter composting, the composting process was accelerated in the summer, even though there was more noticeable microbial activity. This led to a shorter time (only 40 days) for compost maturation. This study highlights the seasonal fluctuations that are inherent in composting mediated by bacterial inoculums, offering important insights into how to best optimize the process for various climates. The results emphasize the possibility of turning abattoir sludge into nutrient-rich, safe compost, but they also highlight the necessity for customized strategies to maximize the advantages of bacterial inoculate in a variety of environmental contexts.

PS007.I

Characterization of feline leishmaniosis presentations based on seropositivity, clinical and clinicopathologic associations

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Introduction: Feline leishmaniosis (FeL) management relies on understanding the parasite, its vector, and the immune response of cats. Although significant progress has been made in updating FeL epidemiology, diagnosis, and clinical features, the non-specificity of clinical signs complicates disease management. Common clinical manifestations, such as systemic signs, dermatological, ophthalmic and respiratory lesions, anemia and hyperglobulinemia, require laboratory confirmation for accurate diagnosis. Current immunological diagnostic tools for leishmaniosis, including IFAT and ELISA, are hindered by variable sensitivity and specificity. The potential of *Leishmania*-specific recombinant antigens for FeL diagnosis has not been explored, despite their efficacy in canine leishmaniosis diagnosis. This study investigates associations between seropositivity to different diagnostic tests and clinical, haematological, and biochemical abnormalities to improve clinical decision-making on FeL.

Materials and Methods: Seropositivity was assessed for 228 feline samples using three ELISA antigens (soluble promastigote *Leishmania* antigens [SPLA]; *L. infantum* recombinant protein kinesin 39 [rK39] and the *L. infantum* cytosolic tryparedoxin peroxidase [LicTXNPx]), IFAT and DAT. Serological cut-offs for ELISA were based on negative controls, and literature recommendations for IFAT (1:80) and DAT (1:100). Associations between serological results and clinical signs or clinicopathological abnormalities (n = 78), were analysed using chi-square (χ^2) and McNemar tests ($P < 0.05$).

Results: Seropositivity to any test was statistically associated ($P < 0.05$) with the presence of overall clinical signs of disease. Seropositivity to rK39 was associated with systemic and gastrointestinal signs, while SPLA positivity was linked to upper respiratory signs. No statistically significant associations were found between LicTXNPx and clinical signs. IFAT and DAT showed a positive association with dermatological lesions. Triple-ELISA seropositivity (SPLA, rK39 and LicTXNPx) identified cases with multiple alterations associated to a worsened prognosis, including leukocytosis, hypoalbuminemia, hyperglobulinemia, hyperproteinemia and low albumin/globulin ratios. The rK39 was most frequently associated with various hematological (e.g. low hemoglobin, leukopenia, thrombocytosis) and biochemical abnormalities (e.g. hypoalbuminemia, hyperglobulinemia, low A/G ratio and high BUN). Hyperglobulinemia, low A/G ratio and high BUN were significantly associated with seropositivity on all tests.

Conclusions: This study highlights the diagnostic potential of rK39 and LicTXNPx in identifying FeL associated disease markers. Antigen rK39 revealed strong correlations with biomarkers indicative of advanced disease. The combination of SPLA, rK39 and LicTXNPx seropositivity may offer a specific diagnostic tool, contributing to detect severe FeL cases, such as those with hypoalbuminemia and hyperglobulinemia.

PS010.I

Efficiency evaluation of scFv antibodies against *Opisthorchis viverrini* cathepsin F for developing as the diagnostic tools

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Introduction: *Opisthorchis viverrini* infection is a significant parasitic infection in Southeast Asia, especially the Mekong subregion. The acute infection causes asymptomatic or mild gastro-hepatic symptoms, while in the chronic phase, the patient presents with cholangiocarcinoma, which is a poor prognosis malignancy. Early diagnosis could limit cancer development and stop the parasite's life cycle, but unfortunately, it''s currently unavailable. Nowadays, the gold standard for diagnosing *O. viverrini* infection is the microscopic-based stool examination, which is sometimes misdiagnosed due to a low number of parasite eggs in the specimen and professional experiences.

Objectives: This study aims to produce and investigate the sensitivity and specificity of our developed scFv antibodies against OvCatF target protein by using immunological assays.

Materials & methods: We developed the single-chain variable fragment (scFv) monoclonal antibodies against *O. viverrini* protein, cathepsin F (OvCatF), using phage display technologies. The developed scFv(s) were verified for the binding efficiency to the recombinant protein of OvCatF (rOvCatF) using ELISA and Western analysis.

Results: The produced scFv(s) could bind specifically to rOvCatF in very low concentrations of less than 0.01 mg. The Western analysis demonstrated that the scFv(s) could bind specifically to rOvCatF at the expected size.

Conclusion: These scFv antibodies that were produced from our study could be developed to be the diagnostic tool for the detection of *O. viverrini* infection in patient specimens which will be other than stool samples further.

PS013.I

Capsule formulation of the standardized extract of *Atractylodes lancea* induces apoptosis in cholangiocarcinoma cells via caspase-3 activation

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Introduction: Cholangiocarcinoma (CCA), the biliary ductal cancer, is reported to have a high prevalence in several countries including Thailand. *Atractylodes lancea* (AL) is a tuber plant in the Asteraceae (Compositae) family. It has been used to treat various illnesses such as rheumatic fever, digestive diseases, night blindness syndrome, fever, and cold. Previous studies are shown that AL extract has anticancer activities in cholangiocarcinoma cells by apoptosis induction.

Objective: This study aims to confirm the process of apoptosis by detecting the level of caspase-3 protein expression induced by the capsule formulation of the standardized extract of AL.

Methods: Two cholangiocarcinoma cell lines including HuCC-T1 and Huh-28 have been treated with the capsule formulation of the standardized extract of AL at different concentrations. The level of caspase-3 expression of treated cells with different concentrations of the capsule formulation of the standardized extract of AL were compared to the control group by western blot analysis.

Results: The caspase-3 was detected in the treated group directly related to the concentration of the capsule formulation of the standardized extract of AL, meanwhile the control group shows otherwise.

Conclusion: The capsule formulation of the standardized extract of AL can induce apoptosis by direct caspase-3 activation in a dose-dependent manner.

PS016.I

Investigation of a piroplasm outbreak in a herd of horses in Southern Germany

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Equine piroplasmosis is a tick-borne disease caused by the protozoan parasites *Babesia caballi* and *Theileria equi*, transmitted by hard ticks (Ixodidae) that causes symptoms such as fever, anaemia, jaundice, and, in severe cases, can be fatal. In October 2022, 18 out of 34 horses in one herd in the district of Rastatt, Baden-Wuerttemberg, were infected with at least one of the two pathogens and three horses had to be euthanized due to severe symptoms. The aim of this case study was to determine the pathogen prevalence in the herd of horses and in the local tick population of *Dermacentor reticulatus*.

Therefore, blood samples were collected in September 2023 and in March 2024 for molecular and serological tests and blood smears were made to screen microscopically for both pathogens. Molecular testing of the blood samples showed infection rates of 53%/26% for *B. caballi* and of 71%/65% for *T. equi* (2023/2024). cELISA revealed infection rates of 74%/88% for *B. caballi* and 62%/65% for *T. equi*. *B. caballi* and *T. equi* stages could both be identified in the blood smears. Moreover, horses could be tested positive by PCR and cELISA in March 2024, which had been tested negative in September 2023 for both pathogens. Additionally, ticks were collected from the horses" pastures from November 2022 until March 2024 periodically and examined for Piroplasms via PCR.

In total over 1000 ticks were collected and 965 adult *D. reticulatus* were examined. 8 ticks were tested positive for *B. caballi*, whereas *T. equi* was not detected in any tick. However, the positive tested ticks came from pastures on which the horses grazed while and directly before the outbreak in 2022.

The origin of equine piroplasmosis could not be finally clarified, but an imported horse from Spain is suspected to have introduced both pathogens into the herd. These results highlight the increasing importance of Piroplasms, which can become established in Germany due to the widespread distribution of *D. reticulatus*.

PS019.I

Long-term insights into host-parasite dynamics: Spatial and temporal patterns of infection and tolerance in threespine stickleback

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Understanding spatiotemporal patterns in parasite occurrences is of major importance, as it allows us to better understand how parasite communities are shaped by their environment and the dynamics underlying host-parasite interactions. Long-term record-keeping of infection prevalence and incidence is particularly valuable, as it enables us to identify patterns that persist over time.

In this study, we analyzed the spatial and temporal patterns of infection prevalence of the cestode *Schistocephalus solidus* in four Alaskan threespine stickleback (*Gasterosteus aculeatus*) populations over 11 years (1996 – 2016). Following the last glacial maximum, oceanic threespine stickleback colonized freshwater lakes and streams, resulting in an adaptive radiation. In these freshwater habitats, stickleback populations first encountered the trophically-transmitted cestode *S. solidus*. Infections with *S. solidus* can significantly reduce stickleback fitness, which should drive the evolution of host genotypes that confer resistance. However, stickleback freshwater populations exhibit substantial variation in *S. solidus* susceptibility, and the underlying mechanisms of these differences are not yet fully understood.

We examined variations in host tolerance through measures such as infection prevalence, the Parasite Index and the presence of fibrotic scar tissue, a specialized form of parasite resistance. We observed fibrosis in both infected and uninfected fish across all populations, with notable year-to-year variation in Cornelius Lake, where prevalence ranged from 9% in 1997 to 63% in 1998, coinciding with the highest recorded infection rates. Willow Lake stickleback exhibited persistently high Parasite Indices, with parasite weights sometimes exceeding host eviscerated weights, and an epizootic event was observed from 2000 to 2005. In contrast, infection prevalence remained relatively stable in Rocky and Cornelius Lakes and consistently low in Lazy Lake.

Our results demonstrate significant variation in infection prevalence, parasite burden, and tolerance mechanisms among host populations and between years. Findings, such as the decoupling of fibrosis formation from infection status highlight the multifaceted nature of host-parasite interactions, driven by a variety of factors. Most importantly, this study emphasizes the value of long-term datasets in revealing patterns in host-parasite dynamics.

Fig. 1

PS022.I

Blended learning in clinical veterinary parasitology: Comparing online and on-farm delivery in livestock infectious disease control to final year veterinary students

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An essential part of livestock veterinary practice is control of infectious diseases in farm populations. As part of a final year farm-animal core rotation, groups of 5-7 veterinary students were tasked to formulate an infectious disease health plan for a beef and a sheep farm. The clinician-supervised activity entailed an introduction, farm visit and classroom time to produce a health plan for discussion. During the on-farm version, students visited 6 key locations on the farm using an app that presented data from diagnostic tests, farmer interviews and "thinking points" to consider. In the online version, students only used the app in a classroom. The aim of this research was to compare online and on-farm (blended) teaching methods to develop confidence and competence of final year students in infectious disease control.

In 2020-21 during the COVID-19 pandemic, beef and sheep health plan activities were conducted online. In 2021-23, beef and sheep health plan activities were conducted in person including farm visits. In addition to act as a control, all cohorts undertook an in-person dairy farm health plan activity including a farm visit. An online questionnaire was used to collate student feedback about learning outcomes, confidence, and teaching methods for all health plan activities. The results of the questionnaire were analysed to compare teaching delivery on student outcomes, with the dairy activity acting as a baseline.

Students felt variably confident discussing different aspects of health plans with farmers, depending on the topic but regardless of teaching method. All students were less confident providing management recommendations compared with understanding farm systems and data. They were less confident in beef systems, possibly because students lacked experience. Consistent sign-posting between activities enabled students to navigate online materials but it would have been less confusing if they had gone to the farm with a clinician. Overall students in their preference of teaching method, often relating to their previous levels of on-farm experience. This study highlights the benefits and pitfalls of blended-learning approaches to teaching applied infectious disease control to veterinary students.

PS025.I

Reconstitution of the essential isoprenoid metabolic pathway of *Plasmodium falciparum* in *E. coli* facilitates functional screens on it *in situ*

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Introduction: New and highly selective drug targets for the malaria-causing parasite *Plasmodium falciparum* are still needed. The ferredoxin redox system (Fd-FNR), located in a plant-derived organelle called apicoplast, is essential for parasite survival due to its involvement in the methylerythritol phosphate pathway (MEP) for isoprenoid biosynthesis. However, *in vitro* screens for inhibitors are challenging due to the labile nature of the recombinant iron-sulfur proteins involved.

Objective: To develop an *insitu* model in *E. coli* which reports essential functions of PfFd and its downstream electron acceptors (i.e. the terminal enzymes of the MEP pathway, shared by both organisms) as growth/intermediate/no growth.

Materials & Methods: Deletion of the two essential genes of *E. coli,* flavodoxin (EcFldA) and MEP enzyme EcIspH, and inducible expression of the respective *P. falciparum* proteins (PfFd, PfFd reductase (PfFNR) and PfIspH), followed standard procedures.

Results: We report for the first time a double mutant of *E. coli* FldA and IspH. It is dependent on the mevalonate bypass system which provides the essential metabolite IPP. Removing mevalonate makes the strain entirely dependent on the simultaneous expression of plasmid-encoded PfFd, PfFNR, PfIspH, which documents that the three parasite proteins are required functionally active to replace the respective endogenous *E. coli* proteins. This system will allow initial functional studies to be performed within a cellular context rather than in vitro. As a proof of concept, several amino acids of PfFd suspected to be involved in the interaction with PfIspH were individually mutated and the respective redox function could be evaluated by simple growth assays. This allowed us to define several amino acids being crucial for PfFd-PfIspH interaction. Metabolomic studies confirmed the mutants' consequences on the MEP. Finally, several of the thus defined essential mutations were transferred to *Toxoplasma gondii* Fd (TgFd) and used to complement our previously described conditional TgFd knockout strain (Henkel et al 2022). They mirrored the findings of the respective *E. coli* strain, thereby allowing the transfer of findings from the surrogate bacterial system to the parasite.

Conclusions: We describe an engineered *E. coli* strain dependent on three *P. falciparum* proteins, PfFd, PfNR and the terminal MEP enzyme PfIspH, which will be very useful for functional as well as drug screening purposes of this essential parasite pathway before findings are validated in the parasites (*P. falciparum* or *T. gondii)*.

Reference

Henkel, S. et al. (2022) Toxoplasma gondii apicoplast-resident ferredoxin is an essential electron transfer protein for the MEP isoprenoid-biosynthetic pathway. J. Biol. Chem. 298, 101468.

PS028.I

Multiple liver and jejunal abscesses due to fasciola flat worm: An uncommon case report from Iran

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Fascioliasis is a zoonotic condition precipitated by the trematode known as Fasciola spp. This parasite affected almost 2.4 to 17 million individuals in the world. The occurrence of liver abscesses is an infrequent phenomenon, potentially arising as a secondary outcome of cholangitis or hepatic inflammation. In this study, we have reported a case of a hepatic abscess induced by Fasciola spp. A 29-year-old male presented with generalized abdominal discomfort, primarily concentrated in the right upper quadrant, which had progressively worsened over the past seven months. The patient did mention a weight loss of approximately 10 kg during the course of their illness, along with increasing fatigue. Eosinophilia was seen too (35%). The stool exam test was negative. An enteroscopy was conducted, revealing no pathological abnormalities. Following this, the surgeon prescribed a surgical biopsy of the mass, which yielded a diagnosis of multiple eosinophilic granulomatous abscesses within the jejunal wall and mesentery. The Fasciola IgG test returned a positive result. After of follow-up, the patient fully recovered. Fascioliasis should be considered in the differential diagnoses of liver abscesses. Because of earlier diagnosis, can be avoided of invasive diagnostic tests and therapeutic interventions can be started earlier.

PS031.I

A 5-year study on visceral *leishmaniosis* in children hospitalized at Tehran children's hospital: Exploring clinical and demographic characteristics alongside nursing interventions

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Introduction: Visceral leishmaniosis (VL) in Iran is predominantly of the Mediterranean type and commonly affects children under ten years old, particularly in the northern and western provinces. This disease is endemic in over 70 countries worldwide. In 2020, the World Health Organization reported 13,000 new cases. The prevalence of VL is on the rise in Iran, with cases now appearing in provinces like Alborz. Without proper treatment and nursing care, VL can lead to fatal complications, causing up to 90% of children's deaths due to issues like infection and bleeding.

Method and Materials: This retrospective descriptive study took place at Tehran Children's Hospital. Over a 5-year period, the medical records of all children diagnosed with VL and admitted to this hospital were reviewed. Data was collected using a questionnaire with two sections: the first part focused on the clinical characteristics and demographics of the patients, while the second part included a checklist developed from established standards and literature reviews to document any instances of failure to provide standard care.

Results: During this period, a total of 16 patients were hospitalized. The results revealed that the majority of the children (52%) were boys, with most falling within the 2-3 year age range (33.8%). Patients came from various geographical areas and provinces across the country (26 provinces), with a higher percentage from Tehran province (23.25%). The majority of children were born and resided in Tehran (23.68%), Karaj (4.63%), and Qazvin (2.26%). Clinical symptoms included fever in 97% of children, with splenomegaly (52.1%), diarrhea (40%), vomiting (23.6%), abdominal pain (5.26%), and abdominal enlargement (4.32%) being the most common. The level of compliance with nursing interventions standards was average (49.8%), with the highest adherence seen in temperature control (94%) and the lowest in child and family education (4%).

Conclusion: The study findings revealed the demographic and clinical profiles of children with visceral leishmaniasis, highlighting substandard nursing interventions. Thus, there is a critical need to enhance the quality and quantity of care provided.

Keywords: Visceral leishmaniasis - Children - Nursing care

PS034.I

Early responses of monocytes and macrophages to *Toxoplasma gondii* tachyzoites

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Introduction: Different immune cell types including monocytes and macrophages are known to release extracellular traps (ETs) in response to pathogens. ETs have the function of pathogen trapping, immobilization and eventually killing. *Toxoplasma gondii* is an apicomplexan parasite and the etiological agent of toxoplasmosis. As a polyxenous parasite, *T. gondii* infects almost any homeothermic animal, including approximately one third of the human population. The aim of the present study was to characterize both *T. gondii* tachyzoite-induced ETosis and pro-inflammatory cytokine release from monocytes and macrophages. Therefore, we used primary monocytes from bovine and human blood, monocytic THP-1 cells and THP-1 cell-derived macrophages (M0-, M1- and M2-like), the latter of which show non-specific, inflammatory, or anti-inflammatory immune responses in a phenotype-dependent manner.

Methods: *T. gondii* tachyzoites (RH-strain) were grown in MARC-145 cells. Scanning electron (SEM) and immunofluorescence microscopy (IF) were used to visualize ETosis. Extracellular DNA was measured to quantify the release of monocyte or macrophage extracellular traps (METs). Pro-inflammatory cytokines (IL-1β, IL-6) into cell culture supernatants after exposure with *T. gondii* tachyzoites was determined by commercial ELISAs. All experiments were performed on tachyzoite-immune cell-co-culture of 4 h.

Results: SEM analysis revealed a parasite-driven activation of all cell types. In addition, co-localization of extracellular DNA, histone and myeloperoxidase (MPO) in released fibers in principle confirmed classical features of ET structures for monocytes and macrophages, but did not reach quantifiable levels. In primary monocytes and lipopolysaccharide- (LPS) primed THP-1 cell-derived macrophages, tachyzoite exposure led to enhanced secretion of IL-1β, while no changes were detected for monocytic THP-1 cells. IL-6 secretion by primary monocytes and LPS-primed monocytic THP-1 cells was significantly boosted by tachyzoite exposure, but was not changed in THP-1 cell-derived macrophages.

Conclusions: Our data suggest that MET formation does not play a major role in the early phase of infection with *T. gondii* tachyzoites. The parasites rather induce an IL-1β response which may help to raise an appropriate immune response.

PS037.I

Development of tools to assess the risk of *Leishmania* transmission – II. the influence of the protein expression system on the reactivity of recombinant antigen

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Leishmaniases are diseases of importance in human and veterinary medicine caused by *Leishmania* (Kinetoplastida), protozoan parasites transmitted by blood-feeding sand flies (Diptera: Phlebotominae). To evaluate the risk of *Leishmania* transmission, host exposure to *Leishmania* vectors can be assessed by measuring antibodies against vector salivary proteins. These anti-sand fly saliva antibodies are elicited by repeated exposure of the mammalian host to sand fly salivary proteins deposited into the host skin during the blood feeding. Such antibodies are species-specific, reflect well the intensity of host exposure to sand fly bites and serve as a reliable marker of host exposure to sand fly bites and consequently as a marker of risk for *Leishmania* transmission. Anti-sand fly saliva antibodies can be measured using sand fly salivary glands manually dissected from sand fly females and homogenised to provide an antigen for serological assay (salivary gland homogenate, SGH). However, the use of recombinant proteins offers more standardised and practical alternative, eliminating the need for colonised sand flies. This concept has been validated e.g. in studies on *Phlebotomus papatasi*, the vector of *Leishmania major*. Recombinant *P. papatasi* salivary protein 32 (PpSP32) has been evaluated and validated in human studies in Tunisia (PMID: 23209854, 26368935). In the present study, we compared the reactivity of recombinant PpSP32 produced in three different expression systems – (1) mammalian HEK cells, as used in previous studies, (2) the widely used and cost-effective *Escherichia coli*, and (3) insect Sf9 cells, which should provide the most natural option for producing recombinant insect proteins. Expression systems differ in post-translational modifications, including glycosylation, which can affect protein reactivity with antibodies. The reactivity of these three proteins has been assessed using sera from naturally exposed hosts – humans and dogs from endemic areas in Turkey - and experimentally exposed mice. The latter model allowed us to test also the specificity of the three recombinants with antibodies directed against salivary proteins from various sand fly species.

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PS040.I

The impact of environmental factors on arbovirus vector behavior and insecticide susceptibility in Cameroon

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Introduction: Arboviruses represent a serious concern in tropical/subtropical countries. In Cameroon, port cities and cities surrounded by forests constitute the main hot-spot of dengue and yellow fever. To date, little is known about the bionomics of their main vectors *Aedes aegypti* and *Aedes albopictus* in such environments.

Objectives: Assess the bioecology, distribution and susceptibility profiles to insecticides of these vectors in three cities of Cameroon.

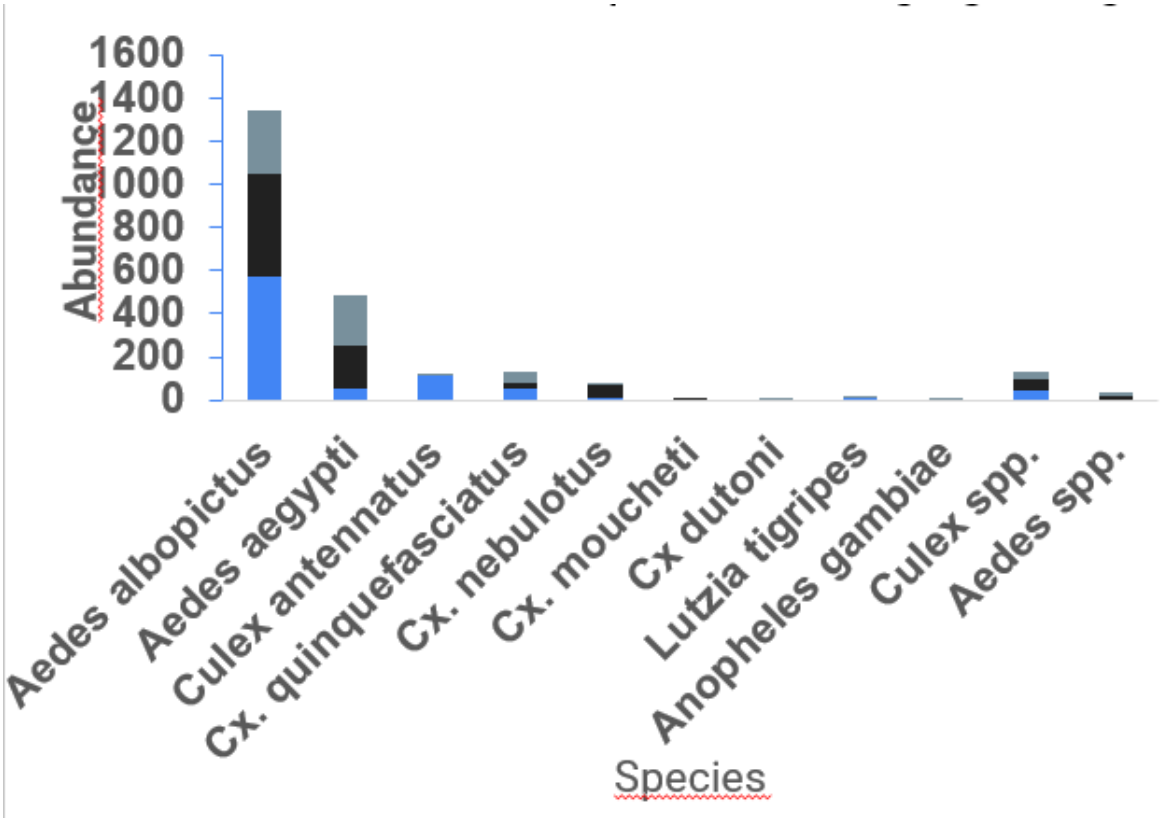
Materials & methods: Entomological surveys were undertaken from September 2021 to October 2022 in Bertoua (savanna area), Kribi (city port) and Sangmelima (forested area). Immature stages of *Aedes* spp. were collected by deeping and Stegomyia indexes were estimated. After emergence and morphological identification, the F1 progeny of *Ae. aegypti* and *Ae. albopictus* were tested using WHO bioassays and mortality rates were assess according to the different insecticides tested.

Results: A total 475 breeding sites in Sangmelima (41.47%), Kribi (33.47%) and Bertoua (25.05%) were identified. Tires and plastic containers were most abundant. A total of 2364 mosquitoes belonging to 4 genera and 9 species were collected. *Aedes* species recorded (1864) included *Ae. albopictus* (72%), *Ae. aegypti* (25.91%), and *Aedes* spp. (2.09%). These main vectors were present in all the study sites. *Ae. albopictus* was predominant in Sangmelima and Kribi while *Ae. aegypti* was mostly found in Bertoua. According to house indexes, transmission of dengue and yellow fever were high in Kribi and Bertoua. *Ae. aegypti* across study sites were found to be resistant to permethrin, deltamethrin and DDT, while *Ae. albopictus* was resistant to bendiocarb in Kribi and Bertoua. However, all these species were susceptible to malathion.

Conclusion: Inadequate control measure against vectors of arboviruses seem to enhance their proliferation and insecticide resistance. This data highlights the need for alternative strategies in vector control interventions in Cameroon.

Fig. 1

Fig. 2


1600
1400
1200
1000
800
600
400
200
0
Abundance
Aedes albopictus
Aedes aegypti
Culex antennatus
Cx. quinquefasciatus
Cx. nebulotus
Cx. moucheti
Cx dutoni
Lutzia tigripes
Anopheles gambiae
Culex spp.
Aedes spp.
Species

PS046.I

Evolution of malaria parasitological parameters in the population of Emana, a rural village in the Centre Cameroon region

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Introduction: Knowledge of all aspects of the disease is needed for effective, controlled control today. The aim of this study was to assess the trend in parasitemia due *to plasmodium falciparum* in traditional houses in the village of Emana, in order to supplement data on malaria transmission in this type of house at local level.

Material & Methods: A longitudinal study was carried out from March 2023 to March 2024. Only residents of Emana, residents of the houses to be studied and those who had given their consent were included in the study. 03 types of houses were selected (earth, cement and houses combining the two). 104 inhabited houses make up the village of Emana. 21 households were selected for monitoring on the basis of material type, physical structure and distance from each other. 64 people living in these houses were monitored during the study period. The parameter studied was paraistemia, measured by a thick drop, and the frequency of malarial access in the different types of house (Fig. 1)

Results: The mean densities of *Plasmodium falciparum* parasites fluctuated seasonally throughout the year (Fig 2). A decrease in parasite densities was observed during the warmer months (corresponding to the dry season, mean=484.2 ± 464.5 trophozoites /µl blood) compared with the months corresponding to the rainy season (mean=544.3 ± 248.4 trophozoites /µl, $p>0.05$). On average 0.53 ± 0.43 gametocytes were detected per year in the population studied, with a maximum of 38 gam/µl of blood counted in one individual in the cold season, and 6.2% to 12.5% of individuals were gametocyte carriers (Fig 3 & Fig 4). Individuals aged >20 years were the most frequent group to find the parasite (5.25 ± 1.9), but children aged 2 to 10 years represented the group with the highest parasite densities (from 0 to 11602.3 trophozoites/ µl) (Fig 1). Residents of mud houses were more affected (4.41 ± 1.44 persons, $p>0.05$) (Fig 5). (Bamou et al 2019 ; Brutus et al, 2022 ; Huho *et al*., 2013 and Jatta et al., 2018).

Conclusion: malaria is maintained in this village by gametocyte carriers throughout the year. questionnaires show a lack of impregnated mosquito nets, so the government should intervene in these high-transmission areas.

Fig. 1

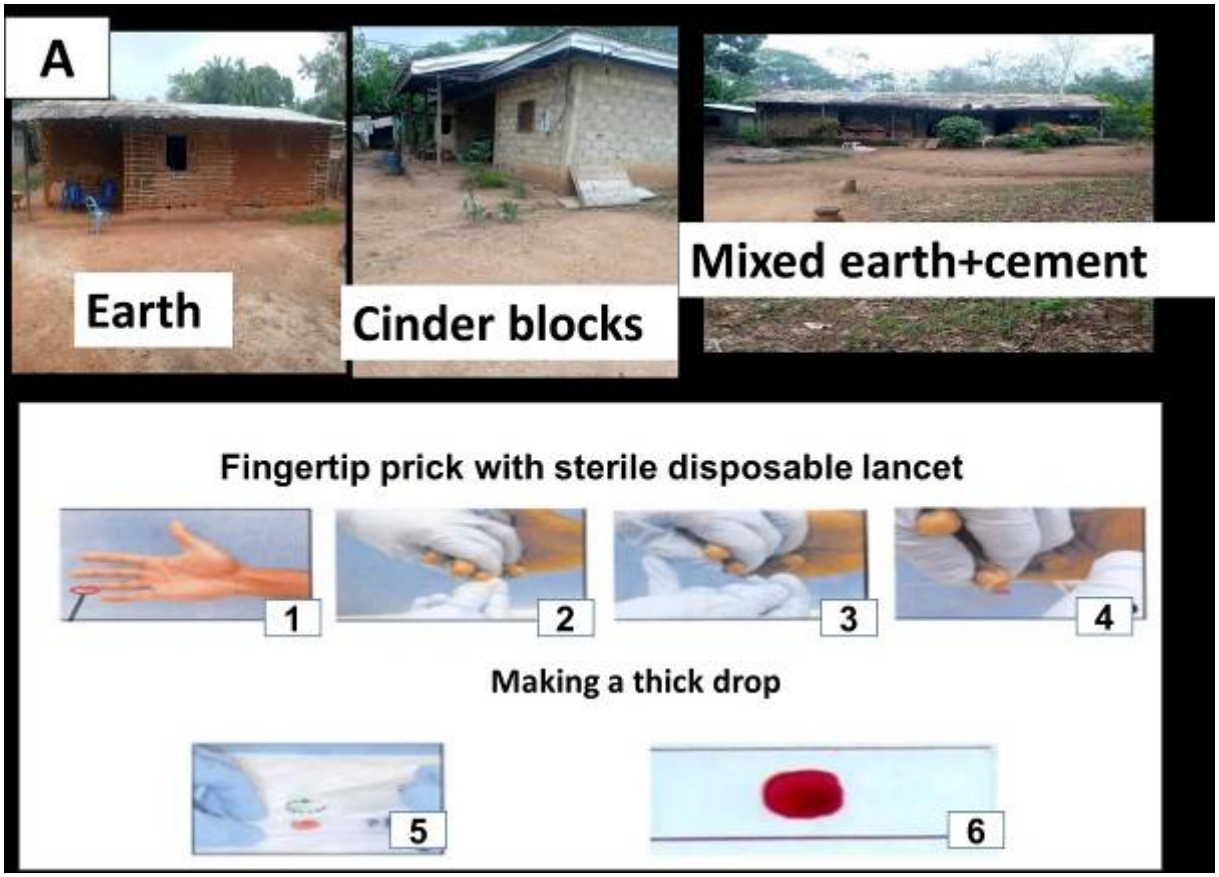
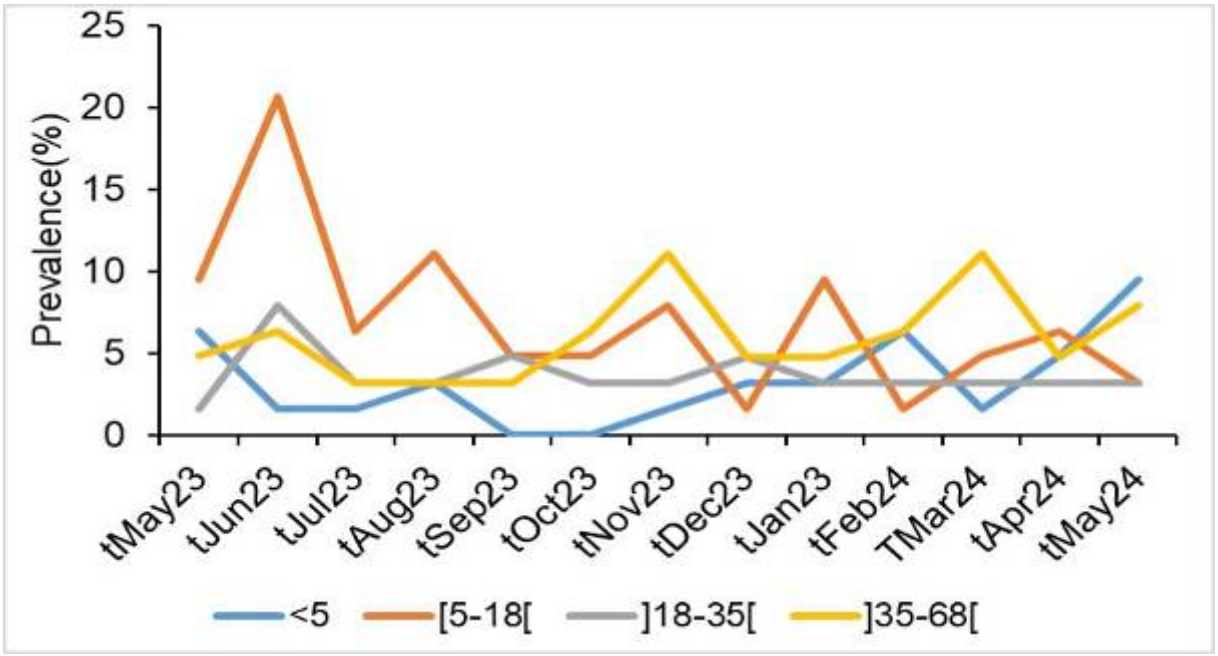

A
Earth
Cinder blocks
Mixed earth+cement
Fingertip prick with sterile disposable lancet
1
2
3
4
Making a thick drop
5
6

Fig. 2


Prevalence(%)
25
20
15
10
5
0
tMay23
tJun23
tJul23
tAug23
tSep23
tOct23
tNov23
tDec23
tJan23
tFeb24
TMar24
tApr24
tMay24
<5
[5-18[
]18-35[
]35-68[

PS049.I

Spatial factors associated with *Schistosoma* infections in sub-Saharan Africa: A systematic review

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Background: The elimination of schistosomiasis as a public health problem is a key goal set by the World Health Organization (WHO) to be achieved by 2030. The highest burden of schistosomiasis is found in sub-Saharan Africa, where individuals from 41 countries require preventive chemotherapy. This study aims to characterize the spatial factors that significantly impact *Schistosoma* infections and infection intensity in sub-Saharan African countries and quantify their effect through a systematic review.

Methods: In December 2024, a comprehensive database search was conducted in PubMed, Embase, Web of Science, and the Global Index Medicus. The search strategy combined the word concepts of "schistosomiasis," "spatial factors," and "sub-Saharan Africa" using Boolean operators. Articles retrieved from the databases will be screened for inclusion and exclusion criteria. Following the screening, a meta-analysis will be conducted on the selected articles to quantify the influence of spatial factors on schistosomiasis prevalence and intensity.

Results: From the initial search, 6496 articles were retrieved, with 3066 duplicates removed. After screening, approximately 50 articles meet the inclusion criteria for the meta-analysis. Initial findings suggest that environmental factors, such as the number and density of safe water access sources, proximity to and density of transmission sites, and access to healthcare facilities, are likely to have significant associations with the chance of *Schistosoma* infections and final findings will be presented here.

Conclusions: The review will provide actionable insights on interpreting the role of identified spatial factors and their implications for achieving the WHO"s 2030 elimination targets, highlighting gaps for future research and potential interventions tailored to the micro-epidemiology of countries and regions. The results will contribute to the evidence base needed for policymakers to design effective, localized strategies for schistosomiasis control and elimination.

PS052.I

The hidden threat: Exploring the parasite burden and feeding habits of raccoon dogs (*Nyctereutes procyonoides*)

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Background and aims: The raccoon dog *Nyctereutes procyonoides* is native to Asia but increasingly occurs in Europe. The introduction in Europe was caused by anthropogenic influence which, among other factors, classifies the raccoon dog as an invasive alien species (IAS). These IAS are known for having an impact on native ecosystems based on their role as vectors of parasites and pathogens as well as potential predation of different native and protected species. The aim of this study was to reveal carried parasites as well as pathogens of raccoon dogs from a defined area in Germany through dissection and to analyze their diet composition. The results are used to assess the raccoon dog"s impact on native ecosystems.

Methods: Totally, 73 raccoon dogs were examined by dissection. Stomach content was separated and identified genetically. Isolated ecto- and endoparasites were identified morphologically as well as genetically. Based on the results, the prevalence, intensity and abundance of parasite infestation was calculated.

Results: Based on the dietary composition, a predation on native animal species such as the protected common frog *Rana temporaria* could be shown. In total, 9 ectoparasite and 11 endoparasite species could be identified morphologically and genetically. Highest prevalence of infestation was calcualted for the nematode species *Toxocara canis* and *Uncinaria stenocephala*, the highest intensitiy of infestation was found for the zoonotic cestode *Echinococcus multilocularis*.

Conclusions: The present study shows that raccoon dogs in Germany could play an important role in the spread of zoonoses as they serve as hosts for a high number of parasite species. In addition, the raccoon dog can cause a decline in native animal species and therefore has a negative impact on native ecosystems as well as on animal and human health.

PS055.I

Diagnosing malaria with a fluorescence flow cytometer (XN-31, Sysmex Deutschland GmbH): A 4 years prospective study of diagnostic accuracy of XN-31 in comparison to microscopy or PCR in a private laboratory

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Question: Suspected malaria is a medical emergency that requires rapid and accurate diagnosis. In a non-endemic country such as Germany, it is difficult to have well-trained laboratory staff available at all times for reliable microscopic detection of the parasites. Fluorescence flow cytometry using the XN-31 offers the possibility of fast and easy-to-perform diagnostics for the detection of malaria, including the quantification and differentiation of *Plasmodium falciparum* from the other species, fully automated with a limit of detection (LoQ) of 20 parasites/µl.

Methods: 891 samples were measured using the XN-31. Thick film, prepared manually and after Giemsa staining and automatic thin film with Pappenheim staining (SP-10, Sysmex) were subsequently performed. All samples that could not be clearly evaluated in the thin film were confirmed by PCR. In addition, an intralaboratory comparison with 10 measurements was performed.

Results: Over a period of almost 4 years, 823 negative and 45 malaria positive blood samples were examined. The thick film showed a high sensitivity of 91%, the thin film of 80% and the XN-31 an excellent sensitivity of 100% in comparison to the event "malaria". The specificity and PPV was 100% for all three methods. The NPV was 99.5% for the thick film, 98.9% for thin film and 100% for XN-31. The agreement was high with Cohen's kappa of 0.92 for thick and 0.81 for thin film. In 23 cases, the XN-31 result was not evaluable (2.6%). None of the samples were positive for malaria. The XN-31 showed a correct differentiation between *P. falciparum* and *P. non-falciparum*. In 8 out of 45 cases a positive result with species unknown (UNC) was indicated. The mean parasitaemia was 0.0014. In all these cases thin film was assessed as negative, the PCR was able to detect *P. falciparum* in all cases. The intralaboratory comparison showed the same evaluation of the result, with a mean density of 0.0208 (SD 0.0003). The coefficient of variation was 0.014.

Conclusions: Malaria diagnostics can be improved in non-endemic areas with the use of the XN-31. The XN-31 offers a reliable exclusion of malaria, as well as an equally reliable suspected diagnosis with the differentiation of *P. falciparum* and *P.non-falciparum* including determination of parasitaemia within a few minutes. Positive results should be confirmed by microscopy of the thin film or PCR. Microscopy of the thick film as an enrichment method can be dispensed with when using XN-31.

PS058.I

Environmental modulators on the development of the raccoon roundworm (*Baylisascaris procyonis*): Effects of temperature on the embryogenesis

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Background and Aims: The family Ascarididae includes parasitic nematodes of the genus *Baylisascaris*, with the raccoon roundworm *Baylisascaris procyonis* being the most well-known representative. Sexual reproduction of the adult nematodes occurs in the small intestine of raccoons (*Procyon lotor*). Eggs are excreted into the environment through feces, where they develop into the infectious stage in a few days to weeks under suitable conditions. The infection of primary and paratenic hosts occurs through oral ingestion of eggs containing the infectious larval stages. *Baylisascaris procyonis* can utilise a broad range of mammals and birds as paratenic hosts and raccoons can also become infected by ingesting infected paratenic hosts. Humans act as accidental hosts. Both, paratenic and accidental hosts, can suffer significant damage to organ tissues, the visual system and the central nervous system. The study was carried out to document the embryogenesis and furthermore to investigate the effects of ambient temperature on the embryonic development.

Materials and Methods: Live *Baylisascaris procyonis* were collected from the small intestines of raccoons and incubated in a culture medium. Single-celled eggs were removed from the culture medium daily and subsequently preserved for testing. The eggs were decorticated, transferred to well plates and incubated at 5 °C, 10 °C, 15 °C, 20 °C, 25 °C, 30 °C, 35 °C and 38 °C. The developmental stages were monitored at 24-hour intervals and detailed photographic documentation was conducted.

Results: An increase in ambient temperature led to a reduction in development time. The temperature range within which embryogenesis proceeded to the L1 larval stage was between 10 °C and 30 °C. Incubation at 5 °C did not produce L1 larvae even after 11 months. Incubation at 35 °C and 38 °C resulted in the complete degeneration of the eggs before reaching the L1 larval stage.

Conclusions: The results indicate a clear effect of temperature on the rate of development of *Baylisascaris procyonis*. Increasing ambient temperatures in the range of 10 °C – 30 °C leads to an acceleration of embryonic development and thus to a shortening of the life cycle of the raccoon roundworm. Our results suggest that the temperature increase caused by climate change could significantly increase the frequency of infective stages of the raccoon roundworm by accelerating embryonic development in the environment.

PS061.I

A new method for rapid identification of fish parasites through whole-fish homogenization

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The global biodiversity crisis, driven by anthropogenic activities, signals the onset of the sixth mass extinction in Earth"s history. The biodiversity decline is particularly evident in freshwater ecosystems, where multiple species are at risk including several fish taxa. Given their integral role in fish health and ecosystem functioning, fish parasites are also of crucial importance. However, the detection and monitoring of these organisms remains challenging, due to methodological difficulties involved. Conventional methods for detecting fish parasites rely on morphological analyses, which involve sampling, sacrificing, and dissecting multiple host organisms. These approaches are ethically debated, time-consuming, labor-intensive, and require specialized expertise.

Here, we tested a new method involving whole-fish homogenization along with DNA-based identification of its parasite biota. Our goal was to validate whether the method is equally effective in terms of identification efficiency while also being more time-efficient than morphological parasite identification. For this, we collected a subset of eel (*Anguilla anguilla*) specimens in the Rhine basin in Germany. The parasites were identified morphologically, and then all the parasites and the entire fish tissue were homogenized together. Molecular identification of the parasites was conducted using parasite-specific primers. The initial results demonstrate that through homogenization, reliable detection of selected parasite species was achieved, even when parasite intensity was low or undetected morphologically.

This new method shows great promise for an efficient and accurate detection of parasites in fish hosts. The employment of this method in biomonitoring campaigns could improve the reliability of parasite assessments, which could aid fish biodiversity conservation efforts.

PS064.I

Environmental conditions trigger a reversible host manipulation in Canada's fluke-infected zombie ants

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Many parasites orchestrate changes in the colour, morphology, and especially behaviour of their hosts. In some cases, the induced changes are spectacular in their expression. In an iconic example, formicid ants infected with even a single larva (brainworm) of the fluke, *Dicrocoelium dendriticum*, attach their mandibles to a plant for hours or days. In a departure from classic examples of parasite manipulation, infected ants do not die on the plant. Rather, they detach, resume normal ant activities, then re-attach. This bizarre "attach/detach/repeat" sequence of fluke-induced altered behaviour likely facilitates transmission into obligate grazing mammals while also preventing desiccation of the ant hosts. In our quest to uncover the mechanisms leading to this reversible manipulation, I monitored the behaviour of infected ants in a parkland region in Alberta, Canada, where the fluke has been introduced from Europe.

One aim is to determine if the timing of attachment and detachment is associated with changes in environmental conditions that ants experience near their nest. To address this objective, I followed ants on plants from 4 fluke-infected nests and continuously assessed temperature, relative humidity, and light intensity with data loggers. Ants were monitored for consecutive days in the summers of 2023 and 2024, resulting in 296 total observations.

My results indicate that the proportion of ants attached varied throughout each day. GLMM analyses indicated that variation in ant attachment is best explained by variation in light intensity, relative humidity, and time of day. I observed increases in attachment at the same time most days, regardless of temperature, indicating a complex association with other environmental factors.

Overall, there was a highly significant negative relationship between the proportion of ants attached to plants and light intensity. These results indicate that combinations of key environmental factors trigger both the attachment and detachment phases of host manipulation. This work provides the foundation for definitive manipulative lab and field experiments to pinpoint how factors such as light intensity and temperature interact to cue host manipulations.

Fig. 1

PS067.I

Evidence of *in vivo* resistance against allopurinol in a dog infected with *Leishmania infantum* by reduction in copy numbers of the S-adenosylmethionine synthetase (*METK*) gene

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Objectives: Allopurinol is the main drug for long-term management of canine leishmaniasis. Clinical relapses of *Leishmania* (*L.*) *infantum* infection treated with allopurinol are described. Resistance to allopurinol has been demonstrated in-vitro, but there is little knowledge on in-vivo resistance in dogs.

Patients & methods: A two-year-old Akita Inu from Nice (France) was diagnosed with leishmaniasis based on the cytological finding of *Leishmania* amastigotes, positive PCR testing, and positive serology. The dog was treated with allopurinol over a period of 1316 days and additionally received two cycles of meglumine antimoniate.

Results: The laboratory work-up revealed mild thrombocytopenia, mild hyperproteinemia with hyperglobulinemia, a marked elevation of the c-reactive protein, and decreased iron concentration. Serum protein electrophoresis showed a polyclonal peak in the gamma globulins. Serology was positive by both ELISA (21.5 laboratory units) and IFAT (1:1024). Quantitative PCR testing of blood was positive with low numbers of *Leishmania* (10/ml blood) at the timepoint of suspicion for resistance. Resistance to allopurinol was associated with chromosome and gene copy number (CN) variations including a decreased S-adenosylmethionine synthetase (*METK*) gene CN. A CN level of below 3 is considered as suspicious for resistance, as revealed in the described dog.

Impact/Clinical significance: Relapse of *L. infantum* infection after applying allopurinol for 1316 days due to resistance was suspected clinically, backed up by the reduction in the *METK* gene CN. Recognizing resistance in relapses is crucial for the management of dogs with leishmaniasis. Dogs infected with allopurinol resistant *L. infantum* strains may represent a great risk for infection of naïve dogs, cats, and humans.

PS070.I

Vector dynamics and onchocerciasis transmission risk in first-line communities near Arinta and Olumirin waterfalls, Southwest Nigeria, and implication for tourism and elimination target

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Introduction: Arinta and Olumirin waterfalls are prominent tourist destinations in Southwest Nigeria, where blackfly biting activity has been observed based on a recent breeding site assessment. However, detailed information on vector dynamics and *Onchocerca* infectivity is lacking.

Objectives: This study aimed to investigate vector dynamics and *Onchocerca* infectivity in *Simulium* flies and human skin snips, while also assessing community knowledge of onchocerciasis and levels of participation in mass drug administration programs.

Methods: *Simulium* flies were collected monthly from September 2023 to August 2024, with 20% dissected for parity and infectivity, and 80% preserved for O-150 pool screening. Skin snips microscopy was performed on 236 participants in two front-line communities to the waterfalls, and questionnaires assessed knowledge of onchocerciasis and ivermectin participation.

Results: A total of 1,090 blackflies were collected, with higher but statistically insignificant abundance during the rainy season ($p > 0.05$). Only forest *Simulium* flies were observed breeding at both sites, with no evidence of savanna flies which are typically associated with the blinding strain of *Onchocerca volvulus*. No *Onchocerca* infectivity was detected through either fly dissection or O-150 pool screening, and skin snip examination revealed no presence of microfilariae. Results from questionnaire showed that knowledge of onchocerciasis is low, however, ivermectin uptake was high at 75.7% and 76.7% in Erin-Ijesha and Ipole-Iloro, respectively, but still below the 80% therapeutic coverage recommended by World Health Organization.

Conclusion: Findings suggest low transmission risk of onchocerciasis at both waterfalls and in first-line communities, with flies mainly posing biting nuisance. We advocate the use of protective wears and repellent by tourists and improved sensitization in first-line communities by organizing more health worker outreach to achieve optimum therapeutic coverage.

PS073.I

Cytosine base editing in *Trypanosoma brucei*

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Molecular tools in *Trypanosoma (T.) brucei* have revolutionized the study of this protozoan parasite, enabling detailed investigations into its biology and infectivity. Techniques such as RNA interference (RNAi), CRISPR-Cas9 genome editing, and advanced molecular tagging systems have facilitated functional genomic studies, offering a better understanding of this versatile parasite on a molecular level.

In addition to existing molecular tools, we want to introduce here the Cytosine Base Editor (CBE) in bloodstream form (bsf) *T. brucei*. The CBE complex enables the precise conversion of cytosine to thymine within targeted genomic sequences of the parasite.

This technology can, for instance, facilitate the introduction of early stop codons in a gene of interest. Hereby leading to a functional mutation by truncating the gene of interest.

We demonstrate the reliability of the CBE in bsf *T. brucei* through a proof-of-principle experiment targeting the tdTomato fluorescent gene in a tdTomato expressing cell line. The successful truncation of tdTomato highlights the CBE"s reliable functionality here.

The CBE"s ability to directly edit DNA in general expands the scope of genetic manipulation in *T. brucei*. Unlike RNAi, which is limited to targeting transcribed regions, the CBE can be employed to edit promoters, regulatory elements, or other non-transcribed regions. Furthermore, the CBE system offers a versatile platform for applications beyond gene truncations, e.g. enabling precise introduction of specific amino acid substitutions to study protein function. We are confident that in addition to existing molecular tools, the establishment of the CBE will help to study the biology of *T. brucei* further.

PS076.I

Spotlight on neglected lineages: A path to understanding the diversity and evolution of parasitism strategies in apicomplexa

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Apicomplexa include highly successful parasitic protists occurring in a wide range of invertebrates and vertebrates. However, knowledge of Apicomplexa is based primarily on studies of etiologic agents of significant diseases (malaria, toxoplasmosis), belonging to evolutionarily advanced lineages. Here we place our findings on neglected groups (e.g. gregarines, agamococcidia, protococcidia) into a broader context, offering new insights into parasitism strategies in Apicomplexa. Conclusions presented herein are based on our published and unpublished data obtained using parasitological and protistological approaches, *in vitro* experiments, light and electron microscopy, immunocytochemistry, and molecular phylogeny.

Ancestral apicomplexans parasitising marine annelids most likely spread to other marine invertebrates, then to freshwater and terrestrial invertebrates, and finally to vertebrates. They have evolved unique adaptations for invading and surviving within hosts. This is especially true for enormously diversified basal groups that in various ways realise the extracellular, epicellular and intracellular parasitism in different organs or cavities of invertebrates and vertebrates [1-3]. Basal lineages differ from other Apicomplexa in that their trophozoites tend to be motile, utilising several motility mechanisms that represent specific adaptations to parasitism in different environments and differ from substrate-dependent actin/myosin-based gliding described for apicomplexan zoites [3,4].

Apicomplexa demonstrate two major determinative evolutionary trends: i) origination of epicellular parasitism, observed mostly in gregarines and cryptosporidia, with significant modifications to the trophozoite attachment apparatus and motility mode; and ii) origination of intracellular parasitism accompanied by rejection of trophozoite polarity and motility (coccidia, Aconoidasida). Evolution progressing from myzocytotic predation to myzocytotic extracellular parasitism and finally to intracellular parasitism appears to be one pathway for the emergence of parasitism in Apicomplexa [1-3].

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PS079.I

Intermediate insights – Tracing amphibian trematodes via their first intermediate snail hosts

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Amphibians are a prime example of the global biodiversity crisis, representing the most threatened group of vertebrates, with major population declines and two out of every five species facing extinction. For amphibians classified as critically endangered, diseases pose the most significant threat of extinction, such as exemplified by the chytrid fungus genus *Batrachochytrium* (e.g. *B. dendrobatidis* or *B. salamandrivorans)*. Nevertheless, amphibian macroparasites constitute one of the most poorly described groups of parasites, with the majority of species lacking detailed morphological, molecular, or ecological data. Among these, digenean trematodes constitute one of the most dominant groups and feature multi-host life cycles. This study investigates the first intermediate hosts of trematodes - aquatic mollusks - to assess the occurrence, prevalence, and seasonality of amphibian trematodes at three sites in North Rhine-Westphalia, Germany. From the survey of 4,335 lymnaeid snails, three species of trematodes infecting amphibians as definitive hosts were identified (*Cephalogonimus retusus*, *Lecithopyge rastellus, Opisthioglyphe ranae*). Distinct seasonal shedding patterns were observed for *L. rastellus*, which provided valuable insights into the species' life cycle. Detailed information on morphology was provided via light microscopy and SEM. Comprehensive molecular analyses were conducted and novel sequences generated to elucidate the phylogenetic relationships of the species (28S rRNA gene, ITS1-5.8S-ITS2, ITS2, *cox*1, *nad*1), with a particular focus on the position of *L. rastellus* within the Plagiorchiidae. Our findings address significant gaps in knowledge regarding amphibian macroparasites and highlight the value of life cycle implementation as a non-invasive method for monitoring parasite biodiversity in amphibians. By focusing on the first intermediate host, this study provides detailed insights into the distribution and ecology of amphibian trematodes and facilitates further research in this field.

PS082.I

Preliminary data of a time-controlled splitCas9 genome wide screen in *Toxoplasma gondii*

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CRISPR/Cas9 technology has revolutionized genome editing and gene knockout studies across various organisms. Its successful application in *Toxoplasma gondii* has provided crucial insights into this parasite's biology. Previously, we utilized splitCas9, an inducible Cas9 system relying on rapamycin-induced dimerization of FRB and FKBP domains fused to either the N- or C-terminal domain of Cas9, for targeted gene knockout in *T. gondii*. This approach facilitated a time-controlled induction of gene knockouts through continuous co-expression of suitable guide RNAs (gRNAs), leading to the identification of essential egress genes in a small-scale phenotypic screen.

Here, we present preliminary data of a genome-wide screen employing the splitCas9 system, targeting all annotated genes of *T. gondii*. After integrating a genome-wide gRNA library into a strain constitutively expressing splitCas9, we induced dimerization of the N- and C-terminal Cas9 domains. We collected samples at various time points post-induction, allowing for the assignment of genes to their respective "dropout times" and providing first insights into their potential roles in the cell cycle. Comparison of the library complexity over the course of the experiment to the starting library allowed the calculation of so-called phenotypic scores for each gene, indicating its essentiality for parasite survival. Based on each gene's score progression over time, we computationally clustered all genes into distinct groups, allowing for further conclusions about their role in tachyzoite proliferation. Of particular notice was the group of so-called "adapting" genes, which, upon disruption, cause a temporary growth defect that is recovered at a later time point. This indicates plasticity of the involved pathways, a process with significant relevance to drug development.

PS085.I

Molecular characterization and functional analysis of two isoforms of nAChR-α6 subunit from a tick (*Ixodes ricinus*)

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Nicotinic acetylcholine receptors (nAChRs) are ligand-gated ion channels from the cys-loop receptor superfamily that mediate fast synaptic signalling in the nervous system and at neuromuscular junctions. nAChRs respond to the natural neurotransmitter acetylcholine in addition, nicotine and neonicotinoid pesticides, commonly used to control ectoparasites, arthropod vectors and agricultural pests, target these receptors. Functional nAChRs are homo-or hetero-pentameric complexes with five homologous subunits arranged around a central ion-conducting pore. These subunits can be divided into alpha (α) and non-alpha (β, γ, δ, and ε) groups, based on the presence (alpha) or absence (non-alpha) of two adjacent cysteine residues in loop C. Functional homomeric nAChR consists of only α subunits such as α7 from mammals or α5, α6 and α7 from insects or related organisms, while heteromeric nAChRs comprises α and β alone or with other subunits. In many animals, including humans, the essential chaperone RIC-3 is required exclusively for nAChR expression. Additionally, ancillary proteins such as UNC-50 and UNC-74 have also effectively expressed these receptors in the Xenopus model.

nAChRs have been functionally studied in several mammals and arthropods to understand their biological and pharmacological functions. Still, detailed characterization of different nAChR subunits in tick species is lacking. We have investigated the nAChR-α6 subunit from the brown castor tick (*Ixodes Ricinus)* at the molecular level and functionally in *Xenopus laevis* oocytes. Phylogenetic analysis placed the Ir_nAChR-α6 in the monophyletic group which includes α5, α6, α7 and α9 from different arthropods. Protein 3D models confirmed the presence of all features of nAChR α subunits. Two alternative splice variants of the α6 subunit were present in all the developmental stages of the ticks. Cloning and expression of both isoforms in *Xenopus laevis* oocytes confirmed that only one of the isoforms can form a functional homomeric receptor and this was obtained without the use of ancillary proteins. Moreover, the pharmacological properties of this functional receptor, for acetylcholine and other compounds such as neonicotinoids, were investigated by two-electrode voltage-clamping. To our knowledge, no functional nAChR from an arthropod has been expressed in the *Xenopus laevis* model without the use of ancillary proteins previously.

Fig. 1

PS088.I

Implementation of immunofluorescence staining of the female reproductive tissue – A technique to analyze the influence of helminths on the vaginal immunity against sexually transmitted viral infections

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Given the co-endemic occurrence of sexually transmitted infections (STIs) and helminth infections in Sub-Saharan Africa, both with high prevalence, it is of great interest to investigate the potential interplay between these diseases. Indeed, previous studies have shown that underlying helminth infection can increase the risk of STIs, like Human Immunodeficiency Virus (HIV) and Human Papillomavirus (HPV). However, the influence of helminths on the immunological mechanisms of the female reproductive tract (site of viral infection) remains unclear. Thus, we aimed to provide methods for qualitative and quantitative data acquisition using immunofluorescence (IF) microscopy of vaginal tissue. Hence, we compared antigen retrieval methods of formalin-fixed paraffin-embedded vaginal tissue from filarial and Herpes Simplex Virus (HSV)-infected mice and tested two antigen retrieval methods suitability for automated image analysis, facilitating reproducible quantitative microscopic data acquisition. Heat-based antigen retrieval at 80°C in citrate buffer showed increased antibody binding to distinct immune cells like eosinophils, HSV, and improved tissue morphology, and was the most efficient method for sample processing and quantitative analysis. Thus, heat-based antigen retrieval treatment with a citric acid buffer of vaginal tissues is a vital method to analyze immunological mechanisms in the female reproductive tract and might allow elucidation of how helminths influence vaginal immunity against STIs. Moreover, the technique might also provide a platform for the diagnosis and research of other parasitic infections that affect the female reproductive tract like female genital schistosomiasis.

PS091.I

Diagnosis and treatment monitoring of strongyloidiasis patients using coprology, serology, and real-time PCR techniques in Southwestern Iran

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Introduction: *Strongyloides stercoralis* is a soil-transmitted helminth affecting over 100 million people in tropical and subtropical regions. The gold standard for diagnosis is larval observation, but many patients remain undiagnosed due to low parasite loads and the low sensitivity of conventional direct smear methods.

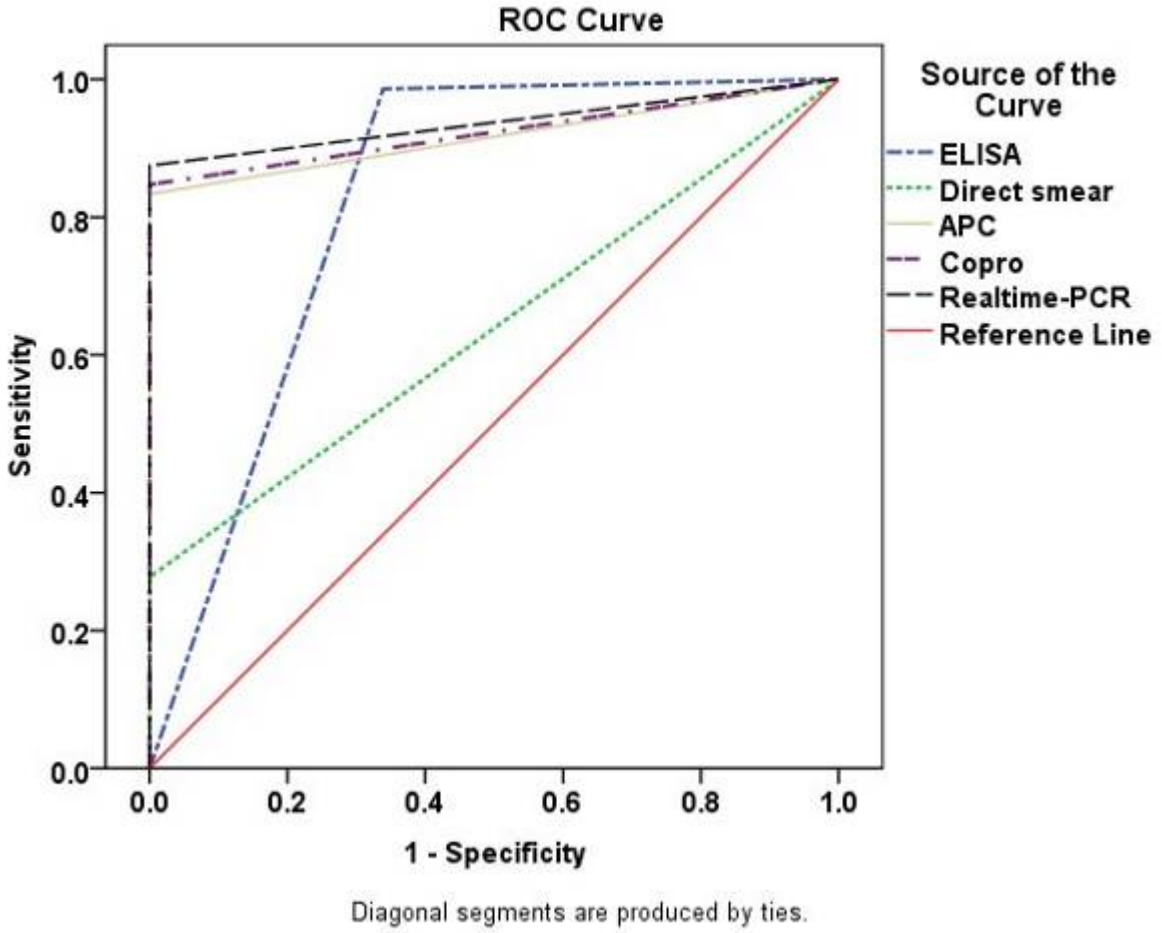
Objectives: This study aimed to evaluate Real-Time PCR for diagnosing and monitoring treatment in strongyloidiasis patients in southwestern Iran, comparing it with coprology and serology methods.

Methods: In this study, fecal samples from 91 ELISA-positive and 40 ELISA-negative individuals in Abadan County, Iran, referred to 17 Shahrivar Hospital between 2023 and 2024, were analyzed using direct smear (DS), agar plate culture (APC), and real-time PCR. Additionally, among the ELISA and larva-positive cases, 20 patients were analyzed 3 to 6 months post-treatment similarly. The sensitivity and specificity of the tests were assessed using coprology and real-time PCR as the reference standard.

Results: The DS, APC, and COPRO (combined DS and APC) tests detected 20(15.3%), 60(45.8%), and 61(46.6%) strongyloidiasis cases, respectively. All but one positive case were ELISA positive. Real-time PCR identified 63 positive cases (48.1%), of which 19(30.15%) were positive by DS, 51(80.95%) by APC, 52(82.53%) by COPRO, and 62(98.4%) by ELISA. Based on the reference standard results, the frequency of positive cases increased to 72(55%). Among the 20 treated patients, 5 cases (25%) were positive by real-time PCR. Of these, one case was positive by both DS and APC methods, while the others were negative. Accordingly, ELISA demonstrated the highest sensitivity at 98.6%, followed by real-time PCR with a sensitivity of 87.5%. In contrast, direct smear exhibited the lowest diagnostic sensitivity at 27.8%. Except for ELISA, which exhibited a specificity of 66.1%, all other tests demonstrated a specificity of 100%. The highest positive predictive value (PPV) was attributed to DS, APC, and real-time PCR, all achieving 100%. Conversely, the highest negative predictive value (NPV) was observed for ELISA, at 97.5%. The AUC comparison showed that real-time PCR had the highest value (0.938), while direct smear had the lowest (0.639). The lowest agreement coefficient (Kappa= 0.147) was found between direct smear and ELISA, while the highest agreement coefficient (0.985) was observed between APC and COPRO. The real-time PCR and APC, with AUC values of 0.938 and 0.917, respectively, demonstrated the highest efficacy in detecting positive cases of *Strongyloides* infection.

Conclusions: Given the absence of a gold standard method for diagnosing and monitoring strongyloidiasis, ELISA is considered a viable option for screening due to its sensitivity and high negative predictive value; however, combining molecular and traditional parasitological techniques can significantly enhance diagnostic accuracy and support better clinical decision-making.

Fig. 1


ROC Curve
Sensitivity
1 - Specificity
0.0
0.2
0.4
0.6
0.8
1.0
Source of the Curve
ELISA
Direct smear
APC
Copro
Realtime-PCR
Reference Line
Diagonal segments are produced by ties.

PS094.I

Molecular tools for determining the causative agents of cercarial dermatitis: LAMP, qPCR, and end-point PCR

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Cercarial dermatitis (CD), or swimmer"s itch, is a hypersensitive reaction caused by the penetration of larval stages (cercariae) of schistosomes into the skin. The avian schistosomes, especially the genus *Trichobilharzia*, are primarily responsible for CD. Penetration by cercariae provokes an inflammatory skin reaction characterized by intense itching. This condition is particularly problematic in recreational water bodies, impacting public health and local economies. Therefore, monitoring *Trichobilharzia* in environmental samples is critical for predicting outbreaks and designing control strategies. Since the currently used methods are labor-intensive and often lack sensitivity, there is a need for a modern and effective approach.

This study aimed to design and assess *Trichobilharzia*-specific LAMP and qPCR assays for detecting and identifying the CD causative agents in Europe. Additionally, species-specific end-point PCR was developed to identify the most common *Trichobilharzia* species (*T. franki*, *T. szidati*, and *T. regenti*). We aimed to compare the specificity and sensitivity and evaluate the possibilities of these assays.

We developed *Trichobilharzia*-specific LAMP, qPCR assays, and species-specific end-point PCR for identifying *T. franki*, *T. szidati*, and *T. regenti*. The assays were tested for specificity using non-*Trichobilharzia* DNA of other trematodes and for sensitivity by determining the minimum detectable DNA quantities. These assays were also applied to samples of whole cercariae without prior DNA isolation.

We confirmed the specificity of all three assays for the genus *Trichobilharzia*. The end point PCR was species-specific for *T. franki*, *T. szidati*, and *T. regenti*. Sensitivity tests revealed that qPCR was able to amplify 1-10 copies of the target DNA sequence, while LAMP reliably amplified 1000 copies but only occasionally succeeded with 10 copies. These results suggest that both methods are suitable for detecting *Trichobilharzia* in environmental samples. In addition, the LAMP assay can be used on whole cercariae without DNA extraction.

Trichobilharzia-specific LAMP, qPCR, and species-specific end-point PCR assays provide a comprehensive molecular toolkit that can be used in various scenarios, ranging from environmental monitoring to species identification. These assays offer an effective and sensitive alternative to traditional monitoring methods, improving the detection and management of CD outbreaks in recreational water bodies.

PS097.I

First report of *Trypanosoma evansi* in animals from small farms on the Ecuadorian Amazon

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[2]Universidad Central del Ecuador, Instituto de Investigación en Zoonosis, GIBCIZ, Quito, Ecuador
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Introduction: *Trypanosoma evansi* is a widely distributed hemoparasite that affects both wild and domestic animals, impacting the productivity of rural and semi-rural production units. The presence of this parasite has been reported in some South American countries, including Amazonian regions. Despite the importance of livestock, there are scarce studies on trypanosomosis in Ecuador, and no previous reports of *T. evansi* in the country.

Objective: This work provides molecular evidence of *T. evansi* in canines and equines from two provinces in the Ecuadorian Amazon.

Materials & methods: We used DNA biobank samples collected between 2015 and 2016 to detect hemoparasites in domestic animals relevant to their transmission cycle, in the provinces of Orellana and Sucumbíos. For molecular identification, PCR assays were performed using four molecular markers (*ITS1*, *ESAG6/7*, *MaxiCyt0.2/2*, and *RoTat 1.2 VSG*).

Results: *T. evansi* was consistently detected with three sets of primers *ITS*, *ESAG*, and the specie-specific *RoTat*, with an overall molecular prevalence of 11.5% (3/26). No amplification was obtained with *MaxiCyt* primers targeting mitochondrial genes. DNA sequences from the amplicons were analyzed using maximum likelihood phylogenetic methods, confirming the presence of *T. evansi*.

Conclusion: This is the first report of this parasite in Ecuador, suggesting a silent circulation of trypanosomatids in farms of the country, and indicating a potential zoonotic risk for the human population.

Figure 1. The *RoTat 1.2 VSG* phylogenetic tree showing the location of the sequences obtained from Orellana and Sucumbios provinces.

Figure 2. Molecular detection of *T. evansi* using *RoTat 1.2 VSG* primers. Lanes: 1, ladder. 2, Non-template control. 3, postive control (TeAp-Mantecal01). 4-10, field samples from canines and equines.

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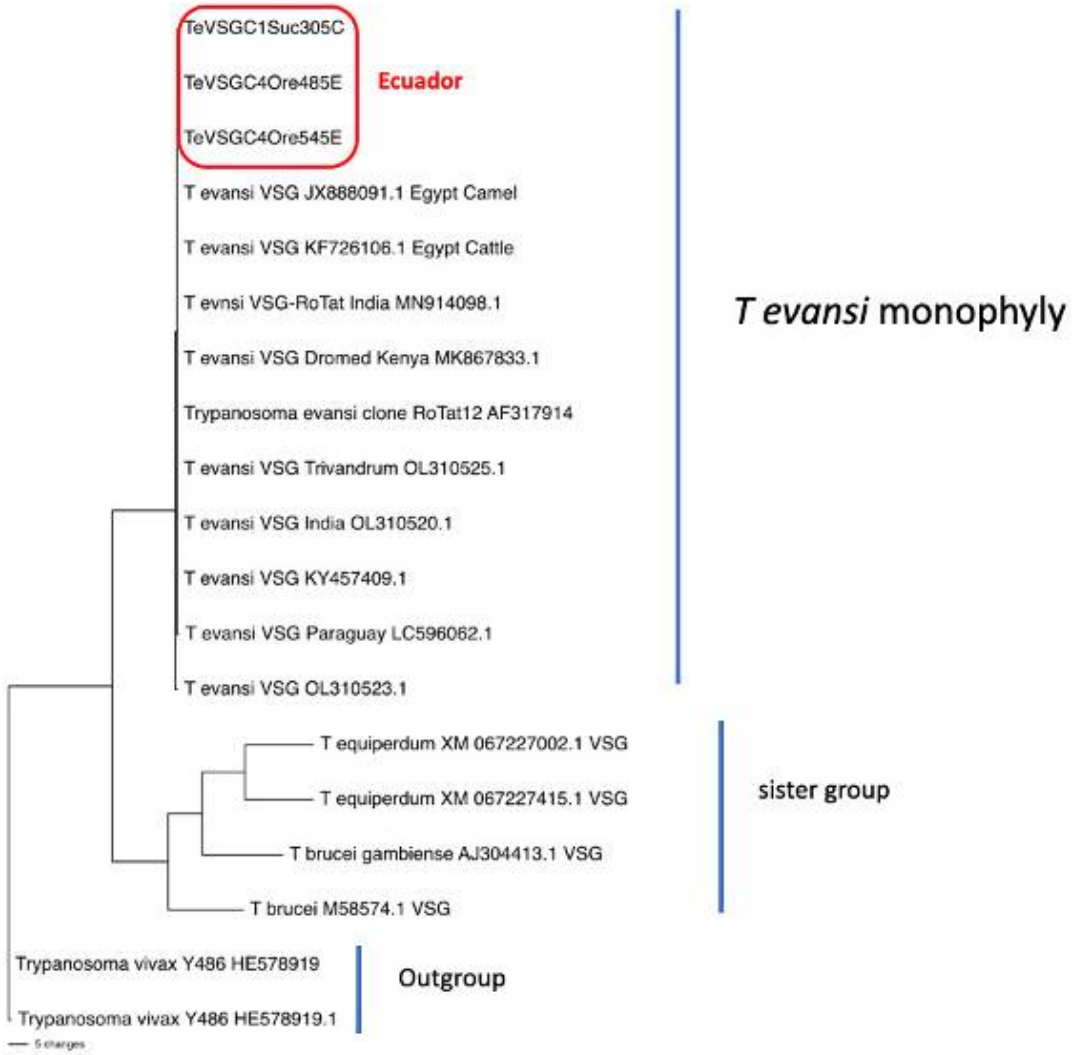
Fig. 1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TeVSGC4Ore545E
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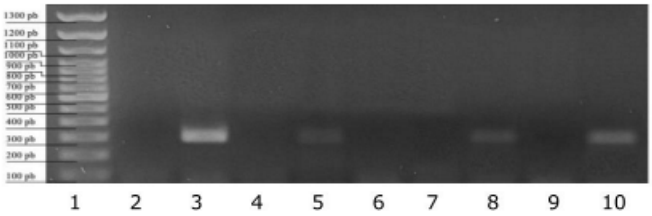
Trypanosoma evansi clone RoTat12 AF317914
T evansi VSG Trivandrum OL310525.1
T evansi VSG India OL310520.1
T evansi VSG KY457409.1
T evansi VSG Paraguay LC596062.1
T evansi VSG OL310523.1
T equiperdum XM 067227002.1 VSG
T equiperdum XM 067227415.1 VSG
T brucei gambiense AJ304413.1 VSG
T brucei M58574.1 VSG
Trypanosoma vivax Y486 HE578919
Trypanosoma vivax Y486 HE578919.1
5 changes
T evansi monophyly
sister group
Outgroup

Fig. 2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PS100.I

Echinococcus multilocularis contamination of commercially grown berries and infection of foxes in the Dutch province of Limburg

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Introduction: *Echinococcus multilocularis*, the fox tapeworm, has been recognized as the most important food-borne parasite in Europe. Foxes are the main definitive hosts and humans can accidentally get infected by ingestion of eggs. This may cause the life-threatening disease alveolar echinococcosis (AE), where the larval stage of the parasite develops into a tumor-like structure in the liver with the potential to metastasize. After the first detection of *E. multilocularis* in foxes in the provinces of Limburg and Groningen in 1996, the prevalence in foxes and the number of human cases have increased. Since ingestion of eggs might cause AE, it is important to not only monitor the presence of *E. multilocularis* in foxes, but also in food. This is especially important in produce that is eaten raw, since cooking will inactivate the eggs. Results from the European-wide MEmE project (2022) showed that some soft fruit samples from the province of Limburg contained *E. multilocularis* DNA, so a study was set up in 2024 to investigate the contamination rate on a larger scale.

Materials & methods: To assess the prevalence and associated risk factors of *E. multilocularis* on fresh produce in the Dutch province of Limburg, 220 soft fruit samples consisting of 100 grams of fruit were collected directly from 22 farm plots on 14 different farms during the harvest season. Additionally, fruit producers were interviewed and 2 camera traps were placed on each farm. The fresh fruit was tested for the presence of *E. multilocularis* DNA using a washing and centrifugation protocol, followed by DNA isolation and an *E. multilocularis*-specific qPCR targeting the 12S mitochondrial gene. Results were confirmed by COX1 PCR and sequencing.

Results: In the 2022 MEmE study, *E. multilocularis* DNA was found on 1/8 strawberry and 1/6 blueberry samples from Limburg. In our 2024 study, 10/220 (4.5%) soft fruit samples tested positive for *E. multilocularis* DNA, but only in low concentrations. Additionally, foxes were captured by the camera traps on 6/14 farms and seem to deliberately enter farm plots to forage fruits. These results confirm the potential contamination of commercially grown soft fruits in Limburg, but the implications for food safety remain unclear.

Follow-up: Soil samples (50g) were taken from the same locations as the fruit samples used in this study. These soil samples will be tested for *E. multilocularis* DNA as well to estimate the contamination rate of the environment in which the produce grows. Data analysis, using the questionnaire data combined with qPCR results, will follow when all samples have been analyzed. Additionally, the current endemic area as well as the prevalence and worm burden of *E. multilocularis* in the fox population will be estimated by a large field study sampling foxes in Limburg in 2024-2026. These results will contribute to estimating the public health risk of *E. multilocularis* and the development of prevention strategies.

PS103.I

Impact of behavioural interventions on schistosomiasis-related knowledge, attitudes and practices of primary schoolchildren in Pemba, Tanzania: A three-year study

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Introduction: Schistosomiasis is a debilitating disease, mostly affecting children in Africa. In addition to preventive chemotherapy and other interventions, behaviour change communication (BCC) is recommended to reduce transmission and reach elimination.

Objective: We evaluated the impact of BCC interventions on schistosomiasis-related knowledge, attitudes and practices (KAP) of schoolchildren who were exposed to BCC for a varying number of intervention periods during the 4-year SchistoBreak project in Pemba, Tanzania.

Materials & methods: Annual school-based surveys were conducted from 2020–2024, where schistosomiasis-related KAP were assessed in randomly selected children from grades 3–5. BCC interventions were implemented in schools for one period (4 schools), two periods with no gap (3 schools), two periods with a one-year gap (1 school) or never (10 schools). Mixed-models with random effect were applied to assess associations between BCC exposure categories as predictors and knowledge or attitude scores, or unsafe washing practices, respectively, as the outcome variable in 2024.

Results: A total of 4196 children participated in the surveys. Knowledge and attitude increased with repeated exposure and children who received BCC intervention once or twice had considerably higher schistosomiasis related knowledge and attitude scores, respectively, compared with those who never received BCC interventions. A substantial decline in the use of natural open waterbodies for washing across the 4 study years was found in children from all schools. However, in the final survey, children who were exposed to BCC interventions once or twice had significantly lower odds of using unsafe water compared with children who never received BCC interventions. The washing platforms installed in hotspot areas were known by up to half of the children exposed to BCC interventions, but their use varied between 7.5-43.1%.

Conclusion: For a maximum and lasting effect on KAP that can support schistosomiasis elimination through behaviour change, BCC interventions should be applied for long-term and consolidated by easy access to improved water infrastructure. Only when people know and are reminded repeatedly about the transmission route and consequences of schistosomiasis and when they have easy access to adequate alternatives, they may change behaviour to prevent infection and transmission, which will ultimately support elimination.

PS106.I

Structural analysis of variant surface glycoproteins of *Trypanosoma congolense*

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African trypanosomes are the causative agents of Human African Trypanosomiasis (HAT) and Animal African Trypanosomiasis (AAT) in sub-Saharan Africa. In their bloodstream form life cycle stage, these parasites have cell surfaces coated with a dense layer of glycosylphosphatidylinositol (GPI)-anchored proteins known as Variant Surface Glycoproteins (VSGs). The VSG coat enables the parasite to evade the host"s immune response through antigenic variation, by switching the expression of antigenically distinct VSGs from a repertoire of over 1,200 VSG genes. In addition, the VSG layer acts as a physical barrier, protecting invariant surface proteins from immune system attacks.

VSG monomers in *Trypanosoma brucei* have a molecular mass of 45–55 kDa and consist of a larger N-terminal and a smaller C-terminal domain. The VSG family exhibits relatively high sequence diversity, with only 15–25% sequence identity between most VSGs. However, despite this variability, they share a conserved overall structure. Although the repertoire and structure of VSGs in *T. brucei* has been extensively studied, knowledge of the repertoire of functional VSGs in other African trypanosome species is sparse and no structural data are available. The VSGs of *Trypanosoma congolense* are smaller, comprising only a single domain that resembles the N-terminal domain (NTD) of *T. brucei* VSGs. Only a few VSGs have been characterised well with the vast majority of VSGs identified from genome sequences. Based on bioinformatic analysis VSGs from *T. congolense* have been shown to resemble B-type VSGs in *T. brucei* and they belong to two families, Fam 13 and Fam 16.

Here, we have focused on Fam13 *T. congolense* VSGs. We have successfully used *T. brucei* as an expression system for expressing a number of selected Fam 13 VSGs. However, not all Fam13 proteins supported growth of *T. brucei* cells.

PS109.I

Prevalence of *Toxoplasma gondii* antibodies in domiciliated cats of the city of Sao Paulo, Brazil

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[2]University of Santo Amaro, Posgraduación Program in One Health, São Paulo, Brazil

Toxoplasmosis is a zoonosis, caused by the protozoan *Toxoplasma gondii*, which is of great importance in public health. Felids are the definitive hosts of the parasite and eliminated oocysts that can infected different animal species and humans. The present study aimed to determine the seroprevalence of anti-*T. gondii* antibodies in domesticated cats treated at four Public Veterinary Hospitals, from different regions of the city of São Paulo, Brazil. The detection of antibodies was performed by the Indirect Immunofluorescence Antibody Test (IFAT) with a cutoff of 16. A total of 804 samples of cats, with ages from 3 months to 22 years, were obtained and 61 (7.6%) presented *T. gondii* antibodies. The prevalence of positive cats was 7.8% (20/256) from east region; 5.9% (12/202) from west region, 10,2% (19/187) from south region and 6.3% (10/159) from north region. Of the total samples analysed, 7.5% (32/427) of the males and 7.7% (29/377) of the females were positive. Positive samples were serially titrated on base two up to the maximum reagent dilution. The IgG antibody titres of the analysed cats were 32 (n=5), 64 (n=6), 128 (n=12), 256 (n=8) and 512 (n=30). No association ($p>0.05$) was observed between the gender and regions of the city where the cats lived and occurrence of *T. gondii* antibodies. The results of the present study indicate the presence of domestic cats infected with *T. gondii* in the city of São Paulo.

PS112.I

Novel kinetoplast genome structure and RNA editing patterns in the trypanosomatid *Vickermania*

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[2]Moscow State University, Moscow, Russian Federation
[3]Comenius University, Biochemistry, Bratislava, Slovakia
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The study of trypanosomatid flagellates has continually broadened our knowledge of eukaryotic biology and highlighted the limits of our assumptions based on model organisms. These parasites possess in their single mitochondrion a highly complex kinetoplast (k) DNA, which is composed of interlocked circular molecules of two types. Dozens of maxicircles represent a classical mitochondrial genome, and thousands of minicircles encode guide (g)RNAs, which direct the processive and essential uridine insertion/deletion mRNA editing of maxicircle transcripts. While the details of kDNA structure and this type of RNA editing are well-established, our knowledge mostly relies on a narrow foray of intensely studied human parasites of the genera *Leishmania* and *Trypanosoma*. Here, we analyzed kDNA, its expression, and RNA editing of two members of the poorly characterized genus *Vickermania* with very different cultivation histories. In both *Vickermania* species, the gRNA-containing HL-circles are atypically large with multiple gRNAs each. Examination of *V. spadyakhi* HL-circle loci revealed a massive redundancy of gRNAs relative to the editing needs. In comparison, the HL-circle repertoire of extensively-cultivated *V. ingenoplastis* is greatly reduced. It correlates with *V. ingenoplastis*-specific loss of productive editing of transcripts encoding subunits of respiratory chain complex I and corresponding lack of complex I activity. This loss in a parasite already lacking genes for subunits of complexes III and IV suggests an apparent requirement for its mitochondrial ATP synthase to work in reverse to maintain membrane potential. In contrast, *V. spadyakhi* retains a functional complex I that allows ATP synthase to work in its standard direction.

PS115.I

The histone acetyltransferase *Pf*GCN5 is essential for *Plasmodium falciparum* survival and transmission and regulates Pf H2B.Z acetylation and chromatin structure

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Plasmodium falciparum is the protozoan parasite responsible for the most severe form of human malaria. Its developmental stage transitions and its virulence factors are regulated in part by epigenetic mechanisms. *P. falciparum* GCN5 (*Pf*GCN5) is an epigenetic regulator that functions as an acetyltransferase and can also bind to acetylated lysine residues on histones via its bromodomain (BRD). Here, we created a parasite line allowing the inducible deletion of the *Pf*GCN5 BRD to show that *Pf*GCN5 is essential for parasite survival in the human blood, for the normal differentiation of gametocytes, and for sporozoite development in mosquitoes. Genome-wide chromatin immunoprecipitation (ChIPseq) and RNAseq revealed that *Pf*GCN5 regulates genes important for metabolism and development, and its BRD is required at euchromatic gene promoters for their proper expression. Loss of correct *Pf*GCN5 targeting by BRD deletion resulted in reduced acetylation of the variant histone Pf H2B.Z at target gene promoters, suggesting that Pf H2B.Z is a key target of *Pf*GCN5 acetyltransferase activity. However, *Pf*GCN5 was most abundant in the heterochromatin compartment and loss of the *Pf*GCN5 BRD de-repressed heterochromatic genes and increased levels of acetylated Pf H2B.Z in heterochromatin. The *Pf*GCN5 BRD-binding compound L-45 phenocopied deletion of the *Pf*GCN5 BRD, identifying *Pf*GCN5 as a promising drug target for BRD inhibitors. In conclusion, *Pf*GCN5 mediates Pf H2B.Z acetylation and appears to directly activate euchromatic genes, but *Pf*GCN5 is also critical for maintaining repressive heterochromatin structure.

PS118.I

A CRISPR cytosine base editor toolbox for functional genomics in *Leishmania*

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Large-scale functional genomics in the protozoan parasite *Leishmania* is constrained by challenges such as a repetitive genome architecture, limited DNA repair mechanisms, and the absence of RNA interference in most species. Here, we present a cytosine base editor (CBE) toolbox designed to overcome these limitations in *Leishmania* species.

We demonstrate how CBEs enable the introduction of functional mutations, including homopolymers and STOP codons, through cytosine-to-thymine conversion. Co-expression of the CBE with T7 RNA polymerase (T7 RNAP) and a modified T7 RNAP promoter ensures stable and efficient CBE-sgRNA expression without affecting parasite growth. The additional expression of an AsCas12a ultra variant facilitates DNA double-strand breaks at a safe-harbor locus, enabling the integration of CBE-sgRNA expression constructs with up to one transfectant per 70 transfected cells. This approach supports the efficient transfection of large sgRNA libraries and enables genome-wide loss-of-function screening in *Leishmania* species.

To further optimize our method, we introduce BEAN (Base Editor Activated Normalization), which uses an editing reporter that undergoes simultaneous editing to that of the targeted endogenous locus. This allows for the evaluation of CBE-sgRNA editing efficiency during screens in *Leishmania*. Additionally, we developed a Split-CBE system that permits inducible functional mutations upon rapamycin addition.

Overall, our CBE toolbox provides a robust platform for efficient loss-of-function screening in *Leishmania* and holds significant potential for identifying genes associated with drug resistance, fitness, and other phenotypes across the *Leishmania* life cycle and on a genome-wide scale.

PS121.I

Phenotype and interactions analysis of lncRNAs in *L. braziliensis* parasites

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Leishmaniasis is a neglected tropical disease caused by digenetic protozoan parasites from the genus *Leishmania*. *Leishmania braziliensis* is the most important causative agent of cutaneous and mucocutaneous leishmaniasis in South America. *Leishmania* parasites lack canonical promoters for genes, emphasizing post-transcriptional gene expression by Ribonucleoprotein (RNP) complexes as key regulators. Despite not coding for proteins, non-coding RNAs (ncRNAs) are modulatory elements in gene regulation in all domains of life. In *L. braziliensis*, we previously reported two long ncRNAs (lncRNAs) differentially expressed between parasite lifecycle stages (Ruy, Teles et al., RNA Biology, 2019). Further investigation demonstrates both lncRNA_380 and lncRNA_243 Knockout parasite cell lines present reduced infectivity to macrophages in vitro that is rescued to the wild-type levels by ectopic lncRNA expression. RNA pull-down experiments using these lncRNAs as baits yielded cofactor protein candidates. Key lncRNA:Protein interactions were confirmed and expanded upon by the endogenously-tagging and RNA immunoprecipitation of filtered selected cofactor candidates. Our outcomes confirm implicit roles for lncRNA_380 and lncRNA_243 in infectivity with RNP complexes for both involving nucleic acid binding, protein secretion and post-translational regulation processes. In summary, we further demonstrate the importance of lncRNAs to the infectivity of *Leishmania* parasites and reveal key protein interactions. These results are the first to validate the stage-regulated expression, pertinence to infectivity and key interacting proteins of *L. braziliensis* lncRNA candidates that we previously identified. This study contributes significantly to the growing research field of non-coding RNAs in *Leishmania* and other Kinteoplastid parasites.

PS124.I

Leishmania parasites species-specifically modulate macrophage migration, morphology, and F-actin dynamics

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Leishmania parasites pose a significant health risk to over 350 million people worldwide, with transmission occurring via sand fly bites. Once delivered into the skin, these parasites either remain localized, causing cutaneous leishmaniasis (CL), or disseminate internally, resulting in visceral leishmaniasis (VL). We hypothesize that these distinct clinical manifestations result from *Leishmania's* ability to hijack host cell migration, facilitating their dissemination within the mammalian host.

Here we employed 2D motility assays to investigate the migration patterns of THP-1 and BLaER1 derived macrophages infected with different *Leishmania* species. Using a low infection ratio to minimize external influences on macrophage behaviour, we conducted a detailed tracking analysis of 360 individual macrophage infection experiments. Our findings reveal that VL-causing species, *L. donovani* and *L. infantum*, enhance macrophage motility, whereas the CL-causing species *L. mexicana* suppresses it. These motility differences are associated with parasite-specific alterations in macrophage morphology and actin cytoskeletal dynamics. *L. donovani*-infected macrophages displayed reduced circularity, increased actin-based protrusions, and higher total actin content. Conversely, *L. mexicana*-infected macrophages were more rounded and exhibited diminished actin signals. Importantly, these parasite-driven effects were distinct from those observed in bead-bearing macrophages. These species-specific manipulations were consistent across various conditions, including temperatures mimicking skin and organ environments (34°C and 37°C), differing parasite loads, and varying *in vitro* passage numbers.

Collectively, these findings suggest the ability of *Leishmania* to species-specifically regulate macrophage migration through actin cytoskeletal remodeling and underscore the utility of *in vitro* assays in elucidating the mechanisms underlying the diverse clinical manifestations of leishmaniasis.

PS133.I

Role of NIF interacting factor-like phosphatase-4 (NIF-4) during the erythrocytic stage development of *Plasmodium falciparum*

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Protein phosphorylation plays a vital role in the life cycle of *Plasmodium falciparum*. The cAMP-dependent protein kinase catalytic subunit (PKAc) is an essential kinase required for merozoite invasion of host erythrocytes. To better understand its regulatory mechanism, we previously examined the impact of PKAc on the parasite"s phosphorylation profile. We identified 39 high confidence targets of PKA that were significantly regulated following conditional knockout of three key components of the cAMP signalling pathway: adenylyl cyclase beta (ACβ), phosphodiesterase beta (PDEβ) and PKAc. One of these potential substrates of PKA was the protein phosphatase NIF-4. To investigate its function, we used a conditional knockout system to study its role during the asexual stages of parasite development. Our findings demonstrated that NIF4 is crucial for parasite survival within red blood cells. Parasites lacking NIF-4 expression arrested at the ring stage and were unable to progress further. Using RNA sequencing, we showed that NIF4 influences transcription affecting the expression of numerous genes including those encoding DNA polymerases, kinases and phosphatases, highlighting its wide-ranging effect. Through pull-down experiments, as expected we identified the RNA polymerase II complex and the transcription initiation factor IIF as binding partners. Surprisingly, we also identified NIF-4 as an interactor of both the catalytic and regulatory subunits of PKA. Notably, interaction of PKA subunits with NIF4 has not been reported in other organisms, suggesting a unique feature of *P. falciparum*.

PS136.I

Birago project – What has happened thirty years after the introduction of macrocyclic lactones in a historically endemic area for *Dirofilaria immitis*? Preliminary data

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Introduction: The first observation of *Dirofilaria immitis* in dogs was made by Birago in 1626, a nobleman living in Northern Italy. Since the 1980s, scientific studies have identified Northern Italy, particularly the area around the Po River, as a highly endemic region for heartworm disease, with prevalences ranging from 31% to 98% (Martini et al., 1996). After the introduction of ivermectin in 1988 and other macrocyclic lactones in the following years, parasitic pressure gradually decreased, leading to a reduction in the prevalence and incidence of heartworm disease in this area. However, in recent years, this reduction has led to some practitioners to pay less attention to the disease, including in terms of diagnosis and prevention.

Objectives: The aim of the study was to assess, approximately 30 years after the introduction of macrocyclic lactones preventives, the prevalence and distribution of *D. immitis* in Northern Italy, a historically endemic region for heartworm disease.

Materials & methods: The study population consisted of 500 dogs. The inclusion criteria were stray dogs or those without microchips referred to shelters or veterinary facilities, older than 10 months of any breed, and untreated with macrocyclic lactones for heartworm prevention. Approximately 2 ml of blood was collected from each dog and stored at +4°C in tubes with anticoagulant. An identification form was completed for each animal, including the location of recovery, age, breed, sex, weight, and the presence of any clinical signs. Each blood sample was analyzed using Knott's test (Genchi et al., 2021) and a antigen test (Idexx 4DX).

Results: Preliminary data from 328 dogs examined with Knott"s test, from 10 provinces of Lombardy and Emilia-Romagna, showed a *D. immitis* prevalence of 25% (fig.1). In particular, 14% were positive for *D. immitis*, 8% for *D. repens* and 11% mixed infection. The antigen prevalence was 29%, 9 dogs were antigen-positive but microfilariae negative, while 2 dogs were antigen-negative but microfilaremic.

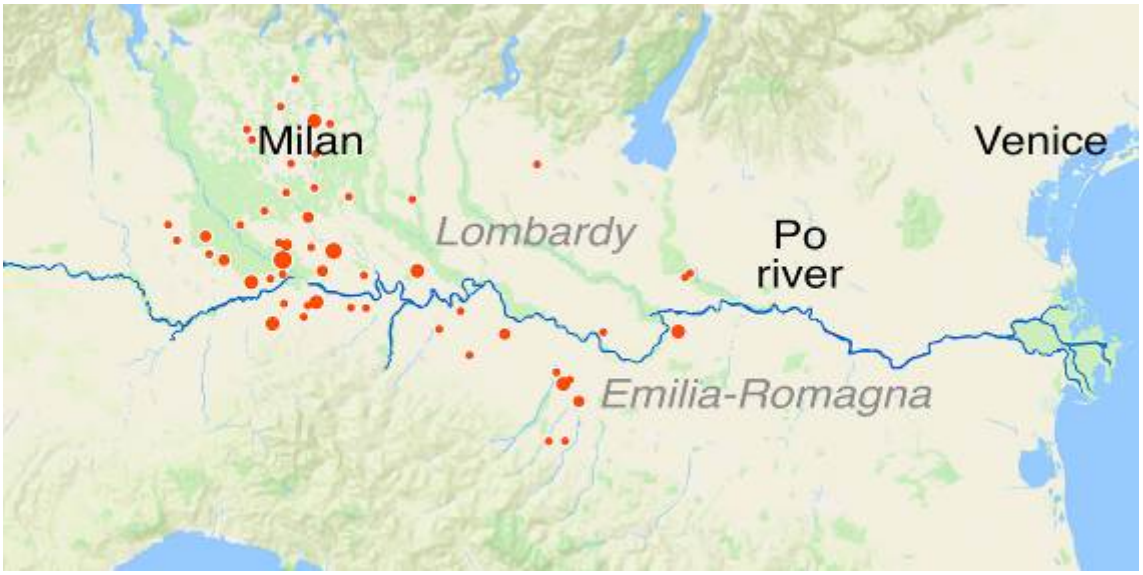
Conclusion: The data show that even 40 years after the introduction of chemoprophylaxis, in a historically endemic area such as Northern Italy, parasitic pressure has remained high. These data underline the importance of continuing to make a correct diagnosis and prevention not only for *D. immitis* but also for *D. repens* and how practitioners must not lower their attention towards these parasites.

Birago F. Trattato cinegetico, overo della caccia. Milano: GB Bidelli, 1626:59

Genchi M, Ciuca L, Vismarra A, Ciccone E, Cringoli G, Kramer L, Rinaldi L. Evaluation of alternative reagents on the performance of the modified Knott's test. Vet Parasitol. 2021 Oct;298:109555.

Martini M, Capelli G, Poglayen G, Bertotti F, Turilli C. The validity of some haematological and ELISA methods for the diagnosis of canine heartworm disease. Vet Res Commun. 1996;20(4):331-9

Fig. 1



PS139.I

Characterisation of the interaction of specific *Pf*EMP1 variants and endothelial cell receptors during cytoadhesion of *Plasmodium falciparum* infected erythrocytes

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One of the major difficulties in the development of treatments and vaccines against *P. falciparum* malaria is the ability of the parasites to alter the expression of antigens on the surface of infected erythrocytes (iEs). These antigens enable the iEs to evade recognition by the immune system and at the same time facilitate adhesion to other iEs and the endothelium of the blood vessels. This process, known as cytoadhesion, is mainly mediated by members of the *P. falciparum* erythrocyte membrane protein 1 (*Pf*EMP1) family. The *Pf*EMP1 proteins are encoded by approximately 60 *var* genes in the parasite genome. To date, at least 24 endothelial cell receptors (ECRs) have been identified as binding partners for iEs, including CD36, ICAM-1 and EPCR. *Pf*EMP1s are mutually exclusively expressed, with a small proportion of the population switching to other *Pf*EMP1s to evade the immune response. Until recently, this antigenic variation posed a significant challenge in the study of *Pf*EMP1-mediated cytadhesion. Using the selection linked-integration (SLI) approach, we have developed a method to generate transfectants that express only the *var* gene of interest and therefore present the corresponding *Pf*EMP1 population on the surface of iEs. With a library of SLI-*var* transfectants, it is now possible to further characterise endothelial binding partners under static and flow conditions as well as using single cell force spectroscopy. Static binding assays with transgenic CHO cells displaying ECRs on the surface and with parasite transfectants expressing *var01*, *var16* or *var19* have already led to the identification of new binding partners (e.g. TNFR2, CD55, and VCAM-1) and ICAM-1 as a new binding partner for Var19. Using an *in vitro* flow system, the type of binding (static or rolling) of the different interaction partners was analysed in the presence of different shear forces. Furthermore, this system will be used in the future to analyse the influence of the different *Pf*EMP1s presented on endothelial cells of different organs.

PS142.I

Trypanosoma brucei moving in microchannels and through constrictions

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Trypanosoma brucei (*T. brucei*), a single-celled parasite, is responsible for fatal sleeping sickness in infected mammals, including humans. As a natural microswimmer, it performs a peculiar helical swimming motion due to its helically attached and beating flagellum. Understanding how *T. brucei* interacts with fluid environments and navigates through confining spaces is crucial not only for medical and clinical applications but also for a fundamental understanding of how life organizes in a confining microscopic world [1,2]. Using an *in silico* model coupled with multi-particle collision dynamics (MPCD) as a fluid-flow solver [3], we scrutinize the locomotion of *T. brucei* in bulk fluid, in microchannels, and across constrictions.

We first present an elaborate analysis of the helical swimming path of *T. brucei* in bulk fluid, including the average helix diameter, swimming velocity, and rolling motion about its body axis. In straight cylindrical channels, we observe that the helical trajectory of *T. brucei* becomes rectified compared to bulk fluid. The diameter of the helical trajectory governs the swimming speed in channels: it first increases as the channel narrows and then decreases as the helix diameter is compressed. Optimal swimming speed occurs when the channel width is approximately twice the bulk helix diameter. This behavior results from an interplay between the parasite's hydrodynamic interactions with the cylindrical channel walls and its high deformability. In microchannels with constrictions, we characterize the motions of the anterior and posterior ends, the end-to-end distance, and the log-rolling motion of the cell body. These motions show salient differences from the straight-channel case. Depending on the constriction length and width, we report distinct slip, stuck, and stuck-slip motions of *T. brucei* within the constriction [4]. Our results may provide some mechanical insights into how *T. brucei* moves through tissues [5] and blood vessels, and across the blood-brain barrier.

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PS145.I

Casein kinase 1α regulates *Echinococcus* body axis formation by controlling β-Catenin degradation

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Introduction: The lethal zoonosis Alveolar echinococcosis (AE) is prevalent in the Northern Hemisphere and is caused by the infiltrative growth of the metacestode larval stage of the fox tapeworm *Echinococcus multilocularis*. The tumor-like development of the metacestode results from an *Echinococcus*-specific modification of the anterior-posterior (AP) body axis, which is regulated by Wnt/β-Catenin signaling. This signaling pathway is activated by the binding of Wnt proteins to receptors and leads to the stabilization of β-Catenin, which regulates gene expression. Controlling β-Catenin levels is crucial for Wnt signaling, with Casein kinase 1α (CK1α) acting as a negative regulator that mediates the degradation of this Wnt transcription activator. Objectives: To analyze CK1α in *Echinococcus* and its contribution to Wnt signaling and parasite development. Materials & methods: We used bioinformatics to identify *Echinococcus* CK1α and in vitro cultivation systems, combined with inhibitor assays were used to study its role in *Echinococcus* and development. Results: We identified three homologs of CK1α which are expressed by differentiated and stem cells in *Echinococcus* larvae. By applying the CK1α agonist pyrvinium, which is known to enhance CK1α activity, to metacestode vesicles, protoscoleces, and primary cells we observed significant effects on larval morphology indicating alterations in Wnt signaling. Particularly, pyrvinium induced complete loss of the posteriorized neck region in protoscoleces leading to parasite death, and re-differentiation of protoscoleces to metacestode vesicles. Furthermore, stem cell proliferation was affected in all larval stages. Conclusions: Our data indicate that CK1α modifies the AP-body axis in *Echinococcus* by controlling β-Catenin degradation and therefore Wnt-signaling. In addition, control of Wnt signaling by CK1α contributes to proliferation dynamics of germinative cells. Our data are relevant for understanding *Echinococcus* developmental processes and may be exploited for the development of novel anti-parasitics.

PS148.I

Deep learning-based quantitative behavior analysis in trematode cercariae

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The use of AI in parasitology offers new opportunities for studying host-parasite interactions and environmental cues. In this study, we applied deep learning-based pose estimation software to analyze the behavior of two trematode species, Cryptocotyle lingua and Himasthla elongata. These species share the snail host Littorina littorea but differ in their swimming patterns and strategies for infecting the next host.

We developed and trained models to recognize key body parts in these cercariae, enabling the quantitative assessment of behavioral and morphological changes. This approach allowed us to explore the effects of pharmacological substances and environmental pollutants on their swimming behavior. In addition to traditional metrics such as speed and trajectory, we identified subtle body shape changes, providing a more detailed understanding of their responses.

Our findings demonstrate the potential of deep learning-based methods for quantitative behavioral analysis in parasitic systems. This approach shows promise for advancing studies in both neuroethology and environmental toxicology in parasites.

PS151.I

Fatty acids are an important carbon source in low oxygen environments for *Toxoplasma gondii*

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The intracellular parasite *Toxoplasma gondii* undergoes diverse oxygen tensions over its life cycle. Parasites sporulate in the environment, where they are exposed to environmental oxygen. However, upon ingestion, parasites infect through the gastrointestinal tract, wherein oxygen levels can be as low as 0.5%. How does such a divergent organism respond to this change in its surroundings? Using transcriptomics we demonstrate a shift in metabolic genes – low oxygen conditions result in an upregulation of fatty acid degradation genes, and a down regulation of fatty acid synthesis genes, as well as genes in the central carbon pathway. This phenomenon was identified through KEGG maps and GO term enrichments. Consequently, lipidomics was employed, revealing substantial differences in lipid composition and abundance between *Toxoplasma* cultured in low or high oxygen conditions. Other than cholesteryl esters and monoacylglycerol (which increased in abundance), abundance of all other lipids decreased in low oxygen conditions. Additionally, in hypoxic conditions, free fatty acids decreased both intracellularly and in the media. Oxygen concentration also impacted the composition of lipids - saturated fatty acids decreased in hypoxia, with a concomitant increase in unsaturated fatty acids. Furthermore, there is a shift in phospholipids: in low oxygen, phosphatidylcholines increase, with an accompanying decrease in phosphatidylethanolamines. Finally, plaque assays using delipidated media reveal that lipids are essential for growth in low oxygen conditions, but not in high oxygen conditions. Our data shows a novel, essential role for fatty acids and fatty acid metabolism in low oxygen environments for *Toxoplasma gondii*.

PS154.I

Targeting host-pathogen interaction in *Leishmania donovani*: Insights from iMAC-based screen

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The current treatment options for the leishmaniasis are limited by side effects, high costs, and emerging drug resistance. There is an urgent need to identify new antileishmanial compounds to ensure safe and effective treatment in the future. Screening campaigns are most effective against the disease-relevant intracellular amastigotes, complicating the assay set-up. Here, we used induced pluripotent stem cell (iPSC)-derived macrophages (iMAC) as host cells. iMACs were obtained through both 2D-and 3D-differentiation and infected with *L. donovani*, ensuring infection rates up to 80%. By screening the Novartis Mode of Action Discovery set, a library containing 5632 compounds with annotated human protein targets, we identified four compounds which specifically affect the intracellular amastigotes. We could validate the annotated target of one identified hit, a vacuolar ATPase (V-ATPase) inhibitor, indicating that V-ATPases are potential therapeutic drug targets. Additionally, we picked up multiple LXE408-derivates, underscoring the potential of the iMAC-based assay for drug screening. We also confirmed the use of both 2D- and 3D-derived iMACs for drug screening. Additionally, using GFP-expressing *L. donovani* enabled us to use flow cytometry to measure the dose-response activity of compounds. In short, our study confirms that iMACs can be used for antileishmanial drug discovery and studying host-pathogen interaction. Their human origin, stable genetic background, potential for genome editing and high yields make iMACs attractive alternatives to immortalised cell lines and primary murine macrophages.

PS157.I

The effect of *Phlebotomus duboscqi* saliva on the ongoing *Leishmania major* infection in a murine model

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The timing and frequency of exposure to sand fly saliva significantly affect the host immune response, leading to different outcomes of *Leishmania* infection. This study investigates the effect of various exposure schemes of BALB/c mice to *Phlebotomus duboscqi* prior and after *Leishmania major* infection, with particular focus on the conditions underlying the establishment of protective versus enhancing effect of sand fly saliva on *Leishmani*a infection. These exposure scenarios aim to simulate conditions potentially occurring in leishmaniasis-endemic regions. Mice repeatedly exposed to sand fly bites were intradermally infected with *L. major* (obtained from the infected sand fly midguts) together with sand fly saliva and further exposed to sand fly bites also post-infection. Lesion development was monitored, and the experiments were terminated after 11 weeks of infection. At this time point, disease outcome was assessed based on (i) lesion size and (ii) *L. major* parasites load in tissues by qPCR and further subjected to a thorough immunological analysis by (iii) determination of cellular immune response including myeloid and lymphoid cell populations by flow cytometry and immunohistochemistry and (iv) levels of antigen-specific antibodies against *L. major* and P*. duboscqi* by ELISA test. Preliminary results showed that pre-infection exposure to sand fly saliva delays lesion development. In addition, continuous post-infection exposure to sand fly saliva did not diminish the protective effect induced by prior exposure, as reflected in lesion size and parasite load in the infected ear and the corresponding lymph node. Moreover, an immunological analysis of infected tissue microenvironment showed the significant effect of sand fly saliva on myeloid cells populations, particularly eosinophils and neutrophils, correlating also with the lesion size and phenotype.

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PS160.I

Synergistic and competitive interactions of tick-borne pathogens in cattle and ticks maximize transmission dynamics in Ogun state, Nigeria

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Background: Tick-borne diseases (TBDs) are a significant public health concern in tropical regions, where co-infections and pathogen interactions complicate disease control and management. Understanding how these interactions vary between vertebrate hosts and tick vectors, and how they influence disease transmission, is crucial for developing effective intervention strategies. This study investigates the impact of pathogen interactions within vertebrate hosts and tick vectors on the transmission dynamics of tick-borne pathogens in Ogun State, Nigeria. It specifically tests the hypothesis that these interactions are influenced by the species involved and that they subsequently affect pathogen transmission.

Methods: A comprehensive field and laboratory study was conducted across three main agroecological zones of Ogun State, collecting 1771 ticks from 11 cattle breeds and 21 herds, and analyzing 225 cattle blood samples. Pathogen prevalence was assessed using genus-specific end-point PCR assays for Anaplasma, Rickettsia, Babesia, Borrelia, and Coxiella. Interaction dynamics were studied using Yule"s Q statistic for correlation assessment and a network approach to map interactions and determine their nature across different species. Additionally, mathematical modeling was employed to simulate disease transmission dynamics under various scenarios of pathogen interactions, enhancing our understanding of how these interactions could potentially influence broader epidemiological trends.

Results: Significant differences in pathogen prevalence and interaction patterns were observed between vertebrate hosts and tick vectors. Ticks displayed a lower overall pathogen prevalence (25.35%) and simpler co-infection patterns, whereas cattle showed nearly universal infection rates with complex co-infection dynamics. Notably, synergistic interactions among pathogens such as Anaplasma, Rickettsia, and Babesia were prevalent in cattle, enhancing pathogen load and potential for transmission. In contrast, ticks exhibited competitive interactions that appeared to reduce co-infection rates and potentially act as a bottleneck in disease transmission. The modeling results further highlighted how these dynamics could influence the spread of TBDs in the region.

Conclusions: The findings underscore that pathogen interactions are heavily influenced by the species involved, significantly affecting the epidemiology of TBDs. These interactions have important implications for understanding and managing the spread of these diseases in tropical settings. The integration of empirical data and mathematical modeling provides a comprehensive framework for predicting disease spread and formulating targeted control measures.

PS163.I

Detection of *Phleboviruses* in sandflies from Northern Italy: Insights into toscana, fermo and other arboviruses

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Numerous arboviruses transmitted by hematophagous arthropods are actively monitored due to their widespread distribution and public health relevance. Here are reported the results of regional surveillance for *Phleboviruses* in sandflies captured in northern Italy, including Toscana virus (TOSV) and Fermo virus (FERV). The captures were conducted during 2022 and 2023 using CDC miniature light traps placed in the hilly regions of Emilia-Romagna. The female sandflies were grouped into pools of 50 insects and stored at -80°C until further analysis. RNA was extracted using the BioSprint® 96 One-For-All Vet kit (Qiagen) in the KingFisher™ workstation (Thermo Scientific™) reverse transcribed using RNase(H-)M-MLV reverse transcriptase (Promega) along with random primers (Roche)and then tested for Toscana virus (TOSV) and Fermo virus (FERV) using specific PCR assays, in addition to a Pan-phlebovirus PCR. Amplicons were sequenced using the Sanger technique. A total of 98,886 sandflies were captured. The species identified include *Phlebotomus perfiliewii* (87.2%) and *Phlebotomus perniciosus* (12.2%). A total of 70,398 sandflies were analyzed, divided into 1,712 pools tested for the presence of TOSV, revealing 74 positive pools (4.32%), and for the presence of FERV, with 337 positive pools (19.68%). Three sites recorded the highest number of positive pools for TOSV, all located in the province of Forlì-Cesena. Several sandfly pools (36) also tested positive for both viruses, indicating co-presence of TOSV and FERV. Additionally, positivity for other *Phleboviruses* was detected, specifically 35 sequences related to the Ponticelli virus (21 from the province of Forlì-Cesena), 12 sequences from the Corfou virus (8 of which are from the province of Parma), and 2 sequences related to the Punique virus (from the province of Forlì-Cesena).

The presence of significant captures in certain lowland sites, an environment commonly considered unfavorable for the presence of sandflies, has been showing increasing numbers in recent years. The detection of at least 7 different *Phleboviruses* is surprising and is likely partly related to the high mutation rate observed in these viruses (Daoudi *et al*., 2023), thus confirming the abundant presence and circulation of these viruses in the study area (Calzolari *et al*., 2018). Furthermore, the use of specific real-time PCR protocols for TOSV and FERV has effectively allowed for the detection of a higher number of positive pools than what was evidenced by Pan-phlebovirus PCR. The epidemiology and potential pathogenic capability of these viruses for humans and animals remain to be investigated, both for those already known to cause disease in humans and for those recently described with a less understood cycle.

Daoudi *et al*., 2023 Viruses 15(2):422

Calzolari *et al*., 2018 Infect. Genet. Evol. 64, 131-134

PS166.I

Tracking *Trypanosoma brucei d*evelopment with CITE-seq: A multi-omic approach

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Trypanosoma (T) brucei alternates between the Tsetse fly and mammalian host, undergoing dramatic morphological transformations and extensive changes to their transcriptome and proteome. We are investigating these changes by adapting the multi-omic approach: Cellular Indexing of Transcriptomes and Epitopes by Sequencing (CITE-seq) to track the parasite"s development within the Tsetse fly. This technique enables simultaneous profiling of both RNA and protein and so correlating transcript to protein through the developmental cycle. Given the significant changes in surface antigens during T. brucei's developmental stages, we have used antibodies targeting these stage-specific proteins to capture transitions with unprecedented precision. By establishing a CITE-seq protocol with both in vitro and in vivo derived T. brucei cells, we can correlate the transcriptome with the expression of stage-specific surface proteins, aiming to provide new insights into trypanosomatid biology and mammalian infectivity. Our results could lead to similar methodologies for studying other vector-transmitted protists with complex life cycles, such as Plasmodium, Leishmania, and T. cruzi.

PS169.I

Investigating the role of human miRNAs in the disruption of blood brain barrier integrity during *Plasmodium falciparum* infection

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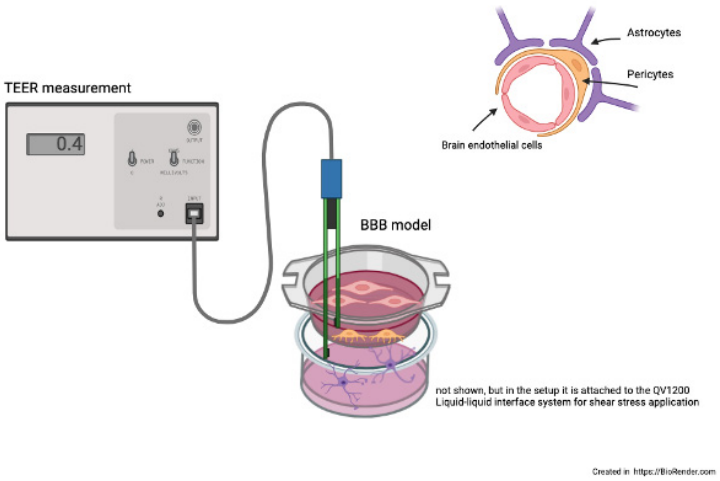
Plasmodium falciparum (P. falciparum), the malaria parasite species associated with the highest death rate, is known to cause severe complications which seem to differ within the age range of patients. Cerebral malaria, most common from ages 2-16, leads to a dramatic loss of blood brain barrier (BBB) integrity, which can cause brain edema, hypoxia and an elevation in intracranial pressure, ultimately leading to coma and death.

As the role of miRNA as a signaling and regulatory molecule becomes more and more relevant for therapeutic and diagnostic prospects, the question of its specific role in the pathogenesis of *P. falciparum* infection arises. While cytoadhesion has long been known to play a significant role in the cellular pathways that lead to severe complications in multiple organs, there also seem to be other factors at play, which could perhaps be explained through miRNA and its impact on mRNA translation.

This project focuses on unraveling the alteration in miRNA profiles under *P. falciparum* infection in a dynamic *in-vitro* model of the human BBB.

Methods: The first step was to build up the blood brain barrier model under static conditions. For this, we established the co-culture of primary human astrocytes, pericytes and brain endothelial cells in a 24 well plate using a cell culture tissue insert. Then, a dynamic flow system (QV1200) will be used to simulate the physiological microvascular shear stress. TEER measurement is used to control the integrity of the barrier. After establishment, *P. falciparum* HB3 infected red blood cells will be added to the model to study the pathogenic effects of these parasites. This will be assessed using TEER, immunohistochemistry, as well as tight junction protein assays. mRNA and miRNA of the BBB cells will be isolated and NGS sequencing will be performed to characterize the miRNA profiles in response to the different stimuli.

Fig. 1



PS172.I

Characterisation of antimicrobial peptide profiles in nematode excretory secretory products

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Antimicrobial resistance (AMR) driven by the overuse of antibiotics, particularly in agriculture, is a global health challenge. Rapidly escalating AMR, and few novel therapies reaching the market, drive the need for new approaches to tackle AMR. Nematode-derived antimicrobial peptides (AMPs) are nematode innate immune effectors and may offer a promising alternative to traditional antibiotics. Indeed, AMPs have shown potential as antimicrobial agents. The identification and characterisation of nematode-derived AMPs will reveal their role and importance in nematode biology and inform their potential as novel antimicrobial therapies. This work aims build upon previous research, identified putative AMP-encoding genes from 134 nematode genomes through *in silico* computational analyses. While this revealed that nematodes possess an abundance of putative AMPs, we do not know which, if any, are secreted into the host environment, or their potential role and importance in host-parasite interactions.

This study aims to develop a pipeline for the enhanced detection of AMPs in nematode biofluids of key livestock gastrointestinal nematode parasites that are host-facing. This involves (i) building improved, comprehensive, peptide libraries e.g. for peptides such as neuropeptide-like proteins (NLPs) which have the potential to be antimicrobial; (ii) improving mass spectrometry analysis pipelines and (iii) the detection of AMPs in nematode excretory-secretory products (ESPs). Our *in silico* analysis has identified 107 NLPs from the predicted protein data sets of 138 nematodes, representing the most comprehensive NLP analysis to date. An initial LC-MS/MS analysis of the ESP peptide profile of the free-living nematode *Panagrellus redivivus* has established an optimised pipeline to maximise AMP detection. These ESP analyses have identified 36 peptides in *P. redivivus* ESPs and tissue, including representatives of the FMRFamide-like peptides (FLPs), NLPs, and AMPs (AMP-like-Peptides, Nemapores, and GRSPs) (Figure 1). These data highlight an advance in our understanding of the NLP facet of nematode peptidomes, and *P. redivivus* ESP profiles, that will underpin the discovery of novel antimicrobial agents. Our optimised nematode biofluid analysis pipeline will be translated to the analysis of ESPs from agriculturally relevant parasitic nematodes including *Haemonchus contortus* and *Teladorsagia circumcincta* to advance in our understanding of the host-facing facet of parasite peptidomes.

Fig. 1

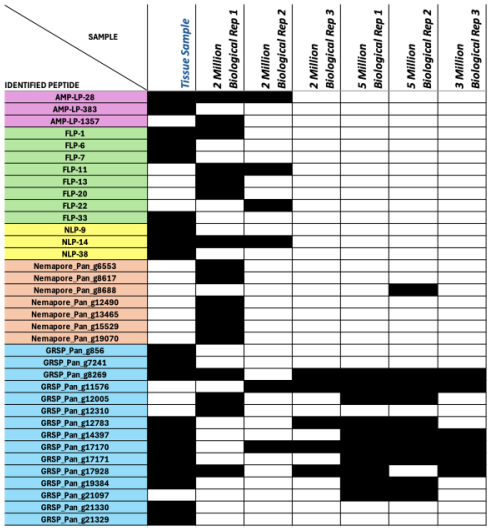

Figure 1. Block distribution table of peptides identified in samples of *P. redivivus* ESP or tissue by TimsTof mass spectrometry. Black boxes are indicative of peptide presence.

PS175.I

Methylation meets metabolic labelling: Studying RNA modifications in *T. brucei*

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The protist parasite *Trypanosoma brucei* (*T. brucei*) relies on posttranscriptional mechanisms to regulate gene expression. Methylation of mRNAs plays a significant role in their overall expression. For instance, a hypermethylated cap enhances translation, while methylated poly(A) tails in the mRNAs of variant surface glycoproteins (VSGs) are associated with increased stability. However, the molecular details underlying the stabilizing effect, including the mediating enzymes and methylation stoichiometry, remain elusive.

Although antibody-based approaches have provided valuable insights into RNA modifications, they suffer from limitations such as high variability. To address these challenges and advance our understanding of RNA methylation in *T. brucei*, we developed a metabolic labeling approach for systematic characterization of RNA methylation. This method leverages the chemical properties of a methionine analog that is incorporated into RNAs in place of methyl groups, enabling conjugation to functional biomolecules like biotin. We used Nanopore direct RNA sequencing of the biotin-labeled RNA to map and quantify RNA methylation of bloodstream form transcripts. This methodology will allow us to investigate the role of two conserved motifs of the VSG 3'UTR in the methylation pattern of this key virulence factor.

In summary, we established a novel metabolic labeling approach for studying RNA methylation in *T. brucei.* This method has the potential to be extended to investigate methylation of DNA and proteins not only in Trypanosomes, but also in also other organisms.

PS178.I

Flow and force microscopy assays to investigate the mechanism and physics of attachment and adhesion of *Giardia duodenalis*

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Giardia duodenalis trophozoites colonize the hosts" small intestine and adhere to the epithelial tissue. Motility and attachment are essential for the establishment and maintenance of the parasite infection. The key organelle in the attachment process is *Giardia"s* unique ventral disc, but the mechanism and the actual adhesion forces by which it confers attachment remain disputed.

Single cell force spectroscopy revealed unique retraction-force curves for *Giardia* that are unlike those of other eukaryotic cells, and are consistent with ligand independent attachment. Based on the concept that Giardia proceeds trough attachment – detachment cycles, we propose a theoretical framework for interpretation of measurable physical forces that takes the parasite"s stage in the attachment cycle into account.

To this end and because *Giardia* experience normal and shear forces in their habitat, we developed flow assays to complement force spectroscopy. Analysis of initial quantitative data on forces resisted by parasites in these assays is used to illustrate the interpretation framework and its usefulness to disentangle physics and biology of attachment.

PS181.I

Generation of a peroxiredoxin based H_2O_2 sensor to study the redox status of heat treated *Trypanosoma brucei*

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African trypanosomes experience drastic temperature changes as they circulate between their insect host and the mammalian vector. The response and adaptations of the parasites to this stress is not fully understood. 2-Cys-Peroxiredoxins (Prxs) are ubiquitous enzymes responsible for H_2O_2 scavenging, involved in redox signaling and acting as chaperones. *Trypanosoma brucei* (*T. brucei*) expresses a cytosolic (cPrx) and a mitochondrial (mPrx) peroxiredoxin. Next to its peroxidase activity, mPrx but not cPrx functions as a heat-activated chaperone in vitro. Therefore, mPrx has been proposed to play a role in the adaptation of *T. brucei* to increased temperatures encountered by the parasites during infection of the mammalian host. Recent findings from the Hellmich and Krauth-Siegel groups indicate that a temperature shift from 37 to 39 °C might cause redox stress in the mitochondrion of mammalian stage *T. brucei*. The parasites expressing a peroxidase-inactive mPrx in a mPrx knock-out background die after transfer to 39 °C, similarly to cells with depleted mPrx (Bogacz, unpublished; Bogacz et al. 2020). The goal of this work is to investigate the redox changes occurring in the mitochondrion of trypanosomes upon heat stress. To this end, I generated a new genetically encoded redox sensor, namely cPrx-roGFP2, which is based on the existing Tpx-roGFP2 (Ebersoll et al., 2020). Both probes were expressed in the cytosol of *T. brucei* WT cells. Current work is focused on the analysis of the changes in the redox status of trypanosomes upon transition from 37 to 39 °C, using both sensors. Furthermore, the probes will be expressed in the mitochondrion of mPrx-depleted parasites in order to better understand the role of mPrx in the adaptation of *T. brucei* to fever conditions. Additionally, this approach provides a new tool to study H_2O_2 generation within living parasites in real time, in response to various stress factors, drugs and environmental cues.

PS184.I

Molecular characterization of *Schistosoma haematobium*, *Schistosoma bovis* and their hybrid in human and snail populations in Simiyu region, North-western Tanzania

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Introduction: Schistosomiasis is a neglected tropical disease, yet it remains highly prevalent in many parts of sub-Saharan Africa. The hybridization of Schistosomes poses a significant challenge to current Schistosomiasis control strategies. While *Schistosoma haematobium-bovis* hybrids have been reported in West Africa, there is limited data on Schistosoma hybrids in humans and snail populations in Tanzania. This study aimed to identify and quantify *S. haematobium/S. bovis* hybrids in human and snail populations across four districts in the Simiyu region of Northwestern Tanzania.

Methods: A cross-sectional study was conducted in the Simiyu region of Northwestern Tanzania. Urine samples were collected from schoolchildren and examined using urine filtration to detect the presence of Schistosoma eggs. The eggs were hatched, and miracidia were collected on Whatman® FTA cards for molecular analysis. Additionally, snails were collected using a standard scoop and subjected to a cercarial shedding experiment. Cercaria were then collected on Whatman® FTA cards for molecular analysis. Individual miracidia and cercaria were molecularly characterised using rapid diagnostic multiplex mitochondrial cox1 PCR and Sanger sequencing of the nuclear internal transcribed spacer (ITS1+2) rDNA region.

Results: Out of 5,265 urine samples collected from schoolchildren in four districts, 16.9% (888/5,265) tested positive for Schistosoma eggs. A mitochondrial cox1-based diagnostic PCR was performed on 508 miracidia, and it was successful for 273 miracidia, all of which (100%) exhibited an S. haematobium cox1 profile.

A subsample of 249 cercaria revealed 64.3% (160/249) with an *S. bovis* cox1 profile and 10.4% (26/249) with an *S. haematobium* cox1 profile. The ITS1+2 amplicons of the miracidia and cercaria were Sanger sequenced from a random sample of 97 miracidia and 89 cercaria. Among the miracidia, 80.4% (78/97) presented a pure *S. haematobium* genetic profile, while 19.6% (19/97) exhibited a *S. haematobium/S. bovis* hybrid genetic profile. No miracidia displayed a pure *S. bovis* genetic profile.

From a subsample of 89 cercaria, high-quality sequences were obtained for 84 cercaria. Among these, 29.8% (25/84) exhibited a hybrid genetic profile of *S. bovis/S. haematobium*. Meanwhile, 58.3% (49/84) displayed a pure *S. bovis* genetic profile, and 11.9% (10/84) showed a pure *S. haematobium* genetic profile.

Conclusion: These findings provide evidence that *Schistosoma haematobium-bovis* hybrids are present in both human and snail populations in Tanzania, with a notably high prevalence in the snail intermediate hosts. The higher prevalence of *Schistosoma bovis* presents a potential threat to animal health. Further studies are necessary to investigate the possibility of zoonotic transmission in Tanzania.

PS187.I

Active site cysteine positioning in a *Trypanosoma brucei* dual-function peroxiredoxin enables adaptation to a parasitic life style

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2-Cys-Peroxiredoxins (Prxs) are ubiquitous hydrogen peroxide (H2O2) level sensors and cellular redox signaling hubs. Redox activity is contingent on a highly conserved peroxidative (CysP) and a resolving (CysR) cysteine, which form a disulfide bond after H2O2 reduction. Prxs often assemble into ring-shaped homodecamers, which in some cases can accommodate a less explored chaperone activity. *Trypanosoma brucei* encounters drastically changing environments, circulating between its mammalian and insect hosts. The parasites express a cytosolic (cPrx) and a mitochondrial (mPrx) peroxiredoxin, and depending on the life cycle stage, mPrx function differs. In the insect stage, mPrx acts as an essential H2O2 scavenger in the parasite's highly branched and metabolically active mitochondrion. The mammalian bloodstream form *T. brucei*, characterized by a rudimentary mitochondrion that lacks an active respiratory chain, require mPrx only when cultured at elevated temperatures. We speculated that these distinct life cycle and temperature dependent activities can be ascribed to the dual function of the enzyme as a peroxidase and a putative chaperone. We have determined the structure of *T. brucei* mPrx and show through functional assays that it indeed exhibits a temperature-activated chaperone activity. Furthermore, despite the presence of conserved resistance motifs, mPrx becomes inactivated through overoxidation by submolar H2O2 concentrations. This hypersensitivity is caused by the unusual positioning of the CysR. A "Cysteine-shifted" mutant that is more resistant to overoxidation suffers from "loose" decamer assembly with decreased chaperone activity. The dual functionality of *T. brucei* mPrx thus seems to strike a fine balance between its redox and chaperone capabilities. Indeed, our recent studies using a trypanosoma-specific mitoTpx-roGFP2 sensor suggest that mitochondrial matrix of temperature-stressed bloodstream *T. brucei* becomes more oxidizing. This finding goes in line with our observation that expression of mPrx CysP mutant in a mPrx KO background only partially rescued growth of the parasites at elevated temperatures that mimic fever of the mammalian host. Thus, the unusual positioning of the resolving cysteine of mPrx may have originated as part of the adaptation of *T. brucei* to parasitic life style.

PS190.I

A search for commercial alternatives to detect IgG antibodies to *Strongyloides stercoralis*

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Introduction: Despite a growing support for neglected tropical diseases (NTD), strongyloidiasis is still an underappreciated disease. The number of and indication for immunomodulatory drugs is growing and *Strongyloides* hyperinfection is still a clinically relevant problem. Diagnosis of strongyloidiasis is complex; optimally a combination of fecal examination and antibody testing is advised. Due to the recent extensions of the IVD regulation, there have been shifts in the diagnostic landscape; laboratory testing in non-endemic settings needs more standardization and optimal scientific confirmation.

Objectives: We aimed to assess the performance of two commercially available antibody tests compared to our in house assay for IgG antibodies to *Strongyloides stercoralis*.

Patients & Methods: From the laboratory information system, we selected sera from patients previously positive or negative for IgG antibodies to *Strongyloides stercoralis* in the reference test. Initially, 20 positive samples were selected from patients with at least two positive sera (n=11) or a positive PCR result on a fecal sample (n=9). Negative sera (n=18) were from individuals with multiple negative results in the reference test. Based on the outcome, the negative group was supplemented with 20 sera from patients who were only screened before immunosuppression, were (assumed) native Dutch and who also had a PCR-negative fecal sample. The reference test is an in-house developed ELISA, aimed at subclasses IgG1 and IgG4. The antigen mix in this test was based on larval extract from L3 *S. stercoralis* larvae. Two commercially available CE-marked tests were tested, the *Strongyloides ratti* enzyme immunoassay (Bordier Affinity; commercial test 1) and the NovaLisa® Strongyloides (NovaTec; commercial test 2).

Results: The initial validation for commercial test 1 resulted in three (of n=20) false negative and four (of n=18) false positive results. Following extension of the number of samples in the test panel with additional criteria, the same pattern was seen. This resulted in an overall sensitivity of 85% and specificity of 84% compared to the reference test. Commercial test 2 was applied on the same sample set, which showed a sensitivity and specificity of 81% and 61%, respectively. The concordance between the two tests was low, with 17 of 23 positives (test 1 vs test 2; 74%) and 19 of 35 negatives (54%).

Conclusion: Compared to the reference test, an in-house developed ELISA based on larval antigens of *S. stercoralis*, two commercially available test performed poorly to detect infection with as well as to exclude exposure to *Strongyloides*. Although the reference test may be confined in sensitivity and specificity, including a group with very low a priori risk for infection as well as concomitant fecal PCR results did not improve the test performance. Cooperative efforts are needed to optimize serological diagnosis of strongyloidiasis.

PS193.I

Investigation of the contamination of raw milk by *Cryptosporidium* spp. and evaluation of contamination risk factors

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Introduction: Cryptosporidiosis is a zoonotic disease affecting both humans and animals, and in particular young calves. Infectious neonatal diarrhea accounts for over 25% of veterinary visits to the farm, and is the main cause of mortality in calves under one month of age. Among etiologic agent of such infective diarrhea, *Cryptosporidium* spp. are one of the most frequent. Besides, outbreaks linked to raw-milk dairy products have been reported worldwide and also in France (2017, Nantes). This raises the questions of the probability of milk contamination when parasites are circulating on farms, and the practices that could lead to such contamination.

Objectives: The objectives were: i) to investigate the potential *Cryptosporidium* contamination of milk on dairy farms in a context of parasite circulation in calves; ii) identify any practices that may pose a risk of milk contamination

Materials & Methods: Sampling was performed in volunteer dairy farms by veterinarians based on the detection of cryptosporidiosis in calves feces. Stool samples from calves and cows were sampled concomitantly with milk production. Samples (both stools and milk) were analysed by qPCR and positive samples were genotyped on the *gp60* gene. If necessary, multi locus variant analysis (MLVA) were performed to distinguish same *gp60* subtypes. A survey questionnaire based on risk factors" hypothesis was designed and implemented in participating farms to compare the practises in farms depending on the milk contamination status (univariate statistical analysis).

Results: To date, 69 dairy farms were sampled representing 225 stool samples and 85 milk samples analysed. *Cryptosporidium* DNA was detected from stool samples in 94% of the 69 sampled dairy farms. *C. parvum*, *C. bovis*, *C. andersoni*, *C. ryanae* and *C. hominis Cryptosporidium* species and 17 different *gp60* genotypes were identified. *Cryptosporidium* DNA was detected in 13% (9/69) of sampled milk from the 69 dairy farms investigated with frequency depending on milk destination. From the 9 positive samples, all identified *gp60* genotypes were different. Genotypes characterized in milk were also different from ones simultaneously identified in calves. According to DNA detection in milk, positive farms differed from negative farms in practices linked to milking hygiene, calf managements, and cleanliness of the cows bedding.

Conclusion: *Cryptosporidium* DNA was occasionally detected in milk, with a significant lower frequency in farms delivering/processing raw milk, indicating a higher control of pathogens from fecal origin. *Cryptosporidium* DNA detection does not inform on the infectivity of the parasite and the risk for consumers. Genotypes were mainly different from those circulating in calves, suggesting other routes of contamination or role of biofilms in the contamination of milk. Better control of hygiene measures in raw milk sector contribute to a better management of Cryptosporidium contamination.

PS199.I

Trypanosoma brucei cattle infections contain cryptic transmission-adapted bloodstream forms at low parasitaemia

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Tsetse-transmitted *Trypanosoma brucei* parasites infect a wide host range and cause Human African Trypanosomiasis and Animal African Trypanosomosis in sub-Saharan Africa. The primary hosts of *Trypanosoma brucei* in tsetse fly endemic regions are non-human mammals, including agriculturally important cattle. In rodent infection models, *T. brucei* transitions from proliferative slender to tsetse-transmissible stumpy forms at high parasitaemia in a density-dependent quorum sensing type process. Recently, we have found that in *Trypanosoma brucei* chronic stage infections of mice (several weeks) when parasitaemia in the blood remains high, most parasites in the bloodstream are non-proliferating forms pre-adapted for transmission (Larcombe et al 2023, PNAS). Additionally, "intermediate" forms that have undergone cell cycle arrest and express stumpy-associated transcripts but had not undergone full morphological change, were evident in chronic infection. In contrast, chronic bovine infections are characterised by markedly lower blood parasitaemia levels; in most cases substantially below the density assumed to trigger slender-to-stumpy differentiation. This challenges the current (rodent-based) assumptions and quantitative parameter estimations around the generation of stumpy forms in the mammalian bloodstream by quorum sensing. Thus, cattle infections are an essential foundation for exploring the roles of slender, stumpy and "indeterminate" forms in chronicity and providing insight into the infection status dominant in the field.

By combining scRNA-seq and microscopy we conducted a molecular characterisation of pleomorphic *T. brucei* forms in cattle blood over 60 days infections of two calves. Single cell transcriptomics was performed at four discrete time points from early, mid and late infections, together capturing the transcriptomes of 37,602 individual parasites, and paired with microscopy. Throughout infection, we observed mixed populations of parasites with slender and stumpy-like transcriptomes. The appearance of stumpy-like forms coincided with fewer proliferating parasites and parasites exhibited a shortened flagellum indicative of differentiation, despite the absence of an extreme stumpy morphology or developmental marker protein expression. Comparisons with slender and stumpy form transcriptomes from murine infection and *in vitro* culture demonstrated conserved transcriptomic signatures for both slender and stumpy-like forms in bovines, as well as host specific differences. These similarities and differences are key to understanding parasite development and transmission in its natural host.

PS289.I

Molecular Detection of Pathogens in Ectoparasites Collected from Companion Animals in Sylhet City Corporation (SCC) Bangladesh

Introduction: Vector-borne pathogens (VBPs) represent a significant threat to both animal and human health, with ticks and fleas serving as primary vectors for these pathogens. In Bangladesh, the increasing population of free-roaming dogs and cats poses a potential public health risk. This study aimed to investigate the diversity of tick and flea species infesting dogs and cats in Sylhet, Bangladesh, and to identify the associated VBPs through morpho-molecular techniques.

Methods: A total of 112 dogs and 48 cats were examined for tick and flea infestations in Sylhet. Ticks and fleas were collected and identified morphologically. DNA was extracted from the ectoparasites, and polymerase chain reaction (PCR) was performed using gene-specific primers to amplify VBPs. The socio demographic information collected from questionnaires was analyzed using the R software to identify any significant associations with ectoparasite infestations. Results: Among the dogs, 46 individuals (41.1%) were infested with ticks, 19 (16.9%) had flea infestations, and 12 (10.7%) were co-infected with both ticks and fleas. In cats, 9 individuals (18.7%) had ticks, 14 (29.2%) were infested with fleas, and 3 (6.2%) were co-infected with both. Tick species identified in dogs included Rhipicephalus sanguineus, Ixodes ricinus, and Haemaphysalis longicornis, while flea infestations were primarily caused by Ctenocephalides canis and Ctenocephalides orientis. In cats, R. sanguineus was identified in 7 cases, and I. ricinus in 3 cases. Flea infestations in cats were predominantly due to Ctenocephalides felis, with fewer instances of C. orientis and Xenopsylla cheopis. Among dogs, Babesia gibsoni was the most prevalent pathogen, followed by Hepatozoon canis, Anaplasma platys, Ehrlichia canis, Babesia canis, and Rickettsia felis. In cats, Rickettsia felis was the predominant pathogen with single detections of Rickettsia massiliae, Rickettsia conorii, and Bartonella elizabethae. Sequence analysis of B. gibsoni from Sylhet showed high genetic similarity to sequences from Assam and Siliguri, India, while B. canis sequences from Sylhet were also closely related to those from India. Similarly, Hepatozoon canis from ticks in Sylhet showed significant genetic similarities with sequences from India, confirming the presence of shared genetic strains across regional boundaries.

Conclusion: This study provides valuable baseline data on the distribution of ectoparasites and VBPs in dogs and cats in Sylhet, Bangladesh. The detection of a variety of pathogens highlights the zoonotic potential of these ectoparasites, posing a serious public health concern. The molecular characterization of Babesia and Hepatozoon species indicates a high degree of genetic similarity between the Sylhet isolates and those from neighboring regions in India, emphasizing the need for integrated cross-border control efforts. These findings underline the necessity for enhanced surveillance and the development of coordinated control measures to mitigate the risks of vector-borne diseases in both animals and humans in Bangladesh and the surrounding regions.

PS202.I

Role of the nucleotide excision repair endonuclease XPF in the kinetoplastid parasite *Trypanososoma brucei*

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The nucleotide excision repair (NER) mechanism is responsible for the removal of bulky DNA damage such as pyrimidine dimers induced by ultraviolet light. The NER repair pathway detects such lesions and excises the damaged strand through incisions at 5 ' and 3' regarding the damage. The 5' incision is catalyzed by a heterodimeric endonuclease composed of XPF (catalytic subunit) and ERCC1 (non-catalytic). Here, we show that the genome of *Trypanosoma brucei*, the causal agent of human African trypanosomiasis or sleeping sickness, codes for an XPF ortholog. Consistent with a role in DNA repair the protein localizes to the cell nucleus and is found preferentially associated to the nucleolar region. RNAi silencing of TbXPF, which encodes an essential component of the NER pathway, sensitizes cells to UV irradiation, thus providing evidence that NER operates in these parasites. In addition, TbXPF confers protection against intra- and inter-strand crosslinks induced by cisplatin and mitomycin C respectively. Furthermore, TbXPF is likely involved in the processing of camptothecin-derived DNA lesions suggesting a role in single-strand break repair. The presence of a functional NER pathway in trypanosomes suggests that *in vivo*, they are susceptible to damage that blocks replication and transcription. The results obtained with various antitumor agents provide proof of concept for the potential of NER inhibition as a means to improve antiparasitic therapies.

PS205.I

Experimental design of a genome-wide CRISPR/CAS9 knockout screen in *Toxoplasma gondii* to elucidate gene expression during invasion in porcine cells

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Toxoplasma gondii, an obligate intracellular protozoan parasite, is one of the most prevalent parasitic pathogens worldwide, infecting nearly all warm-blooded animals, including humans. This parasite poses significant health risks, particularly to pregnant women and immunocompromised individuals, and contributes to substantial economic losses. While murine models are traditionally used for studying *T. gondii*, the course of toxoplasmosis in pigs shares greater similarities with humans, including comparable severity of infection and immune responses. This makes pigs a highly relevant model for studying host-parasite interactions. Our previous research using human host cells employed the splitCas9 system for inducible, time-controlled gene knockouts in *T. gondii*. This approach integrated a genome-wide gRNA library into a strain constitutively expressing splitCas9 and enabled the identification of essential genes through phenotypic screening. Computational clustering provided insights into genes roles during the parasite's cell cycle, reveling distinct groups of genes, including "adapting" genes, which caused temporary growth defects that recovered over time, highlighting the plasticity of critical biological pathways. Building on these findings, this study aims to establish a CRISPR-Cas9-based platform for genome-wide screening in porcine cells to identify the genetic determinants of *T. gondii* invasion. The splitCas9 methodology will be adapted to porcine cells, facilitating a direct comparison of genetic factors involved in parasite invasion and proliferation in both human and porcine systems. This comparative approach will uncover differences in *T. gondii* adaptation across hosts This approach may represent a significant step in validating pigs as a model for human toxoplasmosis, enhancing our understanding of host-parasite interactions, and contributing to the development of novel strategies to mitigate the impact of this globally significant parasite.

PS208.I

From nose to nose: Prevalence, lifecycle, and health impact of *Halarachne halichoeri* in grey seals of the North and Baltic Sea

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Harbour and grey seals in the German North and Baltic Seas harbor a diverse array of parasites, including the endoparasitic nasal mite *Halarachne halichoeri*, which underwent a long evolutionary parasite-host adaption in the marine environment. Absent from Central Europe for over a century, *H. halichoeri* occurred in 2014 in grey and harbour seals from the Baltic and North Sea. Little is known about its prevalence, lifecycle, or ecological implications.

Between 2019 and 2023, 60 grey seals stranded along the coasts of Schleswig-Holstein and were examined during post mortem investigations in the frame of a health monitoring. Prevalence of parasitic infections was documented, and the intensity of infection was assessed semiquantitative. Mucosa was inspected for macroscopic changes, subsequently histological analysis were conducted. Parasites were collected and preserved, and live mites were cultivated in vitro under varying conditions.

Results revealed a high prevalence of *H. halichoeri* with 56% (n = 34) of grey seals from the North and Baltic Sea infected. Infection rates were significantly higher in adult seals, with the Baltic Sea coast showing the highest number of infected seals.

Survival times for larvae kept in 0.9% sodium chloride solution were up to 103 days, while larvae cultivated in mucous under in vitro conditions lasted only 24 days. For the first time, moulting of *H. halichoeri* was observed, with larvae progressing through protonymph, deutonymph, and adult stages. Nymphal stages exhibited high motility compared to sessile adults, indicating their role in parasite transmission. Histological examinations revealed rhinitis of varying severity, linked to infection intensity.

The recovering grey seal population in the North and Baltic Sea leads to an increase of interspecific contact on sandbanks during haul-out, this may be driving the dispersal of *H. halichoeri* to new host species, potentially with severe ecological consequences. This study highlights the emerging prevalence of this nasal mite but also provides critical insights into its lifecycle and transmission dynamics, underscoring the need for further investigation into its impact on marine ecosystems.

PS211.I

Towards nucleoside analogues with potent activity against *Trypanosoma* and *Leishmania* species

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One of the main barriers between the identification of potential lead compounds against parasite-born diseases and clinical development of them as potential new drugs is that the development cost for a possible drug against a single neglected disease is deemed to be too high relative to the potential benefits of the manufacturer. Over the last few years, adenosine analogues with potent activity against trypanosomes have been identified, but mostly they were much less active against *Leishmania* species. We reasoned that the most likely reasons for the uneven activity were (a) differences in activation by adenosine kinase (AK) or (b) differences in adenosine transporters, which are NT1 in Leishmania and P1 and P2/TbAT1 in *Trypanosoma brucei brucei*.

As such, we have created a number of investigative cell lines, including *L. mexicana* ΔLmexAK, and ΔLmexAK expressing TbbAK to investigate whether *Leishmania* AK could be the factor limiting sensitivity to adenosine analogues. Equally, we created ΔLmexNT1 (null for adenosine uptake) and *T. brucei* ΔTbAT1 as well as ΔLmexNT1 expressing *T. congolense* and *T. vivax* P1-type adenosine transporters. The structure-activity relationships (SAR) of the AKs and transporters were investigated by determining the EC50 values for numerous adenosine analogues against the various modified and control cell lines. In addition, docking studies were conducted comparing the poses of a subset of analogues in the *Leishmania* and *Trypanosoma*adenosine kinases and we are conducting detailed transport assays of nucleoside analogues as inhibitors of LmexNT1.

PS217.I

Uncovering the role of SNARE proteins for nutrient uptake in mosquito stages of the malaria parasite

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The malaria-causing parasite *Plasmodium* is an evolutionary outlier compared to commonly studied model organisms. It exhibits unique modes of replication in both the vertebrate and mosquito host, relying on highly divergent proteins for essential biological processes. Within the vertebrate host, *Plasmodium* invades red blood cells (RBCs) and feeds on the host cell cytoplasm to facilitate intraerythrocytic growth. Central to this process is the endocytic uptake of RBC cytoplasm, which is trafficked to the parasite's digestive vacuole (DV), where host proteins are digested to provide the parasite with amino acids for protein synthesis. Upon entering the mosquito, the parasite undergoes sexual replication, culminating in the formation of extracellular oocysts in the mosquito midgut. These oocysts grow substantially to ultimately produce thousands of infective sporozoites. Preliminary data from our laboratories suggests that there is a highly active endocytic pathway in oocysts driving the nutrient uptake and degradation required for this extensive production of biomass. Yet the cellular and molecular characteristics of such a pathway remain largely unknown. Here, we set out to study the function of two mediators of endosome-DV fusion during oocyst development, aiming to elucidate endocytic vesicle trafficking at this parasite life cycle stage.

We recently identified two putative SNARE proteins that colocalise to the DV membrane in *P. falciparum* blood stages. Conditional inactivation of these membrane fusogens during intraerythrocytic development resulted in the accumulation of fusion-incompetent endosomes, highlighting the importance of these SNAREs for endosomal trafficking to the DV. To study the function of these SNARE proteins in oocysts, we endogenously tagged them with mNeonGreen in the rodent malaria parasite *P. berghei*, which allows the study of the entire parasite life cycle in a laboratory setting. In blood stages, both SNAREs localised to vesicles and multiple small DVs. Notably, we found that the SNAREs were also expressed during oocyst development, indicating that these proteins also function at this stage. In developing oocysts, the SNAREs colocalised with the residual hemozoin crystals to compartments that might be equivalent to the blood stage DV, while upon sporozoite formation, the localisation changed to a more spotted, punctuated pattern. A single SNARE-positive structure was also observed in each sporozoite, indicating the presence of a DV-like structure in these cells. We are currently using a promoter swap approach to specifically silence SNARE expression in oocysts to further explore their putative functions in endosome-DV fusion during mosquito infection. By elucidating the role of these SNARE proteins in oocyst development, this study aims to uncover the molecular mechanisms of nutrient uptake in these understudied parasite stages, paving the way for novel insights into the divergent biology of the malaria parasite.

PS220.I

Establishment of the Roslin vector-borne-disease research facility: Enabling whole system studies on tsetse flies, ticks, and cattle pathogens

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Vector-borne diseases exert a significant global health burden, particularly in tropical regions, where livestock diseases caused by parasitic vectors and vector-borne pathogens lead to significant economic losses and threaten food security. These diseases are perpetuated by the complex interactions between vectors, pathogens, and hosts, with research efforts often constrained by a lack of facilities capable of supporting integrated, whole-system studies.

The Roslin Vector-Borne-Disease Research Facility (RVRF) at the Roslin Institute, University of Edinburgh, provides a cutting-edge facility permitting the study of major cattle disease arthropod vectors, primarily *Glossina morsitans* (tsetse flies), *Rhipicephalus microplus*, and *Rhipicephalus appendiculatus* ticks, and the pathogens they transmit. Situated at the Easter Bush campus, RVRF combines state-of-the-art equipment, multidisciplinary expertise, and importantly access to the Large Animal Research and Imaging Facility (LARIF), with infection models in the clinically relevant cow host. This enables a holistic exploration of host and vector biology, pathogen transmission dynamics, and host-vector-pathogen interactions. While Roslin focuses on livestock disease, the RVRF can also support mouse model research, further broadening the capacity to investigate disease systems in a controlled and scalable manner. This versatility currently allows the study of livestock diseases including trypanosomosis and tick-borne diseases such as East Coast fever (caused by *Theileria parva*) from multiple biological perspectives.

The facility"s team of experienced researchers in parasitology, livestock immunology, vector biology, and disease ecology offer tailored support for collaborative projects, fostering advancements in understanding vector borne disease systems. Furthermore, one of the facility’s aims is to provide high-quality vectors and associated pathogens for external research, promoting global partnerships in vector research. By providing opportunities for *in vivo* studies in cattle and mice, the RVRF aims to facilitate significant advancement in understanding vector-borne disease systems, and the development of novel control strategies. We invite researchers to collaborate and engage with the facility to drive forward the fight against vector borne diseases and their complex impacts.

PS223.I

Neuropeptide-growth interplay in juvenile liver fluke

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The liver fluke, *Fasciola hepatica*, places a significant disease burden on ruminant livestock worldwide and is a causative agent of fasciolosis / fascioliasis, a neglected tropical zoonosis. Notably, a single drug, triclabendazole (TCBZ), is efficacious in the treatment of acute fasciolosis caused by pathogenic juvenile fluke. Increasing reports of TCBZ resistance underscores the need for novel drug target identification. Given its role in a myriad of fundamental biological processes, the parasite nervous system has long been considered a source of potential novel drug targets. We employed *in situ* hybridisation to localise neuropeptides and selected processing enzymes, knockdown of which yielded significant growth and motility phenotypes. Notably, *Fh*NPF4 knockdown resulted in significantly enhanced growth. Single cell RNA sequencing analyses expose a range of neuropeptidergic neuronal cell clusters. Transcriptomic analyses of *Fh*NPF4-silenced juveniles demonstrate the upregulation of metabolic pathways and genes involved in the cell cycle. Functional genomics screening of putative neuropeptide GPCRs identified reduced growth phenotypes associated with a small cohort of *Fh*GPCRs. Taken together, these data highlight the diverse functions governed by neuropeptides in *F. hepatica* and underscore their dysregulation as an attractive therapeutic strategy.

PS226.I

ZKBS risk assessments of genetically modified parasites

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The Central Committee on Biological Safety (ZKBS) is a German honorary expert panel with a legal mandate providing opinions in the field of genetic engineering. The administrative office of the ZKBS is located at the Federal Office for Consumer Protection and Food Safety (BVL) in Berlin.

The main task of the ZKBS is to conduct risk assessments of microorganisms or to assign containment levels for genetic engineering operations and assessment of required safety measures in genetic engineering facilities. Donor and recipient micro-organisms that already have been assigned to a risk group are listed in the ZKBS organism database (https://zag.bvl.bund.de/). The database includes viruses, bacteria, archaea, parasites, fungi and other eukaryotic unicellular organisms. The administrative office of the ZKBS also provides databases on recipient strains for biological safety measures, oncogenes plasmid vectors and cell lines.

In Juli 2024, the ZKBS published a position statement on genetic engineering activities using parasites. The statement summarizes the risk groups of parasite-infected insect vectors as well as infectious and non-infectious parasite life stages. Furthermore, the ZKBS provides general position statements and specific non-public statements on parasites used in genetic engineering activities.

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PS229.I

Tracking *Angiostrongylus malaysiensis* and *cantonensis*: Food market and wild gastropod survey in Lao PDR

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Neuroangiostrongyliasis, a food-borne zoonotic disease, is caused by a metastrongylid nematode *Angiostrongylus cantonensis*, but possibly also by a closely related species, *Angiostrongylus malaysiensis*. In Southeast Asia, the two species co-occur in many regions. The morphological similarities of the two species and a similar life cycle including rats as definitive hosts and gastropods as intermediate hosts can lead to misidentification and bias in favor of *A. cantonensis* over *A. malaysiensis* in prevalence studies without employing proper methodology.

In the present study, we aimed to determine occurrence of *A. cantonensis* and/or *A. malaysiensis* DNA in animal products on Lao food markets and in terrestrial gastropods captured from peri-domestic environment. The sampled species were chosen based on their ability to harbour infective L3 of the parasites. Material obtained from wet markets included freshwater snails *Pomacea canaliculata, Pila ampullacea, Filopaludina filosa,* freshwater shrimp *Macrobrachium lanchesteri* and freshwater bivalve *Corbicula fluminea*. Terrestrial gastropods, such as *Lissachatina fulica, Valiguna siamensis* and *Cryptozona siamensis,* were collected from three locations: Vientiane, Vang Vieng and Luang Prabang.

In the first step, a highly sensitive AcanR3880 qPCR was used to detect samples positive for *A. cantonensis/malaysiensis* DNA. As a next step, a species-specific SYBR Green qPCR targeting the mitochondrial cytochrome b gene was used to distinguish between the two species.

Overall, 157 terrestrial wild-caught gastropods and 278 samples from markets were analyzed. Positive results were obtained from 25 wild-caught gastropods (1 *L. fulica,* 24 *V. siamensis)*, while all of the samples from the markets proved to be negative for *Angiostrongylus cantonensis/malaysiensis* DNA. Subsequent SYBR Green assay showed 20/25 samples positive for *Angiostrongylus malaysiensis* DNA, while the remaining 5 samples showed dubious results, most probably due to the low parasite DNA concentration within the gastropod tissue, pointing out the SYBR Green method limitation.

This study further confirmed presence of *A. malaysiensis* in gastropods in Laos and the importance of a slug *Valiguna siamensis* as an intermediate host in this area. For future studies in regions with overlapping distribution of the two parasite species we suggest increased attention on species classification for the correct evaluation of public health risk, especially for the evaluation of *A. malaysiensis* pathogenic potential for humans and animals.

PS232.I

Development of an IgE-based reporter system essay for diagnosis of cystic Echinococcosis in dogs

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Introduction: Echinococcus sp. is one of the 20 Neglected Tropical Diseases (NTDs) targeted by the WHO for control and eradication. Clinical diagnosis mostly based on the presence of eggs (or proglottids) in faecal samples cannot morphologically be distinguished from frequently co-endemic Taenia sp. eggs. Copro-PCR and Copro-ELISA have shown good results, but rely on handling of potentially hazardous biological materials. Therefore, cheaper and safer tests such as serology would be an advantage. However, current serological tests based on IgG detection are unreliable due to low specificity.

Aim: Despite the central role of IgE responses in most metazoan parasite infections, this antibody isotype has not been used for diagnosis of Echinococcosis. We intend to develop a highly sensitive and specific serological assay by "caninizing" rat basophil leukaemia (RBL)-derived IgE reporter cell lines for diagnosis of dog echinococcosis.

Materials & methods: Starting from an existing rat basophilic leukemia (RBL) reporter cell line (NPY-mRFP), which contains preformed red fluorescent proteins in granules, released upon activation, we developed three dog IgE reporter cell lines. Each cell line expressed a variant of the canine alpha chain of the high affinity IgE receptor (FcεRIα). The first construct encoded wildtype dog FcεRIα, the second expressed a chimeric dog/rat FcεRIα consisting of the extracellular FcεRIα dog sequence and the transmembrane/cytosolic rat sequence, and the third expressing a wild type dog FcεRIα chain in which three potential endoplasmic reticulum retention Lys signals have been changed to Ala. After several months of culture in the presence of a selective antibiotic, the cells were cloned by limited dilution using FACS. For functional testing, the cloned cells were sensitized with dog serum and polyclonal stimulation with an anti-dog IgE antibody.

Results: All three stable transfectants were put through clonal selection process. Several hundred clones were obtained, enriched and assessed for surface expression of dog FcεRIα by flow cytometry. Clones with the highest dog FcεRIα expression were expanded and tested for functionality. All three constructs sensitized with dog sera showed release of RFP reporter upon polyclonal stimulation with anti-dog-IgE, cross linking all surface-bound IgE. The released fluorescence was significantly increased compared to the parental cell line which did not express dog FcεRIα, while the signal to noise ratio between cell lines ranged between 2 and 3. Among the three cell lines the chimeric dog/rat cell line showed the highest RFP release.

Conclusion: We have successfully shown proof-of-principle that a rat-derived cell line is amenable to use with IgE contained in dog sera. However, the obtained signal to noise ratio of 2-3 is lower than expected and may result in a lack of sensitivity when used in combination with individual IgE binding antigens rather than the polyclonal stimulation used in this study.

PS235.I

Mechanisms of metronidazole resistance in *Trichomonas vaginalis*: Roles of efflux pumps and nitroreductases

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Introduction: *Trichomonas vaginalis* infections are a significant public health concern, linked to an increased risk of HIV transmission, prostate cancer, and pregnancy complications. Current treatments rely heavily on 5-nitroimidazoles, particularly metronidazole, but emerging drug resistance threatens treatment efficacy.

Objectives: This study aimed to assess the efficacy of metronidazole and tinidazole against metronidazole-resistant *T. vaginalis* strains, evaluate the potential of efflux pump inhibitors to reverse resistance, and investigate the roles of nitroreductases in metronidazole resistance.

Methods: Metronidazole and tinidazole were tested on both sensitive and resistant strains of *T. vaginalis*. Checkerboard assays evaluated potential synergy between nitroimidazoles and efflux pump inhibitors. Nitroreductase activity and ferric iron reduction assays were used to explore the functions of nitroreductases.

Results: Tinidazole exhibited greater efficacy against metronidazole-resistant strains, with lower minimal lethal concentration values. Efflux pump inhibitors, including zosuquidar, elacridar and cimetidine, did not significantly enhance metronidazole or tinidazole efficacy. While pyrimethamine showed limited activity, it failed to improve nitroimidazole efficacy in combination. Nitroreductase activity assays revealed no direct metronidazole-reducing function, but nitroreductase 8 demonstrated ferric iron reduction, suggesting a potential role in resistance mechanisms.

Conclusion: Tinidazole is a more effective alternative for treating metronidazole-resistant *T. vaginalis*. However, efflux pump inhibitors are not effective in reversing resistance. The discovery of nitroreductase 8"s ferric iron-reducing activity highlights a possible resistance pathway, underscoring the need for further studies to develop novel treatment strategies and enhance trichomoniasis management.

PS238.I

Exploring the 'dehydration hypothesis' of host behavioural manipulation by the nematomorph *Paragordius varius*

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Nematomorphs are parasitic worms of arthropods. To complete their lifecycle, they manipulate host behaviour to seek out and enter water. The mechanism of this manipulation is unknown, though light attraction, water attraction and erratic behaviour are all thought to play a role. Here we focus on the 'Dehydration Hypothesis', which hypothesises that the nematomorph is mimicking or inducing dehydration in the host to induce water seeking behaviour. We investigated if dehydration-like behaviour and proteomic signatures drive water attraction in crickets (*Acheta domesticus*) exposed to the nematomorph *Paragordius varius*.

As expected, crickets infected with a nematomorph were more likely to enter water than uninfected controls. Dehydrated, uninfected crickets were also more attracted to water than hydrated control crickets. However, dehydrated crickets preferred to submerge their heads in the water, whereas infected crickets fully entered it. This indicates a level of consciousness of the danger of water in the dehydrated group, which is presumably overridden by the parasite.

Quantitative mass spectrometry was carried out on cricket haemolymph to identify and compare proteomic signatures of infection and dehydration. Similarity in the signatures would suggest a similar molecular mechanism is involved between dehydration and nematomorph host manipulation. Twenty-seven out of 134 identified proteins were differentially abundant in infected compared with uninfected control crickets and include an increase in proteins related to energy storage, humoral immune and cellular defence, and allergens in infected cricket haemolymph, and proteins related to disease resistance, stress response, pathogen defence and egg proteins in uninfected haemolymph. Proteins of unknown function were identified which could have further functions in the manipulation by nematomorphs. Seventeen out of 388 identified proteins were differentially abundant in dehydrated crickets compared to hydrated control crickets, including an increase in proteins related to stress, DNA repair and cellular defence in dehydrated cricket haemolymph, and enzyme-related proteins in hydrated control crickets. There was no overlap of differentially abundant proteins between infected and dehydrated individuals, suggesting the molecular mechanism is different. However, the proteins had broadly similar functions, with an increase in abundance of energy storage, stress response and cellular defence related proteins, which may represent responses of the cricket to stress.

In summary, infected and dehydrated individuals are more attracted to water than their respective controls, though infected fully enter the water. Protein signatures of infection and dehydration in the haemolymph are different. These results suggest that dehydration may play a role in attracting infected crickets to water but cannot fully explain this behaviour.

PS241.I

Characterisation of antibody clearance from the surface of *Trypanosoma brucei*

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Previously, antigenic variation of variable surface glycoprotein (VSG) was thought to be the sole mechanism underpinning persistent survival of *Trypanosoma brucei*. However, recently it was found that persistent infection by African trypanosomes also relies on the efficient clearance of surface bound antibodies, as trypanosomes with compromised antibody clearance cannot survive in mice unless antibody synthesis is inhibited. In this study, the interactions between the four stages of antibody clearance were investigated; namely, hydrodynamic sorting of antibody-VSG complexes to the flagellar pocket, endocytosis, degradation of antibodies, and recycling of VSG to the surface. This study also investigates the proportion of IgM that can bind VSG on the surface of live cells. By incubating live cells with IgM *in vitro* and allowing them to clear any binding antibody, it has been shown that a large amount of non-binding antibody remains in culture. The remaining antibody has been shown to not aggregate live cells, indicating that a large amount of antibody produced by the host is ineffective against the live trypanosome.

PS244.I

Comparison of diagnostic methods for the detection of schistosomiasis in a clinical cohort – Key takeaways and future perspectives

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Schistosomiasis is a neglected tropical disease caused by the parasitic flatworms of the genus *Schistosoma*. It is the second most impactful parasitic disease after malaria, with over 250 million people infected, with substantial associated morbidity and mortality. The WHO roadmap for NTDs to 2030 targets schistosomiasis for elimination as a public health concern in all countries, key to achieving this target is developing novel diagnostics. Current diagnostic approaches are inadequate for mapping, monitoring treatment and post-treatment surveillance, with the goal being to develop sensitive point-of-care tests that aid in decision-making and mapping. To this end we have explored comparing in-house PCR testing to urine CAA and urine filtration microscopy on a clinical cohort; to evaluate the suitability of these diagnostic methods and guide future development.

The clinical cohort used for testing consisted of 379 participants in the DFG HelmVit study, which recruited pregnant women in Lambaréné, Gabon. Each participant was tested for *Schistosoma hematobium* infection using several methods including urine filtration microscopy, UCP-LF CAA, POC-CCA and finally PCR on cell-free DNA from plasma samples. We used statistical methods to evaluate the performance of each of these tests and evaluate the suitability of plasma PCR as a screening or diagnostic method. The PCR approach relied on the detection of the DraI repeat region of *S. hematobium* (or Sm1-7 of *S. mansoni*) whereas we also employed a crude DNA extraction method (Quantabio Extracta) to minimize sample input to only 20µl and keep equipment to a minimum. This yielded a test with a reduced sensitivity, though one that was still a good predictor of infection. Additionally, owing to the little sample input and high throughput, as well as potential adaptation to POC settings, such a test could offer a useful screening tool after some development. An additional benefit of such a molecular test, is the ability to detect pre-patent infection, which we have so far validated using a cohort of *S. mansoni*-infected mice. However, in general we found several discrepancies between the different test methods, suggesting the need for more sensitive and specific diagnostics approaches.

Such studies are essential for defining the current gaps and limitations to elimination of schistosomiasis. In this study, the need for more sensitive and specific, high-throughput molecular testing was highlighted. To this end, further research and development must be dedicated to take advantage of technological developments to deliver such POC tests. In the case of schistosomiasis, molecular tests to diagnose female genital schistosomiasis (FGS), detect drug resistance to praziquantel and for xeno-monitoring and surveillance should also be of priority.

PS250.I

Impact of two *E. histolytica* clones with different pathogenic properties on intestinal epithelial cell barriers

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Entamoeba histolytica is a protozoan parasite which causes the disease amoebiasis in humans. In 90 % of infections, the parasite persists in the intestines without causing symptoms, with the amoebae becoming invasive in 10 % of cases. *E. histolytica* can reach other organs, mainly the liver, through the bloodstream and form abscesses there (1 % of cases). If left untreated, these abscesses can lead to the death of the host. The factors that trigger the invasive phenotype of *E. histolytica* are only partially understood. To investigate processes behind the tissue invasion, we are using intestinal epithelial cell barrier models composed of Caco-2 cells or derived from human colon spheroids (colonospheres).

Colonosphere-derived cells or Caco-2 cells were seeded onto a filter membrane in a transwell system and grown in differentiation medium until a tight cell monolayer has formed.

Intestinal epithelial cell monolayers (iECMs) were then incubated with two *E. histolytica* clones differing in virulence (non-pathogenic A1np clone and pathogenic B2p clone), while sharing the same genetic background. The impact on barrier integrity during co-incubation was analyzed by measurement of the transepithelial electrical resistance (TEER) and immunofluorescence assay. Dual RNA sequencing (RNAseq) was used to investigate transcriptomic changes on the host and parasite side. TEER measurements showed differences in the ability to disrupt iECMs between A1np and B2p trophozoites. RNAseq data revealed a different expression profile of the intestinal cells after contact with A1np and B2p trophozoites.

Overall, the results suggest, that the two amoeba clones A1np and B2p stimulate epithelial cells of the human colon differently. Further analysis of the sequencing data could provide hints for the detection of new pathogenicity factors involved in intestinal tissue invasion of *E. histolytica*.

PS253.I

Análisis de prevalencia y riesgo de geohelmintos humanos en comunidades rurales de Ilalo, Ecuador

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Introducción: Las helmintiasis transmitidas por el suelo (STH) se encuentran entre las infecciones parasitarias más comunes a nivel mundial y afectan a poblaciones empobrecidas con acceso limitado a servicios sanitarios y agua potable. Los principales agentes son *Ascaris lumbricoides, Trichuris trichiura* y anquilostomas [1, 2]. En Ecuador, se ha informado de una prevalencia de STH de aproximadamente el 40%, con tasas más altas en comunidades rurales y marginadas [3]. La pandemia de COVID-19 alteró las condiciones de vida y los recursos sanitarios, lo que podría afectar a la prevalencia de STH y los factores de riesgo asociados [4].

Objetivos: Este estudio tuvo como objetivo evaluar la prevalencia pospandémica de STH y los factores de riesgo asociados en las comunidades rurales de Ilaló, Pichincha, Ecuador, proporcionando información para intervenciones de salud pública específicas.

Materiales y métodos: Se realizó un estudio transversal de junio de 2021 a mayo de 2022 en cinco comunidades rurales de Ilaló. Se seleccionaron 320 participantes mediante un muestreo aleatorio estratificado de una población de 2.432 individuos. Las muestras de heces se analizaron mediante las técnicas de Kato-Katz, McMaster y Mini-FLOTAC [5, 6]. Los datos epidemiológicos y sociodemográficos se recogieron mediante cuestionarios estandarizados. Los análisis bivariados y multivariados identificaron factores de riesgo y protección, con una significación estadística establecida en $p < 0,05$.

Resultados: La prevalencia general de STH fue de 22,81% (73/320). *Ascaris lumbricoides* fue el parásito predominante (74,73%), seguido de *Trichuris trichiura* (13,19%) y anquilostomas (12,09%) [7]. Se identificaron infecciones mixtas en 19,18% de los casos positivos. Se observó una prevalencia más alta entre personas de 17 a 65 años (56,16%) y en hogares dedicados a la cría de cerdos (OR: 4,16; IC del 95%: 2,34-7,42) y al cultivo de hortalizas (OR: 11,66; IC del 95%: 4,32-41,08) [8]. Los factores protectores incluyeron el acceso al agua potable (75,31%) y las prácticas de lavado de manos (OR: 0,15; IC del 95%: 0,037-0,53) [9].

Conclusion: The study reveals a reduction in STH prevalence compared to pre-pandemic estimates, likely influenced by enhanced hygiene practices during COVID-19. However, the persistence of significant risk factors, such as inadequate sanitation and agricultural exposure, underscores the need for integrated public health interventions. Promoting sanitation infrastructure, community education, and access to healthcare are critical to sustaining and further reducing STH transmission.

PS256.I

Annotation and comparative analysis of animal trypanosome kinomes defines a core kinome and reveals that animal trypanosome kinomes are smaller than that of their free-living ancestor *Bodo saltans*

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Protein kinases are key signalling molecules with significant potential as drug targets. While the kinomes of the "TriTryps" (*Trypanosoma brucei*, *Leishmania major* and *Trypanosoma cruzi*) were annotated 20 years ago[1], multiple additional kinetoplastid genomes have since become available at TriTrypDB[2]. Here, we sought to annotate the kinomes of the animal trypanosomatids *T. congolense, T. evansi, T. equiperdum, T. grayi, T. melophagium, T. theileri, T. vivax, Blechomonas ayalai* and *Paratrypanosoma confusum,* and to compare them with those of the TriTryps with the aims of defining a core trypanosome kinome as well as identifying species-specific kinases that might reflect unique aspects of biology in each organism. Putative protein kinases were identified through their orthology to the previously annotated kinases of the TriTryps and through PFAM and Interpro protein kinase domain searches. Protein kinases were also identified in the free-living bodonid, *Bodo saltans*, to provide an outgroup for subsequent phylogenetic analyses. Multiple sequence alignments of orthologous protein kinases were performed to determine if key motifs required for kinase activity were present. Where orthologous groups of kinases did not contain annotated representatives from the TriTryps, BLAST and Kinomer[3] searches were performed to assign the likely kinase class, with this being confirmed through the generation of phylogenetic trees of their kinase domains. Results to date indicate that the kinome shared by all kinetoplastid species analysed comprises ~80 protein kinases. Further, there appear to be very few species-specific kinases encoded within most of the animal trypanosome genomes, while *Bodo saltans* encodes dozens of protein kinases for which no orthologue is present in any of the sequenced Euglenozoa species available at TriTrypDB, indicating that these kinases have been lost in animal-infective species.

[1] Parsons *et al*., 2005 BMC Genomics 6:127

[2] Alvarez-Jarreta *et al.*, 2024 Nucleic Acids Research 52:D808–D816

[3] Martin *et al.,* 2008 Nucleic Acids Research 37:D244-D250

PS259.I

New benzhydroxamic acid derivates as a potential antinematodal drugs

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Parasitic nematodes are the most prevalent group of helminth parasites affecting both humans and livestock worldwide. Several classes of anthelmintics are currently used for the chemical control of nematodes; however, the global emergence of resistant and multiresistant strains in recent years calls for intensified drug development. In this study, we explore the potential of newly designed and synthesized benzhydroxamic acid derivatives (OMKs) as novel drugs. Using the barber's pole worm (*Haemonchus contortus*) as a model organism, we compared the nematocidal potential of several OMKs with that of 'traditional' anthelmintics, such as levamisole. Larvae and adults of drug-susceptible and drug-resistant strains of *H. contortus* were incubated with OMKs, and their viability was measured using multiple methods. Demonstrating a significant nematocidal effect, OMK211 was selected as the most promising derivative and subjected to further investigation. It was tested for mammalian cytotoxicity *in vivo* and *in vitro*, yielding negative results. Finally, thermal proteome profiling analysis was successfully employed to identify the molecular target in *H. contortus*. These findings reveal the promising potential of OMKs as a future novel class of antinematodics

PS262.I

Plasmodium berghei oocysts possess fatty acid synthesis and scavenging routes

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Malaria parasites carry out fatty acid synthesis (FAS) in their apicoplast organelle via a bacterially related (type II) enzymatic pathway. In the vertebrate host, exoerythrocytic *Plasmodium* stages rely on FAS, whereas intraerythrocytic stages depend on scavenging FA from their environment. In the mosquito, *P. falciparum* oocysts express and rely on FAS enzymes for sporozoite formation, but *P. yoelii* oocysts do not express, nor depend on, FAS enzymes and thus rely on FA scavenging to support sporogony. In *P. berghei*, FAS enzymes are similarly expendable for sporogony, indicating it conforms to the *P. yoelii* scenario. We show here that *P. berghei*, unexpectedly, expresses FAS enzymes throughout oocyst development. These findings indicate that *P. berghei* can employ FAS alongside FA scavenging to maximise sporogony and transmission, and is more similar to *P. falciparum* than previously assumed with respect to FA acquisition by the oocyst. The ability of oocysts to switch between FAS and scavenging could be an important factor in the non-competitive relationship of resource exploitation between *Plasmodium* parasites and their mosquito vectors, which shapes parasite virulence both in the insect and vertebrate.

Fig. 1

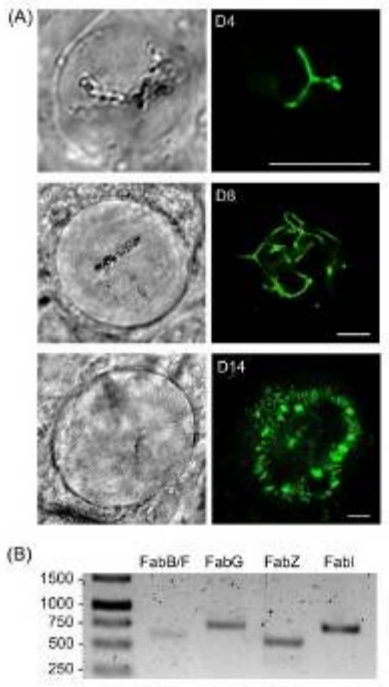

Figure 3. Expression of FAS enzymes in *P. berghei* oocysts. (**A**) Confocal GFP fluorescence and brightfield images of unsporulated (4 and 8 days post-infection) and sporulating (14 days post-infection) oocysts of parasite line FabG/GFP (clone 1). Scale bar 5μm. (**B**) Reverse transcription PCR of unsporulated wildtype oocyst samples with primers specific for mRNA of genes encoding FabB/F, FabG, FabZ and FabI, amplifying 639, 769, 562 and 750bp fragments, respectively. A full-size image of the DNA agarose gel is available in the Supplementary Information.

Fig. 2

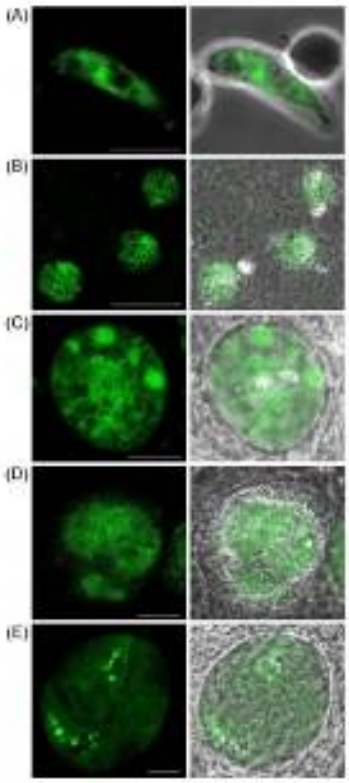

Figure 4. Expression of KLH in *P. berghei*. Confocal GFP fluorescence and brightfield images of parasite line KLH/GFP (clone 1), showing (A) a mature ookinete, (B) group of three young oocysts at 4 days post infection, (C) an unsporulated oocyst at 8 days post infection, (D) a sporulating oocyst at 14 days post infection, (E) a fully sporulated oocyst at 14 days post infection. Scale bar 10 μm.

PS265.I

Drug repurposing: The benzimidazole anthelmintic flubendazole as a potential treatment for pancreatic cancer

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Flubendazole (FLU), a benzimidazole anthelmintic, that acts through inhibition of polymerization of β-tubulins in parasites, has demonstrated promising antitumor activity in various cancers, primarily through the inhibition of microtubule polymerization. In this study, we investigated the therapeutic potential of FLU in pancreatic ductal adenocarcinoma (PDAC) cells, a highly aggressive malignancy with a poor prognosis, as only 5-10% of patients survive beyond five years post-diagnosis. We assessed the effects of FLU on PDAC cell viability, proliferation, and apoptosis. Our results show that FLU significantly inhibited PDAC cell proliferation and induced apoptosis in a dose-dependent manner, suggesting its potential as a novel therapeutic agent. These findings highlight the need for further investigation into FLU"s role in PDAC treatment and its potential to contribute to the development of more effective therapeutic strategies for this challenging cancer.

Stabilized PDAC cell lines (BxPC-3, PANC-1, MIA-PaCa-2, Patu-8902) were used to assess FLU"s efficacy. Cell viability was determined using the WST-1 assay for 2D cultures and the CellTiter-Glo® 3D assay for 3D cultures derived from the BxPC-3 line and prepared with Geltrex. Morphological changes were observed using phase-contrast and fluorescence microscopy. Significant differences in gene and protein expression were identified across the tested cell lines via qPCR and western blotting.

Our findings demonstrate that FLU exerts potent antitumor effects on PDAC cell lines by inhibiting microtubules, highlighting its potential as a promising therapeutic candidate for further investigation in both, 2D and 3D cancer models.

PS268.I

Measuring the impact of different treatment regimes on the genomic evolution of anthelmintic resistance

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Anthelmintic resistance in sheep is a significant concern in the UK since it poses serious challenges to sheep health and farm productivity. Sheep are known to be infected by a wide rage of parasitic helminths, however, *Teladorsagia circumcincta* is the most prevalent and the most resistant parasitic gastrointestinal nematode in sheep in the UK. It causes parasitic gastroenteritis that results in huge production losses to farmers in terms of milk, meat & wool as well as compromising animal welfare. The control of *Teladorsagia circumcincta* infections in sheep largely relies on the use of anthelmintic drugs. Currently, there are five broad-spectrum anthelmintics classes used in the UK to control parasitic helminths and ivermectin from macrocyclic lactones class is the most widely used. However, *Teladorsagia circumcincta* has developed resistance to 4 out of the 5 available anthelmintic classes including ivermectin. The effects of anthelmintic resistance are so dire that some sheep farms in the UK, Australia, South Africa and even New Zealand have been shut down after they were unable to control multiple anthelmintic drug resistance. It is estimated that UK spends over £42 million yearly to control these parasitic worms in sheep sector alone, out of which £3 million is attributed to anthelmintic resistance. My PhD project therefore aims to provide a more detailed & quantitative understanding of how different treatment regimes influence the evolution of candidate genetic markers involved in ivermectin resistance in *Teladorsagia circumcincta*. The plan is to leverage on modern genomic tools such as whole genome sequencing and targeted genotyping to estimate the relative strength of selection acting on candidate ivermectin resistance loci under different treatment regimes.

PS271.I

In vitro anthelmintic activity of methanolic extracts of *Allium sativum*, *Zingiber officinale* and *Ruta graveolens* against *Haemonchus contortus*

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Background: Helminth parasitism is among the main challenges in livestock production. However, anthelmintic resistance becoming an important constraint an the effort to minimize the impacts of the problem. In vitro experimental trail were conducted to investigate the anthelmintic activities of methanolic extracts of Allium sativum (Garlic), Zingiber officinale (Ginger) and Ruta graveolens (Rue) against Haemonchus contortus.

Methodology: The plants were purchased from local markets in Bishoftu town (Central Ethiopia). Methanol extract of the selected medicinal plants were prepared by maceration technique. Serial dilutions (100, 50, 25 and 12.5mg/ml) of the extracts were prepared and evaluated for in vitro anthelmintic effects using the larval motility and egg hatch inhibition tests. The commercial drugs albendazole at 5% and ivermectin at 0.5% concentrations were used as positive control while DMSO was used as negative controls. Dead worms were counted at an interval of three hours, for a total period of nine hours for adults and at an interval of six hours for 48 hours for the larvae. For the egg hatch inhibition test, the wells containing about 100eggs/ml were exposed to various concentration of the plant extracts (50, 25, 12.5 and 6.25mg/ml) and incubated at 27°C for 48 hours and evaluated based on the characteristics such as dead and hatched eggs.

Results: On the 3rd hour of observation, all higher concentrations (>25mg/ml) of extract of ginger and rue have killed 100% of the adult worms. A similar level of efficacy for garlic was observed on the 9th hour of exposure. Nine hours after treatment, only 20% of worms incubated with DMSO alone were found dead and the difference between treatment and control groups was significant ($P<0.001$). The effect of methanolic extract of the three plant species against adult H. contortus worms at all extract concentrations was equivalent to the efficacy of 5% Albendazole ($P>0.05$). Extract concentrations lower than 50mg/ml of all the three plants had significantly lower effects on H. contortus infective larvae compared to Ivermectin which has killed 100% of the larvae. At higher concentrations, however, almost all extracts had greater than 80% efficacy and the difference was significant between treatment and both positive and negative control wells ($P <0.005$). Extract of Ruta graveolens had inhibited less than 60% of nematode egg hatchability at all concentrations tested whereas ginger extract had shown a concentration dependent increasing efficacy which goes beyond the effect of Albendazole although the difference was not statistically significant ($P>0.05$).

Conclusion: The present study revealed the potential nematocidal and ovicidal effects of extracts from the three plant species. Further study is required to fractionate active compounds, support the finding by in vivo test and evaluate any toxic effect that may hamper the use of the products as potential anthelmintics.

Keywords Allium sativum; anthelmintic; extract; in vitro; Ruta graveolens; Zingiber officinale

PS280.I

Pyrethroid-resistant A*nopheles coluzzii* from central Côte d'Ivoire are susceptible to clothianidin

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Introduction The increasing insecticide resistance in the key malaria vector, *Anopheles gambiae* s.l., has been reported across Côte d"Ivoire, potentially compromising the current ITN-based control strategy. To assess the feasibility of supplementing control efforts with IRS using an alternative insecticide in areas with high malaria prevalence, this study examined the intensity and molecular mechanisms of insecticide resistance in wild *An. gambiae* s.l. populations with a focus on pyrethroids and the neonicotinoid clothianidin in Sakassou, Central Côte d"Ivoire.

Methods: Using the World Health Organization insecticide susceptibility test, we assessed the intensity of insecticide resistance in the local *Anopheles* population from Sakassou against the pyrethroids alpha-cypermethrin, deltamethrin and permethrin, as well as the neonicotinoid clothianidin and the organophosphate pirimiphos-methyl. The tested mosquitoes were two- to five-day-old females, reared from larvae and morphologically identified as *An. gambiae* s.l. We ran subsequent diagnostic PCR to further determine the sibling species. To characterise the mechanisms underlying insecticide resistance, we conducted synergist assays using piperonyl butoxide in combination with the pyrethroids. Additionally, we molecularly characterised resistance mechanisms through PCR diagnostics targeting the knockdown loci L995F, L995S and the insensitive acetylcholinesterase G280S locus. We also compared expression levels of the cytochrome P450 monooxygenases (P450s) CYP6M2, CYP6P3, CYP6P4 and CYP6P5 between field-collected mosquitoes and those from a laboratory insecticide-susceptible colony.

Results: Diagnostic PCR revealed that the field-sampled *An. gambiae* s.l. specimens were all *An. coluzzii*. *Anopheles coluzzii* showed resistance to all three pyrethroids, even at 10 times the discriminant concentrations, yet they remained susceptible to clothianidin. Pre-exposure to PBO increased mortality rates to pyrethroids but did not fully restore susceptibility. We found CYP6M2 overexpressed in the field-sampled *An. coluzzii* compared to the susceptible laboratory colony. Together, these results suggest the partial involvement of metabolic resistance mechanism. In addition to metabolic resistance, we detected all three target-site resistance alleles.

Conclusion: *Anopheles coluzzii* from Sakassou shows strong resistance to pyrethroids but remains susceptible to clothianidin. Therefore, clothianidin-based IRS may serve as an effective complementary strategy for malaria control in Sakassou.

PS283.I

A paternal lactate dehydrogenase critically enhances male gametogenesis and malaria transmission

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Malaria blood stage parasite development relies on glycolysis to generate ATP, which requires pyruvate to lactate conversion by an essential lactate dehydrogenase enzyme (LDH1). Conversely, parasites developing in the mosquito employ mitochondrial chemiosmosis for ATP production. The source of ATP during transition from vertebrate to insect is less clear; gametes form in the mosquito midgut lumen within minutes of gametocyte ingestion, and while female gametes possess a mitochondrion, this organelle is absent from male gametes (microgametes). Here, we investigate a second LDH enzyme (LDH2) found exclusively in male gametocytes and microgametes. Knockout of *Plasmodium berghei* LDH2 expression reduces the number and size of exflagellation centres and radically diminishes oocyst development in *Anopheles stephensi* mosquitoes. Our data indicate that LDH2 supplements LDH1 activity to facilitate the cytokinesis step of male gametogenesis, while LDH1 alone is sufficient for motility of free-swimming microgametes. Our results confirm a key role for glycolytic ATP production in microgamete formation and function and identify LDH activity as a new malaria transmission-blocking drug target.

PS286.I

Establishment and validation of red fox (*Vulpes vulpes*) and domestic dog (*Canis lupus familiaris*) airway epithelial cell cultures at the air-liquid-interface as a platform to investigate host-parasite interactions

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The airway epithelium serves as a critical barrier against infectious pathogens and toxins while playing a key role in modulating immune responses in the upper respiratory tract. This is particularly important in red foxes (*Vulpes vulpes*), which are known reservoirs for zoonotic pathogens such as the fox tapeworm (*Echinococcus multilocularis*). In this study, we developed, established, and validated an air-liquid interface (ALI) model of the respiratory tract using primary airway epithelial cells isolated from the tracheas and main bronchi of hunted red foxes. For comparison, we applied the same methodology to develop a similar ALI model for domestic dogs (*Canis lupus familiaris*), enabling a cross-species evaluation of airway epithelial properties. The resulting ALI cultures for both species exhibited structurally differentiated, pseudostratified epithelia characterized by ciliated cells, mucus secretion, and tight junctions, as confirmed by histological and immunohistochemical analysis. Functional assessments, including paracellular permeability assays and transepithelial electrical resistance measurements, demonstrated the formation of tight epithelial barriers. To evaluate the models' utility for studying innate immune responses against respiratory parasites, cultures were exposed to lipopolysaccharide, phorbol-12-myristate-13-acetate, ionomycin, and nematode antigens. Quantitative PCR analysis revealed significant changes in the expression of pro-inflammatory cytokines, including TNF and IL-33 when ALI cultures were exposed to lipopolysaccharide of *E. coli*, phorbol-12-myristat-13-acetat/ionomycin, or *Angiostrongylus vasorum* first-stage larval antigen.

By comparing red fox and dog ALI models, this study provides novel insights into the respiratory biology of a canid wildlife reservoir and a corresponding domesticated species evidencing potential differences that may develop during co-evolution between the animal hosts and the parasite. These models represent an important advancement for studying innate immune responses and therefore host pathogen interactions and disease transmission and can also be applied for investigations on zoonotic diseases.

PS289.I

Prevalence of scabies and community knowledge, attitudes, and practices on the disease in the middle belt of Ghana

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Background: The development of a global scabies strategy has been hampered by inadequate epidemiological data from most countries including Ghana. This study investigated the prevalence of scabies infection and community knowledge, attitudes, and practices on the disease in the middle belt of the Bono Region of Ghana.

Methods: A cross-sectional study was conducted in five communities in the Wenchi and Tain District, Bono Region in Ghana. Written consent was obtained from participants, with young children's consent obtained from parents or legal guardians. A standardized body examination of the entire regions of the skin for scabies manifestations such as burrows, vesicles, papules, and body distribution of rash was done. Microscopy was done on skin scrapings to determine the prevalence rate by identification of mites, eggs, or fecal pellets in scraped samples. A questionnaire was then administered to assess participants knowledge, attitudes, and practices on the disease.

Results: A total of 164 people participated in the study [median age: 27.9, range (4–95) years]; 101(61.6%) were females. The overall prevalence of scabies was 14.6 % (15/103, 95% CI: 9.0-22.7) with the Abekwai community having the highest prevalence of 21.9% (7/32, 95% CI:11.0-38.8). Scabies was more prevalent in participants aged 31-40 years (22.2%, 2/9; 95% CI:6.3-54.7) and lowest in those aged above 40 years (10.8%, 4/37; 95% CI:4.3-24.7). The infection was more prevalent in males (28.5%, 18/63; 95% CI: 18.9-40.7) than in females (25.7%, 26/101; 95% CI: 18.2-35.1). Interestingly, only half of the participants (54.3%) had good level of knowledge, 40.6% had positive attitudes and 58.7% had good practice towards yaws and its control in the study communities. Being >30 years old and having a higher level of knowledge were significantly associated with higher odds of increased risk perception of the disease.

Conclusion: This study revealed a significant prevalence of scabies in the study communities in the Wenchi and Tain Districts in the middle belt of Ghana. Unfortunately, community dwellers had poor levels of knowledge, attitudes and practices concerning the disease. This highlights the need for intensive and targeted educational and behavioral change campaigns by the National Yaws Eradication Programme in Ghana to remedy the situation. There is a further need for research to assess the scope of this challenge in other communities in Ghana to help inform programmatic decisions.

Keywords: Scabies, prevalence, Ghana, Wenchi, endemicity, scrapping, perceptions, neglected tropical disease.

PS292.I

Molecular Detection of Pathogens in Ectoparasites Collected from Companion Animals in Sylhet City Corporation (SCC) Bangladesh

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Introduction: Vector-borne pathogens (VBPs) represent a significant threat to both animal and human health, with ticks and fleas serving as primary vectors for these pathogens. In Bangladesh, the increasing population of free-roaming dogs and cats poses a potential public health risk. This study aimed to investigate the diversity of tick and flea species infesting dogs and cats in Sylhet, Bangladesh, and to identify the associated VBPs through morpho-molecular techniques.

Methods: A total of 112 dogs and 48 cats were examined for tick and flea infestations in Sylhet. Ticks and fleas were collected and identified morphologically. DNA was extracted from the ectoparasites, and polymerase chain reaction (PCR) was performed using gene-specific primers to amplify VBPs. The socio demographic information collected from questionnaires was analyzed using the R software to identify any significant associations with ectoparasite infestations. Results: Among the dogs, 46 individuals (41.1%) were infested with ticks, 19 (16.9%) had flea infestations, and 12 (10.7%) were co-infected with both ticks and fleas. In cats, 9 individuals (18.7%) had ticks, 14 (29.2%) were infested with fleas, and 3 (6.2%) were co-infected with both. Tick species identified in dogs included Rhipicephalus sanguineus, Ixodes ricinus, and Haemaphysalis longicornis, while flea infestations were primarily caused by Ctenocephalides canis and Ctenocephalides orientis. In cats, R. sanguineus was identified in 7 cases, and I. ricinus in 3 cases. Flea infestations in cats were predominantly due to Ctenocephalides felis, with fewer instances of C. orientis and Xenopsylla cheopis. Among dogs, Babesia gibsoni was the most prevalent pathogen, followed by Hepatozoon canis, Anaplasma platys, Ehrlichia canis, Babesia canis, and Rickettsia felis. In cats, Rickettsia felis was the predominant pathogen with single detections of Rickettsia massiliae, Rickettsia conorii, and Bartonella elizabethae. Sequence analysis of B. gibsoni from Sylhet showed high genetic similarity to sequences from Assam and Siliguri, India, while B. canis sequences from Sylhet were also closely related to those from India. Similarly, Hepatozoon canis from ticks in Sylhet showed significant genetic similarities with sequences from India, confirming the presence of shared genetic strains across regional boundaries.

Conclusion: This study provides valuable baseline data on the distribution of ectoparasites and VBPs in dogs and cats in Sylhet, Bangladesh. The detection of a variety of pathogens highlights the zoonotic potential of these ectoparasites, posing a serious public health concern. The molecular characterization of Babesia and Hepatozoon species indicates a high degree of genetic similarity between the Sylhet isolates and those from neighboring regions in India, emphasizing the need for integrated cross-border control efforts. These findings underline the necessity for enhanced surveillance and the development of coordinated control measures to mitigate the risks of vector-borne diseases in both animals and humans in Bangladesh and the surrounding regions.

PS002.II

First mentions of torymus (Hymenoptera:Chalcidoidea) of the bedeguaris group in oaks gall wasps in America

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We reported here the two new species of Torymus (Hymenoptera: Torymidae) belonging to Torymus bedeguaris species group. Both species emerged from oak galls wasps (Hymenoptera: Cynipidae: Cynipini) in Mexico. In North America, this species group has been mainly reported either from Cecidomyiidae galls (Diptera) on numerous plants, or from Tephritidae galls (Diptera), Psyllidae galls (Hemiptera: Sternorrhyncha) and cynipid galls on Rosa and Rubus so far. The two new species (not formally described yet) correspond to the first records of Torymus from the Torymus bedeguaris species group in oak gall wasps in North America. We illustrate and discuss here main morphological characters that are used for differentiation of these new species from the other species of the Torymus bedeguaris species group. Biology of both species is discussed.

PS005.II

The European researchers' night: An example of community engagement in controlling vector-borne diseases

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The European Researchers" Night (ERN) is the largest science outreach event in Europe. The ERN brings research and researchers closer to the public, promotes research projects across Europe and beyond, increases the interest of young people in science and research careers and shows how researchers" work affects our daily lives. On September 27, 2024, the University of Parma participated in the ERN as part of the "heaL thE plAnet"s Future" (LEAF) project, with the involvement of numerous Italian universities and research institutions and funded by the HORIZON-MSCA-Citizens-2022. Our contribution to the event was an interactive seminar titled *Mosquitoes and the Monsters Inside Them*, which was divided into three parts. The first part provided an introduction on how to distinguish mosquitoes from other insects (*dipteri nematoceri*), highlighting their main anatomical features and briefly touching upon mosquito ecology. This was followed by an in-depth exploration of the morphological identification of the most common mosquito species in Italy. The second part focused on the potential health risks associated with various pathogens transmitted by vectors (mosquito bites), with particular attention to the *Dirofilaria* spp. parasite. It also provided guidance on how to protect oneself and pets from mosquito bites, prevent ecological niches, and control mosquitoes during their larval and adult stages. Finally, the third interactive part consisted of quizzes, a coloring book with take-home messages, direct observation of *Dirofilaria* spp. adult worms and mosquitoes through a stereomicroscope.By providing basic knowledge and practical tips for prevention, this initiative led to empower citizens to protect themselves and their communities from mosquito-borne diseases, allowed the citizen scientist to have opportunities to create spaces to discuss, to communicate and build trust through interaction. These forms of citizen science communication and engagement need openness from all involved, the ability to accommodate the roles that all partners involved in a citizen science activity take, and commitment to the responsibilities and tasks that come with these roles (Hecker & Taddicken, 2022; Salmon *et al*., 2021). At the same time, acting together in citizen science projects allows researchers to rethink who they are in a scientific endeavor; not only what citizen scientists might be able to learn (Bela *et al*., 2016; Pandya & Dibner, 2018) but also what we can and have to learn.

Hecker & Taddicken, 2022 *JCOM 21*(01), A07

Salmon *et al*., 2021 *Diversity* 13(7), 309

Bela *et al*., 2016 *Conservation Biology* 30(5), 990-999

Pandya & Dibner, 2018 doi:10.17226/25183

PS008.II

Molecular detection of the infectivity status of *An. gambiae* s.l in Osun State, Nigeria

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The present study reports the infectivity of *An. gambiae* s.l with sporozoite of *Plasmodium. falciparum*.

Adult mosquitoes were caught quarterly in three communities across the state between 1800hr – 0600hr using the centre for disease control light trap (CDC) and 0600hr – 0700hr for pyrethrum spray catch (PSC) using protocol by the WHO and identified using morphological keys. Molecular analysis for sibling species identification was conducted using polymerase chain reaction (PCR).

The CDC light trap had a total of ninety (90) catches while the PSC had a relatively low number (1) of catch.

A total of 4 samples were positive for *P. falciparum.* Ido-Osun 2 (50%) recorded the highest infectivity status as compared to Inisa 1(50%) and Ife 1 (50%). Infectivity was found in the first, second and third quarters respectively. The outdoor catch had the highest number of sporozoites detected across the study areas as compared to the indoor catch ($p = 0.59$; $P > 0.05$). Similarly, 1 (33.3%) sporozoite was detected in the outdoor catches across all the study areas. The only sporozoite detected indoor was found at Ido-Osun. No sample was positive for *P. vivax* sporozoite across the study areas.

The present study therefore suggests the possibility of an ongoing transmission of falciparum malaria in the state. Therefore, the need to devise proactive control and preventive measures targeted at containing the transmission.

PS011.II

Prevalence of neglected waterborne parasites in sampled water from Lahore and Faisalabad, Pakistan

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Background: Having a reliable water source is essential to reduce the water-borne illnesses among population. Water borne Parasites are comparatively neglected by health and monitoring bodies. This study aimed to investigate the medically important water borne parasites in sampled water in selected areas.

Materials and methods: Total 420 samples were collected from different sources (river, canal, borehole and filter), 210 samples from each city between the months of April 2024 and July 2024. Different developmental stages of both protozoan and helminthic parasites has been isolated and examined from all the sampled water sources by filtration and centrifugation, then processed by using different parasitological methods.

Results: Variety of parasites were isolated from water samples. The highest prevalence of *Strongyloides stercoralis/* hookworms (3.33%) and *Entameoba histolytica* (2.38%) were observed, while prevalence of *cryptosporidium parvum* 2.86% and *Ascaris lumbricoides* and *Entameoba histolytica* of 2.38% were also found. Recreational water has highest prevalence of 45.71%, followed by borehole 30% and least parasitic contamination has been detected in filtered water 6.42%. Comparison of prevalence among borehole and recreational water samples showed statistically significant difference ($p<0.05$). Highest prevalence of waterborne parasites were observed in the month of April 47.9% followed by May 37%, June 14.7% and July 1.2%.

Conclusion: Parasitic contamination was found in all water sources, while contamination was high in recreational water it should not be used for humans and animal activities. An advanced improvement in investigation and prevention of parasitic infections are required for public and animal health in developing countries. A safe water supply is vital for the existence of living organisms.

Keywords: Waterborne parasites, contamination, recreational water.

Fig. 1

Table : Prevalence (%) of Waterborne Parasites from different areas of Lahore and Faisalabad.

Parasites	Location								
	Lahore				**Faisalabad**				
	Borehole water (n=70)	Filtered water (n=70)	Recreational water (n=70)	Total (n=210)	Borehole water (n=70)	Filtered water (n=70)	Recreational water (n=70)	Total (n=210)	Total (n=420)
Ancylostoma duodenale	1 (1.43%)	0 (0%)	1 (1.43%)	2 (0.95%)	2 (2.86%)	0 (0%)	1 (1.43%)	3 (1.43%)	5 (1.19%)
Ascaris lumbricoides	3 (4.28%)	0 (0%)	2 (2.86%)	5 (2.38%)	3 (4.28%)	0 (0%)	2 (2.86%)	5 (2.38%)	10 (2.38%)
Balantidium coli	2 (2.86%)	0 (0%)	2 (2.86%)	4 (1.9%)	0 (0%)	1 (1.43%)	2 (2.86%)	3 (1.43%)	7 (1.67%)
Blastocytosis hominis	0 (0%)	0 (0%)	3 (4.28%)	3 (1.43%)	1 (1.43%)	0 (0%)	1 (1.43%)	2 (0.95%)	5 (1.19%)
Clonorchis sinensis	0 (0%)	0 (0%)	1 (1.43%)	1 (0.48%)	0 (0%)	0 (0%)	1 (1.43%)	1 (0.48%)	2 (0.48%)
Cryptosporodium parvum	1 (1.43%)	0 (0%)	3 (4.28%)	4 (1.9%)	1 (1.43%)	2 (2.86%)	5 (7.14%)	8 (3.81%)	12 (2.86%)
Cystoisopora belli	1 (1.43%)	0 (0%)	2 (1.43%)	3 (1.43%)	1 (1.43%)	2 (2.86%)	2 (2.86%)	5 (3.81%)	8 (1.90%)
Dracunculus medinensis	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (1.43%)	1 (0.48%)	1 (0.24%)
Entamoeba coli	2 (2.86%)	0 (0%)	2 (2.86%)	4 (1.9%)	1 (1.43%)	0 (0%)	1 (1.43%)	2 (0.95%)	6 (1.43%)
Entamoeba histolytica	0 (0%)	2 (2.86%)	1 (1.43%)	3 (1.43%)	5 (7.14%)	0 (0%)	2 (2.86%)	7 (3.33%)	10 (2.38%)
Enterobius vermicularis	1 (1.43%)	0 (0%)	2 (2.86%)	3 (1.43%)	1 (1.43%)	0 (0%)	2 (2.86%)	3 (1.43%)	6 (1.43%)

Fig. 2

Giardia duodenale	0 (0%)	0 (0%)	1 (1.43%)	1 (0.48%)	1 (1.43%)	0 (0%)	0 (0%)	1 (0.48%)	2 (0.48%)
Giardia lamblia	2 (2.86%)	0 (0%)	1 (1.43%)	3 (1.43%)	3 (4.28%)	0 (0%)	1 (1.43%)	4 (1.90%)	7 (1.67%)
Hymenolepsis nana	0 (0%)	0 (0%)	2 (2.86%)	2 (0.95%)	1 (1.43%)	0 (0%)	1 (1.43%)	2 (0.95%)	4 (0.95%)
Iodameoba butschli	0 (0%)	0 (0%)	1 (1.43%)	1 (0.48%)	0 (0%)	1 (1.43%)	2 (2.86%)	3 (1.43%)	4 (0.95%)
Schistosoma spp	0 (0%)	0 (0%)	2 (2.86%)	2 (0.95%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	2 (0.48%)
Strongyloides stercoralis	1 (1.43%)	0 (0%)	2 (2.86%)	3 (1.43%)	5 (7.14%)	0 (0%)	6 (8.57%)	11 (5.24%)	14 (3.33%)
Taenia saginata	2 (2.86%)	0 (0%)	3 (4.28%)	5 (2.38%)	0 (0%)	1 (1.43%)	3 (4.28%)	4 (1.90%)	9 (2.14%)
Trichostrongylus spp	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (1.43%)	0 (0%)	0 (0%)	1 (0.48%)	1 (0.24%)

PS014.II

Insights into malaria and chloroquine resistance in Southeast Iran

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Introduction: Malaria remains a significant parasitic infectious disease worldwide. In Iran, particularly in the southeastern regions including Sistan and Baluchistan province, malaria persists, primarily attributed to *Plasmodium vivax* and *Plasmodium falciparum*. Over the past decade, substantial efforts in control and prevention led to a significant decrease in malaria cases. However, with the emergence of Covid-19 and its impacts on malaria control measures, coupled with climatic, demographic, and economic changes, malaria resurgence has been observed, leading to a rollback phenomenon.

Objectives: This study aimed to identify the *Plasmodium* species causing malaria and evaluate the sensitivity of *Plasmodium vivax* to chloroquine in southeast Iran in 2023.

Materials & Methods: This study included 250 symptomatic patients with fever and a microscopic diagnosis of malaria who sought care at health and treatment centers in Sistan and Baluchistan province. DNA extraction was performed on all blood samples, and malaria species were identified using the Plasmodium 18S ribosomal RNA gene. *Plasmodium vivax* sensitivity to chloroquine was assessed on the third, fifth, seventh, 14th, and 28th days using the in vivo method. Nested PCR was additionally employed on the 7th and 14th days, alongside microscopic examination, for precise parasite detection.

Results: Nested PCR revealed fragments associated with *Plasmodium* genus infection in all patients. *Plasmodium falciparum* was detected in three patients, while *Plasmodium vivax* was identified in 246 individuals. One patient exhibited mixed infection with both *Plasmodium vivax* and *Plasmodium falciparum*. Following the national protocol, chloroquine treatment was administered for three consecutive days as a blood schizontocide for malaria caused by *Plasmodium vivax*. Parasites were undetectable in peripheral blood from the sixth day onward, and no protozoan forms were observed on the 7th, 14th, and 28th days.

Conclusion: Malaria cases due to *Plasmodium falciparum* and *Plasmodium vivax* were documented among residents and natives of Sistan and Baluchistan villages, including cases of mixed infection. This study found no evidence of *Plasmodium vivax* resistance to chloroquine in the study area.

PS017.II

Isolation, characterization, and pathogenicity assay of *Acanthamoeba* and its endosymbionts in respiratory disorders and COVID-19 hospitalized patients, northern Iran

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Acanthamoeba spp., are common free-living amoebae found in nature that can serve as reservoirs for certain microorganisms. The SARS-CoV-2 virus is a newly emerged respiratory infection, and the investigation of parasitic infections remains an area of limited research. Given that Acanthamoeba can act as a host for various endosymbiotic microbial pathogens and its pathogenicity assay is not fully understood, this study aimed to identify Acanthamoeba and its bacterial and fungal endosymbionts in patients with chronic respiratory disorders and hospitalized COVID-19 patients in northern Iran. Additionally, a pathogenicity assay was conducted on Acanthamoeba isolates. Urine, nasopharyngeal swab, and respiratory specimens were collected from two groups, and each sample was cultured on 1.5% non-nutrient agar medium. The cultures were then incubated at room temperature and monitored daily for a period of two weeks. Eight Acanthamoeba isolates were identified, and PCR was performed to confirm the presence of amoebae and identify their endosymbionts. Four isolates were found to have bacterial endosymbionts, including Stenotrophomonas maltophilia and Achromobacter sp., while two isolates harbored fungal endosymbionts, including an uncultured fungus and Gloeotinia sp. In the pathogenicity assay, five isolates exhibited a higher degree of pathogenicity compared to the other three. This study provides significant insights into the comorbidity of acanthamoebiasis and COVID-19 on a global scale, and presents the first evidence of Gloeotinia sp. as a fungal endosymbiont. Nevertheless, further research is required to fully comprehend the symbiotic patterns and establish effective treatment protocols.

PS020.II

Irritable bowel syndrome associated with *Blastocystis hominis* or without relationship to it?

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Blastocystis hominis (B. hominis) is a protozoan parasite that has a worldwide distribution. Some studies have suggested a link between *B. hominis* and the development of irritable bowel syndrome (IBS). The objective of this study was to determine the prevalence of *B. hominis* in patients with IBS compared to healthy individuals. A total of 65 stool samples from patients with IBS and 65 samples from healthy individuals in northern Iran were examined. The samples were tested using various methods including direct smear, formalin ether sedimentation and culture to detect the presence of *B. hominis*. Additionally, polymerase chain reaction (PCR) was performed on all culture-positive isolates to confirm the results and identify the genotype. *B. hominis* was detected in 15.38% of IBS patients and 9.2% of the healthy group. The culture in RPMI1640 was found to be better than the formalin ether and direct smear methods. Positive samples were confirmed using the molecular method. No significant difference was observed in the order of *B. hominis* infection between the two groups. The results of our study indicate that no significant difference was observed in the order of *B. hominis* infection between IBS patients and healthy groups. Therefore, further study is necessary to determine the potential pathogenic effects of this parasite and its role in causing IBS.

PS023.II

Treatments with c-myc and mTOR inhibitors efficiently block *Cryptosporidium parvum* development *in vitro*

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Introduction: *Cryptosporidium parvum* is a zoonotic intracellular protozoan parasite that causes severe diarrhoeic disease and productivity failures in calves, but also significantly affects the health of young children and immunocompromised humans. Currently, nitaxozanide is the only approved drug by FDA but it is equal to a placebo in immunocompromised patients. No effective drugs are available, neither in case of bovine nor of human cryptosporidiosis. Thus, there is a clear need to improve drugs to treat this disease. Recent reports have indicated a critical metabolic dependence of *C. parvum* intracellular development on both, glycolytic- and glutaminolytic activities of host cells, both being linked to the master regulators mTOR and c-myc.

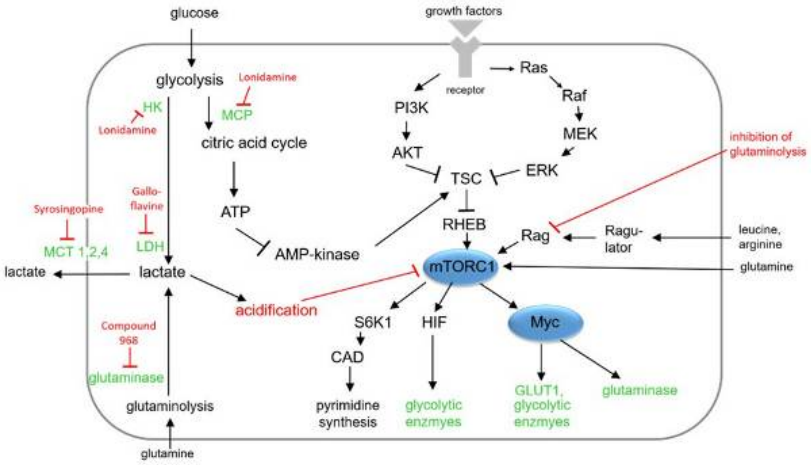
Objective: We here aimed to study the efficacy of different mTOR- and c-myc inhibitors on *C. parvum* development *in vitro.*

Materials and Methods: *In vitro C. parvum* infections in HCT-8 cells were performed using the IOWA strain (P102) at physioxic- (5 % O2) and hyperoxic (21 % O2) oxygen conditions. Appropriate, non-toxic mTOR- (oleocanthal and PP242) and c-myc (KJPyr9 and MYCi361) inhibitor concentrations were evaluated by XTT® cell proliferation assays. HCT-8 cells were pre-treated for 24 h, washed-off before *C. parvum* infection and re-administered 3 h *post infectionem* (p .i.) to avoid drug-driven alteration of sporozoite infectivity. HCT-8 cells were infected with 1 x 105 sporozoites per 24-well. *C. parvum* infection rates were assessed at 48 h p. i. using *Vicia villosa* lectin-based staining of the parasite. To reliably detect single host cells, nuclei and cytoskeletal elements (actin) were stained in parallel by DAPI and phalloidin 594, respectively.

Results: Overall, mTOR (2.5-5 µM oleocanthal; 0.25-0.5µM PP242) and c-myc (1.25-2.5µM [AT1] KJPyr9; 1.25-2.5 µM MYCi361) inhibitors all significantly blocked *C. parvum* development in HCT-8 cells. Thus, parasite reduction rates of 80- 90% were achieved. Overall, the effect of oxygen conditions proved marginal since no significant differences were observed between 5% and 21% O2 conditions.

Conclusion: *C. parvum* infection proved highly dependent on mTOR and c-myc cellular activities. Future *in vivo* studies in appropriate animal models will help to clarify the real relevance of mTOR and c-myc inhibitors as potential anti-cryptosporidial drugs.

Fig. 1


glucose
growth factors
glycolysis
Lonidamine
HK
Lonidamine
MCP
citric acid cycle
receptor
Ras
Raf
PI3K
MEK
AKT
ERK
TSC
inhibition of glutaminolysis
Syrosingopine
Gallo-flavine
MCT 1,2,4
LDH
ATP
AMP-kinase
RHEB
Rag
Ragu-lator
leucine, arginine
lactate
lactate
mTORC1
glutamine
acidification
S6K1
HIF
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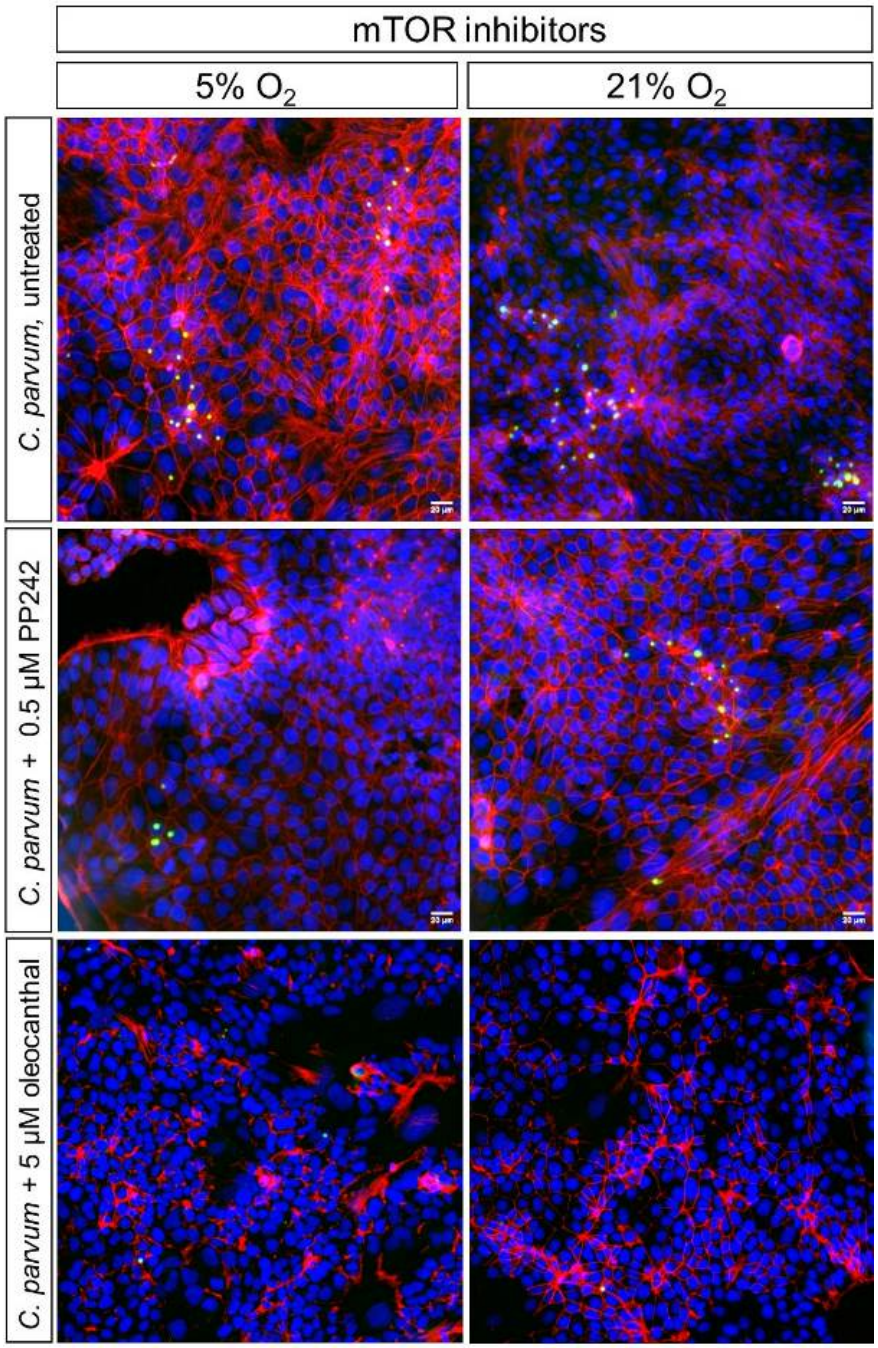
glycolytic enzmyes
GLUT1, glycolytic enzmyes
glutaminase
glutamine

Fig. 2


mTOR inhibitors
5% O_2
21% O_2
C. parvum, untreated
C. parvum + 0.5 µM PP242
C. parvum + 5 µM oleocanthal

PS026.II

Neospora caninum-driven modulation of host cell cycle and DNA damage response

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Introduction: *Neospora caninum* is an obligate intracellular coccidian parasite and the causative agent of neosporosis in cattle and canid hosts. Neosporosis causes abortions and enhanced neonatal mortality, especially in cattle breeding systems.

Objective: Since *N. caninum* modulates its host cell for successful replication, here we focused on parasite-driven effects on host cell cycle progression and DNA damage induction.

Materials & Methods: FACS-based analyses on cell cycle progression were performed in non-infected and *N. caninum*-infected primary bovine endothelial cells (BUVEC) at 24 and 32 h p. i. The expression of key cell cycle regulators cyclin A2 and cyclin B1 were assessed by Western blot. Both, comet assays and immunofluorescence detecting γH2Ax-related DNA damage foci were used to estimate DNA damage in *N. caninum*-infected cells and non-infected controls. Activation of DNA damage repair pathways was studied by Western blot-based quantification of related key proteins. In addition, intracellular ROS concentration was measured by FACS in *N. caninum*-infected BUVEC and non-infected cells, using NAC (N-acetylcysteine) and 4NQ0 (4-Nitroquinolone 1-oxide) as inhibitor and inducer, respectively. Since lamin B1 interacts with proteins of cellular DNA damage response and repair pathways, infected and non-infected BUVEC were analysed for nuclear lamin B1 distribution and total cellular expression via immunofluorescence assays and western blotting, respectively.

Results: *N. caninum* intracellular replication drives an S-phase arrest in primary bovine endothelial cells by modulation of cyclins A2 and B1, which are key cell cycle checkpoint regulating S- and G2-phase transition. Moreover, *N. caninum* infections consistently damage host cell DNA, specifically inducing double-strand DNA breaks. Given that *N. caninum* additionally downregulates key proteins of both, the non-homologous end joining and the homologous recombination pathway, host cells presumably hampered from activating DNA damage responses. However, no changes were detected in ROS production. Furthermore, nuclear lamin B1 shows an irregular nuclear pattern and a downregulation in expression, which is intrinsically associated with inadequate DNA repair in *N. caninum*-infected BUVEC.

Conclusion: Overall, current data suggested that *N. caninum* modulates host cell cycle progression by inducing DNA damage and nuclear lamina deformations, independently of host cell ROS production.

PS029.II

Structural characterization of LiTat 1.3: A major diagnostic VSG of the human parasite *Trypanosoma brucei gambiense*

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Introduction: Variant surface glycoproteins (VSGs) form a protective coat on African trypanosomes. VSGs are highly immunogenic, triggering a strong antibody response. However, trypanosomes evade immune clearance through antigenic variation, leading to successive parasitemic waves.1–3 In human African trypanosomiasis (HAT), caused primarily by *Trypanosoma brucei gambiense (Tbg),* serological diagnostics rely on detecting antibodies against the VSGs LiTat 1.3 and LiTat 1.5.4–10 Early and accurate diagnosis is critical for controlling disease spread, yet the structural understanding of these antigens remains limited.

Objectives: We aimed to investigate the structural properties of the major diagnostic antigen LiTat 1.3 from *Tbg*. Specifically, we sought to determine its oligomeric state and conformational dynamics. This knowledge will contribute to improve our understanding of the essential role of LiTat 1.3 VSG in gambiense-HAT diagnostics.

Methods: AlphaFold-Multimer was employed to predict LiTat 1.3"s 3D structure. Native VSG was purified from *Tbg* bloodstream forms using Concanavalin A affinity chromatography and then analyzed using size exclusion chromatography (SEC), and small-angle X-ray scattering (SAXS). BilboMD was used for SAXS-based ensemble modeling.

Results: AlphaFold-Multimer predictions indicated that LiTat 1.3 adopts a trimeric conformation, as evidenced by various confidence metrics. This was confirmed through SEC and SAXS analyses, which showed that the trimeric structure of LiTat 1.3 is independent of protein concentration. Ensemble modeling further revealed that LiTat 1.3 contains a rigid N-terminal domain (NTD) and a flexible C-terminal domain (CTD).

Conclusion: LiTat 1.3 adopts a concentration-independent trimeric structure and can be best described as a conformational ensemble, with a rigid NTD and a flexible CTD, enabling adaptation to varying protein densities on the membrane. These findings provide the first structural insights into LiTat 1.3, laying the groundwork for refining *gambiense*-HAT diagnostics to improve disease detection.

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PS032.II

Morphological and molecular identification of metazoan parasites in amphibians from Germany and their monitoring through environmental DNA

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Amphibians face significant challenges from climate change, human activities, and invasive species. While current research largely focuses on these threats, another critical issue—parasites—remains relatively understudied. Although invasive Chytrid fungi have received substantial attention in the last years, there is limited knowledge on distribution, abundance, and species composition of metazoan parasites of amphibians, particularly in Germany. We address these knowledge gaps by evaluating amphibian parasite biodiversity using morphological and molecular methods. Beyond understanding parasite distribution and diversity, this approach will help identify cryptic species and assess the status of already established species.

Notably, amphibian endoparasites are seldom included in biomonitoring programs in Germany. Traditional biomonitoring approaches for endoparasites are time-intensive, resource-demanding, and require expertise. A significant limitation is the necessity of sacrificing host organisms for parasite analysis. To overcome those challenges, we explore the use of environmental DNA (eDNA) metabarcoding, a rapid and non-invasive method for species identification from environmental samples. For parasite monitoring, this technique offers a viable alternative for biodiversity assessments and risk evaluations without the need for collecting and harming the host organisms. In preliminary studies, amphibians from selected sites in Germany were dissected to identify endoparasites through morphological and molecular analyses. These findings will guide the development of parasite-specific primers and facilitate the validation of eDNA-based methods for monitoring amphibian parasites. This presentation will also include initial results on amphibian parasite distribution and diversity across multiple regions in Germany, along with progress in developing eDNA-based monitoring techniques.

PS035.II

Variant surface glycoproteins from other African trypanosome species support growth of *Trypanosoma brucei*

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The expression of variant surface glycoprotein (VSG) on their cell surface is a halmark of the bloodstream form of African trypanosomes. Most information available on the repertoire of VSGs within the large family of proteins and their function is, however, restricted to the species *Trypanosoma brucei*. Their genome contains over 1000 VSG genes or pseudogenes and the VSG proteins contain two domains, a larger N-terminal and a smaller C-terminal domain that can be assembled in a modular way. For both domains the family of proteins can be divided into different groups, A1, A2 and B for the N-terminal domain and 1-6 for the C-terminal domain. Whereas expression of a functional VSG is essential for *Trypanosoma brucei* viability we have shown that the C-terminal domain is dispensable under cell culture conditions.

Other African trypanosome species, such as *T. congolense* and *T. vivax*, also express VSGs on their cell surfaces. Both their VSGs contain an N-terminal domain similar to those of *T. brucei* but neither possesses a comparable C-terminal domain.

We have used *T. brucei* as an expression system to express VSGs from both *T. congolense* and *T. vivax*. As a proof-of-principle we could successfully generate *T. brucei* cell lines expressing the *T. congolense* VSG BENat1.3 and the *T. vivax* VSG ILDat1.2. The expressed VSGs were purified and used for structure determination.

PS038.II

ApiMT: An apicoplast methyltransferase essential for toxoplasma gondii viability

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Toxoplasma gondii is an obligate intracellular parasitic protozoan that infects most warm-blooded animals, including humans, and is the causative agent of toxoplasmosis. The parasite"s ability to establish and maintain an infection in its intermediate host depends on the tightly regulated events of the parasite's lytic cycle. Previous studies on methyltransferases in *T. gondii* have highlighted the importance of methylation in ensuring the smooth progression of the lytic cycle.

In our exploration of other crucial methyltransferases, we identified a protein that shares similarity to the SET domain of human SETD3, involved in actin methylation, and the plant LSMT, which methylates Rubisco in chloroplasts. To uncover the function of this protein, we generated an inducible knockout (KO) using the DiCre system and systematically investigated its role in the parasite. Our investigation revealed that the protein localizes to the apicoplast therefore we named it ApiMT. Despite its similarity to SETD3, ApiMT does not appear to methylate actin or significantly affect actin dynamics. Parasites appeared morphologically normal during the first 4-5 days post-induction of KO. However, beyond this point, we observed aberrant apicoplast segregation, culminating in the collapse of parasites within the parasitophorous vacuole.

These findings indicate that ApiMT plays a delayed yet critical role in maintaining apicoplast integrity, which becomes essential for parasite survival over extended replication cycles.

PS041.II

Mass spectacular parasites: Applications of secondary ion mass spectrometry and imaging mass spectrometry in parasitology

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Mass spectrometry imaging is a quickly developing field that can map the spatial chemical and molecular composition of biological sample. It allows users to localise compounds of interest "label free" ensuring no change in molecular kinetics through the introduction of a tag and removing the need for antibody development. This poster will outline 3 mass spectrometry imaging techniques and include a case study of how we are using them to study whipworm.

The Cameca **NanoSIMS** 50L is a high lateral resolution secondary ion mass spectrometry microprobe (beam size can be focused to 50 nm) that can be used to image and measure elemental and isotopic distributions in samples at subcellular scale. A primary ion beam impacts a sample surface which produces atomic and small ionic species which are then detected in a mass spectrometer. We have used the NanoSIMS to identify the feeding mechanism of *Trichuris muris* through the introduction of isotopically labelled nutrients. NanoSIMS can then be used to localise the nutrients at a subcellular scale via measuring enrichment of the stable isotope above the natural abundance.

Time of Flight SIMS (**ToF SIMS**) is a technique similar to NanoSIMS however it has a *gentler* primary ion beam which allows extraction of molecular ions and molecular fragments, these are detected via a Time-of-Flight detector. This has a lower lateral resolution (typically around 1 μm) but gives more molecular information. We have used ToF SIMS to identify the compounds *T. muris* produces from isotopically labelled nutrients.

Matrix Assisted Laser Desorption Time of Flight (**MALDI ToF**) is a mass spectrometry imaging technique (MSI) that utilises a laser to produce ions from the surface of a sample. Whilst this technique has the lowest spatial resolution of the techniques presented it gives the highest molecular information with detection of ions up to 350,000 Da. Further, MALDI ToF analyses can be performed at ambient pressure, which allows analysis of non-vacuum compatible samples, and involves significantly less sample processing. We have used MALDI to identify the molecules secreted by *T. muris* and to identify metabolic changes in the gut induced by whipworm infection.

All of these techniques have different advantages and drawbacks, however, are applicable to many different contexts in parasitic research. Using these case studies, we have shown the power of each of the techniques and demonstrated their applications across parasitology.

PS044.II

RNAi-based exploration of *Schistosoma mansoni* GPCRs in male-female interactions and BRET-based identification of neuropeptide binding partners

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Schistosoma mansoni is a parasitic worm responsible for schistosomiasis, a neglected tropical disease. The reproductive biology of schistosomes is unique, as the female requires constant pairing with a male to achieve sexual maturation. Despite extensive studies on G protein-coupled receptors (GPCRs) in vertebrates, mammals, and plants, their roles in invertebrates like schistosomes remain poorly understood. With Praziquantel (PZQ) being the only drug available for schistosomiasis, identifying new drug targets is important. GPCRs represent promising targets for drug development due to their involvement in various biological processes and their druggability.

To investigate their role(s) for male-female interaction, we focus on the functional characterization of *S. mansoni* GPCRs that are differentially expressed between pairing-experienced males, pairing-inexperienced males, and females. To this end, we applied RNAi for knockdown (KD) of candidate GPCRs. Since previous approaches revealed low KD efficiencies of GPCRs, another goal was improving the RNAi protocol. To monitor physiological effects after RNAi *in vitro*, we additionally performed EdU assays and started establishing Bioluminescence Resonance Energy Transfer (BRET) for analysing GPCR interaction with neuropeptides (NPPs), previously identified using the MALAR-Y2H system.

KD experiments were performed with a novel two-dsRNAs-one-target approach. Effects on pairing stability, attachment capacity, stem-cell activity, and egg production were investigated for 21 days *in vitro*. For BRET assays, a candidate GPCR (GPCR9) was humanized and transfected into HEK cells. Internalization assays were done to obtain first hints for NPP partners. Subsequently, BRET assays have been started including G15, Gq, Gi, and Arrestin to investigate potential downstream signaling processes.

The novel RNAi approach resulted in 92-99% KD for different candidate GPCRs. Interesting phenotypes were observed, including curling, tegumental damage, and decrease of motility. Furthermore, we noted a reduction of EdU positive cells, suggesting effects on stem-cell proliferation including reproductive tissues. This was paralleled by a decrease of mature oocytes in the RNAi groups. These results suggest roles of the selected GPCRs for vitality and reproduction. Internalization experiments provided hints for specific NPP partners (15b, 26b, 31/32.2, and 41) of GPCR9. And the first results from the BRET Gq assays indicated NPP 26b and NPP 41 as potential interaction partners of GPCR9.

In summary, we optimized the functional characterization of GPCRs of *S. mansoni* and identified candidates with potential roles for male-female interaction but also for target-based drug design as alternative for PZQ for fighting schistosomiasis.

PS047.II

Subpellicular microtubules in *Plasmodium knowlesi* merozoites form a divergent 'two-class' arrangement

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The invasive stages of *Plasmodium* parasites are stabilized by subpellicular microtubules (SPMTs). Our understanding of their morphology in merozoites, which drive pathogenic blood stage infection, has been primarily shaped by studies on *P. falciparum*. There, only one to two parallel SPMTs are formed, each spanning the length of the cell. In contrast, we here present ultra-expansion microscopy data on merozoites of the zoonotic parasite *P. knowlesi*, revealing seven to nine apically radiating SPMTs. Unique amongst known SPMTs, these are furthermore divided into two morphologically distinct subclasses during merozoite formation. Here we typically observe two to three adjacent 2.2 µm long SPTMs akin to those in *P. falciparum*, as well as five to six 0.2 µm short ones. These two classes are further distinguished via differences in posttranslational poly-glutamylation. As typical for SPMTs, the long microtubules carry the modification along their entire length, while on short microtubules, this modification was only observed at the apical base. In fully formed merozoites, however, all SPTMs are fully poly-glutamylated and the microtubules of the two classes have converged on an intermediate length. Intriguingly, this suggests opposing growth dynamics of the distinct SPMTs despite a shared cell volume. These results not only reveal several unique features of the *P. knowlesi* cytoskeleton and pose interesting questions on the regulation of microtubule dynamics, but also further highlight the morphological diversity of these structures between different *Plasmodium* species.

PS053.II

Exploring the functional mechanisms of microRNAs in *Fasciola hepatica* host-parasite interactions

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Flatworms such as the liver fluke *Fasciola hepatica* exert a significant global influence on human and animal health, and are associated with considerable economic losses and decreased agricultural productivity. Despite their profound impact worldwide, there are still considerable knowledge gaps regarding the host interaction mechanisms employed by flukes, with our focus being the role played by microRNAs (miRNAs) and extracellular vesicles (EVs). Since there is a notable absence of flatworm vaccines, limited availability of flukicidal anthelminthics, and growing resistance to available flukicides, enhancing our knowledge of RNA driven parasite-host interactions has the potential to lead to novel flatworm controls. To probe the mechanisms of fluke-derived miRNAs and explore the phenotypic effects they may have on parasite hosts, we used size exclusion chromatography (SEC) and tunable resistive pulse sensing (TRPS) to collect 93 billion EVs from ten adult flukes. Western blots were used to detect known RNA binding proteins in these EVs, and miRNA pulldowns identified proteins associated with fluke EV miRNAs. This novel dataset will catalyse an improved understanding of the role of fluke-derived miRNAs in host interactions, which could provide opportunities for the development of disease diagnostics and therapeutics.

PS056.II

Development of an one-pot assay for rapid detection of *Giardia duodenalis* by combining recombinase polymerase amplification and *Pyrococcus furiosus argonaute*

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Background: Giardiasis is a common zoonotic disease of significant public health concern worldwide that is caused by *Giardia duodenalis* (*G. duodenalis*). Consequently, it is essential for the development of an accurate and efficient pathogen detection system that can rapid identify *Giardia* infections. The recombinase polymerase amplification combined with the *Pyrococcus furiosus* argonaute (RPA-*Pf*Ago) system, a novel diagnostic platform is widely used to address the limitations of traditional detection methods.

Methods: In this study, we developed a rapid one-pot detection method of RPA combined with *Pf*Ago. The RPA reaction and *Pf*Ago premix were divided by solid paraffin. Firstly, the RPA reaction was completed at 39℃ for 30min, then the temperature was increased to 95℃, the paraffin was dissolved and raised to the liquid surface. The RPA product was combined with the *Pf*Ago premix to complete the second reaction within 25min. The one-pot detection method can use two different probes, FAM and BHQ labeled probes emit green fluorescence under blue light after completing the reaction, which can be directly observed by the naked eye or the fluorescence intensity can be recorded by qPCR instrument. FAM and Biotin labeled probes can be combined with Lateral flow assay (LFA) to quickly determine the results.

Results: To evaluate the performance of the one-pot RPA-*Pf*Ago detection system, 49 clinical samples were tested. Among the 49 samples examined, 16 were tested positive. The method established in this study is consistent with the results of nested PCR.

Conclusion: The one-pot RPA-*Pf*Ago minimizes the difficulty of operation and aerosol contamination caused by multiple opening of the cap. At the same time, the cleavage of the target sequence by *Pf*Ago also reduces the possibility of subsequent contamination. The reaction is completed within 1h, which is an efficient, sensitive, specific and convenient method. The one-pot RPA-*Pf*Ago provide a quick way for the field detection of *G. duodenalis* on-site detection and furnishes new ideas for detecting other pathogens.

PS059.II

Detection of protozoan parasites and diagnosis of their associated diseases in Ecuador in the period 2016-2023: A scoping review

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Introduction: Epidemiological surveillance and biomedical research on protozoa with pathogenic potential are highly relevant due to the significant impact these microorganisms have on human and animal health. Nevertheless, the prioritization of other high-impact infectious diseases, such as COVID-19 and dengue, combined with limited financial support and low political visibility, highlight the need for targeted studies on these parasites in tropical countries like Ecuador.

Objective: To analyze the scientific literature on protozoa detection in Ecuador from 2016 to 2023.

Materials & Methods: Following a systematic search in PubMed and SciELO databases according to PRISMA guidelines, 56 studies that met the established inclusion criteria were selected.

Results: The findings highlight a predominance of research on *Leishmania* spp. and gastrointestinal parasites, although articles about a wider range of infectious agents were observed from 2020 onward. Frequent use of molecular tools, such as PCR and genetic sequencing, was particularly noted in studies involving humans and animals. However, notable research gaps persist in certain provinces, including Andean and Amazonian regions, and for specific pathogens, such as trypanosomatids and apicomplexans.

Conclusions: Stronger research efforts on vectors, reservoirs, and zoonotic transmission, especially in rural and Amazonian areas, are essential to achieve a more comprehensive ecoepidemiological understanding. Sustaining epidemiological surveillance, developing advanced diagnostic methods, and adopting a comprehensive public health perspective that addresses both human and animal health, aligned with global initiatives, are imperative. Similarly, gaps in epidemiological data and a lack of multidisciplinary biomedical studies indicate the need for further research to improve the management of these infections in Ecuador.

Figures.

Figure 1. PRISMA flow chart and screening.

Figure 2. Scientific production and detection of protozoa by province in Ecuador. A) Total quantification of studies for each year included obtained from the databases. B) Map showing the detection of protozoa, at genus level, by province, described in the studies analyzed.

References

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Fig. 1

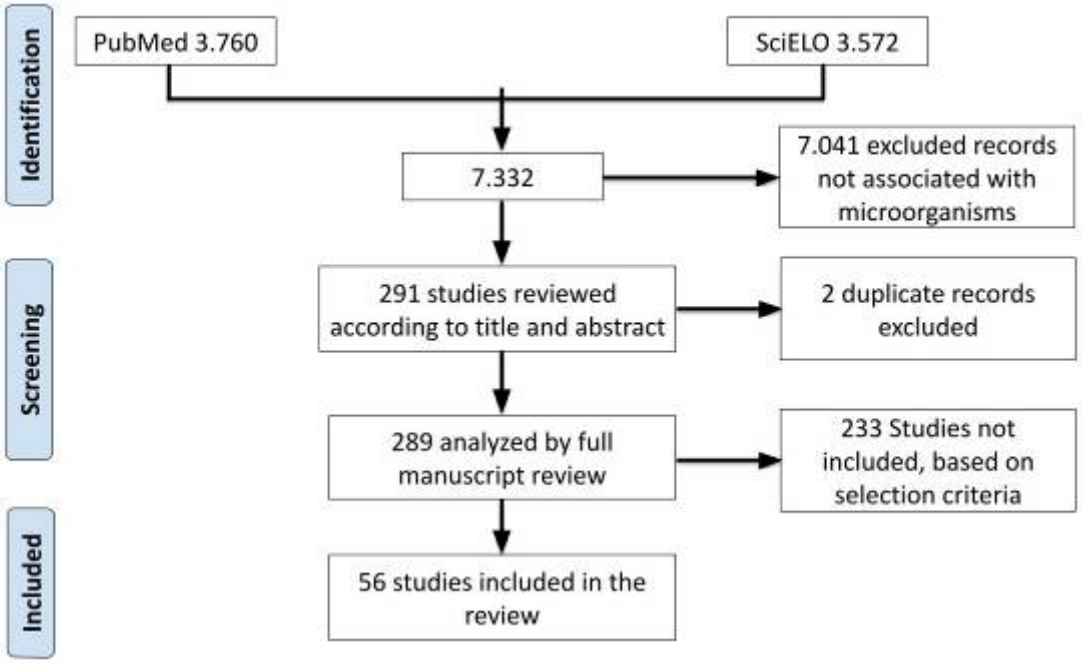
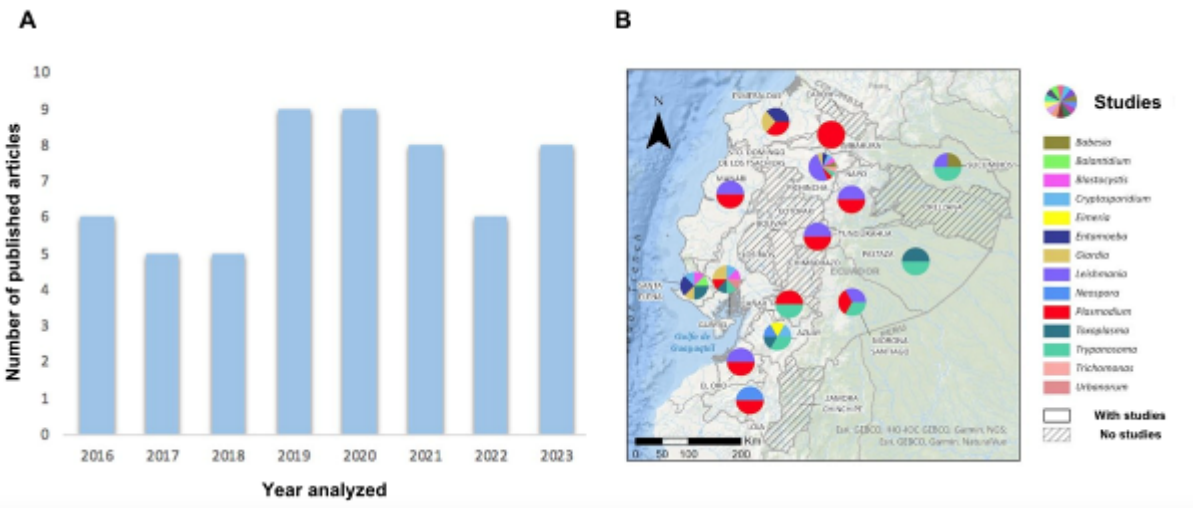

Identification
Screening
Included
PubMed 3.760
SciELO 3.572
7.332
7.041 excluded records not associated with microorganisms
291 studies reviewed according to title and abstract
2 duplicate records excluded
289 analyzed by full manuscript review
233 Studies not included, based on selection criteria
56 studies included in the review

Fig. 2


A
B
Number of published articles
Year analyzed
2016
2017
2018
2019
2020
2021
2022
2023
Studies
With studies
No studies

PS062.II

The AMPK pathway plays a role in *Besnoitia besnoiti* and *Toxoplasma gondii* tachyzoite-induced PMN responses

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Besnoitia besnoiti and *Toxoplasma gondii* are closely related apicomplexan parasites that can infect cattle, but the course of infection and the development of symptoms varies greatly. Polymorphonuclear neutrophils (PMN)-derived effector mechanisms directed against coccidian parasites include reactive oxygen species (ROS) production, degranulation, phagocytosis and neutrophil extracellular trap (NET) formation. The molecular mechanisms of coccidia-driven NET formation are still largely unknown but several findings suggest the PMN metabolism and autophagy as key regulators. The adenosin monophosphate protein kinase (AMPK) controls several PMN effector mechanisms by sensing the metabolic state of a cell and regulating cellular processes, such as autophagy, chemotaxis, ROS production and NETosis.

In the present work, the activation of the AMPK-related signalling pathway by *T. gondii* and *B. besnoiti* tachyzoite exposure was studied in bovine PMN by Western blot. Therefore, calcium fluxes in bovine PMN in response to tachyzoites were studied via flow cytometry. Effects of the AMPK-activator AICAR on both the neutrophil metabolic status and *T. gondii*/*B. besnoiti* tachzoite-driven NET formation was studied by extracellular flux analyses (Agilent, Seahorse) and confocal microscopy, respectively.

Current data showed *T. gondii* tachyzoite confrontation induces a rise in neutrophil intracellular calcium concentrations, thereby correlating with a subsequent increase in calcium/calmodulin-dependent protein kinase kinase (CAMKK) activity, AMPK phosphorylation and activation of the AMPK pathway after 30 min of confrontation. At the same time point, an expression increase of the autophagy-related proteins p-beclin-1 and ULK was stated. In line, *B. besnoiti* tachyzoite exposure also resulted in AMPK and autophagy activation in PMN being accompanied by ULK-1 and p-beclin-1 upregulation. Intriguingly and in contrast to *T. gondii*, *B. besnoiti* tachyzoite exposure did not induce a rise in intracellular calcium concentration thereby indicating alternative upstream mechanisms of AMPK-activation. To further evaluate the role of the AMPK pathway in parasite-driven PMN effector mechanisms, effects of AICAR (AMPK activator) and dorsomorphin (putative AMPK inhibitor) pretreatments on the neutrophil cellular metabolism and NET release were evaluated. Overall, AICAR treatments indeed boosted the oxidative metabolism and NET formation in bovine PMN but failed to trigger additive effects on parasite-driven PMN activation and NETosis. In contrast, dorsomorphin treatments had no effects at all.

In conclusion, the AMPK pathway plays an important role in *T. gondii* or *B. besnoiti* tachyzoite-driven PMN responses. In both cases, autophagy is activated by tachyzoite exposure. However, upstream mechanisms seem to differ between these two parasitic AMPK stimulators. Finally, the role of AMPK activation in NETosis is demonstrated, giving new insights on apicomplexan-induced NETosis.

PS065.II

Host-derived lipid mediators as novel regulators of Treg cell development with distinct features in maintaining immune tolerance during human helminth infection

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Background and question: Distinct tissue-derived signals drive regulatory T cell (Treg) induction and shape their heterogeneity and functionality in chronic inflammation. In the inflammatory parasitic brain disease, neurocysticercosis (NCC), we have recently identified that the lipid mediator PGE2 secreted from parasitic glutamate dehydrogenase (GDH)-modulated monocyte and microglia is a central driver of Treg development, which is essential to control disease in asymptomatic, non-epileptic NCC patients. Here, we characterize the epigenetic and transcriptional determinants of GDH-PGE2-modulated Treg cell development and the clinical implications in brain inflammation and silencing and following anti-helminthic treatment.

Methods: Targeted lipidomics and extensive LC/MS/MS profiling of lipid mediators in controls, NCC asymptomatic and patients with epilepsy and neurological symptoms guided the identification of disease- and GDH-remodeling of eicosanoids, precursors and metabolites. In-depth immunophenotyping and pulsing of controls" and patients" peripheral cells with recombinantly expressed parasite GDH or PGE2 revealed the context-dependent Treg development with unique features in asymptomatic in contrast to symptomatic disease. Subsequent mechanistic pathways of lipid mediator regulation of Treg induction were elucidated by transcriptional profiling of ex vivo sorted monocytes. The epigenetic and transcriptional determinants of Treg development and landscapes in asymptomatic patients were furthermore assessed via ATACSeq and RNASeq as compared to in vitro induced Treg and ex vivo sorted Tconv from healthy individuals.

Results: Targeted lipidomics revealed a bias arachnoid acid metabolism in NCC patients with a pronounced COX-PGE2 pathway in asymptomatic disease, which declined following anti-helminthic treatment. Importantly, the upregulation of the COX-PGE2-Treg axis is associated with distinct features of enhanced CNS migration and endothelial cell adhesion potency (CD69hi, CCR7+, VLA-4hi, LFA-1+) of ST2+Tregs, but absent in brain inflammatory symptomatic disease. The marked increase of PGE2 and precursor metabolites in patients" sera is positively correlated with pronounced EP2 and EP4 receptor expression on peripheral naïve CD4+CD25- T cells. Moreover, integrative sequencing analyses interestingly revealed the non-canonical TNFR2-NF-kB and the JAK-STAT signaling pathways as important regulators controlling GDH-PGE2-driven Treg differentiation and thus a potential role in the regulation of inflammatory NCC.

Conclusions: This work highlights important insights into lipid mediators as novel regulators of Treg cell development with distinct features to maintain immune tolerance in NCC with relevance for other parasite-induced inflammatory brain diseases.

PS068.II

Generation of transgenic Plasmodium falciparum strains to assess the invasion-egress effect of low molecular weight heparins

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Transgenic Plasmodium parasite strains provide valuable tools in the study of these parasites, supporting studies as diverse as investigating cell-host interaction to exploration and evaluation of new parasite inhibitors and vaccines. Genetic modification of blood-stage Plasmodium falciparum is currently limited to manipulation of the intraerythrocytic stages, maintained by continuous in vitro culture systems. This process is made more challenging as delivery of exogenous DNA to the parasite needs to cross four membrane barriers: (i) erythrocyte plasma membrane, (ii) parasitophorous vacuole membrane iii) parasite plasma membrane and (iv) the nuclear membrane barrier. However, In this study, we have successfully generated four independent strains expressing luciferase. These strains were subsequently used in a luciferase-based assay to assess and evaluate the growth inhibition activity of different low molecular weight heparins (LMWHs). LMWHs have shown concentration-depend inhibition activity which is mediated by blocking of merozoite invasion and disruption of egress from erythrocytes. The generation of transgenic human malaria parasite strains with different phenotypic invasion pathways offers an attractive method to explore and study new classes of plasmodial inhibitors and support current efforts in drug development

PS071.II

Molecular characterization of the DEAD-box RNA helicase eIF4A as potential drug target in *Schistosoma mansoni*

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Schistosomiasis, caused by infection with trematodes of the genus *Schistosoma,* has been treated with praziquantel for decades. The absence of a vaccine and the risk of resistance development against praziquantel emphasizes the urgent need to identify new targets and drugs against schistosomes like *S. mansoni*, which is one of several species infectious for humans. A strategy of drug development is to target protein synthesis of the parasite, as it is a crucial process for its survival. One candidate factor is the eukaryotic translation initiation factor 4A (eIF4A), an RNA helicase that unwinds secondary structures in the 5" untranslated region of selected mRNAs. Once the secondary structures are dissolved, ribosomes can access the mRNA and initiate protein synthesis. It has been shown, that inhibition of human eIF4A by natural and synthetic compounds impedes viral replication and may represent a novel antipathogenic strategy.1,2 We investigated the function of schistosomal eIF4A to evaluate its potential as drug target.

In *S. mansoni,* eIF4A isoforms were identified by *in silico* analysis. The isoforms *Sm*eIFAI and *Sm*eIF4AII, which are potentially involved in translation initiation, were selected for further investigations. Functional analyses of both *Smeif4a* isoforms were performed by RNA interference (RNAi). After three weeks of dsRNA treatment, the relative transcript level of *Smeif4a1* was reduced by ≥ 88% in female and male worms. After 10 d and 20 d of RNAi, total egg production and worm attachment were significantly reduced, respectively. Confocal laser scanning microscopy showed disrupted testes of treated males and disorganized ovaries with reduced numbers of immature oocytes of treated females as morphological phenotypes. EdU-staining to examine effects on stem-cell proliferation revealed a reduction of cell proliferation mainly in the reproductive organs of treated worms. *Smeif4a2* RNAi led to a knockdown efficiency of ≥ 61% in male and female worms. After 17 d of RNAi, worm attachment of the treated group was significantly reduced, but no further morphological phenotypes were observed.

In conclusion, *Sm*eIF4AI seems to be involved in worm vitality, stem-cell proliferation, spermatogenesis, and oogenesis. Furthermore, both isoforms appear to be functionally distinct, as the phenotype caused by RNAi of *Smeif4a1* could not be rescued by *Smeif4a2*. Based on the molecular findings, we conclude that especially *Sm*eIF4AI may have target potential.

1Müller et al. (2020), *Antiviral Research*, 175:104706.

2Obermann et al. (2023), *Scientific Reports*, 13:9297.

PS074.II

Intestinal helminth infection: A potential confounder of QuantiFERON-TB gold plus test performance

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Introduction: Timely detection and treatment of Latent TB infection (LTBI) is a promising key strategy for combating tuberculosis (TB). However, helminth infection may affect the detection performance of interferon gamma releasing assay (IGRA) tests.

Objective: Aimed to assess the effect of helminth infection on the QuantiFERON-TB Gold Plus test for LTB detection.

Methods: A cross-sectional study was conducted from October 2022 to March 2023 in Bahir Dar city administration. Kato Katz and wet mount techniques were used to identify participants with helminth infection. About 5 ml of venous blood was collected with Lithium- heparin tube, and subsequently one ml was transferred to each of the four QFT-Plus tubes for stimulation at 37oC for 16-24 hrs. Culture supernatant was collected to determine IFN-γ with ELISA as per the QFT-Plus kit protocol. SPSS version 20 was used for statistical analysis, and p value < 0.05 were considered statistically significant.

Results: A total of 100 study participants were recruited with the mean age of the 28.51 years (SD=9.76). Seven helminth species were detected from the 53 helminth positive participants where *A. lumbricoides* and hookworm were the two predominantly identified helminthes. Based on the QFT Plus test result, the overall proportion of QFT positive tests was 30%. QFT positivity was significantly higher among male participants than female (P= 0.032). The proportions of QFT positivity among helminth positive and negative participants were comparable (30.2% vs 29.8%). Considering participants with intestinal helminth infection, QFT positivity rate was significantly reduced in participants with hookworm infection above the median egg count (P=0.029).

Conclusions: While helminth infection in general did not impact QFT positivity rate, hookworm infection appears to have a significant effect on the performance of IGRA depending on the worm burden. Further studies with larger sample size are warranted to understand the species specific effect of helminth infection on immune modulation of the host.

Key words: Intestinal Helminths, LTBI, QFT-Plus, *MTB*, Ethiopia

PS077.II

Expression of the pro-apoptotic BCL-2 proteins Bak and Bax during *in vitro* infection of murine neurons with *Toxoplasma gondii* tachyzoites

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Parasite survival depends greatly on how well they can exploit their host. *Toxoplasma* (*T.*) *gondii*, a cosmopolitan zoonotic Apicomplexa, is no exception to this rule. Its exploitation mechanisms start very early in its host-invasion process. More specifically, right after the parasite infects the cell. *T. gondii* can not only swift the arrangement of certain organelles but it can also manipulate their signalling pathways (Ikara et al. 2021, Kellermann et al. 2021). One example is the regulation of the apoptotic cascade in the host cell, which can be hindered by *T. gondii* infection (Besteiro S, 2015). This allows the parasite to comfortably replicate within the cell and leave it on its own terms. The understanding of how *T. gondii* delays apoptosis has not been completely elucidated. The objective of this study is to evaluate the expression of Bak and Bax: two pro apoptotic proteins from the BCL-2 family in murine neurons. These proteins normally work together to produce permeabilization of the mitochondrial outer membrane (MOMP) which facilitates the release of cytochrome c and the triggering of the apoptotic cascade.

For this, we used two different *T. gondii* strains: RH (Type I) and Pru (Type II). We separately infected confluent neuroblastoma-derived cell (cell line Na 42/13) monolayers with each parasite strain and 2 infection groups: MOI=1 and MOI =2 for 3, 24, and 24 hours (h). A negative control of non-infected Na 42/13 monolayers was added to the study. Afterwards, we purified RNA from the cells with a commercial kit, and generated a complementary DNA strain through a reverse transcriptase-regulated process. With this, we amplified the mRNA region of both Bak and Bax using quantitative PCR. The double delta Ct method was used to calculate the relative fold gene expression. At 3h post infection (hpi), downregulation of Bax was already present in all infection groups. The major difference was shown at 24 hpi with Bax expression levels significantly low in both RH strain infection groups. Cells infected with *T. gondii* Pru strain show Bax expression levels mildly higher than the negative control at a MOI =1 and mildly lower than the negative control at a MOI = 2. Bak levels were always higher than Bax at 3hpi and 24hpi. At 48hpi all expression levels of both Bak and Bax were lower than the negative control.

It is common to use Bak/Bax as a unit in the apoptotic reaction. However, in the case of neurons, it is considered that Bax is the main excecutor of MOMP (together with other apoptotic proteins). In this study we demonstrate that *Toxoplasma*-derived Bax downregulation could vary amongst strains or virulence, particularly at 24 hpi. How delicate *T. gondii* manipulation of the apoptotic cascade is, would need to be further investigated, particularly across different cell types and strains.

PS080.II

Performance evaluation of the *diaxxoPCR* system for rapid and easy-to-perform stool-based diagnosis of trichuriasis, ascariasis and strongyloidiasis in Mozambique

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Nucleic acid amplification tests have shown promising results for diagnosing soil-transmitted helminths (STH). However, large-scale implementation of real-time PCR (qPCR) in low-resource settings is still hampered by multiple logistical challenges, including the need for a cold chain for essential PCR consumables. In this study, we assessed the diagnostic performance of a portable and affordable cartridge-based real-time PCR system (*diaxxoPCR*) for the detection of DNA of *Trichuris trichiura*, *Ascaris lumbricoides* and *Strongyloides stercoralis* in clinical samples. Initially, a technical validation of the *diaxxoPCR* system in a singleplex pod (cartridge) design was performed, using a selection (n=37) of positive and negative DNA samples (Study A). This was followed by an extended evaluation (Study B), performed in a laboratory located in a rural area of Mozambique by examining 325 DNA samples extracted from stools collected in a clinical trial for the evaluation of a fixed-dose co-formulation of albendazole and ivermectin. These samples were tested independently by the *diaxxoPCR* system (singleplex) and qPCR to assess qualitative (sensitivity, specificity, positive and negative predictive values) and quantitative (agreement) parameters. Finally, one negative and one positive DNA sample were used to demonstrate the performance of a multiplex pod design as a potential use-case for the *diaxxoPCR* system (Study C). Study A showed acceptable outcomes in the technical performance of the *diaxxoPCR* system, with minimal intra- and inter-assay variation and sufficient output reproducibility. Study B showed a positive qPCR outcome in 57.5% (187), 15.4% (50) and 0.3% (1) of the 325 DNA trial samples for *T. trichiura*, *A. lumbricoides* and *S. stercoralis*, respectively. The *diaxxoPCR* system demonstrated sensitivities and specificities above 97% and 94%, for each target, resulting in nearly perfect to perfect qualitative agreements between *diaxxoPCR* and qPCR. Concerning quantitative output, significantly strong correlations were seen between the Ct-values (qPCR) and Cq-values (*diaxxoPCR*). In Study C, the *diaxxoPCR* system correctly detected all 3 targets in the multiplex pod design. In conclusion, with refinements regarding DNA extraction from stool samples, the *diaxxoPCR* system has the potential to provide high-quality, accurate and easy-to-use molecular diagnostics of STH in relatively low resource laboratory settings. The system can be adapted to either STH species-specific population-based surveys or diagnostic management of individual patients.

PS083.II

The *Calicophoron daubneyi* genome provides new insight into mechanisms of feeding, eggshell synthesis and parasite-microbe interactions

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Background: The rumen fluke, *Calicophoron daubneyi*, is the major paramphistome species infecting ruminants within Europe. Adult flukes reside within the rumen where they are in direct contact with a unique collection of microorganisms. Here, we report a 1.76 Gb draft genome for *C. daubneyi*, the first for any paramphistome species.

Results: Several gene families have undergone specific expansion in *C. daubneyi*, including the peptidoglycan-recognition proteins (PGRPs) and DM9 domain-containing proteins, which function as pattern-recognition receptors, as well as the saposin-like proteins with putative antibacterial properties, and are upregulated upon arrival of the fluke in the microbe-rich rumen. We describe the first characterisation of a helminth PGRP and show that a recombinant *C. daubneyi* PGRP binds to the surface of bacteria, including obligate anaerobes from the rumen, via specific interaction with cell wall peptidoglycan. We reveal that *C. daubneyi* eggshell proteins lack L-DOPA typically required for eggshell crosslinking in trematodes and propose that *C. daubneyi* employs atypical eggshell crosslinking chemistry that produces eggs with greater stability. Finally, although extracellular digestion of rumen ciliates occurs within the *C. daubneyi* gut, unique ultrastructural and biochemical adaptations of the gastrodermal cells suggest that adult flukes also acquire nutrients via uptake of volatile fatty acids from rumen fluid.

Conclusions: Our findings suggest that unique selective pressures, associated with inhabiting a host environment so rich in microbial diversity, have driven the evolution of molecular and morphological adaptations that enable *C. daubneyi* to defend itself against microorganisms, feed and reproduce within the rumen.

PS086.II

Vaccine adjuvants boost oxfendazole efficacy in the *Litomosoides sigmodontis* filarial rodent model

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Introduction: Infections with filarial nematodes such as *Onchocerca volvulus*, *Wuchereria bancrofti* or *Brugia* spp. can cause debilitating neglected tropical diseases, affecting millions of people worldwide. Drugs currently used for mass drug administration lack a macrofilaricidal (i.e., adult worm killing) effect and the only macrofilaricidal treatment that is currently available, doxycycline, requires a 4-6 week daily treatment, restricting it for individual therapy. Oxfendazole, a promising clinical candidate, has demonstrated macrofilaricidal efficacy in pre-clinical models and is currently being tested in phase II clinical trials against onchocerciasis, loiasis, mansonellosis and trichuriasis. Previous research has shown oxfendazole efficacy to be dependent on the immune system, and stimulation with IL-5 during oxfendazole treatment to improve the treatment outcome, allowing shorter treatments.

Aim: Here, we investigated the potential of adjuvants to boost the efficacy of oxfendazole against *Litomosoides sigmodontis*, a rodent filarial nematode, and determined whether combination therapy allows a shorter treatment regimen.

Methods: 6-8 week old female BALB/c wild type mice were naturally infected via mite bite with *L. sigmodontis*. 35 days post infection mice were treated for 3 or 5 days either with just the vehicle, oxfendazole or adjuvant monotherapy, or with a combination treatment of oxfendazole and adjuvant. Mice were sacrificed 63 days post infection to determine the effect of the combination treatment.

Results: While three day oxfendazole monotherapy was insufficient for a reduction of adult worms (24%), combination therapy of three day oxfendazole with either MF59 (62%) or CpG-ODN1826 (55%) led to a reduction of total adult worm burden similar to a five day oxfendazole monotherapy (66%). Additionally, the combination treatment led to a near complete clearance of microfilariae from the peripheral blood as well as a significant reduction of all embryonal stages, while the three day oxfendazole monotherapy was insufficient. Furthermore, changes in adult worm length and morphology could be observed.

Conclusions: The results of our study indicate that common adjuvants can boost the efficacy of direct-acting, macrofilaricidal drugs like oxfendazole which allows shorter treatment regimens that may support the success of elimination programs.

PS089.II

The CLIMOS project – Providing a better knowledge and comprehension of climate and environmental drivers of sand fly-borne diseases

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Sand fly-borne pathogens, mainly Leishmania sp. protozoans and phleboviruses, represent a major public health and veterinary concern. The spread of sand fly vector populations and the pathogens they transmit into previously non-endemic regions due to climate and environmental changes in recent decades led several research consortia to improve knowledge, surveillance and control in Europe and neighboring countries.

The CLIMOS project (Climate Monitoring and Decision Support Framework for the Detection and Mitigation of Sand Fly Diseases with Cost-Benefit and Climate Policy Measures; http://www.climos-project.eu), brings together 29 partners, including universities, institutes, research centers and ministries of health from 16 countries within and outside Europe. It aims to characterize the climatic, environmental, demographic, and epidemiological characteristics associated with the presence and abundance of sand flies and domestic animal infection rates at different geographic scales across Europe and neighboring countries.

These data will feed into mathematical epidemiological-climate prediction models of realistic human-induced climate change scenarios to help develop an early warning system for sand fly presence, activity and risk of

infection designed for public use seeking to better prepare for current and future impacts of climate and environmental change on human and animal health.

Funding: The CLIMOS consortium is co-funded by the European Commission grant 101057690 and UKRI grants 10038150 and 10039289. The six Horizon Europe projects, BlueAdapt, CATALYSE, CLIMOS, HIGH Horizons, IDAlert, and TRIGGER, form the Climate Change and Health Cluster.

Fig. 1

PS092.II

The epidemiology and potential impact of gastrointestinal nematodes of the European hedgehog (*Erinaceus europaeus*) accepted into wildlife rescue centres in North-West France

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Aims: Hedgehogs serve a crucial ecological function; however, little is known about their conservation conflicts in European countries. Many hedgehogs that are brought to the wildlife rescue centres are reported to be heavily impacted by gastrointestinal nematodes (GIN). This study aimed to identify parasites affecting hedgehogs in North-West France and their impact on recovery in wildlife rescue centres.

Methods: Admission data of hedgehogs arriving to four wildlife rescue centres in North-West France were collected over one year (May 2023-2024). At entry, faecal egg counts (FEC) were performed using the McMaster coprological technique at one centre (Envol) and direct smears at the other three centres. Simple descriptive statistics described the dataset and generalised multivariable logistic regression models investigated host factors and presence of GIN species on host outcomes.

Results: In total 300 hedgehogs were sampled and 58.3% were positive for GIN infection. Infecting species included *Crenosoma striatum* (25.7%), *Capillaria* spp. (46.3%), *Brachylaemus erinacei* (11%), and *Isospora* spp. (7.3%). Age, season, and the specific wildlife rescue centre significantly influenced GIN species presence.

For the Envol centre sample (n=176), FEC of *C. striatum*, *Capillaria* spp. and *B. erinacei* were correlated suggesting a co-infection relationship within individual hosts. Risk of death appeared to be associated with season admitted (spring: OR=0.17) and presence of *C. striatum* (OR=0.79). Weight gain (Young: OR=1.86; Juvenile: OR=1.70) and length of recovery (Young: OR=2.16; Juvenile: OR=2.02) were associated with age only.

Conclusion: This is the first study in Europe to investigate GIN influence on hedgehog outcomes admitted to rescue centres and the first to describe infecting species in North-West France. In addition, the work highlights the potential influence GIN co-infections which warrants further research.

PS095.II

VEuPathDB: A bioinformatics resource for facilitating data exploration, analysis, and integration for vectors and eukaryotic parasites

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The Eukaryotic Pathogen, Vector and Host Informatics Resources VEuPathDB (https://veupathdb.org) provides access to genomic-scale data-mining resources for >700 species of eukaryotic pathogens and related taxa, including protozoan parasites, fungi & oomycetes, arthropod vectors of disease, and selected host species. VEuPathDB resources empower end-users to leverage diverse multi-Omics datasets, without requiring specialized analytical or computational skills. Our internal pipelines analyze a wide variety of omics data and couple the analysis results to data mining capabilities, data visualizations, and custom tools to facilitate the discovery of meaningful relationships from large volumes of data. Available data types include genome sequence and population-level variation data; manually-curated and automatically generated annotation; epigenetic, transcriptomic and proteomic data; pathway information, including metabolomic datasets; genome-wide phenotypic analyses and information on host-pathogen interactions. VEuPathDB provides a phylogenetic framework to facilitate cross-species functional inference via orthology. These resources merge evidence from diverse data and across species, making the power of bioinformatic analysis accessible to research scientists worldwide. Future advancements are aimed at integrating AI-driven tools to enhance literature curation and metadata annotation and incorporating tools for new data types. Our active user support offers an email help desk, social media, video tutorials, webinars and workshops.

Please email us at help@VEuPathDB.org for more information.

PS098.II

Phenotypic delineation of benzimidazole effects on juvenile *Fasciola hepatica in vitro*

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Fascioliasis is a highly pathogenic foodborne trematodiasis caused by *Fasciola hepatica* infections. This flatworm has significant impact on animal health, and subsequently agricultural productivity and financial sustainability.

Control relies on a small panel of chemotherapeutics, the most effective of which is triclabendazole (TCBZ). TCBZ is unique in that it is effective against both the juvenile liver stage and adult bile duct stage parasites, while the other flukicides are primarily adulticides. Unfortunately, drug resistance has now been reported for all but one of the available flukicides.

Despite juvenile migration resulting in the most significant damage to the host (and sometimes death), most drug trials have considered the adult fluke. Here we use *in vitro* exposure assays to determine the effects of benzimidazole drugs on the juvenile parasite using a range of bioassays. Previous work on TCBZ has found the parent compound can be actively metabolized by fluke/host tissues and the sulfoxide (TCBZ.SO) and sulfone ($TCBZ.SO_2$) metabolites are found in the environment in which the fluke live and may play a key role in control. Also, other benzimidazoles have been mooted as having potential in liver fluke control including fenbendazole and oxfendazole.

We find that like albendazole, fenbendazole and oxfendazole have very limited effects on juvenile fluke *in vitro*. In contrast, TCBZ, TCBZ.SO and $TCBZ.SO_2$ do have a variety of phenotypic impacts, with TCBZ and TCBZ.SO being most potent. We were able to delineate the phenotypic impacts on newly excysted juveniles and 3-week old juveniles. Also, whilst TCBZ and TCBZ.SO treatments impacted motility, cell proliferation and survival, the latter had the most potent effects on growth.

Next, we examined differences in the phenotypic responses to TCBZ and its metabolites in drug susceptible and drug-resistant isolates, noting that the resistance phenotype most closely aligned with the TCBZ.SO response and differential cell proliferation and growth responses.

Some have reported changing drug response profiles within isolates over time. This has been highlighted by our most recent experiments using TCBZ which have shown diminishing differences in the survival of selected TCBZ-S and TCBZ-R strains *in vitro*. However, while $TCBZ.SO_2$ did not have as big an impact on survival, it did undermine growth, particularly of the TCBZ-R worms. Work is continuing to determine if this is due to a change in *in vivo* drug response status.

The potential evolving susceptibility of "lab"-maintained strains highlights how even closed populations can evolve. These isolates are vital in continuing to build the potency profile of TCBZ and other anthelmintics to improve our understanding of the modes of action and mechanisms behind resistance.

PS101.II

Suppression of the growth and metastasis of cancer by tapeworm antigens

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In recent years, the study of the interactions between helminth species and cancer has gained interest. Indeed, there are many ways by which parasitic worms interact with this disease, be it as causative agents themselves or potential preventative agents. Helminths may carry antigens common with certain cancer cells, helping the immune system to fight them off, they may aid in the suppression of potentially carcinogenic inflammation through their immunomodulatory capabilities, or their products could directly affect malignant cells.

Our work expands upon the recently described cancer suppression by the *Taenia crassiceps* and *Mesocestoides corti* tapeworms (Schreiber et al. 2024), whereby the intraperitoneal infection with their larvae significantly reduces the burden of melanoma tumours. We seek to bypass the pathogenic larvae and elicit a similar effect by their antigens only. We utilize whole worm homogenates or excretory-secretory products injected intraperitoneally to induce a milieu of immune cells hostile to the cancer cells.

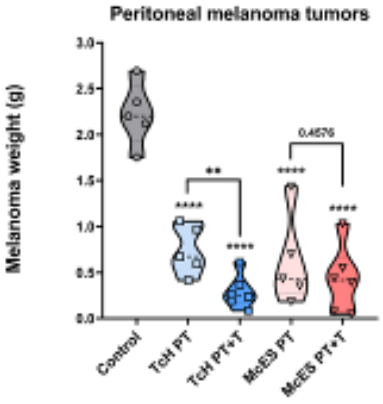
The research focuses on the rapidly-growing B16F10 melanoma and the comparatively slower-growing ID8 ovarian carcinoma, both of which form peritoneal tumours. The melanoma burden is measured by the weight of excised tumours, while the growth of ID8 is measured by luminescence, as this line is genetically modified to produce luciferase. In the case of melanoma, histological examination for metastases is performed. The peritoneal immune cell milieu is examined, with both the lymphoid and myeloid lineages recounted.

Thus far, the *T. crassiceps* homogenate and *M. corti* excretory-secretory products have shown themselves capable of suppressing B16F10 melanoma. Mice inoculated with these antigens have shown massively reduced tumour burden and greater body weight, hinting at better overall health. A continuous inoculation even after cancer cell introduction improves this effect further. When the peritoneal immune cell milieu was examined in a group, which received inoculations, but was sacrificed at the day reserved for cancer cell introduction, a significantly increased number of potentially cancer-killing cells was discovered. An increased amount of Natural killer cells and eosinophils was detected, similar to the immune cells present at the same timepoint in tapeworm larvae infections.

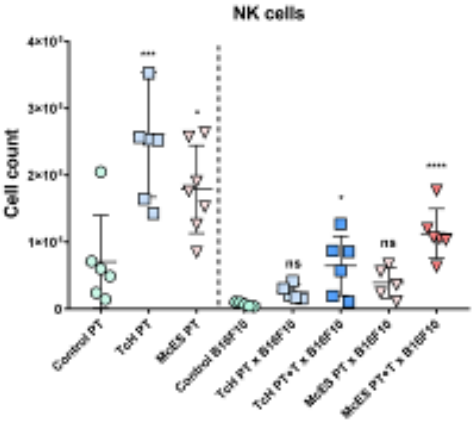
In conclusion, these results point towards an immune-cell mediated cancer-suppressive effect elicited by tapeworm antigens present in these mixtures. The crucial point in time is the creation of a hostile environment for the introduced cancer cells, which are reduced in number, disallowing greater tumour growth in treated mice.

Schreiber, M., T. Macháček, V. Vajs, B. Šmídová, M. Majer, J. Hrdý, O. Tolde, J. Brábek, D. Rösel, and P. Horák. 2024. "Suppression of the Growth and Metastasis of Mouse Melanoma by *Taenia crassiceps* and *Mesocestoides corti* Tapeworms." *Frontiers in Immunology* 15 (March). https://doi.org/10.3389/fimmu.2024.1376907.

Fig. 1



Comparison of peritoneal melanoma tumour weights. This graph shows the significant reduction of the weight of excised peritoneal melanoma tumours in mice, that received *T. crossiceps* homogenate (**TcH PT**) and *M. conti* excretory-secretory products (**McES PT**) inoculation regimens before B16F10 melanoma cell introduction compared to control, which received sterile PBS only. The decrease was greater in mice that received injections following cancer cell introduction as well (**TcH PT+T**, **McES PT+T**).



Increase in Natural Killer cell numbers in the peritoneal cavity. Here, the amounts of NK cells are shown. Following the pre-treatment regimen, the number of NK cells increases both in the case of *T. crassiceps* homogenate injections (**TcH PT**) and *M. conti* excretory-secretory products (**McES PT**). In mice, that were injected with B16F10 melanoma, but did not receive further inoculations following the cancer cell introduction, the number of NK cells decreases after three weeks (**TcH PT x B16F10**, **McES PT x B16F10**), but remains significantly increased if inoculations continue (**TcH PT+T x B16F10**, **McES PT+T x B16F10**). Comparisons are made to respective control groups (vertical line separation).

PS104.II

Chromatin profiling unraveled: Comparing ATAC-seq profiles in monomorphic versus pleomorphic *Trypanosoma brucei*

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Trypanosoma brucei, typically vectored by blood-feeding tsetse flies, is the causative agent of Human and Animal trypanosomiasis. During bloodstream infection, pleomorphic *T. brucei* utilise density dependent quorum sensing (QS) to develop from proliferative slender forms into non-proliferative stumpy forms, preadapted for transmission to the tsetse fly. However, monomorphic trypanosome clades – namely *T. b. evansi* and *T. b. equiperdum*, causative agents of surra and dourine, respectively – do not undergo stumpy development, having become refractory to the QS-signal, and are not reliant on the tsetse fly as a vector as a result. Instead, these parasites are transmitted mechanically via biting flies or sexually between horses, allowing for their escape from the tsetse belt. With tsetse fly numbers declining in some regions of sub-Saharan Africa, the selection pressure to reduce reliance on the tsetse fly as a transmission vector (and thus become monomorphic) may be increasing. Recent work analysing early changes in the development of monomorphism via laboratory-selected monomorphic lines of *T. b. brucei* Antat1.1 bloodstream forms, generated through serial passage in brain-heart infusion (BHI) until parasites become refractory to QS, suggested that monomorphism may initially arise as "proto-monomorphs", whereby reversible changes in the expression of key quorum-sensing genes initiate the progression towards monomorphism (Oldrieve et al., 2024). Subsequent accumulation of deleterious mutations in these genes could then lead to the proto-monomorphic parasites being locked into a monomorphic lifestyle. To investigate these early reversible changes further, we are examining changes in chromatin accessibility during the progression towards monomorphism in laboratory-selected isolates, and subsequently comparing these results to monomorphic field isolates. To achieve this, we have optimised the "assay for transposase accessible chromatin with sequencing" (ATAC-seq) for use in *T. brucei* laboratory-lines and, for validation purposes, used it alongside RNA-seq, to compare changes in chromatin accessibility and gene expression in monomorphic Lister427 bloodstream forms and procyclic forms, as well as pleomorphic Antat1.1 bloodstream forms. Comparisons between monomorphic and pleomorphic bloodstream forms, and procyclic forms, with respect to chromatin accessibility and gene expression profiles will be presented and discussed with respect to the development competence and progression of African trypanosomes through their life cycle.

PS107.II

RNA-based communication between *Strongyloides ratti* and rat immune tissue

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Parasitic helminths secrete a variety of entities into the host environment to facilitate infection and evasion/manipulation of the host immune response. Following seminal studies revealing proteins as helminth excretory/secretory (ES) products, the diversity of secretions has broadened, to also now include extracellular vesicles and various RNA species. These can be internalised by host cells, in some cases dysregulating normal functionality to aid parasite survival. While our understanding of RNA-based communication is growing, it is far from complete, particularly in the context of leveraging this knowledge for parasite control strategies. Using *Strongyloides ratti* maintained in rats as a model system, we investigated interactions between parasites and host intestinal Peyer's patches (PP), the sentinel immune tissues responsible for detecting and responding to intestinal antigens. PP play key roles in initiating and controlling immune responses to helminth parasites in the intestinal lumen. Our hypothesis was that *S. ratti* secreted RNAs would be detectable within host PP. Using small RNA sequencing of PPs from infected rats, we were able to identify 22 parasite-derived miRNAs in PPs, all of which were absent from uninfected control animals. We next used mRNA-Seq to identify potential impacts of parasite miRNA delivery on host PP transcription. While the majority of these data showed a classic anti-*Strongyloides* response characterised by upregulation in mast cell and muscle transcription, we also identified many downregulated host transcripts, some of which were computationally predicted as binding targets for the worm-derived miRNAs. These included targets with immunological significance including the notch ligand *jagged-1*. Interestingly, these data also revealed the presence of 322 parasite-derived mRNAs, 91 of which were full-length, in host PP (which were absent from uninfected PPs), marking, to our knowledge, the first report of this phenomenon. These parasite-derived mRNAs were also detected in cell-free *in vitro* parasite maintenance media. Ongoing work aims to further characterise the cellular targets and functional consequences of *S. ratti* miRNA delivery, driving forward our understanding of the evermore complex host-parasite dynamic.

PS110.II

Histone modification in the development of drug resistance in the parasitic nematode *H. contortus*

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The ever-growing drug resistance in *H. contortus* poses challenges for livestock farming and associated economic losses, and risks of transmission to wildlife like deer and mouflon. Addressing this resistance requires multiple strategies, including developing new drugs targeting known molecular sites, identifying new targets, and exploring drugs outside of the anthelmintic category. Histone modification represents one approach to investigate drug resistance and its transmission to the next generation in the form of so-called transgenerational epigenetic inheritance, which has been previously described in both mice and the model nematode *C. elegans* 1. This epigenetic mechanism alters gene expression by modifying DNA without changing its structure, mainly through N-acetylation and N-methylation of lysine or arginine residues, which affects chromosome condensation and gene transcription of certain genome regions. These modifications are mostly carried out by enzymes of the histone acetyltransferase (HAT) and histone deacetylase (HDAC) family. This project aims to determine whether changes in histone modification might be related to the development of drug resistance in the parasitic nematode *H. contortus*. A bioinformatic *in silico* analysis was performed to select homologous domains of HAT and HDAC compared to *C. elegans*. In addition, transcriptional analysis of selected HAT and HDAC was performed for all developmental stages of *H. contortus* (eggs, larvae, adults), comparing both the susceptible strain (ISE) and the resistant strain (IRE). All developmental stages were exposed to sublethal doses of albendazole, and the expression of the same genes was also determined.

The study was supported by Charles University Grant Agency (231423).

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PS113.II

Dynamics of malaria infection in a child cohort study in Bandiagara, Mali

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Introduction: Malaria remains a major public health issue and significal economic burden. with an estimated 228 million cases and 405,000 deaths, children under the age of 5 are the most vulnerable group affected by malaria, accounting for 67% (272,000) of deaths worldwide in 2018. The incidence rate of malaria fell worldwide between 2010 and 2018, from 71 to 57 cases per 1,000 inhabitants at risk (1). In Mali, malaria is the leading cause of malaria consultations, with more than 2 million cases and 1,700 deaths in 2018. Children under 5 and pregnant women are the groups most affected (4). *Plasmodium falciparum* is the most widespread species in the south of the country (94%) and the most deadly in Mali, followed by *P. malaria* (5%) and *P. ovale* (1%), which are not generally fatal. In the north, the prevalence of *P. vivax* can be as high as 30% in some areas (5,6).

Objectives: The aim of our work was to update epidemiological parameters of malaria transmission in the context of scaling up preventive strategies.

To determine the plasmodium index according to microscopy and PCR in children aged 6 months to 15 years from October 2017 to December 2018 in Bandiagara. To determine the prevalence of asymptomatic carriage of *Plasmodium* according to microscopy and PCR in children aged 6 months to 15 years from October 2017 to December 2018 in Bandiagara. To determine the prevalence of anaemia in children aged 6 months to 15 years from October 2017 to December 2018 in Bandiagara. To assess local time series of malaria episodes and asymptomatic carriage of *Plasmodium* as a function of rainfall.

Patients and methods: We conducted a prospective longitudinal cohort study to assess the dynamics of malarie infection of children from October 2017 to December 2018living in Bandiagara, Mali. the study population included children aged 6 months to 15 years, a total of 300 participants.

Results: The majority of patients were female 51.3 % (154/300), with a sex ratio of 1.01 in favour of females. Children aged 6-10 years predominated in our study 38 % (114/308), followed by children aged 6 months to 5 years 34 % (102/300) and children aged 11-15 years 28% (84/300). The mean age was 7.44 years, ranging from 6 months to 15 years.

The highest plasmodic index on microscopy and PCR was observed at the start of the study in october 2017, 8.9 % (26/292) and 12 % (35/292) respectively.

The overall malaria prevalence by microscopy and PCR during active follow-up was 2.8 % (122/4383) and 4.6 % (203/4383) respectively. The age group over 10 years was the most affected by malaria in our study. The overall prevalence of anemia during active follow-up was 2.4 % (111/4667)

Conclusion: A reduction in malaria transmission in Bandiagara was observed due to the implementation of control strategies including seasonal malaria chemoprevention. However, the permanent presence of asymptomatic *Plasmodium* carries confirms the existence of a parasite reservoir that maintains tramission.

PS116.II

Computational workflow to build a data-driven model of a "virtual parasite"

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In this project, we build "virtual parasites" from high-resolution image data as basis for a precise data-driven mechanical understanding of parasite biophysics. The parasitic life cycle involves a multitude of physical interactions with the host microenvironment during stages of motility and adhesion. The shape and elasticity of unicellular parasites are largely defined by their cytoskeleton. In parasitic kinetoplastids such as Trypanosoma brucei, the cytoskeleton includes a subpellicular array of microtubule filaments that forms a corset around the entire cell. How exactly the interaction between the beat of the flagellum, which is attached to the cytoskeleton and winds around the cell, and the mechanical response of the cell body, gives rise to the intricate rotational motility patterns of T. brucei is not known.

To answer this question, a detailed structural and mechanical model of the microtubule cytoskeleton and the interior of the cell, which together define its elasticity, is needed. We perform a complete semantic segmentation (pixel-level annotation) of electron tomography image volumes of T. brucei using a deep learning workflow. We then apply automated tracing of microtubule filaments and semi-supervised instance segmentation of the cell interior to arrive at a complete 3D structural model of the cell. This model is converted to an annotated volume mesh amenable for finite element analysis and subjected to in-silico deformation test to validate the model against experimental data. By adapting the workflow to other image data, similar mechanobiological phenomena in other model parasites can be studied. Our data-driven approach will enable new ways of understanding the physics of parasitism by connecting imaging with computer simulations.

PS119.II

Experimental evolution in the parasitic worm *Strongyloides* to map ivermectin drug resistance in the lab and field

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Strongyloides stercoralis is a soil-transmitted helminth (STH) that infects approximately 600 million people worldwide causing strongyloidiasis, often described as the most neglected of neglected tropical diseases (NTDs). The World Health Organization (WHO) has recognised the urgent need to address strongyloidiasis by including *S. stercoralis* as a target species for control efforts in the WHO Roadmap for NTDs 2030. Treatment for strongyloidiasis, which presents as skin and gastrointestinal pathologies to severe complications including death, is the anthelmintic drug ivermectin. Ivermectin acts by selectively activating glutamatergic receptors in the parasites" nervous system, leading to paralysis and death. Although highly effective, resistance to ivermectin has become a significant concern for controlling both livestock- and human-infective parasites. The genetic mechanisms underlying ivermectin resistance in *Strongyloides* (and broadly, any parasitic nematode) remain poorly understood. With the expected increase in ivermectin use through mass drug administration, there is a growing concern that the emergence of drug resistance in *S. stercoralis* will threaten the success of control programmes. Here, we aim to use integrated genomic and transcriptomic approaches to (i) determine the mechanisms underpinning anthelmintic drug resistance in an experimental evolution model using rat-infective *S. ratti* and (ii) identify quantitative trait loci (QTL) and specific genetic variants associated with ivermectin resistance by performing genetic crosses between resistant and susceptible *S. ratti* lines. Initial results show that administering ivermectin to rats infected with *S. ratti* at a 500 µg/kg reduces infection by 92%, while a 100 µg/kg dose achieves a 78% reduction. Paired whole-genome, RNA, and small RNA sequencing of individual worms from both resistant and susceptible lines will identify genetic variants and expression changes associated with resistance. The findings from this project will provide insights into the genetic basis of ivermectin resistance and enable the development of molecular tools for monitoring resistance in the field, supporting effective control strategies for *S. stercoralis*.

PS122.II

Avian *Plasmodium* occurrence in mosquitoes in Apulia region (Southern Italy)

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Avian malaria is a worldwide disease affecting birds caused by hemoprotozoa belonging to the genus *Plasmodium* (Apicomplexa: Haemosporida) and transmitted by Culicidae mosquitoes. It is characterized by a great variability of symptoms, including mortality. In this study, the circulation of the parasite is investigated through its detection in natural vector insects to evaluate their use as possible biological indicators.

From May to November 2022-2023, mosquito traps (CDC and BG Sentinel®) were positioned in some farms located in wetlands and in a Wildlife Rescue Center (WRC) in Apulia, South of Italy. Mosquitoes were identified by the morphological keys (1) and pooled according to species, sex, date, and trap locations. Detection of *Plasmodium* spp. from female mosquitoes was performed by a qPCR method (2). The positive specimens underwent nested PCR (nPCR) as previously described (3). Nucleotide sequences from nPCR amplicons were determined by the Sanger methods by using the nested primers as sequencing primers. The sequences were assembled by means of CAP3 and compared by BLAST with those present in GenBank for identification purposes.

A total of 771 adult mosquitoes were identified and classified into 6 genera (*Aedes*, *Anopheles*, *Coquillettidia*, *Culex*, *Culiseta*, and *Ochlerotatus*) and 10 species (*Aedes albopictus, Anopheles algeriensis, Anopheles maculipennis* s.l.*, Coquillettidia richardii, Culex pipiens, Culiseta annulate, Culiseta longiareolata, Ochlerotatus caspius, Ochlerotatus detritus,* and *Ochlerotatus communis*). Out of the 154 pools (size between 1 and 50), 7 were positive for *Plasmodium sp.* in the qPCR. Amplicons obtained from the nPCR were sequenced and identified (Table 1). Considering that no double peaks were observed in the sequencing chromatograms, it is possible to assess that no pools were infected by more than one *Plasmodium* species.

This study represents the first detection of *Plasmodium* spp. from mosquitoes in Southern Italy, confirming the circulation of avian malaria-related plasmodia in wild areas. Notably, all detected plasmodia were associated with *Cx. pipiens*, suggesting its key role as a vector in this region. Furthermore, the application of a qPCR strategy, initially developed for quantification, has proven effective for detection purposes, highlighting its versatility for parasitological studies. (This study was supported by grants of the Italian Minister of Health, Ricerca Corrente IZSPB 01/2021)

Table 1. Identification of plasmodia from the qPCR-positive pools

References

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Fig. 1

Sampling site	Site type	Mosquito species	Pool size	*Plasmodium* identification
A	Farm	*Culex pipiens*	8	*Plasmodium* sp.
B	Farm	*Culex pipiens*	20	*Plasmodium* sp.
C	WRC	*Culex pipiens*	3	*Plasmodium elongatum*
D	Farm	*Culex pipiens*	25	*Plasmodium relictum*
E	Farm	*Culex pipiens*	1	*Plasmodium cathemerium*
F	Farm	*Culex pipiens*	12	*Plasmodium relictum*
G	Farm	*Culex pipiens*	5	*Plasmodium* sp.

PS125.II

Mechanisms of host: Parasite immune regulation during *Schistosoma mansoni* infection

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Introduction: To establish chronic infection, parasites must be able to manipulate their hosts and subvert destruction by the immune system. Extracellular parasite molecules are amongst the prime mediators of these host:parasite interactions but are also often targeted by host protective humoral responses. Schistosomes, like other helminths, can produce molecules with immunomodulatory functions that will allow them to develop and thrive in their host"s vasculature. Yet, infection studies in animal models suggest that it is possible to develop protection to schistosome re-infection and that this protection is likely to be antibody-mediated.

Objectives: Identifying the molecules through which schistosomes manipulate host immune responses but also those that are targeted by protective antibodies could pave the way for the discovery of new vaccine candidates. We have focused our study on cell-surface and secreted *Schistosoma mansoni* proteins expressed during the somule and adult stages.

Materials & methods: To preserve the conformational epitopes present in extracellular polypeptide chains, we have selected a library of over 150 *S. mansoni* proteins and recombinantly expressed their extracellular domains in mammalian HEK293-6E cells. This large library of correctly-folded proteins was used in comparative serological studies between cohorts of mice with different levels of protection from re-infection to try and identify protective antigens, and in SAVEXIS, a technique for detection of protein:protein interactions, against a library of 750 human surface immune receptors to identify parasite proteins with immunomodulatory potential.

Results: The serological study identified sixteen antigens with increased reactivity in the most protected group of animals, including five proteins whose reactivity was also associated with a significant shift in antibody isotypes: from mostly IgG1 in permissive animals to IgG2 subtype in protected mice. Strikingly, three of these five antigens belong to a family of related LU-domain-containing surface proteins.

The protein:protein interaction study identified new host:pathogen receptor:ligand pairs, including one between the *S. mansoni* protein Granulin and the human neutrophil receptor CD177. This interaction was further characterised by mapping the binding domain on each partner and measuring the biophysical parameters of the interaction by surface plasmon resonance. Cellular assays are currently under way to determine the functional impact of this interaction on neutrophils.

Conclusion: Our library of biochemically active recombinant proteins from *S. mansoni* represents a versatile resource for serological, biochemical, cellular and structural analysis of host:parasite interactions and may help shed light on the complex interplay between hosts and parasites during infection.

PS128.II

Molecular evidence of hookworms and *Strongyloides* species in humans and dogs in Bangladesh

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Introduction: Hookworms and *Strongyloides* are two major groups of soil-transmitted helminths (STHs) known to infect humans and animals. Despite the high zoonotic potential, literature on such helminths in Bangladesh is still scarce and nonspecific.

Objectives: This study aimed to identify hookworm and *Strongyloides* species from humans and stray dogs across northeastern Bangladesh.

Materials and Methods: A total of 260 stool samples were screened for the presence of helminth larvae/eggs through the formalin-ether concentration technique (FECT) and, subsequently, the agar plate technique (APT). DNA was extracted from the single worms. The identification was made based on morphometric features and confirmed by amplifying the 18S ribosomal RNA gene (rRNA).

Results: The prevalence of helminth infections based on FECT was 27.7% (36/130) in humans and 50.8% (66/130) in stray dogs. Hookworm-like larvae were observed in 14.6% (19/130) of humans and 33.1% (43/130) of dogs in APT. From a total of 62 worms (one per positive), 18S rRNA sequences revealed *Ancylostoma ceylanicum* in humans and *A. caninum*, *A. ceylanicum*, and *Strongyloides fuelleborni* in dogs. *Ancylostoma ceylanicum* and *S. stercoralis* were identified in both humans and dogs. Two distinct types of *S. stercoralis* (type A and type B) were identified. Type A was isolated both from humans and dogs, while type B was found exclusively in dogs.

Conclusion: This is the first molecular proof of the emerging pathogen *Ancylostoma ceylanicum* from humans in Bangladesh. Detection of another emerging nematode, *Strongyloides fuelleborni,* in dogs highlighted the possibility of zoonotic transmission among humans living with dogs. The source of the infection in humans is unclear. Thus, the implementation of effective control measures should seriously consider this zoonotic implication.

Keywords: Parasites and clinical parasitology, Zoonoses, One Health, Diagnostic Parasitology.

PS131.II

An assessment of knowledge, attitudes and perception of a forestry community about ticks and tick-borne diseases in Nigeria

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Introduction: Ticks are obligate haematophagous arthropods that vectors of bacterial, viral, rickettsia protozoan and helminth disease agents, they pose a significant threat to human and animal well-being as they parasitize every class of terrestrial vertebrates. The best living conditions for ticks are forests with rich and moist undergrowth protecting them against dryness, areas grown with alder and elder trees, and fern-covered places commonly seen at the border of forests, meadows, and pastures. Many groups of humans especially at risk individuals like forestry workers are at risk of tick-bite with attendant infection when they carry pathogens without their knowledge of the risk and possible impact of such pathogens, thus this study was to evaluate the knowledge and risk perception with regards to tick and tick-borne infection among forestry workers.

Materials and Methods: With an interviewer-administered qualitative questionnaire, five clusters of forestry workers: officers, guards, technicians, students (final year and graduate) and others (farmers, hunters, operators, contractors and indigene) that visit the forest reserves, were evaluated for their knowledge and perception about ticks, tick bite and tick-borne disease (TBDs). Their knowledge practices and perception were compared against age, gender, level of education and cluster of the forestry workers using chi-square analysis with significant value set at $p \leq 0.05$ using SPSS.

Results: Of 208 respondents from the five clusters studied; 6.3% (13/208) were forest officers, 20.2% (42/208) forest guards, 13.5% (28/208) forest technicians, 31.7% (66/308) people that visit the forest and 28.4% (59/208) undergraduates and graduate students. Based on their educational level; 21.2% (44/208) of the respondents had no formal education, 15 (7.2%) had primary education, 16.8% (35/208) had secondary education while 54.8% (114/208) had a tertiary education. Of the 208 respondents, 78.4% (163/208) were males and 21.6% (45/209) were females. Of the study population, 93.3% (194/208) knew about ticks and could identify them, 44.2% (92/208) knows that ticks could be found on grasses. Of the 208 respondents, 32.2% (67/208) said they have been bitten by ticks while 67.8% (141/208) had never been bitten by ticks. Protective measures, such as protective clothing, repellents and local herbs were used by 80.8% (168/208) of participants when visiting forests amazingly none of the respondents could name any specific TBD.

Conclusion: From this study, a high level of knowledge of ticks and its habitat with low level of knowledge about tick-borne diseases was demonstrated which calls for public campaign and awareness creation amongst forestry workers on ticks and tick-borne diseases, considering the study group being at risk of tick bites and probable tick infections by the nature of their job of regular contact with tick when in bush.

PS134.II

Evolutionary history and host microhabitat shape the diversity of microbial communities associated with helminths of the bat *Peropterix kappleri*

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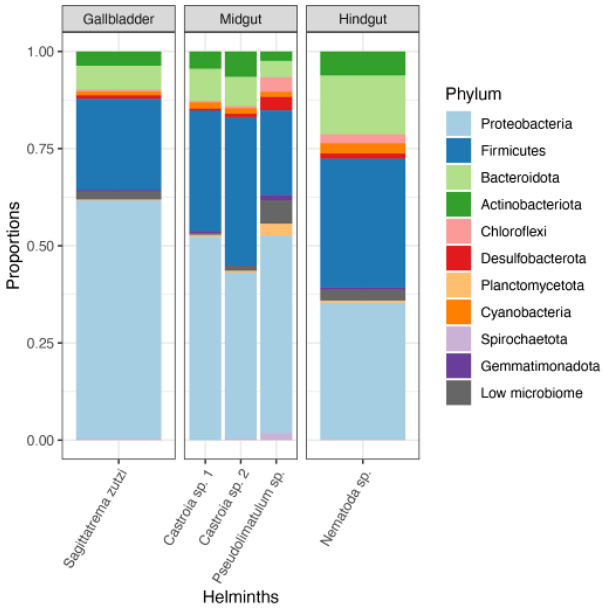
Multiple eco-evolutionary processes work at different spatiotemporal scales and affect vertebrates" development, behavior, and interactions with other organisms. These processes determine the diversity and composition of the intestinal microbiome. Currently, it is known that there is a certain intestinal microbial biogeography, which could be influencing the coexistence of certain groups of metazoan parasites, such as helminths. Helminths, in turn, are also associated with different microbial communities (microbiome), which probably influence host–parasite interactions. However, although helminths represent a large part of the taxonomic diversity of metazoans and have a high value in ecosystems, the factors that influence the structure of their microbiome remain unclear or unknown, mainly those that parasitize wild hosts. Thus, using the V4 region of the 16S rRNA gene, this study examined the microbial diversity of 41 adult helminth individuals from five different species that belong to the Mexican bat *Peropterix kappleri*, which is found in Boca de Chajul, Chiapas. The results show that the biogeography of the gut microbiome of the bat host and the phylogeny of the helminth significantly influenced the diversity and composition of the microbiome associated with them. Microbiome structure dissimilarity increased with increasing intestinal geographic distance and phylogenetic distance from the helminth host. Patterns of phylosymbiosis were observed in helminths belonging to the class Trematoda, which harbor specific microbial taxa at the generic level. The present work provides a robust assessment of the multiple eco-evolutionary mechanisms driving the assembly of microbial community structures associated with bat helminths.

Fig. 1



PS137.II

Target-ligand engagement analysis through cellular thermal shift assay identifies active type-I and -II MAP kinase inhibitors with schistosomicidal effect

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Monitoring target engagement at various stages of drug development is an essential step in drug discovery. Cellular Thermal Shift Assay (CETSA) is an innovative technique used to study protein-ligand interactions within living cells, providing information and insights into the thermal stability of target proteins upon ligand binding. In the context of schistosomiasis, a debilitating parasitic disease caused by pathogenic *Schistosoma*, we have shown that targeting of *S. mansoni* MAP kinases (SmMAPKs) proteins offers a promising therapeutic strategy. Based on *in silico* studies, we identified several active compounds predicted to bind to SmMAPKs (SmJNK, Smp38, SmERK1, and SmERK2). *In vitro* screening assays have confirmed the effects of these compounds on parasite survival, development, and reproduction. However, demonstrating target-ligand engagement becomes a challenge in the study of parasitic proteins, which lack commercially available tools and can be difficult to express recombinantly in sufficient amounts. Here, we applied CETSA to enable the identification of compounds that interact with SmMAPKs by monitoring changes in protein thermal stability upon ligand binding. Since no specific antibody is available against SmMAPKs, we have used transfected Sf21 cells to express recombinant V5-tagged SmMAPKs. This method allowed direct detection of ligand-target engagement on a cellular level without the need for protein purification. We have shown that predicted Type I- and Type II-kinase parasite-specific inhibitors thermally stabilize SmMAPKs similarly to some commercially available human MAPK inhibitors. CETSA's ability to provide direct evidence of target engagement within living cells makes it a powerful tool for drug discovery. In the fight against schistosomiasis, CETSA facilitates the study of novel MAPK ligands, advancing our understanding of parasite biology and contributing to drug development for future therapeutic use. Moreover, this approach exemplifies the integration of such techniques into parasitology research, opening new avenues for combating neglected tropical diseases.

PS140.II

The establishment of long-term cat intestinal organoids allows the study of *Toxoplasma gondii* sexual stages

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Introduction & Objectives: *Toxoplasma gondii* is an apicomplexan parasite whose sexual cycle is restricted to feline hosts. The final sexual stage released into the environment are oocysts which can survive there for extended periods of time and stay infectious. Working on *T. gondii* oocysts is difficult since accessibility to enough material for research is limited and thus animal experiments still remain a requirement. *In vitro* models capable of generating oocysts are therefore urgently needed to advance our understanding of *Toxoplasma*"s environmental life stage.

A decisive breakthrough towards this goal was recently achieved with the identification of two transcription factors (AP2XII-1 and AP2XI-2) responsible for pre-sexual stage formation (Antunes *et al.* 2024). Merozoite formation was observed even in human fibroblast (HFF) cells, but the development of sexual stages appeared to require additional cues. The role of host cell species and specificity has not been investigated in this respect.

Results & Conclusions: Here we report the development of continuous, long-term intestinal organoid (IO) cultures derived from a cat. Compared to other previous reports, our cat IO system is robust, requires no feeder cells and is thus well suited for continuous experiments under constant, comparable conditions.

This system allowed us to test the hypothesis that the cat host cell environment has an influence on the progression of tachyzoites devoid of AP2XII-1 and AP2XI-2 to sexual stages beyond those seen in human fibroblasts. Using a set of stage-specific genes and RT-qPCR as readout, as well as confocal and electron microscopy, we found only slightly elevated levels of these transcripts in cat IOs vs. HFF.

Thus, while sexual stage formation was not (yet) observed in our experiments, this system permits easy modifications of media components and culture conditions to further mimic the physiological situation in the cat gut and to define the missing cues. Our cat IOs will have the potential to shed light on the molecular processes of the pre-sexual and sexual stages in cat intestinal cells.

Reference: Antunes, A.V. et al. (2024) In vitro production of cat-restricted Toxoplasma pre-sexual stages. *Nature*, 625:366-376.

PS143.II

Behind the curtain of the protective effect against leishmaniasis: A new role for eosinophils?

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The protective effect against leishmaniasis is a known phenomenon, as the pathology of cutaneous leishmaniasis, demonstrated in mice and caused by *Leishmania major*, is significantly milder if the mice are repeatedly exposed to the blood feeding of non-infectious *Phlebotomus* sand flies prior to infection. It is generally believed that this protection results from immunization with salivary antigens, which induce a specific cellular immune response characterized by the so-called delayed-type hypersensitivity (DTH) reaction. During DTH, activated proinflammatory macrophages infiltrate the sand fly feeding site, with a characteristic peak at 48 hours after feeding. These activated macrophages produce nitric oxide (NO), a potent leishmanicidal molecule, thereby reducing the parasite load.

However, previous studies assessed this effect only in mice infected at least 7 days after the last exposure to sand fly saliva, not during the peak of the DTH reaction (at day 2 after the last exposure). To fill this gap, we immunized mice twice at weekly intervals and infected them either two (D2) or seven (D7) days after the last immunization. Paradoxically, *L. major* lesions were larger in mice infected at D2, at the peak of expected protective DTH reaction.

In the follow-up study presented here, we focused on the immune response of mice at time points D2 and D7, emphasizing the myeloid leukocyte populations in the ear—macrophages, neutrophils, and eosinophils. Mice underwent three immunization feedings at weekly intervals and were sacrificed either at D2 or D7 post-last feeding.

In line with the above-described paradigm, we demonstrated that leukocytes migrated to the feeding site at D2 in response to the induced delayed-type hypersensitivity (DTH) reaction. Interestingly, using a combination of cytometric and histological approaches, we found that among the three examined cell populations, eosinophils were by far the most abundant. As expected, macrophages, the typical *Leishmania* host cells, were also present in the infiltrate, but surprisingly, they were not polarized proinflammatory as expected in *Leishmania*-protected mice. Neutrophils known for their role in propagating infection, were only rarely present compared to other cell populations. At D7, infiltrated myeloid cells were overall less abundant, but cell population patterns remained consistent.

The dominant presence of eosinophils, coupled with the lack of proinflammatory macrophages, suggests a previously unrecognized role for eosinophils in the protective effect against *Leishmania*. Further experiments investigating this issue are underway in our laboratory.

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PS146.II

A novel strain of *Leishmania braziliensis* harbors not a toti- but a bunyavirus

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Leishmania, a genus of obligate parasitic flagellates in the family Trypanosomatidae, comprises four subgenera: *Viannia, Leishmania, Mundinia,* and *Sauroleishmania*. The first two subgenera are the primary causative agents of leishmaniasis, a neglected vector-borne tropical and subtropical disease with a complex pathology that ranges from self-healing cutaneous sores to terminal failure of visceral organs. Numerous *Leishmania* isolates have been found to harbor Leishmania RNA viruses (LRVs), double-stranded RNA viruses from the family *Pseudototiviridae*. The presence of LRVs has been shown to exacerbate leishmaniasis. Here, we report the first discovery of a virus other than LRV in the *Viannia* subgenus. The novel *L. braziliensis* isolate BO17 harbors a bunyavirus from the family *Leishbuviridae*. Given that viruses can serve as additional virulence factors in pathogenic protists, we encourage researchers to screen *Leishmania* isolates for a broader range of viruses.

Reference:

Kostygov AY, Grybchuk D, Heeren S, Gerasimov ES, Klocek D, Reddy A, Sádlová J, Pacáková L, Kohl A, Stejskal F, Volf P, Dujardin JC, Yurchenko V. A novel strain of *Leishmania braziliensis* harbors not a toti- but a bunyavirus. PLoS Negl Trop Dis. 2024 Dec 27;18(12):e0012767. doi: 10.1371/journal.pntd.0012767.

PS149.II

The role of Schistosoma mansoni MEG proteins during infection

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Schistosomiasis is a parasitic neglected tropical disease affecting over 240 million people annually, primarily in low to middle income countries. One of the main species of Schistosoma responsible for the disease is *Schistosoma mansoni* which is transmitted through infected water. Praziquantel is at present the only drug that effectively kills adult parasites, and no licenced vaccine is currently available. To survive within the vasculature, schistosomes must interact with their host and extracellular parasite antigens are likely to be involved in these interactions.

Micro-exon genes (MEGs) encode for secreted and cell surface protein families, where symmetrical micro-exons (from 6bp up to 81bp) are present in the coding sequence and can give rise to multiple protein isoforms through exon skipping. MEGs also exhibit high non-synonymous/synonymous substitution rates and possibly play a role in immune evasion through alternative splicing and antigenic variation. *S. mansoni* lacking the oesophageal gland (a MEG upregulation hotspot) cannot establish infection in immunosuppressed mice, so it is strongly theorised that the MEGs expressed at the oesophagus play a role in immune evasion or suppression. This theory is supported by the fact that Rhesus macaques exhibit unique *S. mansoni* self-cure and resistance to reinfection through the production of antibodies targeting the oesophageal gland.

Our aim is to characterise the role of *S. mansoni* MEG proteins during mammalian infection. In a first instance, we will aim to identify the MEG transcripts variants expressed at different life stages of the parasite"s life cycle through long read RNA sequencing. These MEGs will then be expressed recombinantly in mammalian cells and screened against a library of human immune receptors using SAVEXIS to identify new host:parasite interactions. The function of these interactions will then be tested in cellular assays and possibly in a murine model of S. mansoni infection.

PS152.II

Development of an episomally expressed membrane marker for *Plasmodium*

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Fluorescence microscopy is a powerful tool for studying *Plasmodium falciparum* parasites, the causative agents of the most severe form of human malaria. Long-term live-cell imaging is often used to analyze the dynamic intracellular processes in the clinically relevant blood-stage of infection. This requires either fluorescent labelling of marker proteins or the use of specific dyes. To stain membranes, Bodipy TR ceramide is commonly used, however, dyes may have adverse effects on cell viability during live-cell imaging. Here, we present FLUMMI (Fixed-and-Live-cell Universal Membrane Marker for Imaging), a 33-amino acid long peptide derived from *P. falciparum* PCNA1, which targets (fluorescent) protein tags to intracellular membranes. Episomally expressed, FLUMMI enables non-invasive, long-term live-cell imaging of membranes without affecting *P. falciparum* viability, when compared to Bodipy TR ceramide staining. Our data indicates that FLUMMI is functional in both, *P. falciparum* and *P. berghei* and it is compatible with advanced techniques such as expansion microscopy. Together, our results suggest that FLUMMI can be used as a versatile and non-toxic tool for membrane imaging in *Plasmodium* research.

PS155.II

Satellite DNA, an alternative diagnostic option for detecting helminth DNA in faeces using loop mediated isothermal amplification (LAMP)

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Introduction: Trichuris trichiura, are one of several parasitic worms termed soil transmitted helminths (STH). The World Health Organisation estimates that 1.5 billion people globally carry an STH infection, with transmission often occurring in impoverished conditions. Arguably the easiest way to detect an STH infection in the field is by microscopy, identifying eggs in faeces, however this method can be insensitive and requires training to analyse samples. Whilst most molecular diagnostics based on DNA amplification are not point of care (POC) friendly due to reliance on a laboratory environment, loop mediated isothermal amplification (LAMP) is becoming increasingly popular as a simpler route for fast detectable DNA amplification in a POC setting. Here we utilise new bioinformatic routes to detect repetitive satellite DNA (satDNA), to see if open-source sequencing data could be used to create a LAMP assay sensitive enough to detect helminth DNA In faeces.

Methodology: To detect satDNA the open-source bioinformatics platform Galaxy, and programme RepeatExplorer2 (RE2) were utilised. Illumina MiSeq T. trichiura paired sequence reads (SRR2968131) were trimmed (fastp), and sub sampled (seqtk) to 2M reads before submission to RE2. Identified high confidence satDNA sequences were screened for LAMP primer design suitability (>200 bp in length, read count >1000, and incorporation of loop primers). Primer sets were designed using the NEB LAMP primer design tool. Primer sets were optimised initially using T. trichiura adult worm DNA with a fluorescent based LAMP, using a standard 65°C reaction for 30 minutes. The most efficient LAMP assay design was then taken on for application in colorimetric LAMP. Lastly the colorimetric LAMP was tested against 55 T. trichiura positive and negative DNA samples extracted from faeces, collected by the University of the Philippines, Los Banos.

Results: Six high confidence satDNA sequences were identified using RepeatExplorer2, two of which were eligible for LAMP primer design. Cluster 19 (C19) (2276 reads, length 361bp) and Cluster 30 (C30) (1498 reads, length of 2535bp). Initial screening and optimisation using fluorescent based LAMP demonstrated C19 as having the higher sensitivity detecting ≤ 3.84 fG/µL and specificity (no detection of Ascaris spp., and Toxocara spp., adult worm DNA). Further to this, the C19 primer set was then applied to a colorimetric LAMP reaction, which was utilised to screen and assess by eye 55 field samples. Of the 55 field samples, the C19 LAMP confirmed detection of 5 T. trichiura positive samples, supporting original real time PCR results. In addition, a further 3 samples tested positive with LAMP, which had not been detected using microscopy or RT-PCR.

Summary: In conclusion it has been demonstrated that satDNA can be mined from publicly available raw sequencing files and utilised successfully for the design of sensitive and specific LAMP assay.

PS158.II

Unravelling *Ascaris*: A pipeline for *de novo* assembly of mitochondrial genomes from low coverage whole genome sequencing data

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Background: *Ascaris lumbricoides* and *Ascaris suum* are intestinal roundworms infecting ~800 million people globally, primarily in low- and middle-income countries. In humans, infections contribute significantly to disability-adjusted life years (DALYs) and malnutrition in children, while in pigs, they reduce feed-to-conversion efficiency. These closely related species are morphologically indistinguishable and capable of cross-transmission and hybridization. Their taxonomic relationship—whether distinct or a single species—remains debated. Studies based on limited mitochondrial and nuclear markers have not resolved this question, emphasizing the need for comprehensive genomic resources.

Due to the low coverage of whole genome sequencing data for *Ascaris*, mitochondrial genomes offer a practical alternative. Unlike single markers or reference-guided approaches, a *de novo* assembly of the entire mitogenome may provide a more comprehensive perspective for characterization of population structure and host associations.

Methods: We generated paired-end Illumina whole genome sequencing (WGS) data from 16 adult *Ascaris* worms from pig and human hosts from the UK, Hungary, Philippines, Thailand, and Uganda. This dataset was supplemented with WGS data from 68 worms obtained from GENBANK representing Kenya.

Reads were subsampled to 40 million per sample and decontaminated using human and porcine genomes, before de novo assembly using MitoZ. Quality control was performed using FastQC and Seqkit. 11 additional preassembled genomes from GENBANK were also included. Mitochondrial genomes were annotated using Mitos2. Additionally, one *Baylisascaris transfuga* and one *Toxocara cati* sample were included as outgroups. Phylogenetic relationships were inferred using MEGA alignments of amino acid sequences to construct phylogenetic trees.

Results: The assembled mitochondrial genomes ranged from 13.8 to 14.5 kb and included all 12 protein-coding genes. Circularization was omitted due to challenges with the high A-T composition of the D-loop, but assemblies were achieved for all samples. Each assembled mitochondrial genome was validated using BLAST, achieving over 95% identity and alignment coverage for all samples.

Phylogenetic trees based on amino acid sequences revealed distinct clades, indicating genetic differentiation between populations. Ongoing analysis aims to further investigate phylogenetic relationships among samples using mitochondrial genome data.

Conclusion: The absence of genomic datasets, coupled with environmental reservoirs, zoonotic transmission, and potential anthelmintic resistance, presents significant barriers to achieving the 2030 elimination targets set by the WHO.

Here, we present a pipeline for the *de novo* assembly of mitochondrial genomes from low-coverage WGS reads, aimed at elucidating *Ascaris* population dynamics and relationships between *A. lumbricoides* and *A. suum*.

PS161.II

Multi-drug tolerance in *Leishmania* persister-like cells

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Persisters are a small fraction of non-proliferative cells with reduced metabolism that are adapted to withstand a variety of environmental assaults, including lethal doses of antibiotics. Here, we present evidence of existence of persister-like cells in protozoan parasite *Leishmania*, induced upon exposure to normally lethal doses of antimonials. We show that *Leishmania* promastigotes survive lethal doses of antimonials by adopting a quiescence phenotype characterised by reduced proliferation, diminished metabolism and a reduced mitochondrial membrane potential. Depending on the presence of genomic pre-adaption to antimonial resistance, two types of persister-like cells were observed for *L. donovani* field isolates. In non-preadapted lines, persister-like cells are transiently tolerant to antimonials and return to the sensitive state upon removal of drug pressure. In contrast, isolates with genetic changes associated with antimony-resistance, resume growth after just a short period of quiescent state and retain their reduced sensitivity to antimonials in drug-free conditions. Both types of persister-like cells display cross-tolerance to a selection of antileishmanial compounds, but the degree of tolerance is drug- and strain-dependent. Our results demonstrate extreme versatility of this eukaryotic pathogen in adaptation to drug pressure and highlight the need for the development of new antileishmanials targeting non-proliferative forms.

PS164.II

Investigating histone lactylation as a novel epigenetic modification in the malaria parasite *P. falciparum*

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In the course of human infection*, Plasmodium falciparum* encounters a dynamic environment influenced by the host"s immune response and metabolic changes. The parasite adapts in part via epigenetic modifications that modulate the transcriptional program, which is essential for *P. falciparum* survival under adverse conditions and results in changes in virulence and differentiation. In severe malaria patients, high parasite density and inflammation contribute to elevated levels of lactate, which may act as a signaling molecule. Recently, histone lactylation has been discovered as a novel regulatory posttranslational modification, but its function in malaria parasites is not well defined.

In this study, we show that high culture density of *P. falciparum* leads to prolonged asexual maturation and a reduced reinvasion rate in comparison to low parasitemia cultures. High density during maturation from the ring to the trophozoite stage resulted in increased histone lactylation and decreased acetylation of the histone variants PfH2A.Z and PfH2B.Z. To explore the role of lactate produced by the parasite metabolism, we inhibited the lactate transporter PfFNT (Lactate/H+ transporter), inducing intracellular lactate buildup in parasites cultured at low density. Indeed, this caused developmental delays and significantly increased histone lactylation. Untargeted metabolomic analyses indicated that high-density and PfFNT-inhibited parasites show disrupted energy metabolism and pathways critical for post-translational modifications, reminiscent of parasites isolated from severe malaria patients. To further investigate the impact of density-related histone modifications on gene expression, we are currently mapping the genome-wide distribution of histone lactylation, acetylation, and methylation under varying growth conditions using ChIP-seq, complemented by RNA-seq to correlate the transcriptional profiles. Additionally, we studied the putative function of the histone acetyltransferase PfGCN5 and the histone deacetylases PfSir2A and PfSir2B in regulating histone lactylation. Conditional knockout of the PfGCN5 bromodomain impaired asexual growth and reduced PfH2B.Z acetylation without affecting histone lactylation. Conversely, knockout of PfSir2A and PfSir2B increased both histone acetylation and lactylation, highlighting their involvement in modulating histone lactylation. These findings give novel insight into the epigenetic changes that occur in response to parasite density during the asexual life cycle and may provide hints as to how parasites survive and adapt under adverse conditions in the host.

PS167.II

Prevalence and risk factors of *Echinococcus multilocularis* infections and other zoonotic parasites in wild living carnivores on the island of Rügen

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The fox tapeworm *Echinococcus multilocularis* is a cestode that lives in the small intestine of its definitive hosts and is known as the causative agent of alveolar echinococcosis in humans. The red fox (*Vulpes vulpes*) contributes significantly to the parasite"s transmission in Europe but other canine species such as raccoon dogs (*Nyctereutes procyonoides*) can also be affected. *E. multilocularis* is endemic in Germany but the current epidemiological situation in Mecklenburg-Western Pomerania especially on the island of Rügen is unknown.

Here we examine foxes and raccoon dogs from Rügen for the presence of *E. multilocularis* with support from the local hunting community over three years. The exact coordinates each animal"s location is recorded in order to enable environmental risk factor analyses using landscape data. Following necropsy, intestinal mucosa, swabs, tissue and serum samples are collected from each animal and examined for various zoonotic pathogens (including also other parasitic infections, e.g. *Trichinella* spp.). Diagnostic testing for the presence of *E. multilocularis* in the carnivore"s intestine is carried out by using microscopic or biomolecular methods.

Since the start of the project in November 2023, 315 foxes and 169 raccoon dogs have already been collected and sampled as part of the study. Results confirm the presence of *E. multilocularis* in foxes on the island of Rügen. The initial analysis of putative risk factors revealed, that increasing proportions of farmland in buffer zones around fox capture sites corelated with an increased risk of *E. multilocularis* infection. By contrast, increasing proportions of wetland protected from infection. These findings uncover the distinct role of landscape composition on *E. multilocularis* infection in carnivores on the island of Rügen.

PS170.II

An update of the epidemiological parameters of malaria in school age children in Kolle, a rural setting, Mali

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Introduction: Kolle is a malaria research site since 1998. Several clinical trials have been conducted at this site. Currently, there is very few of data on the parasitological and entomological parameters of malaria in this area. This suggests a critical need of updating these parameters. We aimed to update the epidemiological and entomological parameters of malaria transmission in Kolle, and to better inform health policy makers and health care providers to fighting against malaria efficiently.

The main objective of this study was to assess the parasitological parameters of malaria transmission during the low-transmission season at the Kollé basic school.

Methodology: A cross-sectional survey was carried out in 205 school age children during the low transmission season (cold dry January 2023) at Kolle Basic School. Parasitological parameters of malaria transmission were assessed. The survey consisted of a complete clinical and laboratory examination.

Results: The prevalence of malaria infection obtained during this cross-sectional survey was 19.0%. Children aged 10 and 12 years were the most affected (24.3%,) followed by children under 5 years (22.2%). The proportion of gametocyte carriage varied with age and was higher in with children under 5 years of age (11.1 %). *P. falciparum* and *P. malariae* were the two most prevalent malaria species with 92.0% and 8% respectively.

Conclusion: *P. falciparum* malaria is still the dominant parasite in endemic areas of Kolle, and older children are becoming more vulnerable to malaria infection.

Key words: parasitological parameters, transmission, malaria, school age, children, Kolle, Mali.

PS173.II

The VEXing question of VSG regulation in the tsetse fly

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Trypanosoma (T) brucei is transmitted between the tsetse fly and the mammalian host. Acquisition of mammalian infectivity occurs in the tsetse fly salivary glands with the expression of a single *variant surface glycoprotein* (VSG) gene in metacyclic (m) cells. Expression of a singular mVSG in mature metacyclics is preceded by low-level expression of multiple mVSG genes in a pre-metacyclic cell. The mechanisms driving the transition from multiple to monoallelic mVSG expression in mature metacyclics, as well the initiation of *VSG* expression as metacyclics differentiated into bloodstream form cells, remain poorly understood.

We have investigated the role of VEX1 during *in vitro* differentiation of metacyclic to bloodstream form cells and observed that constitutive over-expression of VEX1 in the pleomorphic Antat1.1 cell line, promotes a dysregulation of VSG and mVSG expression arguing for a key role of VEX1 in monoallelic VSG expression initiation and maintenance. Interestingly, transcriptomic analysis of these differentiated cells revealed a decrease in S phase related genes and histones when cells differentiate in a VEX1 over-expression background.

Finally, we are using single cell transcriptomics, in combination with *in vitro* and *in vivo* life cycle differentiation, to unravel the transcriptional regulation of *VSG* genes expression. Our data suggests a role of the VEX complex in the metacyclic differentiation process and their capacity to establish infection in the mammalian host.

PS176.II

Cutaneous trypanosome infections drive cell proliferation and tissue remodelling in skin

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In *T. brucei gambiense* endemic areas, high proportions of individuals are serologically positive by CATT test, but have parasitaemia below the detectable threshold by microscopy. However, parasites can be detected in the skin of these seropositive individuals. These carriers of cutaneous trypanosomiasis act as a reservoir of the disease and pose a threat to the WHO elimination roadmap. The prevalence of cutaneous trypanosomiasis, and how host response to chronic infection in the skin remains unknown

Here we have serologically screened 123 individuals from *gambiense* human African trypanosomiasis (gHAT) foci in the Democratic Republic of Congo. Blood samples and skin biopsies were examined for evidence of trypanosomes by molecular and immunohistological methods. RNAseq analysis was conducted on 20 skin biopsies from infected and uninfected individuals to investigate the host dermal response to trypanosome infection.

We found 45% of our cohort were seropositive and had trypanosomes in their skin. Our cohort included 19 individuals with a previous gHAT diagnosis, of whom 73% had trypanosomes in their skin up to 13 years after clinically successful treatment. Host transcriptomic analysis of skin biopsies revealed that individuals clustered by presence of dermal trypanosomes. We found 1207 differentially expressed genes in infected (serology and histology positive) compared to uninfected (serology and histology negative). There were 142 enriched pathways which were predominantly involved in cell proliferation, tissue remodelling, infection and inflammation.

Our results demonstrate that cutaneous trypanosomiasis is widely prevalent in gHAT foci and that "successful" treatment does not guarantee the clearance of dermal trypanosomes. This highlights that skin analysis could be a valuable addition to clinical diagnosis and treatment monitoring of gHAT. This pilot study shows a clear local host response to dermal trypanosomes of hypoxia, wound healing and tissue remodelling.

PS179.II

Investigating the regulation of heterogeneous nuclear accumulation of *Plasmodium falciparum* PCNA1

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Malaria is caused by unicellular eukaryotes of the genus *Plasmodium*. In the pathogenic blood stage, *P. falciparum* proliferates through a process called schizogony. During schizogony, DNA replication and nuclear division occur without cell division, forming a multinucleated cell. Strikingly, the nuclei multiply asynchronously, despite residing in a shared cytoplasm. A hallmark of this asynchrony is the accumulation of the DNA sliding clamp PCNA1 only in those nuclei that replicate their DNA. Our previous data suggest that PCNA1 association with DNA during replication is critical for nuclear accumulation and that this depends on the activity of the kinase CRK4. However, the molecular mechanism regulating nuclear accumulation remains elusive. To better understand the role of CRK4 for the nuclear accumulation of PCNA1, we investigate the localization of replication factor C (RFC). In other eukaryotes, RFC loads PCNA1 onto the DNA during replication, and RFC is likely phosphorylated by CRK4. Unlike PCNA1, RFC appears evenly distributed over time in the nucleoplasm of all nuclei, suggesting that RFC activity, rather than its localization, may be modulated. To test if immediate participation in DNA replication is key for heterogeneous nuclear accumulation, we also investigate the localization of replication protein A (RPA). RPA binds to single-stranded DNA at the replication fork and RPA is also a substrate of CRK4. Interestingly, RPA::GFP shows a focal fluorescence signal and we are currently quantifying its distribution in and among nuclei. Together, our data help to provide deeper insight into how CRK4 regulates nuclear accumulation of PCNA1 and which role its downstream effectors play in asynchronous nuclear multiplication in *P. falciparum* schizonts.

PS182.II

Host phosphoinositide signaling and toxoplasma infection

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Toxoplasma gondii is an intracellular pathogen with an exceptional ability to infect a wide range of nucleated host cells. The parasite subverts the host signaling to ensure efficient infection in human cells. It is known to activate the class I PI3K, and subsequently, the PI3K/AKT pathway, to promote its proliferation within its host cells; however, whether the parasite also co-opts the class II and class III enzymes is unclear. Our work indicates that invading tachyzoites of Toxoplasma recruit specific host phosphoinositide species. Indeed, the lytic cycle of tachyzoites was impaired upon treatment with selected inhibitors of PIK enzymes. To better understand our findings, we are determining the functional landscape and spatiotemporal signaling of PIK in the host cell during the parasite infection.

PS185.II

Prevalence of malaria parasitemia, malnutrition and anemia at the end of the malaria transmission season in Koumantou (Mali) in 2018

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Introduction: Malaria, malnutrition and anemia constitute a major public health problem that states are confronted with and which give rise to numerous interventions. These pathologies are still a major cause of morbidity, mortality and poverty, especially in sub-Saharan Africa, particularly in Mali in the health district of Bougouni.

Methodology: The purpose of this study was to determine the Prevalences of malaria parasitemia, malnutrition and anemia at the end of the malaria transmission season in Koumantou, Mali, in 2018.We conducted a single cross-sectional descriptive epidemiological study in November 2018 with one week of field collection. Anthropometric parameters and hemoglobin levels were collected to assess nutritional status and anemia in children. Thick drop was performed to determine Plasmodium falciparum carriage.

Results: The study included data from 507 children aged 24 to 37 months. The prevalence of malaria parasitemia and moderate acute malnutrition was 6.1 and 1% respectively. The prevalence of anemia was 64.1%. There were no cases of severe acute malnutrition. The gametocyte index was 1.5% in our study population.

Conclusions: At the end of this study we can say that anemia remains a major health problem in our study population. This is in spite of the free and integral medical care of children and the low prevalence of malaria and malnutrition; compared to national data and the classical epidemiology of the study area.

Key words: malaria, anemia, malnutrition, prevalence, Koumantou.

PS188.II

Identification of potent single-domain antibodies against the malaria sporozoite through a synthetic single-domain antibody library containing unconventional diversification strategies

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Malaria, caused by *Plasmodium* parasites, is one of the "Big Three" infectious diseases, together with HIV and TB. With an estimated 263 million cases and 597,000 deaths in 2023, malaria remains an immediate health threat for almost half the world"s population. Problems are worsened due to low-efficacy vaccines and drug-resistant parasites. This clearly indicates that novel intervention strategies are still direly needed. Antibodies (Abs) are potent tools for parasite neutralisation. Besides conventional Abs, the natural immune repertoire of mammals contains so-called unconventional diversification strategies, which extend the coverage of antigen space. Interestingly, unconventional Abs appear to excel in neutralising highly sophisticated pathogens. Camelid single-domain Abs (sdAbs aka nanobodies) are prime examples of such unconventional Ab fragments. Extensive knowledge on the camelid sdAb structure-function relationship enables the construction of highly diverse synthetic libraries that offer several advantages over immune libraries obtained through immunisation. This project aims to harness the potential of synthetic sdAb libraries with unconventional diversification strategies to tackle the malaria sporozoite through an interdisciplinary research approach combining molecular, structural, and parasitological methods. The synthetic sdAb libraries will be constructed by grafting bovine VH CDR3 "stalk-and-knob" and human LAIR1 domains onto the CDR3 of a camelid sdAb scaffold. Key positions in these grafted domains will be varied to generate two synthetic sdAb libraries called "SyCAbs-KNOB" and "SyCAbs-LAIR1". The design of SyCAbs-LAIR1 is finished. Non-randomised representatives from the SyCAbs-LAIR1 library with different linkers were tested for their recombinant production in and subsequent purification from *E. coli,* thermostability by thermal shift assay (TSA), flexibility and structural integrity by small-angle X-ray scattering (SAXS), and antigen interaction by isothermal titration calorimetry (ITC). The design of SyCAbs-KNOB is currently ongoing.

PS191.II

Identification of molecular targets of anthelmintics – Optimization of new procedures

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Infection of ruminants with parasitic nematodes poses a significant challenge on agricultural establishments. The limited number of approved drugs, i.e. anthelmintics, are ineffective due to the emergence of drug resistance, which complicates the treatment of helminthiasis. Therefore, there is an urgent need to identify new molecules with a novel mechanism of action and new targets that will also be effective against nematodes resistant to the drugs currently in use. The present project aims to introduce and refine a methodology for identifying molecular targets of anthelmintics. To optimise the process, albendazole, an anthelmintic with a known molecular target (β-tubulin), has been selected as a model drug. The detection of interacting proteins, including the predicted β-tubulin, will be achieved through a pull-down approach, employing a probe with a bound alkyne for capture. Following binding to the protein, the alkynated drug can be captured using the so-called click chemistry principle on azide-bound biotin and subsequently captured on magnetic beads coated with streptavidin, which exhibits a high affinity for biotin. Advances in mass spectrometry and bioinformatics have made it possible to identify the actual interacting partners of such a probe. The identification of the molecular target of the used or potential drugs can facilitate the development of new drugs and modifications to existing drugs to increase their effectiveness.

PS194.II

The role of Ku80 and TERT in telomere dynamics in *Leishmania mexicana* and alternative lengthening mechanism

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Telomeres are essential protective "caps" in eukaryotic chromosome ends, by preventing chromosome degradation, misrecognition as DNA breaks thus ensuring proper chromosomal integrity. While telomeres are typically maintained by telomerase reverse transcriptase (TERT), some organisms, such as *Leishmania mexicana*, can employ the Alternative Lengthening of Telomeres (ALT) pathway, a telomerase-independent mechanism based on homology-directed repair. Our study reveals that ablation of *Ku80*, *TERT*, or both genes in *L. mexicana* triggers a transition to ALT without a critical telomere shortening phase. This transition is likely facilitated by the species" high recombination propensity and the complex arrangement of terminal chromosome repeats, comprising tandem arrays of telomere and sub-telomeric satellite sequences. Such a telomere structure is characteristic of ALT survivors in other organisms and underscores *L. mexicana*"s unique ability to switch seamlessly between telomerase-dependent and -independent telomere maintenance pathways.

PS197.II

Murine schistosomiasis associated with horizontal gene transfer in the gut microbiota

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Schistosomiasis is a neglected tropical disease that affects over 250 million people worldwide. Recent evidence suggests that Schistosoma mansoni infection influences Horizontal Gene Transfer (HGT) in the gut microbiota. To test this hypothesis, we investigated the impact of S. mansoni infection on Insertion Sequence (IS) elements - one of the key mediators of HGT - and the functional potential of the gut microbiota in both Wild-Type (WT) and Human Microbiome-Associated (HMA) mice. Using the pseudoR metagenomics pipeline (Kirsch et al., 2024), we analysed IS element activity and its tentative association with changes observed in the gut microbiome in the context of schistosomiasis its contribution to the changes observed in the microbiome. Illumina short-read data were assembled into contigs using MEGAHIT (Li et al., 2015), with bacterial and viral species identified using Kraken2 (Wood et al., 2019). IS elements were identified by mapping unmapped reads to the ISOSDB database, while open reading frames (ORFs) were predicted using Prodigal (Hyatt et al., 2010). Statistical analyses quantified IS activity and its role in HGT across experimental groups. Our results suggest that IS elements drive HGT by targeting accessory genes that may be essential for bacterial adaptation. HMA mice infected with S. mansoni exhibited significantly higher IS activity (1775 IS events) compared to uninfected controls (1149 IS events), whereas WT mice displayed lower IS activity, with 780 and 675 IS events in infected and uninfected animals, respectively. IS insertions were stable and transmissible between microorganisms, supporting their critical role in bacterial genome diversification. The number of unique IS insertions per sample was higher in WT mice, with infection exerting a limited effect (24.25 in infected vs. 24.33 in uninfected). In contrast, HMA mice showed a reduction in unique IS elements with infection (20.63 vs. 19.67). Additionally, IS activity was observed in viral populations, suggesting cross-domain HGT events. This study establishes IS elements as key facilitators of HGT and bacterial genome evolution within the microbiota, providing a framework for understanding how microbiota dynamics are influenced by S. mansoni infection.

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PS200.II

Trypanosoma brucei collectives as active fluids

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We have studied the motility of unicellular, flagellate parasites in dense collectives on hydrogel surfaces. The microswimmers exhibit a highly motile swimming behaviour in tightly packed swarms. The collective migration of these swarms on the semi-solid surfaces produces striking patterns reminiscent of viscous fingering or swarming instabilities of bacteria.

The analyses of single cell motility in relationship to collective migration, showed it to be unlikely that trypanosome motility alone is responsible for the specific higher-order swarm behaviour. Rather, we observe physiochemical parameters to have a major influence on the collective behaviour. Hydrophobic interactions caused by the lipid composition of the environment, for example, need to be considered when analysing swarm migration in social motility assays.

The concentrated suspension of cells spreading on the hydrogel surface can thus be treated as a drop of active fluid, that is driven by the general, energy consuming, persistently motile nature of the trypanosomes, but directed by environmental factors that dominate the collective motion. We control different states of cell order (e. g. nematic) in the fluid by changing the gel surface and thus the fluid characteristics in the swarm.

Social motility assays are regarded as a proxy for parasitic success in the host, i.e. infectiousness, therefore a better understanding of the physical nature of these biologically active fluids is fundamental.

PS203.II

Role of human miRNAs in the pathogenesis of human malaria caused by *Plasmodium falciparum* infection

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Malaria infection is known to severely compromise endothelial cells (EC). The pathogenic consequences of ECs activation might lead to organ failure and death. The organ-specific response of the endothelium has not been described in detail. It is not known whether microRNAs play a specific role in the complications of severe malaria, although microRNAs control 60% of the genes expressed in the human body.
In this study, we report microRNA candidates that are specifically expressed in both ECs types and their secreted extracellular vesicles (EVs). We were able to show that shear stress plays a role in the switching on of variable signaling pathways in brain ECs, such as IL-8 signaling and tight junctions. Specific miRNAs that we found to be differentially expressed in the ECs seem to control these pathways. Incubation with ring stage infected red blood cells (iRBCs) results in activation of endocytic pathways in brain ECs. In contrast, in lung cells, the most prominent activated pathway was the electron transport pathway which was found to be activated in immune cells during inflammation. Data analysis showed that endocytosis and electron transport pathways were targeted by some miRNA candidates. These miRNAs were significantly altered after 8 hours of coincubation with ring stage iRBCs at a shear stress of 1.5 dyne/cm2.
We hypothesize that EC dysfunction is a precursor to severe malaria complications. This disrupts the balance between vasoconstriction and vasodilation, predisposing to cytoadhesion of iRBCs, endothelial proliferation and blood brain barrier leakage. Certain miRNA candidates may be involved in the control of these events within ECs. Through cell-to-cell communication between iRBCs and ECs, these miRNAs can sense the presence of iRBCs and thus stimulate the initiating signaling pathways to respond to infection.

Fig. 1

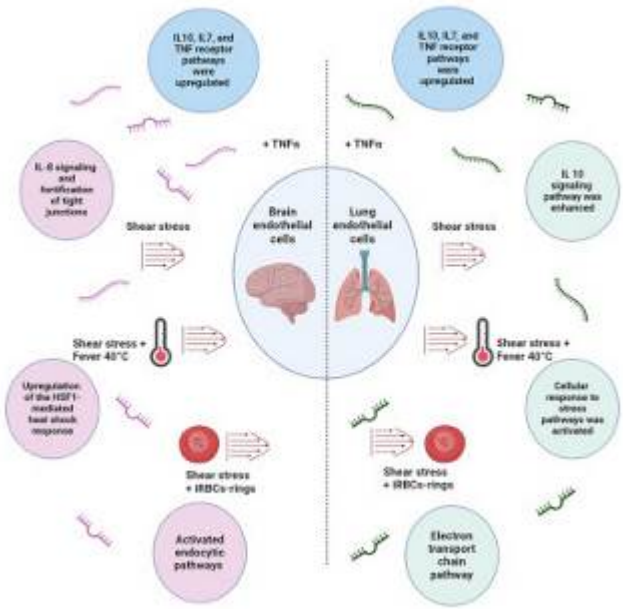
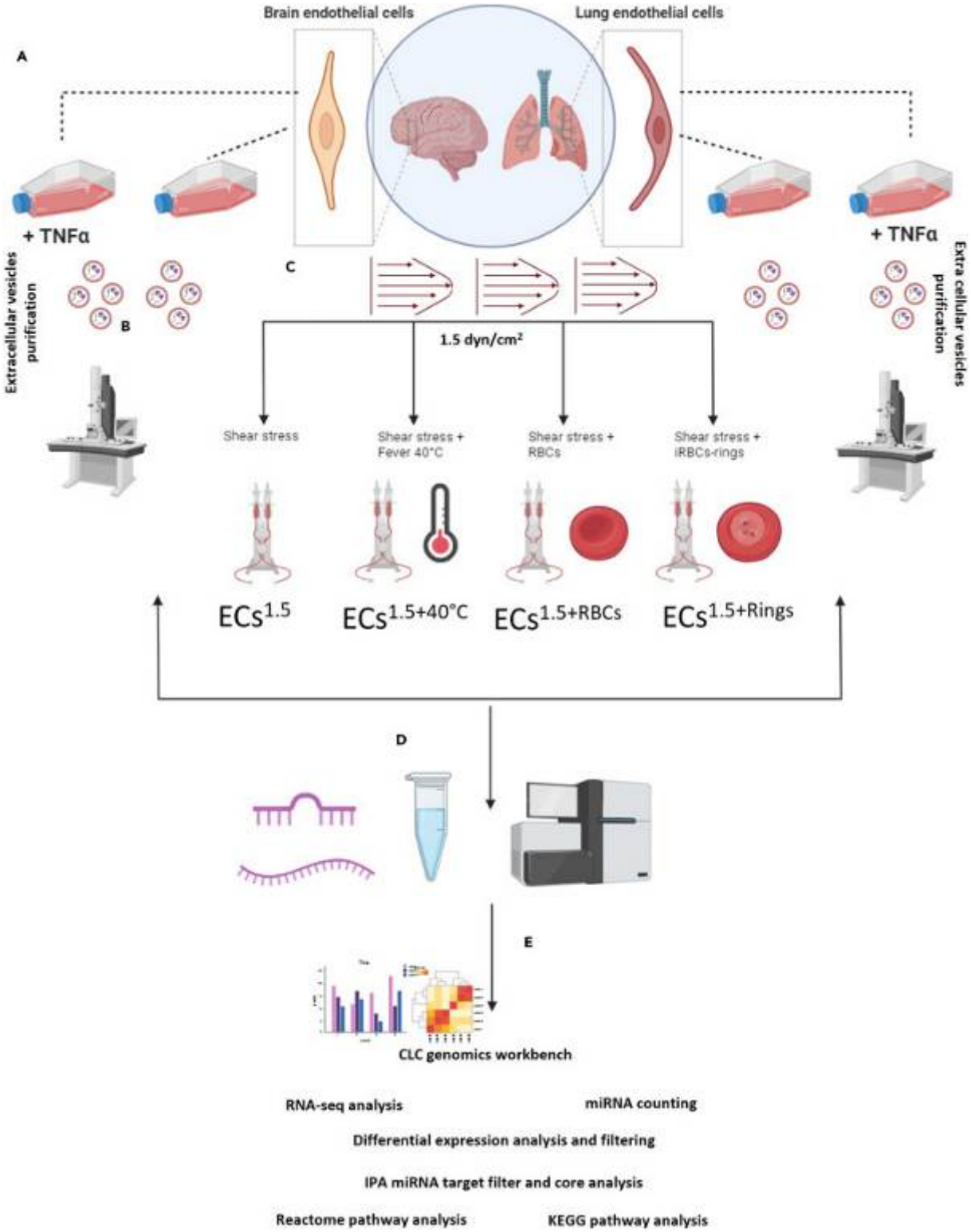


Fig. 2


Brain endothelial cells
Lung endothelial cells
A
+ TNFα
+ TNFα
Extracellular vesicles purification
Extra cellular vesicles purification
B
C
1.5 dyn/cm²
Shear stress
Shear stress + Fever 40°C
Shear stress + RBCs
Shear stress + iRBCs-rings
ECs1.5
ECs1.5+40°C
ECs1.5+RBCs
ECs1.5+Rings
D
E
CLC genomics workbench
RNA-seq analysis
miRNA counting
Differential expression analysis and filtering
IPA miRNA target filter and core analysis
Reactome pathway analysis
KEGG pathway analysis

PS206.II

Biophysical analysis of an oligomerization-attenuated variant of the *Leishmania donovani* dynamin-1-like protein

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Chemotherapy is a cornerstone in the battle against leishmaniasis, a neglected tropical disease caused by *Leishmania* parasites that affects millions worldwide. Unfortunately, an alarming number of reports are describing treatment failure with currently available drugs, which can be traced back to three main mechanisms employed by the parasite to cope with the exposure to chemotherapy: drug resistance (DR), hiding in so-called "sanctuary sites" and parasite quiescence. Given that the current number of anti-leishmanial treatment options is limited and that those available are unsatisfactory, there is a dire need for the discovery of novel compounds, preferably with yet unexplored modes of action. In this quest, a promising lead (TCMDC-143345) from the GlaxoSmithKline (GSK) "Leishbox" was identified and recently explored in a prospective *L. donovani* DR study. The study revealed a clear role for the *L. donovani* dynamin-1 like protein (*Ldo*DLP1) as a resistance marker. It is our aim to further biophysically and functionally characterize *Ldo*DLP1 through a combination of biochemical, structural, biophysical and parasitological methods. We were able to show that wild-type *Ldo*DLP1 has a strong inherent propensity to self-assemble into higher-order oligomers and attempted to produce self-assembly impaired mutants to obtain *Ldo*DLP1 dimers and/or tetramers. A selection of nine-point mutations, including resistance markers, were screened for oligomerization behavior through analytical gel filtration, leading to the identification of a double mutant (G354D/R357S) in the stalk domain. This mutant exhibits significantly reduced (yet not completely abolished) oligomerization behavior. Further biophysical characterization of the *Ldo*DLP1 G354D/R357S double mutant using mass photometry and small-angle X-ray scattering (SAXS) reveals that the protein predominantly occurs as a dimer in solution. Additionally, SAXS analysis experimentally confirms that the *Ldo*DLP1 pleckstrin homology domain is intrinsically disordered, which appears to be a feature typical of dynamins involved in mitochondrial fission, thereby strengthening the hypothesis that *Ldo*DLP1 is most likely involved in regulating the dynamics of fission in the parasite"s single large mitochondrion.

PS209.II

Species-specific diagnosis of porcine cysticercosis using Loop-mediated isothermal amplification (LAMP)

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Background: Cysticercosis is a neglected zoonotic disease caused by larval stages of *Taenia solium* with a significant impact on public health (neurocysticercosis in the brain) and the livelihoods of small-scale pig farmers in many low-middle-income countries (LMIC). Diagnosis of porcine cysticercosis is mainly based on tongue palpation and carcass inspection, complemented by IgG-based serological diagnosis. However, the latter has serious drawbacks, particularly regarding the specificity at the species level. The WHO has called for the development of point-of-care (POC) diagnostic tests for field use to control *T. solium* cysticercosis in endemic countries by 2030. Loop-mediated isothermal amplification (LAMP) is based on isothermal technology, which can be run with minimal settings for POC test development.

Objective: In our study, we targeted the cytochrome c oxidase subunit 1 (*cox*1) gene of *T. solium* and *T. hydatigena* to develop a loop-mediated isothermal amplification (LAMP) for species-specific diagnosis of porcine cysticercosis (PCC).

Methods: A LAMP assay was developed for PCC in the Giessen laboratory, and 47 experiments were carried out initially using cyst DNA and then spiking negative pig serum/human saliva samples with cyst-derived DNA of *T. solium* or *T. hydatigena* with species-specific *cox*1-LAMP primers. The lab-based LAMP assay was then used to screen 118 archived samples (serum, saliva, and cyst fluid) from pigs in endemic areas at the CDL, Makerere University, Uganda, to evaluate its sensitivity and specificity. All archived pig sera were previously tested with a genus-specific commercial apDia Ag ELISA kit, while cyst fluid and pig saliva were collected from carcass dissection-positive pigs. An additional 35 field samples (whole blood, serum, plasma, and saliva) were collected from tongue cyst-positive pigs from Lamwo, Uganda, and tested by PCC-LAMP assay.

Results: From the lab-based study, we found species-specific results for cyst materials and negative pig serum/human saliva spiked with *T. solium* and *T. hydatigena* DNA without false-positive reactions. LAMP can detect up to 0.1 ng/µL DNA for *T. solium* and 0.01ng/µL DNA for *T. hydatigena*. All cyst fluid showed positive reactions with species specificity. However, LAMP showed negative results for apDia ELISA-positive serum, and serum, saliva, whole blood, and plasma from tongue cyst-positive pigs.

Conclusion: While our *cox*1-based LAMP can differentiate *T. solium* from *T. hydatigena*, the negative response using apDia kit/tongue cyst-positive pig samples suggests that either no parasite DNA is present in these fluids or low quantity of DNA that a DNA enrichment step is needed prior to LAMP testing.

PS212.II

Prevalence of waterborne protozoa: Insights from drinking water and stool samples in Faisalabad and Sheikhupura, Punjab, Pakistan

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This study was conducted to evaluate the prevalence of waterborne protozoa in drinking water and stool samples collected from patients suspected of intestinal infections in Faisalabad and Sheikhupura districts of Punjab, Pakistan. A total of 200 water samples were collected from various sources, including domestic supplies and canals, in the selected study areas and analyzed at the KBCMA College of Veterinary and Animal Sciences, Narowal. Simultaneously, 200 stool samples from suspected patients were processed in the Parasitology Laboratory. Stool samples were examined microscopically using iodine and modified acid-fast staining techniques. The overall prevalence of protozoa in water samples was 15%, with Sheikhupura showing a higher prevalence (20%) compared to Faisalabad (10%). Among the water sources, canal water showed the highest prevalence (40%). In stool samples, an overall prevalence of 22% was observed, with a higher rate in Sheikhupura (27%) compared to Faisalabad. Children aged 11-20 years were more susceptible to infection compared to adults. A seasonal variation was noted, with the highest prevalence in August (27.5%) and the lowest in July (17.5%). *Entamoeba histolytica* was the most prevalent protozoan (17%), followed by *Cryptosporidium* (7.5%), and G*iardia lamblia* (5.5%). Statistical analysis indicated no significant difference ($P > 0.05$) between the prevalence rates in water and stool samples across the two districts. The findings highlight the need for effective measures to ensure the safety of water sources and improve hygiene practices to control the transmission of waterborne protozoa, particularly in areas reliant on untreated water supplies.

PS215.II

Integrating high-resolution DIA-based proteomics and locus-specific approaches to uncover chromatin dynamics across the life forms of *Trypanosoma cruzi*

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Trypanosoma cruzi, the causative agent of Chagas disease, undergoes a complex developmental program, transitioning between replicative forms (epimastigotes and amastigotes) and non-replicative forms (metacyclics and trypomastigotes). Each developmental form displays distinct morphological and biochemical traits critical for the parasite's adaptation, survival, and infectivity in both its insect vector and mammalian hosts. Proteomic profiling provides valuable insights into gene regulation and protein function, helping to unravel the molecular mechanisms underlying its differentiation, adaptation, and pathogenicity. In this study, we aimed to identify stage-specific proteins in *T. cruz*i by employing a Data-Independent Acquisition (DIA)-based proteomics approach to analyze its distinct forms: epimastigotes (E), metacyclic trypomastigotes (MT), and tissue-culture-derived trypomastigotes (TCT). We identified 6,828 protein groups from the annotated *T. cruzi* genome (TcDM28C/2018). A total of 1,172 differentially expressed proteins (DEPs) were identified across the stages, with the most significant differences observed between E vs. MT and E vs. TCT. In both comparisons, epimastigotes exhibited enrichment in Gene Ontology (GO) terms related to biosynthesis and cell proliferation, while non-replicative forms were more associated with survival adaptations, preparation for infection, and host interaction mechanisms. Proteomic profiling not only provides insights into stage-specific protein expression but also illuminates the regulation of transcriptional networks, as many of the identified proteins are involved in key regulatory processes. While gene expression regulation in *T. cruzi* is primarily driven by post-transcriptional mechanisms, recent evidence suggests that genome organization may play a role in modulating transcription at specific loci. To explore this further, we are integrating proteomics, nascent RNA analysis, and locus-specific chromatin immunoprecipitation using dCas9-Flag protein. Additionally, we have developed *T. cruzi* expressing dCas9 to target key loci such as the spliced leader (SL) locus and divergent strand switch regions (dSSRs), using enChIP/CLASP. This approach will allow us to identify proteins and chromatin features associated with transcriptional hotspots. These findings will provide valuable insights into the interplay between chromatin and transcriptional regulation, enhancing our understanding of gene regulation in *T. cruzi*. The use of DIA-based proteomics is particularly advantageous in this context, offering high-resolution, quantitative data that can uncover key regulatory proteins and pathways involved in cellular processes. This approach provides a robust platform for studying gene expression regulation and its impact on the parasite"s biology, ultimately advancing our understanding of the molecular mechanisms governing *T. cruzi* development.

PS218.II

Species diversity and host preferences of phlebotomine sand flies in Algeria

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Leishmaniases are diseases caused by parasitic protozoans of the genus *Leishmania*. All *Leishmania* species infecting humans have a digenetic life cycle, circulating between blood feeding sand flies (Diptera: Phlebotominae) and vertebrate hosts, including domestic animals and humans, thus establishing complex and locally varying transmission cycles. Species identification and the determination of blood meal sources in hematophagous insects is an essential parameter to understanding the interaction between sand fly and host and to design correct and efficient disease control strategies.

In the present study [1], entomological surveys were conducted in two leishmaniasis foci in Algeria, Ghardaia and Illizi, located in the north and central Sahara, respectively. Algeria is reported as one of the most affected countries by human cutaneous leishmaniasis. To reveal the role of the local sand fly fauna in the transmission of *Leishmania* parasites, the collected sand flies were identified by a combination of morphological analysis and molecular techniques such as DNA sequencing and MALDI-TOF protein profiling. The engorged females were screened for *Leishmania* infection and also analyzed by peptide mass mapping using MALDI-TOF mass spectrometry to determine their blood meal sources.

In total, 640 sand fly specimens were collected in the two endemic areas; 430 specimens were from Illizi and 210 from Ghardaia province. Altogether, 14 different species were recorded in the study, 6 belonging to *Phlebotomus* and 8 to *Sergentomyia* genus. *Sergentomyia antennata* and *Se. fallax* were the most abundant species in Ghardaia, and *Ph. papatasi* and *Ph. alexandri* were the most common in Illizi. Blood meal analysis of the engorged females revealed various animal hosts such as camel, sheep, goat, donkey, horse, dog, and chicken, but also humans for *Phlebotomus papatasi* and *Ph. alexandri*, suggesting that these vector species are opportunistic feeders. In addition, the integrative approach that combined morphological analysis, sequencing of DNA markers, and MALDI-TOF protein profiling enabled the recognition and formal description of a new *Sergentomyia* species, *Se. imihra* n. sp., raising the number of the Algerian sand fly fauna to 27 species. The study showed the potential of combining traditional morphological analysis and complementary molecular approaches in field surveys of sand flies, which might be extended to other arthropods of medical interest.

[1] Benallal et al. Parasites & Vectors (2024) 17:449

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PS221.II

Establishing a Cas13 knockdown system in the malaria-causing parasite *Plasmodium berghei*

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The malaria-causing parasite *Plasmodium* circulates between vertebrate and mosquito hosts during its lifecycle. The early transmission stages in the mosquito are a bottleneck in the parasite life cycle and therefore might hide valuable targets for antimalarial strategies. However, gene annotations in *Plasmodium* remain incomplete and genetic tools to study gene function are limited, especially during the diploid mosquito stages. In contrast, in vertebrate-infecting, haploid blood stages, gene knockout is established utilising for example CRISPR/Cas9 gene targeting. Yet genes that are essential for blood stage development cannot be targeted this way, and thus also cannot be functionally characterised in subsequent mosquito stages. Forward genetic screens are additionally hampered by the fact that *Plasmodium* lack the non-homologous end joining machinery, and homology directed repair templates are therefore required to knock out genes or introduce mutations. Furthermore, the sexual replication in the mosquito midgut precludes the characterisation of pooled libraries of knockout parasites in mosquito stages. To overcome these hurdles, we here aim to establish a Cas13-based knockdown system in the rodent-infecting malaria parasite *Plasmodium berghei* that should enable translational gene silencing both in haploid blood stages and in the diploid mosquito stages.

Cas13 is a guide RNA-directed endonuclease that targets mRNA for cleavage, resulting in mRNA degradation and consequently reduction of protein levels. Besides cleaving its direct target, Cas13 proteins are also known to non-specifically cleave surrounding RNA, causing collateral damage that can affect cell viability. Recently, high-fidelity variants of Cas13 have been described that show reduced collateral damage making them potentially suitable for a precise knockdown system. To identify a Cas13 variant that is tolerated by *Plasmodium*, we established multiple Cas13-expressing parasite lines and characterised them throughout the life cycle. We identified a high-fidelity Cas13 variant that could be expressed at high levels in the parasite without affecting life cycle progression. Using this parasite line, we successfully generated a proof-of-concept knockdown of a constitutively expressed fluorescent marker by targeting it with three guide RNAs simultaneously. We are currently investigating the presence of collateral damage in this system and further testing the knockdown of endogenous genes. Establishing a Cas13 knockdown system would enable gene silencing in diploid mosquito stages without the need for homology repair templates, which will help to understand the genetic determinants of parasite development in the mosquito host.

PS224.II

Argonaute proteins in the Nematoda phylum

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Argonaute proteins are central to RNA interference (RNAi) across all domains of life. In the Nematoda phylum, the Argonaute gene family has undergone substantial expansion compared to other eukaryotic phyla, likely to support the amplification of RNAi silencing through the production of secondary small RNAs by RNA-dependent RNA polymerases. These secondary small RNAs are bound by worm-specific Argonaute proteins (WAGOs), which are unique to nematodes and lack direct homologs outside of this phylum.

To investigate the expansion of the Argonaute gene family in nematodes, we collected amino acid sequences annotated with functional Argonaute domains from across the phylum and inferred their phylogenetic relationships. Our study includes 2,605 sequences from 157 nematode species, along with four non-nematode outgroup species and three nematomorph species. Using the well-characterized RNAi pathways in the model organism *Caenorhabditis elegans*, we annotated uncharacterized Argonaute proteins into clades corresponding to these pathways.

Our results highlight a significant expansion of Argonaute proteins in species belonging to clades IV and V, while RNAi pathways involving WAGOs appear absent in clade I species. Notably, the ALG-3/ALG-4 pathway, associated with spermatogenesis in *C. elegans*, has undergone significant expansion in the genera *Trichinella* and *Trichuris*. To explore whether this expansion in *Trichuris suis* moderates processes related to spermatogenesis or represents a novel functional adaptation, we utilized RNA and small RNA sequencing datasets from male and female *T. suis*.

This study provides insights into the evolutionary diversification of RNAi pathways in nematodes, shedding light on lineage-specific adaptations and the functional roles of expanded Argonaute proteins in parasitic nematodes.

PS227.II

Association of the bovine leukocyte antigen major histocompatibility complex exon II *DRB3*015:01* to host susceptibility to *Candidatus Mycoplasma haemobos* infection in Bali cattle

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The bovine leukocyte antigen (BoLA) gene is a major genetic component of the immune system and has been used as a disease marker and immunological trait in cattle due to its major function in pathogen recognition. In this study, the 16SrRNA gene of *Candidatus Mycoplasma haemobos* was detected in 37 out of 40 (75 %) Bali cattle. The allelic association of the *BoLA-DRB3* gene with resistance and susceptibility to *C. Mycoplasma haemobos* infection was evaluated in the affected cattle. The association between an allele and *C. Mycoplasma haemobos* were evaluated by Fisher"s exact and Cochran Mantel Haenszel (CMH) test. The odds ratios (OR) and their 95% confidence intervals for susceptibility or resistance were calculated for *each* allele. The Bonferroni correction procedure was performed to adjust the false-positive rate. Association tests having a significance level of $p < 0.0125$ (corrected *p*-value) were considered statistically significant. The PCR-sequence based typing of the *BoLA-DRB3.2* gene from Bali cattle revealed that the gene is highly polymorphic. Six novel alleles were detected (*BoLA-DRB3*007:07, *015:10, *017:06, *018:05, *166:01, *167:01)* in the Bali cattle*,* and these alleles shared about 90.7-95.8% and 85-92% nucleotide and amino acid identities respectively, with the *BoLA-DRB3*016:01* cDNA clone NR-1. Three alleles were identified in the *C. Mycoplasma haemobos* infected cattle namely *DRB3*.2**015:01, *017:01* and **036:01.* The associated allele of *Candidatus Mycoplasma haemobos* infection susceptibility in the Bali cattle was *DRB3*015:01* (OR = 10.500; PCMH = 0.008). None of the alleles were classified as *C. Mycoplasma haemobos* resistance allele. Therefore, this study identified *BoLA-DRB3.2* alleles associated with susceptibility to *Candidatus Mycoplasma haemobos* infection and suggests that genetic selection favouring *BoLA-DRB3.2* susceptibility alleles could be used as candidate markers for culling in the cattle livestock industry. Also, these susceptibility alleles could be used to maintain pathogen-specific memories in the herd.

PS230.II

Elucidating the molecular basis for the recognition of human basigin by *Plasmodium vivax* tryptophan-rich antigens

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Malaria, caused by *Plasmodium* parasites, is one of the 'Big Three' infectious diseases. Each year more than 200 million cases are documented, including more than half a million deaths (>76% of the deceased are children under the age of five). *P. vivax* is the most widespread human-infective malaria parasite and severe cases are increasingly reported. Despite having a severe socio-economic impact on large parts of the world, the progress in battling *P. vivax* is slow. Problems are worsened due to low-efficacy vaccines, drug-resistant parasites and global disease (re-) emergence. This calls for active research into *P. vivax* biology. Invasion of a host reticulocyte (retic) by the merozoite (MRZ) is an essential event in the parasite"s life cycle. Yet, our understanding of interactions at the MRZ-retic interface is limited. The PvTRAgs are MRZ surface antigens mediating retic binding. PvTRAg35.2 and PvTRAg38 are known to interact with basigin. Many aspects of these basigin binding PvTRAgs are yet to be investigated: i) the structural basis for basigin recognition is unknown, ii) the molecular determinants underlying the versatility displayed by PvTRAg-basigin interactions remain enigmatic, and iii) how these events relate to retic invasion is unclear. Given the knowledge gap in *P. vivax* biology and the importance of PvTRAgs in MRZ biology, tackling these issues is expected to generate many novel findings that may support *P. vivax* specific vaccine design efforts. The recombinant production and purification of these PvTRAgs and the human basigin ectodomain (hBSG) have been established within our research group. Furthermore, the first steps towards unraveling the molecular basis for the interaction between PvTRAg.38 and hBSG have been taken, by grating coupled interferometry (GCI), isothermal titration calorimetry (ITC) and macromolecular X-ray crystallography.

PS233.II

A predictive approach to understanding *Angiostrongylus cantonensis* in the Canary Islands

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Angiostrongylus cantonensis is a zoonotic nematode causing neurological disorders in mammals including humans, and birds. Originally endemic to Southeast Asia, this parasite has expanded its range across tropical and subtropical regions worldwide and is now approaching Europe. Tenerife has emerged as one of the most significant European hotspots for this species.

Through extensive field research on Tenerife, we investigated key host groups and detected the nematode across the island. It was found in both endemic and introduced gastropods (intermediate hosts; overall prevalence detected 25.6% (95%CI 22.5-29.1) 179/698), as well as in definitive hosts (rats; prevalence 21.5% (95%CI 13.7-32.2) 14/79) and paratenic hosts (lizards; prevalence 24% (95%CI, 17.4-32.1) 31/129). Prevalence varied significantly across localities, ranging from 41.60% in Anaga in the humid north to 2.44% in Valle San Lorenzo in the arid south. Using the data from the field survey, we developed MaxEnt and Boosted Regression Trees (BRT) models to predict conditions for the parasite"s presence and prevalence across the Canary Islands.

Our models revealed that the distribution of *A. cantonensis* is driven mainly by precipitation seasonality, while its prevalence by Tree Cover Density, followed by precipitation and temperature seasonality. These conditions are observed not only in northeastern Tenerife but also on La Gomera, La Palma, and El Hierro. We also identified areas where the predicted presence of the nematode overlaps with dense human populations (urban areas), highlighting locations with the greatest potential risk of human contact with the parasite. These areas overlap only to a small extent in the northeastern outcrop of the island. This limited overlap could be a contributing factor to the absence of reported human cases of eosinophilic meningitis in Tenerife to date.

To evaluate the similarity of the Canary Islands climate to Hawaii (another island ecosystem with *A. cantonensis* imported, but with numerous reported human cases), we conducted a MESS (Multivariate Environmental Similarity Surface) analysis using selected bioclimatic variables to identify areas of environmental similarity and novelty relative to training data from Hawaii. The analysis revealed moderate to high environmental similarity on Tenerife, with the exception of its northeastern tip, where *A. cantonensis* presence is highest; this area exhibited novel conditions outside the range of the training data. High similarity was also observed on El Hierro and Gran Canaria.

Figure legend: Projection of MaxEnt model results predicting the presence of *A. cantonensis* on Tenerife. Predicted presence is visualized with urban areas overlaid in gray.

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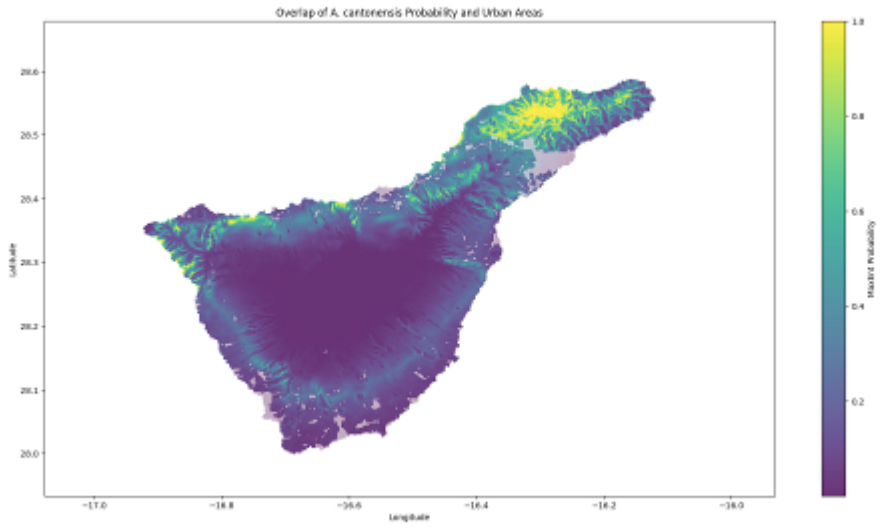
Fig. 1


Overlap of A. canterensis Probability and Urban Areas
Latitude
Longitude
Habitat Probability

PS236.II

Rising threats to Antarctic penguins: Prevalence and implications of *Ixodes uriae* infestations

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Antarctica is increasingly affected by climate change, with rising temperatures and prolonged summers creating favourable conditions for parasitic vectors such as the seabird tick *Ixodes uriae*. These ticks pose a threat to Antarctic wildlife and the health of personnel stationed in the region. This study investigated trends in tick prevalence among penguin colonies and the factors influencing their distribution.

Fieldwork was conducted over two austral summer seasons (2022/23 and 2023/24) across the Antarctic Peninsula, focusing on accessible colonies in the South Shetland Islands. Ticks were collected from beneath stones within rookeries of *Pygoscelis* penguins (Adélie, chinstrap, and gentoo). Prevalence was compared with prior surveys, and colony characteristics such as size and species composition were evaluated.

All developmental stages of ticks were identified in surveyed colonies. Female ticks infested both adults and chicks, whereas nymphs and larvae were predominantly found on chicks. The prevalence of ticks was highest during the guard phase of host species, with gentoo (58%) and chinstrap (50%) penguins exhibiting the greatest infestations, while Adélie colonies showed lower prevalence (28%). Tick abundance ranged from 1 to over 2,000 individuals per stone.

Significant increases in tick prevalence were observed compared to earlier studies, particularly among chinstrap penguins (from 26% to 32%). Additionally, ticks were detected in new locations, including Cierva Cove, Penguin Island, Yalour Island, Lions Rump, Turret Point, and Patelnia/Uchatka Point. As *I. uriae* is a competent vector for various pathogens, including *Borrelia*, *Babesia*, *Rickettsia*, and viruses, these findings highlight potential risks to penguin fitness and survival.

Future research should focus on the prevalence of vector-borne pathogens and their impacts on Antarctic ecosystems to inform conservation strategies.

This study received funding from the Polish National Science Centre (Grant No. 2022/44/C/NZ6/00142) and The Spanish National Research Council (Grant No. PERPANTAR: PID2019-8597R.

Keywords: Antarctica, climate change, Ixodes uriae, penguins, Pygoscelis, ticks

PS239.II

Assessing the impacts of foodborne and waterborne parasites on food tourism

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Tourism is a vital modern activity that drives economic, cultural, and lifestyle changes, fostering sustainable development. Food tourism, which centers on cuisine as a travel motive, is growing rapidly but faces significant challenges from parasitic infections. Foodborne and waterborne parasites, such as *Fasciola hepatica*, *Echinococcus granulosus*, *Giardia intestinalis*, *Cryptosporidium* spp., and *Toxoplasma gondii*, negatively impact tourism economies, particularly in endemic regions. These infections affect tourist perceptions, local food safety, and overall tourism income. Globalization, increased travel, dietary shifts, and lifestyle changes have contributed to the re-emergence of foodborne parasitic diseases, especially in areas with high tourist activity.

Objectives: The study highlights the global prevalence of these parasites and their economic implications for food tourism.

For instance, human fascioliasis has a global prevalence of ~5%, affecting 2.4 million people across 61 countries, with higher rates in South America (up to 9.09%), Egypt (7%-17%), and Bolivia (up to 70%). Livestock prevalence is 17% in cattle and 13% in sheep globally, with some regions reporting up to 90% infection rates. In Iran, human fascioliasis prevalence is ~3%, with higher rates in northern provinces, while livestock rates range from 13% to 32.1%.

Hydatidosis, caused by *Echinococcus granulosus*, remains a public health concern, with infection rates reaching 50-70% in sheep populations in endemic areas. In Iran, animal infection rates can be as high as 50%. *Taenia saginata*, the beef tapeworm, has a global prevalence of up to 5.3%, with rates in Iran ranging from 0.0028% to 3%, and higher rates in northern provinces. *Giardia intestinalis* and *Cryptosporidium* spp. are common waterborne parasites, with prevalence rates in Iran ranging from 5% to 20% and 1% to 15%, respectively, particularly among children and in rural areas. *Toxoplasma gondii* has a global prevalence of 30% to 50%, with seroprevalence in Iran ranging from 20% to 40%, especially in rural areas.

The findings underscore the need for improved food safety measures, public health interventions, and awareness campaigns to mitigate the economic and health impacts of parasitic infections on food tourism. Addressing these challenges is crucial for sustaining the growth of tourism and ensuring the safety and well-being of travelers and local populations.

Keywords: Food Tourism, Waterborne Parasites, Foodborne Parasites, Sustainable Tourism,

Public Health

PS242.II

H. diminuta extracellular vesicle-induced changes to macrophage phenotype and metabolism

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Introduction and Objectives: Helminth-derived extracellular vesicles (EVs) are a research focus of host-helminth interactions. These EVs have effects on a variety of host immune cells, including macrophages. However, little work has been done on EVs from the model helminth *Hymenolepis diminuta*. Our objectives were to characterize *H. diminuta*-derived EVs (Hd-EVs) and then determine their effects on murine macrophage metabolism and phenotype, expanding knowledge of how helminths can affect host immunity.

Materials and Methods: *H. diminuta* were isolated from IL-4 receptor-$\alpha^{-/-}$ mice and cultured for 6h in serum-free RPMI media. Conditioned media was added to an Exoeasy Qiagen EV isolation kit. Hd-EV isolations were then dialyzed using a 10,000 MWCO dialysis cassette in PBS. Hd-EV size and quantity were measured using Nanosight Tracking Analysis. Protein content was measured using a Pierce Micro BCA kit and RNA content via Nanodrop. Endotoxin content was measured using a Pierce Endotoxin kit. Murine (C57Bl/6) bone marrow-derived macrophages were treated with 5 μg/mL protein Hd-EVs for 24h. Cells were lysed and qPCR performed for CCL2, 5, 7, and 8. TNF-α, IL-6, and CCL5 in the culture supernatant were measured via ELISA. Metabolic analyses were conducted using a Seahorse XF analyzer, with treated cells given fresh media and metabolism measured using a Mito Stress test.

Results: The majority of Hd-EVs were 100-350 nm in size, with protein and RNA content after dialysis equaling 93 ± 13 μg/g worm wet weight and 16 ± 4 μg/g worm wet weight respectively (n=8). Endotoxin contamination was <13 pg/mL (n=3). Hd-EVs evoked an upregulation of the chemokine mRNA: CCL2 (60 ±14 fold increase, n=13), CCL5 (312 ±154 fold increase, n=5), CCL7 (36 ±9 fold increase, n=14), and CCL8 (21 ± 6 fold increase, n=12). Hd-EV treatment also induced TNF-α (control = 9±2 pg/mL, Hd-EV = 545 ± 187 pg/mL, n=15) and IL-6 (control = 5±4 pg/mL, Hd-EV= 103 ± 12 pg/mL, n=7), and CCL5 protein production and release (control = 11 ± 4 pg/mL, Hd-EV = 3283± 399 pg/mL, n=8). Regarding metabolism, 24h after exposure to Hd-EVs, macrophages displayed a 62% and 64% increase in baseline oxidative phosphorylation and glycolysis, respectively (n=6).

Conclusions: Hd-EVs affect murine macrophages in a variety of ways, causing a limited production of inflammatory cytokine and a more marked increase in chemokine output and upregulation of energetics. We speculate that exposure to Hd-EVs results in a regulatory macrophage with an enhanced capacity to attract T cells: whether this protects the host from infection and immunopathology or is of pathophysiological significance remains to be determined.

PS245.II

Investigating the role of aromatic ketoacids in trypanosomiasis and their potential to modulate host responses

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The Trypanosomatidae family evolved from an ancestral insect parasite over 100 million years ago. These obligate protozoan parasites, transmitted by hematophagous invertebrate vector, cause diseases in both humans and livestock across the world. As exclusively extracellular organisms, trypanosomes have developed effective mechanisms to evade and modulate host immune responses. One such strategy employed by *Trypanosoma brucei* is the transamination of aromatic amino acids – phenylalanine, tyrosine, and tryptophan – into their corresponding aromatic ketoacids (AKAs) phenylpyruvate, hydroxyphenylpyruvate, and indole pyruvate. These AKAs have been shown to exhibit immunomodulatory effects, promoting anti-inflammatory activity, prolonging host survival and increasing their transmission. However, both hydroxyphenylpyruvate and indole pyruvate are relatively unstable, spontaneously breaking down after production. It is believed that this breakdown is essential for their anti-inflammatory effects during trypanosomiasis. A deeper understanding of the mechanisms involved in AKA degradation and their anti-inflammatory effects could lead to new therapeutic approaches for modulating inflammation in trypanosomiasis and other inflammation-related pathologies.

PS248.II

Angiostrongylus cantonensis in Mallorca: Current situation and detection of hyperendemic hotspots within the island

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In recent years, the rat lungworm *Angiostrongylus cantonensis* has been rapidly spreading worldwide. In Mediterranean Europe, it was first detected in Mallorca in 2018 and later on the Spanish mainland in 2022. Since then, research has primarily focused on identifying its definitive and accidental hosts, while data on its intermediate hosts have remained limited. This study aims to pinpoint the main hotspots of neuroangiostrongyliasis, the disease caused by *A. cantonensis*, in Mallorca through extensive rat sampling across the island. Additionally, we surveyed wildlife and gastropod species in 2 km "buffer zones" surrounding sites where infected hedgehogs had been identified. Using a molecular-based approach, we have identified at least 12 endemic hotspots. Notably, a highly endemic area was found in Mallorca"s largest wetland, near one of the island's main ports, with a prevalence exceeding 40%. Furthermore, some infected species included edible snails commonly consumed in Mediterranean cuisine. We present here the current situation of neuroangiostrongyliasis in Mallorca. These findings highlight the need for further research across the continent to better understand the true distribution of *A. cantonensis* and its potential impact on public health and biodiversity.

PS251.II

Breaking the chains: How *Babesia divergens* unlocks host iron

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The study investigates the mechanisms of iron acquisition and utilization by *Babesia divergens*, a protozoan parasite responsible for bovine and human babesiosis. Iron is essential for the parasite's survival, facilitating processes such as electron transport, protein cofactor activity, and gene regulation. Given the parasite's inability to synthesize heme de novo, it relies on host-derived iron sources, including hemoglobin and serum proteins like transferrin. This research elucidates key pathways and proteins involved in iron uptake, metabolism, and detoxification.

Experiments revealed that *B. divergens* utilizes a reductive mechanism to internalize ferric iron from various sources. This process includes reducing insoluble ferric iron to soluble ferrous iron before active transport into the cell. The highest rates of iron uptake were observed from transferrin, with significantly reduced activity at lower temperatures or in the presence of ferrous iron chelators. These findings highlight an active, energy-dependent iron acquisition pathway in the parasite. Blue native polyacrylamide gel electrophoresis confirmed iron incorporation into protein complexes, and enzymatic assays demonstrated the parasite"s robust ferric reductase activity.

Additionally, the study identified biliverdin production as evidence of heme degradation in *B. divergens*-infected red blood cells, indicating the presence of parasite-derived heme oxygenase-like activity. This enzymatic pathway facilitates the release of iron from the porphyrin ring, enabling the parasite to exploit hemoglobin as a critical iron source.

Proteomic analysis under iron-limiting conditions revealed differential expression of proteins associated with iron homeostasis. Two proteins, exhibited significant upregulation. Structural modeling suggests the hypothetical protein may function as a heme receptor, anchored to the parasite surface, and involved in heme acquisition. The TPR protein shares characteristics with bacterial hemophores, hinting at a role in scavenging host heme. Both proteins represent potential targets for therapeutic interventions and vaccine development, given their surface localization and immunogenic properties.

This comprehensive analysis enhances our understanding of iron metabolism in *B. divergens* and underscores the parasite's reliance on host iron sources for survival. These insights pave the way for developing novel strategies to combat babesiosis through targeted disruption of iron acquisition pathways.

PS254.II

The helical repeat protein HPR3 is required for maturation of ribosomal RNA fragments in *Toxoplasma gondii* mitochondria

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Mitochondrial function in apicomplexan parasites critically relies on expression of the mitochondrial genome, which encodes only three respiratory chain subunits and highly fragmented ribosomal RNAs (rRNAs). The mitochondrial genome of *Toxoplasma gondii* is exceptionally complex, consisting of multiple sequence segments that undergo high frequency recombination. Various mitogenomic arrangements are thought to be transcribed into long, polycistronic precursors. However, the nature of these precursors as well as the post-transcriptional processing mechanisms that generate mature mRNAs and rRNA fragments remain largely unexplored. Proteins of the heptatricopeptide repeat (HPR) family are candidate RNA processing and stabilization factors and particularly abundant in apicomplexans. We discovered that HPR3, a member of the HPR family, localizes to *T. gondii* mitochondria and is important for parasite proliferation and for the integrity of complex IV of the mitochondrial electron transport chain. Moreover, small RNA sequencing and RNA gel blot hybridization experiments strongly suggest that HPR3 is involved in mitochondrial RNA metabolism. Specifically, HPR3 is relevant for stabilization and 5'-end maturation of rRNA fragments, which is prerequisite for their assembly into mitochondrial ribosomes. In conclusion, our study provides the first mechanistic insights into the post-transcriptional steps of *T. gondii* mitochondrial gene expression and highlights the importance of HPR proteins in these processes.

PS257.II

Schistosoma mansoni granulin: Novel ligand of neutrophil CD177 with immunomodulatory potential

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Objectives: *Schistosoma mansoni*, a parasitic helminth that infects millions worldwide, evades the human immune system by secreting specific proteins that modulate immune cell function. This allows the parasite to survive for years in blood vessels. The current analysis follows up on a protein interaction study searching for novel binding partners for extracellular parasite proteins amongst a collection of over 700 human receptors.

Methods: Utilizing an ELISA-based method, SAVEXIS, we identified new host:pathogen interactions. This was achieved by screening for binding partners between cell surface and secreted proteins from *S. mansoni* somules and adults against a library of human leukocyte receptors. Surface plasmon resonance confirmed and characterized the binding kinetics of a selected interaction. Flow cytometry assessed the impact of this interaction on human neutrophil activation.

Results: Our analysis revealed a novel interaction between *S. mansoni* granulin (SmGRN) and CD177, a GPI-anchored glycoprotein predominantly expressed on neutrophils. CD177 plays a crucial role in inflammatory response activation, serving as a receptor for proteinase 3 (PR3). Our findings suggest that SmGRN has the potential to inhibit the binding of PR3 to CD177. However, a direct effect of SmGRN on neutrophil activation was not observed within the scope of this study.

Conclusion: 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. These findings provide new insights into the mechanisms of *S. mansoni* immunomodulation, particularly its manipulation of neutrophil function, and highlight the complex strategies employed by helminths to establish and maintain chronic infection.

PS260.II

The impact of an integrated control approach on the prevalence, intensity of infection and morbidity due to Schistosoma mansoni – The experience from Ijinga Island, Lake Victoria, Tanzania after 8 years of interventions

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Introduction: The Lake Victoria region of Tanzania is known for decades as a high prevalence area for schistosomiasis. Among the rural population living in the proximity of the lake *Schistosoma mansoni* is by far the dominating schistosome species. In 2016 a control project on a Ijinga Island (population 2500 people) in Lake Victoria was launched, aiming to reduce the burden of disease and eventually eliminate schistosomiasis in a defined area by an integrated control approach.

Methods: The integrated control approach consisted of intense counselling, repeated mass drug administration (MDA) with Praziquantel (PZQ) offered to the entire population, provision of safe water by construction of shallow wells and a central, solar-powered water supply system. MDA so far was provided 9x for the adult population and 13x for the school aged children within the past 8 years. The intensity of infection was monitored by repeated parasitological examination of a sentinel group. Abdominal ultrasound using the Niamey protocol was used to monitor the morbidity at baseline and after 7 years. A sanitation survey including group focussed discussions recorded the state of sanitation and toilet use. Drug efficacy was monitored twice acc. to WHO recommendations.

Results: At baseline the rate of periportal fibrosis Stage C-F in adults (n=441) was 43,3% with severe fibrosis (Stage E-F) in 7,7%. The 2023 survey in 474 adults showed a reduction of PPF (Stage C-F) to 4,4% with no stage E,F detected. The intensity of Infection was reduced from 55,2% / 20,4% /12,9% at baseline for light / moderate / heavy infections to 91% / 8,3% / 0% respectively in 2024. Simple monitoring of the prevalence showed a variable effect, depending on the interval between MDA and survey and the method used (Kato-Katz, POCT-CCA-urine-test). 2 drug efficacy trials showed a constantly high egg reduction rate without any evidence of PZQ drug resistance. The sanitation survey revealed substantial deficiencies in sanitation. 79% of the households owned a latrine but 90% of them demanded improvement of the facilities. When leaving home for working 90% of the survey participants did not use public toilets.

Conclusion: The interventions resulted in a significant reduction in the intensity of infection and burden of disease due to S. mansoni. Despite the considerable efforts it was not possible to interrupt the transmission. Prolonged intervals between MDA resulted in a rapid increase in the prevalence indicating a high rate of re-infection. Continued long term efforts and substantial investments in the sanitation infrastructure will be needed to interrupt the infectious cycle in high transmission settings like Ijinga Island.

PS263.II

Aspartyl proteases associated with the role of *Babesia apical* complex during egress and invasion of host erythrocytes

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Introduction: Babesiosis, a tick-borne disease affecting animals and humans, is caused by apicomplexan parasites of the genus Babesia, which invade and egress from vertebrate host erythrocytes during their asexual multiplication cycle, leading to the development of the disease. Similar to distantly related *Plasmodium spp.*, *Babesia* utilizes proteolytic cascades for these processes, which are essential for propagation and pathogenesis. Aspartyl proteases BdASP3a and BdASP3b, homologous to *Plasmodium* plasmepsins IX/X (PfPMIX/X) and *Toxoplasma* ASP3 (TgASP3), are hypothesized to mediate apical complex function in *Babesia divergens*.

Objectives: This study aimed to characterize BdASP3a/b, elucidate their roles in erythrocytic invasion and egress, and evaluate their potential as therapeutic targets.

Materials & Methods: BdASP3a/b were identified through comparative omics analyses. Expression profiling confirmed stage-specific presence, and recombinant BdASP3 enzymes were expressed in bacterial and insect cells. Activity assays using PfPMX substrates, inhibition studies with the hydroxyethylamine inhibitor 49C, and immunomicroscopy were performed. Functional genomic tools, including conditional knockout/knockdown and proteomic analyses, and trans-genera complementation in TgASP3-deficient *Toxoplasma* strains were employed.

Results: BdASP3a/b were expressed exclusively in intraerythrocytic stages, distinguishing them from PfPMIX/X, which are active in vector stages. Recombinant BdASP3s displayed acidic pH optima and proteolytic activity inhibited by 49C.Expansion and TEM Immunomicroscopy both localized BdASP3a to apical complex associated organelles. Inhibition studies implicated BdASP3a/b in invasion, as free merozoites accumulated in serum without invading erythrocytes. Trans-genera complementation linked BdASP3s to protein maturation in the secretory pathway, mirroring TgASP3"s roles.

Conclusion: BdASP3a/b are pivotal to *Babesia* propagation, diverging from PfPMIX/X functions in malarial parasites. They represent promising therapeutic targets, and ongoing proteomic analyses aim to refine their potential for anti-*Babesia* chemotherapy.

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PS266.II

Immunolocalization and 3D modelling of three unique proteins belonging to the costa of *Tritrichomonas foetus*

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Nowadays, even in light of all the massive advances in cell biology, we still find some cellular structures that are not entirely understood. Among those, we highlight the costa, a structure from the mastigont system existent only in some members of the orders Trichomonadida and Tritrichomonadida, including the pathogens of venereal diseases in humans and cattle, *Trichomonas vaginalis* (*T. vaginalis*) and *Tritrichomonas foetus* (*T. foetus*), respectively. The costa is a prominent striated fiber and, although part of the cytoskeleton, differs from its classical components and its molecular composition is still not fully characterized. Using proteomics of *T. foetus*'s costa fraction, we previously identified hypothetic proteins and among these, the protein ARM19800.1, positively localized in the costa and named costain-1. In this study, two other protein candidates were analyzed. To achieve the specific localization of 11810 and 32137 proteins in *T. foetus*'s cells it was used Expansion Microscopy and immunocytochemistry (Figs. 1-2). The immunofluorescence revealed the presence of both proteins throughout the whole costa but with different intensities. Immunocytochemistry using negative staining, LR-White, and Epon embedding revealed a further analysis of the protein's localization. All techniques confirmed the distinct and distributed localization of both proteins: costain-2 (11810) and costain-3 (32137). Also, AlfaFold3 was used to generate 3D models of the three identified proteins, showing a major prevalence of α-helical spans. Nonetheless, identifying and further characterizing these unique proteins can help understand their functional role in the assembled costa and, therefore, better understand the organization and function of this structure in these organisms.

Keywords: ccosta; costain; cytoskeleton; Immunolocalization; *Tritrichomonas foetus*, AlphaFold3

Figure 1. Western blot analysis of the presence of proteins 11810 (116.9 kDa) and 32137 (117.44 kDa) in the protein content of whole cells, an intermediate cell fraction, and an Enriched Costa Fraction (EFC) showing the gradual enrichment of both proteins in the ECF. The protein ladder is shown in the last lane with the respective kilodaltons (kDa) molecular weights. The results were representative of three biologically independent experiments.

Figure 2. Expansion Microscope images from *T. foetus*. The anti-11810 antibody showed positive labeling in the costa (green), and the anti-α-tubulin antibody (red) labeled the axostyle, and the nucleus is labelled with DAPI. The images were obtained in confocal mode on an Elyra PS.1 microscope.

Fig. 1

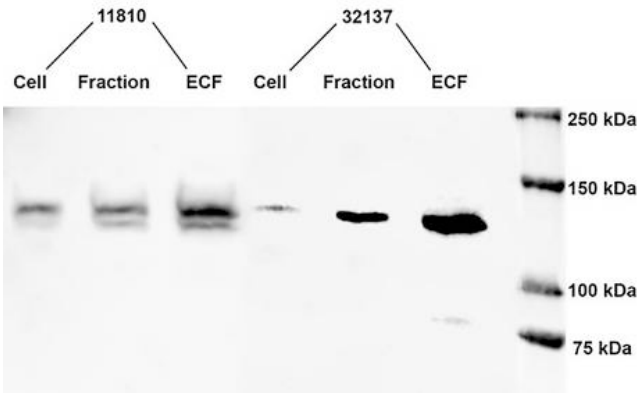
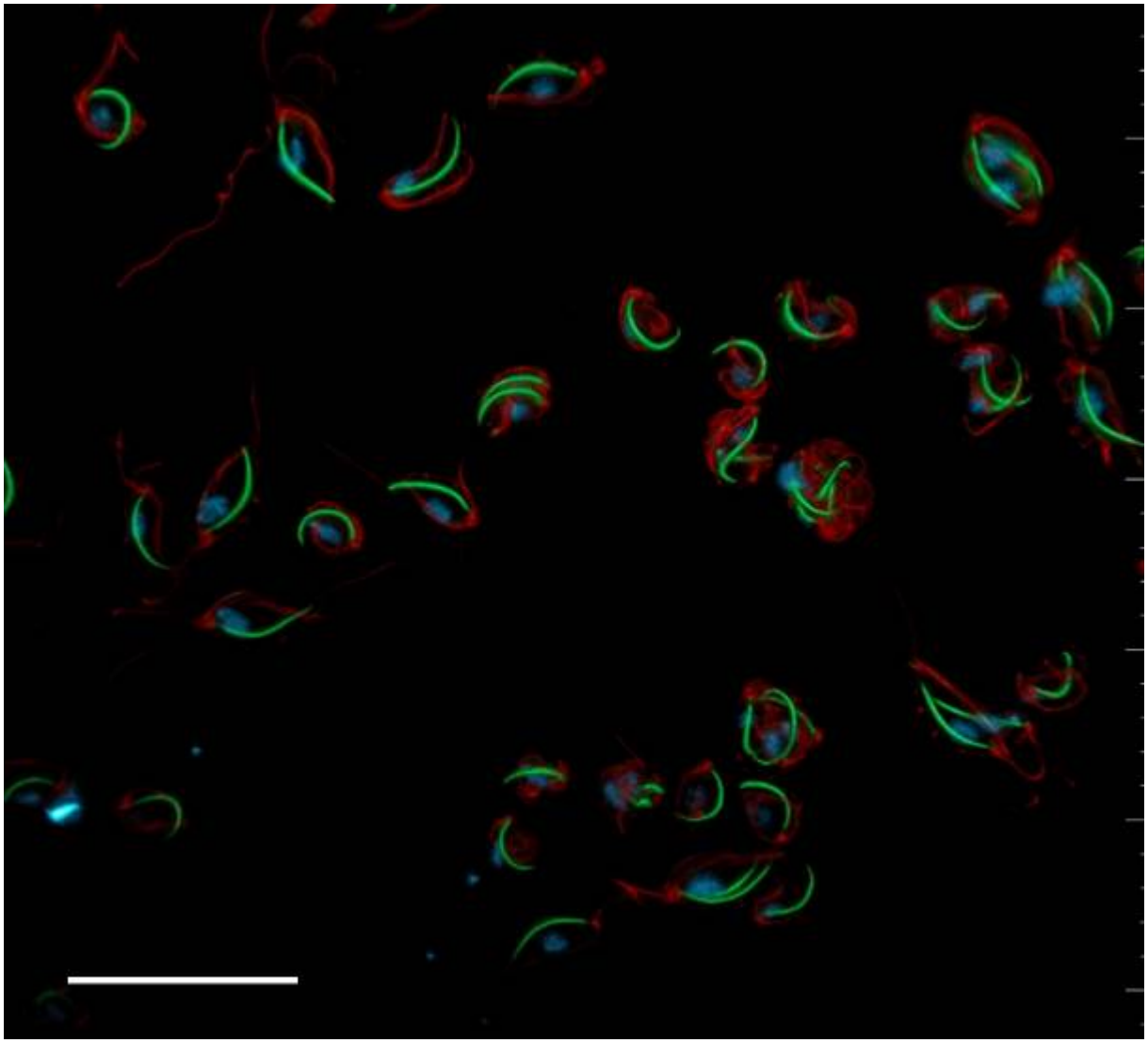


Fig. 2



PS269.II

The microbial *bug*-ground of *Trichuris trichiura* host specificity

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The human whipworm (*Trichuris trichiura*) is a soil-transmitted helminth responsible for infecting hundreds of millions globally, often leading to Trichuriasis, a major neglected tropical disease.

Eggs represent the infective stage of the whipworm life cycle. Upon ingestion, they hatch in the human caecum and proximal colon, releasing larvae that dwell within the caecal epithelium.

Human whipworms are unable to establish a patent infection in non-primate experimental animals such as mice or pigs, suggesting a high degree of host specificity. Since the hatching of eggs and establishment of infection by the mouse whipworm (*Trichuris muris*) is dependent on the intestinal microbiota, we hypothesise that host specificity of *T. trichiura* is determined by the host microbiome.

We first investigated the role of human microbiota on the egg hatching and host colonisation of *T. trichiura* through the experimental infection of humanised microbiota mice. Using caecal samples of our experimental animals *in vitro* egg hatching experiments were also performed by a co-culture with the human whipworm eggs.

Remarkably, we achieved the first successful infection of a non-primate host with *T. trichiura*. Comparative analysis of the bacterial composition in caecal samples from mice harbouring murine *vs* humanised microbiome led to the identification of bacterial species implicated in *T. trichiura* egg hatching. These results were validated with *in vitro* egg hatching assays moreover we experimented with the length of co-culture necessary and involvement of proteases in the mechanism of *T. trichiura* egg hatching.

To investigate whether additional factors related to the composition of the host caecal epithelium could also play a role in host specificity, we exposed *Trichuris trichiura* first-stage larvae (L1) to murine caecaloids cultured in transwells. Successful infection of the non-primate epithelium was observed.

Together, these results suggest that *T. trichiura* host specificity is determined by the host microbiome and not the host intestinal epithelium.

PS272.II

A twenty-year retrospective study of spatio-temporal distribution and prevalence of trypanosomosis among dogs presented for haemoparasite screening at the veterinary teaching hospital, university of Ibadan, Ibadan, Oyo state, Nigeria

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Dogs, often referred to as humans' closest companions, are cherished domestic animals raised within households. Canine trypanosomosis poses a significant health risks to dogs, with potentially fatal outcomes. This study aimed to assess the spatial and temporal patterns of trypanosomosis prevalence among dogs screened for haemoparasite screening at the Veterinary Teaching Hospital, University of Ibadan, located in Oyo State, Nigeria. Data spanning a twenty-year period (2004 - 2023) included 2,354 cases collected from the Department of Veterinary Pathology, the Department of Veterinary Parasitology and Entomology, and the small animal unit of the hospital. The positive and negative cases of Canine Trypanosomiasis were recorded and compiled. Spatial analysis was conducted using QGIS version 3.24.1-Tisler and SatScan v9.7, applying geographic coordinates from the origins of the presented dogs to create thematic maps. Results showed an overall prevalence of 1.87% for canine trypanosomiasis, with the primary cluster located in Akinyele and Lagelu Local Governments within a 6.2 km radius, alongside two secondary clusters. The primary cluster, in a densely vegetated area with expanding residential zones, was situated close (5.9 km) to a cattle market, suggesting that environmental conditions favoring tsetse fly survival likely contributed to the increased trypanosome cases in dogs. In conclusion, the study recommends prioritizing resources toward monitoring areas in Ibadan with ideal environments for Glossina species to better control Trypanosoma species vectors. Additionally, prompt diagnosis and treatment should be ensured for canine cases exhibiting clinical symptoms of trypanosomiasis in these high-risk areas.

PS278.II

Hijacked immune cells traverse microenvironmental barriers by positioning and pushing their intracellular parasite cargo

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The obligate intracellular parasite *Toxoplasma gondii* exploits immune cell motility for dissemination within the host through a "Trojan Horse" mechanism. The amoeboid migration of dendritic cells (DCs) is independent of adhesions, allowing them to efficiently navigate through dense tissues and making them a major candidate for parasite transport. However, *T. gondii* replicates within the host cell forming a parasitophorous vacuole (PV) that can become larger than the host nucleus. It remains elusive, how this large parasitic cargo is transported through complex and confining microenvironments, such as the interstitium. Thus, we investigated the migration of *T. gondii*-infected DCs depending on the size and replication stage of individual parasitic cargos.

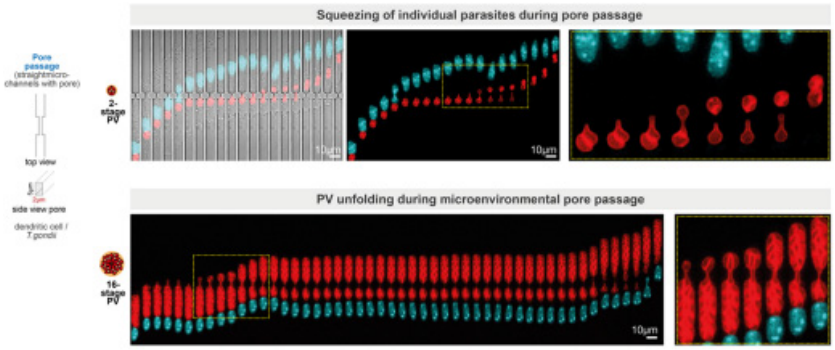
In artificial microchannel assays mimicking the interstitium, DCs migrated efficiently despite large parasitic cargos. To fit in the channels, the parasitic cargo was deformed. Notably, every parasite unfolded from the PV to squeeze through narrow pores one parasite at a time. When pushed against the edges of decision points, the PV split into two or more connected parts, which later rejoined into an intact PV. This demonstrated a high resilience and deformability of *T. gondii* PV, despite being stiffer than the host nucleus.

We uncovered an active positioning mechanism, wherein large parasitic cargos moved to the cell front, overtaking the host nucleus and the microtubule-organizing center. Interestingly, splitting and front positioning of the parasitic cargo were independent of microtubule stability. However, Myosin-II-dependent actomyosin contractility was essential for this positioning and overcoming narrow 2 µm pores. When the host DC was stuck in pores due to inhibited myosin activity, even small PVs with just two parasites egressed from the host cell.

These findings highlight the biophysical adaptability of *T. gondii* during DC-mediated transport in confining microenvironments, emphasizing the critical roles of cargo deformability and actomyosin forces in navigating porous environments. The data suggest a potential "hop-on, hop-off" strategy of parasite dissemination via cycles of immune cell invasion and egress, particularly as the PV grows or encounters mechanical stresses. Further exploration of the "Trojan Horse" dissemination may inform strategies to limit parasite dissemination.

Data in part published as preprint: Ruiz-Fernandez *et al, bioRxiv* (2024) doi:10.1101/2024.05.03.592378.

Fig. 1



PS281.II

Blastocystis sp.-induced oxidative stress and colorectal carcinogenesis: Insights from an _In vivo_ rat model

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Colorectal cancer (CRC) remains a significant global health challenge, with oxidative stress playing a critical role in its pathogenesis. This study investigated the exacerbating effects of *Blastocystis* sp. on azoxymethane (AOM)-induced colorectal carcinogenesis in vivo using a Wistar rat model. Rats were divided into control, AOM-treated, *Blastocystis* sp.-infected, and AOM with Blastocystis sp. co-infected groups. Aberrant crypt foci (ACF) formation, oxidative stress biomarkers, and histopathological changes were analyzed. Co-infected rats exhibited significantly higher ACF counts and oxidative damage compared to AOM-only groups. Elevated levels of advanced oxidation protein products, lipid hydroperoxides, and hydrogen peroxide were observed in the co-infected rats, indicating enhanced oxidative stress. Histological examination revealed severe dysplasia and mucosal damage in co-infected rats. These findings highlight the potential of Blastocystis sp. to aggravate CRC development through oxidative stress and intestinal epithelial damage, underscoring the importance of screening for parasitic infections in cancer management strategies.

PS284.II

Exploring the role of animal reservoirs for the transmission of *Fasciola* spp. in a one health context: A systematic review

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Fascioliasis, caused by *Fasciola hepatica* and *Fasciola gigantica*, is a major global health concern affecting both humans and animals. This systematic review evaluates the role of animal reservoirs in transmitting *Fasciola* spp. to humans. Literature searches were conducted in PubMed, Web of Science, Scopus, and CABI Digital Library without language restrictions, including experimental and observational studies, systematic and narrative reviews, book chapters, reports, and conference abstracts. Additional studies were identified through reference screening of reviews and book chapters. Studies focusing on transmission dynamics involving animal reservoirs were included, while those limited to human or animal prevalence or other liver flukes were excluded. The quality of included studies was assessed using the NIH Quality Assessment and Risk of Bias Tool Repository, specifically the National Heart, Lung, and Blood Institute's Quality Assessment Tool for Before-After Studies with No Control Group and the ROBINS-I tool for non-randomised studies. Findings were synthesised descriptively, emphasising transmission dynamics such as prepatent periods, cercarial shedding intensity, snail infectivity, and host-specific differences. Bayesian meta-analyses compared aspects of parasite biology across reservoir species, while funnel plots and Egger"s tests evaluated publication bias and variability. Of 5,362 screened articles, nine met the inclusion criteria, covering reservoir hosts such as sheep (*Ovis aries*), cattle (*Bos taurus*), pigs (*Sus domesticus*), donkeys (*Equus asinus*), mules (*Equus caballus* × *Equus asinus*), llamas (*Llama glama*), capybaras (*Hydrochoerus hydrochaeris*), black rats (*Rattus rattus*), horses (*Equus caballus*), and goats (*Capra hircus*). Sheep, cattle, and pigs emerged as primary reservoirs. Sheep in particular stood out as principal reservoir host due to their high egg shedding capacity and the high infectivity of *F. hepatica* sheep isolates to the intermediate host. Donkeys, mules, and llamas were identified as secondary reservoirs, while capybaras and black rats contributed to environmental contamination in specific contexts. Horses and goats played minor roles. Despite these findings, research gaps persist, especially for *F. gigantica* and underrepresented regions. This review underscores the complex dynamics of *Fasciola* transmission and highlights the importance of integrated, targeted control strategies tailored to local ecological and socioeconomic conditions. Sustainable interventions are essential to reducing the burden of *Fasciola* spp. on human and animal health globally.

PS287.II

Epidemiology of intestinal parasitosis in a South American hospital and its associated variables

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Introduction: Intestinal parasitosis are infestations caused by protozoa and helminths that reside in the intestine, feeding on their host and causing harm. These infections are typically acquired through the consumption of contaminated food or water. The presence of parasitosis in a community is often linked to poor sanitary and sociocultural conditions, overcrowding, and the presence of vectors. Although these infestations have low mortality rates, they contribute to significant health deterioration, particularly affecting the child population. They are prevalent in tropical regions, especially in socially and economically depressed rural and urban areas.

Objectives: This research aimed to epidemiologically characterize intestinal parasitosis in the community of Mérida, Venezuela.

Materials and Methods: A descriptive cross-sectional study was conducted, collecting 500 stool samples from individuals of all ages and sexes between 2014 and 2018. The samples were analyzed using conventional techniques, including saline solution and Lugol, Kato, Faust, and Baermann methods. Results were analyzed with the SPSS 20 statistical package.

Results: The overall prevalence showed a female predominance (64%). Protozoa (97%) were more common than helminths (3%). Among the protozoa, *Blastocystis spp.* was the most frequently found (82%), while the most common helminth was *Hymenolepis diminuta* (2%). A significant 79% of the population was found to be monoparasitized, with the highest prevalence occurring in the 0 to 20 age group. Additionally, 85% of patients exhibited microcytic and hypochromic anemia. Socioeconomic status, assessed using the Graffar method (modified by H. Méndez-Castellano), categorized the community in stratum IV (relative poverty) (82%). A lack of knowledge about intestinal parasitosis was noted among surveyed heads of households (71.5%).

Conclusions: Intestinal parasitosis was highly prevalent in the studied population, with greater poverty being the most commonly associated factor. These findings underscore the need for improved characterization and awareness within affected communities.

PS003.III

Report of an outbreak of oral transmission by *T. cruzi* in a region of South America and its epidemiological characteristics

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Introduction: Oral transmission of Chagas disease has been infrequent, yet there have been increasing reports from South America. However, epidemiological records have not provided significant data on this phenomenon.

Objectives: This study aims to document an outbreak of acute Chagas disease associated with oral transmission in a South American town in 2020.

Materials and Methods: A retrospective study was conducted to analyze an outbreak in which 20 individuals who consumed food in a community dining room were simultaneously infected with *Trypanosoma cruzi*. This outbreak resulted in severe clinical manifestations, including acute myocarditis in four patients (20%), two of whom died.

Results: Clinical severity among all affected patients was consistent with parasitological, serological, and molecular (PCR) findings, which confirmed the presence of blood-borne trypomastigotes of *T. cruzi*, elevated levels of anti-*T. cruzi* antibodies (IgM and IgG), and *T. cruzi* DNA. The evidence supports the occurrence of oral transmission linked to the community lunch.

Conclusions: This outbreak highlights the potential epidemiological importance of oral transmission of Chagas disease, especially in tropical regions where such infections can have serious acute and long-term consequences. The findings underscore the need for increased awareness and monitoring of this transmission route.

PS006.III

The survivability of *Rhipicephalus sanguineus* s.s. and *Rh. linnaei* in Germany – A controlled outdoor study

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Introduction: *Rhipicephalus sanguineus* sensu lato (s.l.) represents a cosmopolitan tick species complex primarily associated with dogs. Although there are no published endemic occurrences of members of the tick complex in Germany, specimens have been regularly introduced since the 1960s1, which can quickly establish indoors in Germany, but are currently believed not to survive outdoors. Since then, imports of *R. sanguineus* sensu stricto (s.s.), and *R. linnaei* from foreign countries have been introduced regularly with dogs without tick prophylaxis.

Methods: As climate change leads to milder winters, this raise concerns that these introduced tick species may become established outside of buildings. To explore this possibility, a one-year outdoor survival experiment was conducted to assess the viability of all developmental stages (larvae, nymphs, adults) along with egg deposition by engorged females and the hatching of larvae for both introduced *Rhipicephalus* species. Experimental cages containing individuals of both species separately were placed in secure plots at periodic intervals and the number of surviving ticks was recorded weekly.

Results: The results revealed that *R. sanguineus* s.s. exhibited longer average survival time than *R. linnaei* at all developmental stages. During the warmer months (Mar. to Nov.), adults survived for 20–44 weeks, nymphs for 7–20 weeks, and larvae for 2–5 weeks. Engorged females from both species successfully laid eggs from which viable larvae hatched, which survived for 10 to 12 weeks. However, no stage of either species was able to survive the winter period (Nov. to Feb.) for more than 2–4 weeks due to a combination of wet and freezing weather conditions.

Outlook: These findings indicate that outdoor reproduction for both species is indeed possible, as each developmental stage can endure long enough to find suitable hosts during warmer periods and molt. Also, the next generation of eggs was able to develop outdoors. Although the climatic conditions during the winter of 2023/24 were not sufficient to ensure survival, the possibility of established populations remains, at least during milder winters in warmer areas of Germany, if adequate shelter or hiding spots are present.

1 Gothe & Hamel (1973) Zur Ökologie eines deutschen Stammes von *Rhipicephalus sanguineus* (Latreille, 1806) ZF Parasitenkunde

PS009.III

Thermosensitivity of TBE virus strains: Experimental studies

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Introduction: Climate change is altering vector-borne disease dynamics, with rising temperatures increasingly affecting pathogen transmission and virulence. Tick-borne encephalitis virus (TBEV), a significant public health concern across Europe, is primarily transmitted by *Ixodes ricinus* ticks. Understanding the factors that influence TBEV replication and persistence within its vector is crucial for accurately predicting disease risk.

Objectives: Based on the findings of Elväng et al. (2011), who demonstrated a thermosensitive RNA-switch within the 3"-UTR of the TBEV strain Torö-2003 that modulates virus replication in response to temperature changes, this study investigates the thermosensitivity of various TBEV strains to assess the potential impact of rising temperatures on viral persistence and replication in *I. ricinus*.

Material & Methods: Specifically, we examined a meadow isolate and a forest isolate, both from a known hotspot in Bavaria, and the Neudoerfl strain as a control. Infected *I. ricinus* nymphs were incubated at temperatures of 22°C, 27°C, 32°C, and 37°C over a period of up to 12 weeks. Viral load and detectability were assessed using RT-qPCR and plaque assays, while mutations within the 3"-UTR that could potentially influence thermotolerance were analyzed.

Results: Results showed that high temperatures significantly reduced viral loads and detectability of the meadow isolate, whereas the forest isolate displayed thermotolerance similar to Neudoerfl, suggesting a common mechanism. Prolonged exposure of the meadow isolate to 32°C and 37°C indicated increased genetic variability, pointing to a shift toward non-infectious particles.

Conclusion: These findings underscore the need for further studies on climate-induced selection within TBEV strains and the implications for pathogen evolution in tick populations.

Elväng et al., Sequencing of a tick-borne encephalitis virus from Ixodes ricinus reveals a thermosensitive RNA switch significant for virus propagation in ectothermic arthropods. Vector Borne Zoonotic Dis. 2011 Jun;11(6).

PS012.III

Investigating the role of chemokine and glycocalyx collaboration in immune cell recruitment throughout whipworm infection

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The gut dwelling helminth *Trichuris trichiura* currently infects over 600 million people worldwide and is a major cause of global disease burden. In laboratory settings, the murine infective agent of *Trichuris*, *T. muris*, has been used for decades to unpick the immune responses which underpin successful worm expulsion. This extensive body of work includes the key leukocyte populations which are recruited to the site of infection, the large intestine. However, there remains much to be discovered surrounding the mechanisms which facilitate recruitment of these populations from the bloodstream into the gut tissue. Whilst previous studies have implicated certain chemokines, and the receptors which they ligate, within this process, few have been able to attribute specific chemokine-receptor interactions to the recruitment of any given immune cell population. Furthermore, research into a role for the cell surface glycocalyx in leukocyte recruitment throughout *T. muris* infection has thus far been neglected. Using novel transgenic mouse strains, the current project therefore aims to unpick the roles of specific chemokine receptors and glycocalyx components within immune cell recruitment to the *T. muris* infected large intestine. The output of this project will ultimately add to our knowledge of host-parasite interactions, and to the field of chemokine biology in general.

PS015.III

Comparative efficacy on the use of sulfur and copper nanoparticles in controlling ticks as alternatives to ivermectin

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Introduction: Ticks, such as the *Hyalomma anatolicum* and *Rhipicephalus (Boophilus) microplus*, which are common ectoparasites of livestock and wildlife, are responsible for transmitting pathogens that result in significant economic losses.

Objectives: There is an increasing prevalence of resistance by the tick population to the classical chemical acaricides leading to the need for alternative control measures including nanoparticle formulations.

Methods: The Acaricidal effect of sulfur and copper nanoparticles was assessed against various stages of the lifecycle of *R. (B.) microplus* which include eggs, larvae and adults. Nanoparticles were synthesized, and characterization was performed using Scanning Electron Microscopy (SEM) and Fourier Transform Infrared Spectroscopy (FTIR). Efficacy was assessed against ivermectin which has been previously reported to have an LC50 level of 0.5 mg/L.

Results: Copper (LC50 =22.3±3.44 mg/L) and sulphur (LC50 = 36.16 ±6.19 mg/L) nanoparticles killed adult *R. (B) microplus* at 99.17% at a dose of 80 mg/L and 150 mg/L respectively, while S and Cu nanoparticles caused 99.67% and 98.50% larval mortality at 150 mg/L and 80 mg/L, respectively. Considerably lower mortality rates ($P<0.05$) were noted with ivermectin (3 mg/L) at 66.67% adult mortality and 61.50% for the larvae as compared to the larvicidal of sulphur and copper. Copper and sulphur also resulted in causing 99.87% of un-hatched eggs at the concentration of 80 mg per liter and 150 mg per liter, respectively while, the concentration of Ivermectin lead to 90.63% percent un-hatched eggs.

Conclusion: It is important to note that sulfur and copper nanoparticles have a high potential as true substitute for chemical acaricides to target life stages of *R. (B.) microplus*. It is also imperative that further studies are done to evaluate the safety of these compounds and their biological effects on non-targeted species of the environment.

PS018.III

Giardia intestinalis trophozoites trigger NET formation in human PMN

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Introduction: *Giardia intestinalis* is a zoonotic enteric protozoan parasite that causes giardiasis in humans, domestic animals and wildlife, with more than 280 million human cases of diarrhea annually. A typical host innate immune response to *G. intestinalis* includes the activation of polymorphonuclear neutrophils (PMN), which can release neutrophil extracellular traps (NETs). While *G. intestinalis* trophozoites were shown to trigger NET release in bovine PMN, comparable studies for human PMN are lacking, so far. Despite the globally high prevalence of giardiasis, PMN-mediated immune responses against *G. intestinalis* like NET formation, remains poorly investigated.

Objective: The aim of the current study was to evaluate the capacity of vital *G. intestinalis* trophozoites to activate human PMN and to induce NET formation.

Material and Methods: *G. intestinalis* trophozoites were axenically cultured in TYI-S-33 medium. Human PMN (hPMN) were isolated from healthy blood donors (*n* = 4) by immunonegative selection (Stemcell™). NETosis was induced by stimulation with PMA or by exposure of hPMN to *G. intestinalis* trophozoites. NET release was illustrated by scanning electron microscopy (SEM). Typical characteristics of NETs were confirmed by immunofluorescence microscopy by detecting citrullinated histones, neutrophil elastase (NE) and DNA in NET structures. PMN activation was measured on the level of oxygen consumption rates (OCR), extracellular acidification rates (ECAR) and production of reactive oxygen species (ROS).

Results: Microscopic findings indicated that vital *G. intestinalis* trophozoites activate PMN finally resulting in NET release. SEM analysis unveiled both, NET-entrapped trophozoites and phagocytic activities of hPMN. However, trophozoite exposure did not induce oxidative (OCR, ROS) or glycolytic (ECAR) responses in hPMN when compared to unstimulated controls.

Conclusion: This study presents a novel finding on early interactions between the enteropathogen *G. intestinalis* and hPMN demonstrating that motile trophozoites are capable to activate hPMN and to stimulate NET release. Despite contrasting with previous reports, current data suggest that both NET release and phagocytosis are used as effector mechanisms of hPMN in response to trophozoites. The lack of oxidative and glycolytic hPMN responses may reflect E/S-product-based immune evasion strategies of Giardia, however, further research is needed to better understand these mechanisms.

PS021.III

Wolbachia bacteria in *Mansonella perstans* isolates from patients infected in different geographical areas

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[7]Institute of Tropical Medicine, Antwerp, Belgium
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[9]Swiss Tropical and Public Health Institute (Swiss TPH), Allschwil, Switzerland
[10]University of Basel, Basel, Switzerland
[11]Instituto de Salud Carlos III, Madrid, Spain
[12]University of Almeria, Almeria, Spain
[13]Vall d'Hebron University Hospital, Barcelona, Spain
[14]Erasmus MC University Medical Center, Rotterdam, Netherlands
[15]Leiden University Medical Center, Leiden, Netherlands
[16]Careggi University Hospital, Florence, Italy
[17]University of Florence, Florence, Italy
[18]Eberhard Karls University Tuebingen, Tübingen, Germany
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Objectives: *Mansonella perstans* is a filarial parasite widely endemic in sub-Saharan Africa, with sporadic cases in Latin America. Infection is often overlooked due to its inconstant positivity on serology screening for filariasis and unspecific clinical presentation. Treatment of *M. perstans* is not standardized; prolonged courses of mebendazole +/- DEC seem the most effective strategies, but their effectiveness for parasitological cure is uncertain. Anti-*Wolbachia* macrofilaricidal treatment with doxycycline has been applied, but there are scant and contrasting reports about the presence of *Wolbachia* in *M. perstans* from different geographical areas. Taking advantage of a network of European centres specialized in travelers and migrants health, we aimed to expand the knowledge concerning the distribution of *Wolbachia* in *M. perstans*, to contribute to the design of optimal treatment approaches.

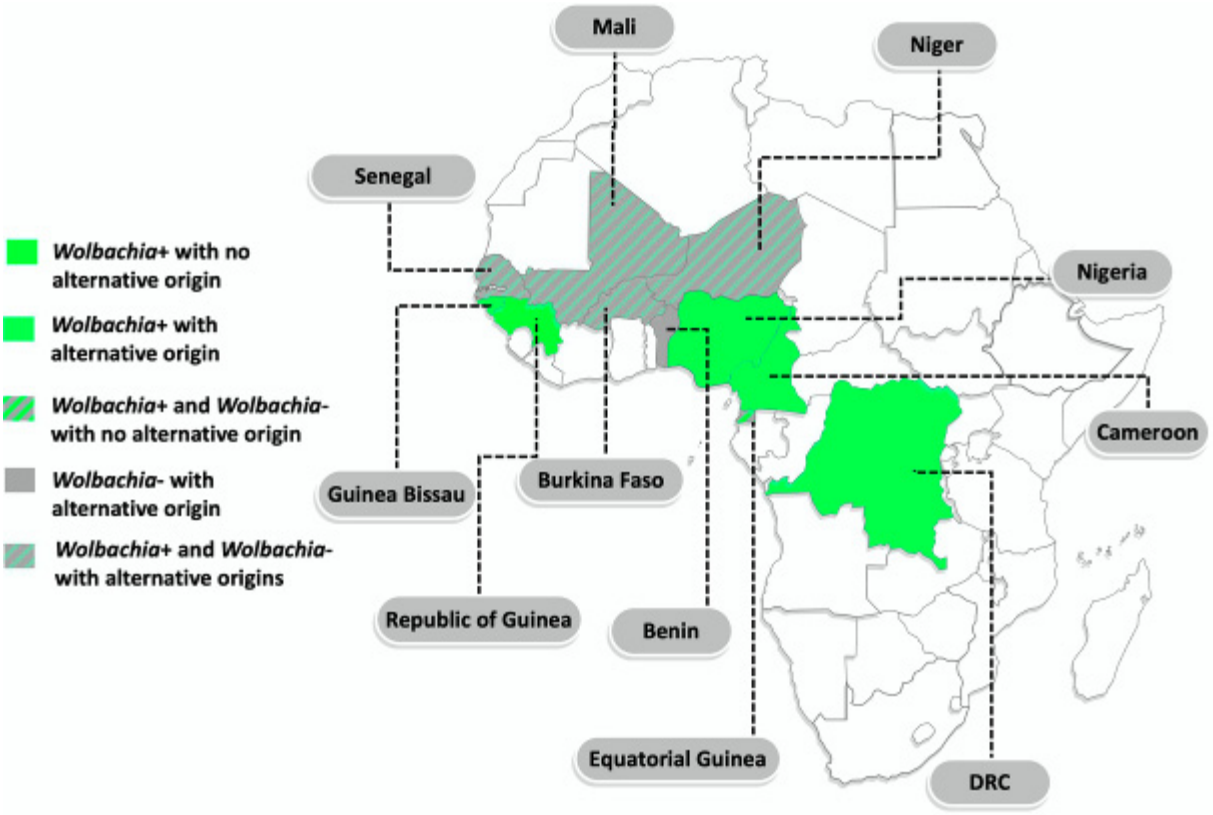
Materials and Methods: We analyzed 19 samples of concentrated *M. perstans* microfilariae or whole blood from *M. perstans*-infected patients who reported having resided or travelled in one or more of 10 West African countries. *Wolbachia* was detected by PCR targeting 16S and ftsZ genes and phylogenetic analysis of *M. perstans* was carried out based on COX1 sequencing.

Results: *Wolbachia* was identified in 14 (74%) samples. With the possible inaccuracy deriving from countries of potential infection being identified retrospectively from routine clinical visit"s documents, this study identified for the first time *Wolbachia* in *M. perstans* isolated from patients who reported only residence/travel in Burkina Faso, Equatorial Guinea, Republic of Guinea, and Senegal. Furthermore, *Wolbachia* might be also present in *M. perstans* from Democratic Republic of the Congo, Mali, Niger and Nigeria. Phylogenetically, *M. perstans* grouped into two branches; with *Wolbachia*-positive and *Wolbachia*-negative samples found in both of them.

Conclusions: The retrieval of *Wolbachia*-positive and *Wolbachia*-negative *M. perstans* samples can be explained by technical limitations or reflect the real existence of *Wolbachia*-positive and *Wolbachia*-negative

M. perstans populations. However, this latter hypothesis was not supported by our phylogenetic analysis. Our results suggest that doxycycline could be used for the treatment of *M. perstans* infection, if possible after ascertaining the presence of *Wolbachia* by PCR performed on concentrated microfilariae using two targets to avoid false negative results.

Fig. 1



PS024.III

Pediatric trichinosis: A case report

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Background: Trichinellosis is a zoonosis, caused by roundworms of the genus Trichinella. Domestic pigs, wild boars, bears, rodents and horses are reservoir animals. We report a case of neck trichinosis presenting as lateral neck swelling. The diagnosis of trichinosis was confirmed by the presence of larvae on muscle biopsy. Furthermore, lateral neck swelling may provide a diagnostic challenge by clinically mimicking a lymphoma or other causes of lateral neck swelling. Due to its rarity and its tendency to pose a clinical diagnostic challenge, we decided to report it.

Case presentation: A-10 yr old male patient presented with a 6 × 4 cm firm; non-tender left lateral neck swelling. Histopathology examination confirmed the diagnosis of trichinosis and the patient was started on albendazole 15 mg/kg/day, divided into two doses and prednisolone 20 mg by mouth, two times daily, for 14 days. Having completed his medication, he had a smooth course and was discharged with appointment scheduled for follow-up after 3 months.

Discussion: Trichinella spp. occur worldwide, most frequently in regions with temperate climates. About 10,000 human infections occur annually worldwide. Cultural factors such as traditional dishes based on raw or undercooked meat or meat-derived products play an important role in the epidemiology of the disease.

Conclusion: In the clinical evaluation of a patient with lateral neck swelling, trichinosis must be considered as a differential diagnosis in subjects from endemic areas for early diagnostic workup and management.

PS027.III

Vulvovaginitis due to *Enterobius vermicularis* in a girl and epidemic enterobiasis in her family

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Vulvovaginitis, an inflammation of the vulvovaginal mucous membranes, is a common reason for pediatric gynecology consultations. One of the causes of this condition is a parasitic worm known as *Enterobius vermicularis (E. vermicularis)*. In girls, adult worms can infiltrate the vagina and release eggs, leading to the development of vulvovaginitis. Furthermore, these worms have the ability to invade the endometrial cavity too. Here we present a case of a 4-year-old girl who suffered from vulvovaginitis caused by *E. vermicularis*. All members of her family were also infected by this parasitic helminth. In the vaginal sample, apart from the eggs, the female adult worm was observed under the microscope. Treatment with mebendazole was administered to all family members, and their progress was followed for a period of 3 weeks, during which it was found that the entire family had been cured. This patient experienced significant improvement in symptoms related to severe anxiety, nervousness, vaginal inflammation, itching, and vulvovaginitis caused by *E. vermicularis*. To prevent infection by *E. vermicularis*, it is crucial to disinfect underwear and bed sheets. In kindergartens, the spread of this parasite should not be underestimated, and asymptomatic individuals who have been exposed to infected persons should receive treatment to prevent an epidemic. Maintaining cleanliness and hygiene, especially after using the toilet, is of the most importance, particularly for girls who are more susceptible to *E. vermicularis* infection. Additionally, it is essential for all family members to be aware of the transmission routes of this parasite.

PS030.III

Achatina fulica as a novel model organism for gastropod-borne diseases in parasitology

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Introduction: The Giant African snail *Achatina fulica* is one of the largest terrestrial gastropods worldwide and is considered a neozoa in numerous countries. *A. fulica* possesses a complex innate immune system with professional phagocytes (haemocytes) capable of releasing invertebrate extracellular phagocyte traps (InEPTs). Despite being a suitable intermediate host (IH) for numerous metastrongyloid parasites, including the zoonotic parasite *Angiostrongylus cantonensis*, gastropod-parasite interactions have rarely been studied, neither in the field of human nor of veterinary parasitology.

Objective: This study aimed to gain knowledge on snail innate immune responses against the metastrongyloid nematodes *Angiostrongylus vasorum* and *Crenosoma striatum*. Furthermore, the *in vivo* migration route of *C. striatum* first-stage larvae (L1) in giant African snails was analysed by PET/CT scanning.

Material and methods: Haemocytes were isolated from *A. fulica* haemolymph and exposed to *A. vasorum* L1. The release of extracellular phagocyte traps (InEPTs) by haemocytes was detected via confocal-, scanning electron and 3D-holotomographic (Nanolive) microscopy. Reactive oxygen species (ROS) production was detected via flow cytometry. For PET/CT scanning, vital *C. striatum* L1 were marked with the radioactive tracer 18F-Fluordesoxyglucose (FDG) and fed to *A. fulica*. The snails were then scanned at different time points p. i.

Results: As demonstrated by confocal microscopy and SEM analyses, haemocytes indeed responded to L1 exposure by releasing InEPTs and immobilized *A. vasorum* L1 by this effector mechanism. In this context, co-localization of DNA-rich ET fibres with MPO and histones confirmed the classical characteristics of ETs. The general capacity of haemocytes to produce ROS after stimulation by A23187 was confirmed by FACS. Finally, early migration routes of *C. striatum* L1 could be tracked by PET/CT scanning. However, due to the short half-life of FDG (i. e. 110 min), only early time points after infection were monitored.

Conclusion: The usefulness of *A. fulica* as a novel model organism in parasitology was here demonstrated. Haemocyte-derived innate immune reactions were analysed in detail via FACS, 3D-holotomography, confocal microscopy and SEM. Via PET/CT scanning, snails were successfully monitored, demonstrating for the first time *in vivo C. striatum* larval migration.

PS033.III

LEISHBLOCK: Exploring an antileishmanial drug toolbox for blocking pathogen transmission in the sand fly vector

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Introduction: Leishmaniases are vector-borne diseases (VBD) caused by *Leishmania* parasites and transmitted through the bite of female phlebotomine sand flies. In the absence of human vaccines, control efforts rely on limited, toxic and costly treatments, and in reducing contact with the insect vectors. However, effectiveness of insecticide-treated nets and spraying is undermined by cost, impracticality and growing insecticide resistance. Mass spraying and traditional toxic sugar baits pose significant risks to non-target organisms, such as pollinators, potentially causing serious ecological imbalances. A new approach targeting parasites within their insect vectors using parasiticides has recently been proposed and has the potential to be combined with antiparasitic sugar baits with minimal toxicity to non-target organisms.

Objectives: LEISHBLOCK overarching aim is to contribute to the development of a novel and better approach for screening anti-*Leishmania* compounds in infected sand flies, with minimal toxicity to the vector and other putative non-target organisms.

Materials & methods: To evaluate whether the development of *Leishmania mexicana* promastigotes could be interrupted within *Lutzomyia longipalpis* sand flies; Amphotericin B - a first line antileishmanial drug used in the treatment of leishmaniases, was probed in a sugar bait and fed *ad libitum* to infected sand flies for 10 days. Treated and non-treated (infected) flies were collected at 14 days post infected blood meal (10 days of treatment) and dissected to analyse parasite burden qualitatively.

Results: Observation of dissected sand fly midguts showed that the majority of treated parasites failed to progress from the vectors" abdominal gut to the stomodeal valve, where normally promastigotes attach and differentiate into infectious metacyclic forms, without any impact on the mortality of the insect. Interestingly, even when treated parasites reached the stomodeal valve, they appeared immotile, suggesting their viability was impaired.

Conclusion: Together, these proof-of-concept experiment, suggests that promastigote development, specifically stomodeal valve colonisation may be interrupted through the use of a toxic antileishmanial sugar bait. Building on this data, the LEISHBLOCK project will focus on the development of a BAR-seq drug screening strategy enabling the evaluation of multiple putative antileishmanial compounds *in insecta*, while testing in parallel their toxicity in sand flies and pollinators.

PS036.III

The internal transcribed spacer 1 sequence polymorphism brings updates to tsetse species distribution in the northern Cameroon: Importance in planning efficient vector control

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Introduction: Vector control remains one of the best strategies to prevent the transmission of trypanosome infections in humans and livestock and, thus, a good way to achieve the elimination of human African trypanosomiasis and animal African trypanosomiasis. A key prerequisite for the success of any vector control strategy is the accurate identification and correct mapping of tsetse species.

Objectives: In this work, we updated the tsetse fly species identification and distribution in many geographical areas in Cameroon.

Materials & methods: Tsetse flies were captured from six localities in Cameroon, and their species were morphologically identified. Thereafter, DNA was extracted from legs of each tsetse fly and the length polymorphism of internal transcribed spacer-1 (ITS1) region of each fly was investigated using PCR. ITS1 DNA fragments of each tsetse species were sequenced. The sequences obtained were analysed and compared to those available in GenBank. This enabled to confirm/infirm results of the morphologic identification and then, to establish the phylogenetic relationships between tsetse species.

Results: Morphologic features allowed to clearly distinguish all the tsetse species captured in the South Region of Cameroon, that is, *Glossina palpalis palpalis*, *G. pallicera*, *G. caliginea* and *G. nigrofusca*. In the northern area, *G. morsitans submorsitans* could also be distinguished from *G. palpalis palpalis*, *G. tachinoides* and *G. fuscipes*, but these three later could not be distinguished with routine morphological characters. The ITS1 length polymorphism was high among most of the studied species and allowed to identify the following similar species with a single PCR, that is, *G. palpalis palpalis* with 241 or 242 bp and *G. tachinoides* with 221 or 222 bp, *G. fuscipes* with 236 or 237 bp. We also updated the old distribution of tsetse species in the areas assessed, highlighting the presence of *G. palpalis palpalis* instead of *G. fuscipes* in Mbakaou, or in sympatry with *G. morsitans submorsitans* in Dodeo (northern Cameroon).

Conclusion: This study confirms the presence of G. palpalis palpalis in the Adamawa Region of Cameroon. It highlights the limits of using morphological criteria to differentiate some tsetse species. Molecular tools based on the polymorphism of ITS1 of tsetse flies can differentiate tsetse species through a simple PCR before downstream analyses or vector control planning.

Fig. 1

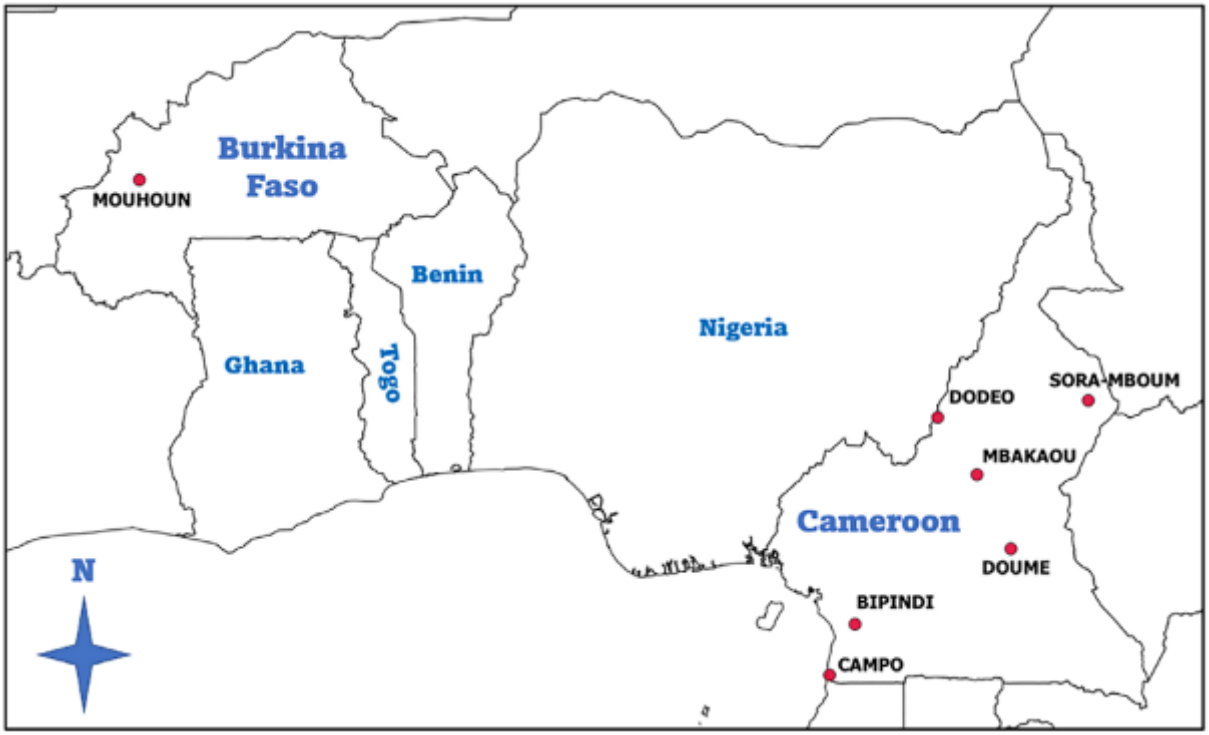

Fig. 2

Tsetse fly species	Study sites (ecological niche)	Number of individuals
Glossina fusca congolensis	Dodéo (Forest gallery)	8
G. morsitans submorsitans	Sora-Mboum (Savannah grassland)	28
	Dodéo (Forest gallery)	14
G. fuscipes fuscipes	Mbakaou (Forest gallery)	7
	Doumé (Forest area)	54
	Dodéo (Forest gallery)	1
G. nigrofusca nigrofusca	Campo Forest area	12
G. pallicera pallicera	Campo (Forest area)	20
	Bipindi (Forest area)	1
G. palpalis palpalis	Bipindi (Forest area)	12
	Campo (Forest area)	100
G. caliginea	Campo (Forest area)	22
G. tachinoides	Sora-Mboum (Savannah grassland)	156
	Dodéo (Forest gallery)	184
	Mouhoun (Forest gallery)	41
Total	/	660

PS039.III

Long-term study on identifying *Sarcocystis* species in environmental samples from Lithuania

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Various protozoan parasites can cause numerous illnesses each year with the help of asymptomatic animals transmitting diseases. Even though, protozoa can be transmitted by cysts through contaminated water or food, most studies are still using animal carcass samples. There is a great need to develop a precise methodology for identifying these parasites in environmental samples. This study aims to develop suitable methods for detecting *Sarcocystis* in environmental samples and compare the dynamics of its occurrence over three years.

From 2022 to 2024, environmental water, hay, and soil samples were collected every summer from nine livestock farms in Lithuania. On each farm, 1 l of hay, 200 ml of soil, and 1 l of water were collected from the animal grazing areas and a nearby water body. Sporocysts of *Sarcocystis* spp. from environmental samples were collected and concentrated using a previously developed water filtration method. A nested PCR using species-specific primers targeting the COX1 gene was selected for the detection of eight *Sarcocystis* species" DNA in the samples.

Analysis of the results showed that the highest overall detection rate of *Sarcocystis* spp. DNA was detected in the first year of the study (63.9%), while in the second and third year, the detection was about twice lower. The greatest diversity of *Sarcocystis* species was found in water and hay samples, where 2-3 different species were mostly identified, and in some cases even 4-5 different species. Interestingly, the results from the soil samples varied wildly, with either no parasite DNA being detected (48.1%) at all in the farm soil samples or 4 to 7 different species being identified. The highest species diversity was detected in 2022, when an average of 5 different *Sarcocystis* species were found per farm, while in 2023-2024 an average of 3 species were identified.

In summary, it can be stated that the situation on each farm under study is very different and can be determined by many different factors: domestic animals kept, possible contact with wild predators, weather conditions, etc. However, it is important to conduct environmental studies on farms to understand possible routes of parasite spread and take measures to prevent infection of animals and humans.

Acknowledgement: The project has received funding from the Research Council of Lithuania (LMTLT), agreement (S-MIP-23–7).

PS042.III

Assessment of dengue seroprevalence and *Aedes* mosquitoes distribution in Bafoussam, West Region, Cameroon

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Introduction: Dengue fever remains a significant public health issue in sub-Saharan Africa, with numerous cases reported in Cameroon since 2006. Although major outbreaks have not occurred, positive cases continue to rise across the country. This study aims to assess the seroprevalence of dengue and identify associated risk factors for infections in Bafoussam, Western Region of Cameroon.

Methods: This study was conducted from March to June 2024 at the Central Regional Hospital of Bafoussam, where consent was obtained from febrile patients (temperature ≥38 °C) for blood collection (3 ml in dry tubes) to perform assays using the SD Bioline Dengue Duo™ rapid diagnostic test, which detects immunological markers (IgG, IgM, and NS1). Additionally, an entomological survey of Aedes breeding sites was conducted in six clusters of Bafoussam to assess their ecology, distribution, and disease transmission risk. Data were analyzed using R software version 4.0.4.

Results: A total of 100 participants were recorded, with 30% testing positive for dengue: IgG (19%), IgM (14%), and NS1 (2%). No correlation was found between sociodemographic factors and dengue seropositivity ($p > 0.5$). The 1st district of Bafoussam had the highest number of positive cases (12/100), with 52 male participants. A total of 292 breeding sites were observed: 57 (19.52%) in the 1st district, 70 (23.97%) in the 2nd, and 165 (56.50%) in the 3rd, with only a high Container Index (>20%) recorded. Tires (89.38%) were the most prevalent breeding sites. A total of 3,517 *Aedes* mosquitoes were collected, with *Aedes albopictus* (89.59%) being more abundant than *Aedes aegypti* (10.41%). A positive correlation was noted between the presence of Ae. albopictus larvae and tires ($p = 0.002$).

Conclusion: This study revealed, for the first time, the circulation of dengue in Bafoussam. The primary vector species, ***Aedes albopictus*** and ***Aedes aegypti***, were widely distributed, followed by high risk of arbovirus diseases transmission.

PS045.III

Dynamics of malaria prevalence and co-infection with helminthiasis among children aged 1 to 12 years in the Olama locality in the forest zone of Cameroon

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Background: Malaria and helminth co-infection is common in tropical regions. Malaria, a major health concern caused by Plasmodium parasites, often coexists with helminth infections. Poverty-stricken areas with poor sanitation are breeding grounds. This dual burden significantly affects health, especially children. Our study tracked malaria, helminthiasis, and co-infection trends in Olama, Cameroon.

Materials and methods: a longitudinal study was conducted from February to November 2023, involving a hundred children. Blood samples were collected through finger pricks, and thick smears were prepared, stained with Giemsa, and examined under a microscope to determine the presence of malaria parasites. Stool samples were also collected and examined using the Kato-Katz technique for the identification of helminth eggs. Demographic, socioeconomic, and knowledge data regarding malaria and helminth infection transmission were collected using a structured questionnaire.

Results: A hundred children aged 1 to 12 years participated in the study. The prevalence of malaria and helminthiasis varied depending on the collection periods. In April, August, and November 2023, the percentage of children infected with malaria was 33.05%, 24.48%, and 10%, respectively, with a significant difference between the periods ($P<0.05$). The prevalence of helminthiasis was 23.42%, 13.58%, and 15.29%, respectively, with *Ascaris lumbricoides* being the predominant species identified. The prevalence of malaria-helminthiasis co-infection was 5.08%, 4.08%, and 1.81% in April, August, and November, respectively, with a significant difference ($P<0.05$). An increased parasitic load of malaria was observed in children under 5 years old in cases of co-infection compared to those solely infected with malaria.

Conclusion: This study demonstrated a decreasing trend in the prevalence of malaria, helminthiasis, and co-infection among children in Olama. The presence of co-infestation with intestinal helminthiasis appears to influence the predisposition to develop a severe form of malaria in children under 5 years of age.

Fig. 1

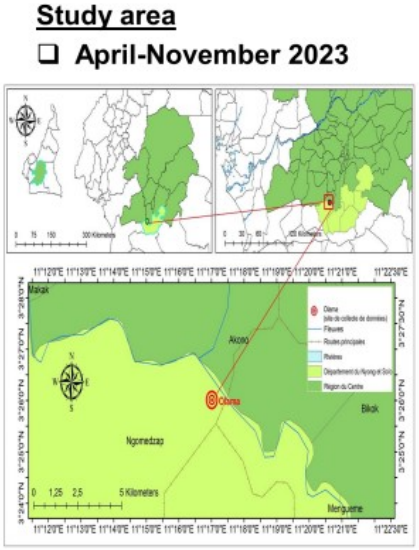
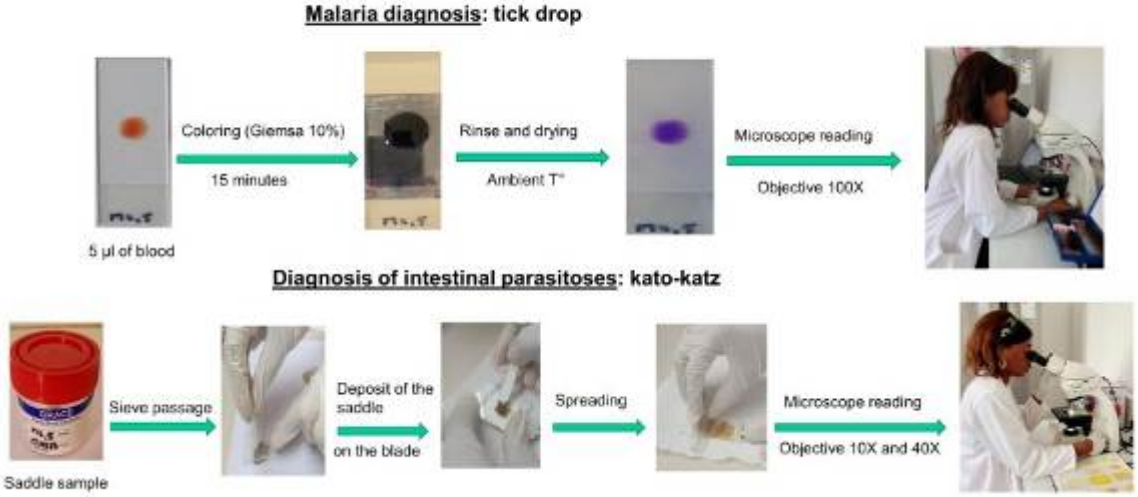


Fig. 2


Malaria diagnosis: tick drop
Coloring (Giemsa 10%)
15 minutes
Rinse and drying
Ambient T°
Microscope reading
Objective 100X
5 µl of blood
Diagnosis of intestinal parasitoses: kato-katz
Sieve passage
Deposit of the saddle
on the blade
Spreading
Microscope reading
Objective 10X and 40X
Saddle sample

PS048.III

Toxicity testing of anti-trypanosomatids using the model organism c. *Elegans*

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Introduction: *Caenorhabditis elegans* are non-parasitic nematodes which are capable of generating a culture rapidly via self-fertilisation as well as their short lifespan allowing for multigenerational testing. Assays using *C. elegans* have been found to consistently rank toxicity in mammals due to genes and signalling pathways being well-conserved in the nematodes.

Objectives: The aim of the project is to use the nematode *C. elegans* as a 3R-compliant model organism for testing the toxicity of potential anti-trypanosomatids which will achieved through both lethality assays and monitoring the effects of sub-lethal doses.

Materials and Methods: Changes to the reproductive rate, movement (can be measured using head thrashing, body bends, etc.) and pharyngeal pumping would all be indicative of potential toxicity. Trypanosome culture assays will also be used to rank the potency of the molecules. The molecules being tested will be aminopiperidine derivatives building off previous work by Dardonville *et al.* (2009).

Results: Preliminary findings from these toxicity investigations will be presented at the conference.

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PS051.III

Intra-specific variations in *Schistosoma mansoni* and their possible contribution to inconsistent virulence and diverse clinical outcomes

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Background: *Schistosoma mansoni* was introduced from Africa to the Americas during the transatlantic slave trade and remains a major public health problem in parts of South America and the Caribbean. This study presents a comprehensive comparative analysis of three *S. mansoni* strains with different geographical origins—from Liberia, Belo Horizonte and Puerto Rico. We demonstrated significant variation in virulence and host-parasite interactions.

Methods: We investigated the phenotypic characteristics of the parasite and its eggs, as well as the immunopathologic effects on laboratory mouse organ systems.

Results: Our results show significant differences in worm morphology, worm burden, egg size, and pathologic organ changes between these strains. The Puerto Rican strain showed the highest virulence, as evidenced by marked liver and spleen changes and advanced liver fibrosis indicated by increased collagen content. In contrast, the strains from Liberia and Belo Horizonte had a less pathogenic profile with less liver fibrosis. We found further variations in granuloma formation, cytokine expression and T-cell dynamics, indicating different immune responses.

Conclusion: Our study emphasizes the importance of considering intra-specific variations of *S. mansoni* for the development of targeted therapies and public health strategies. The different virulence patterns, host immune responses and organ pathologies observed in these strains provide important insights for future research and could inform region-specific interventions for schistosomiasis control.

PS054.III

De novo genome assembly and structural comparison of eimeria species using nanopore sequencing

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Eimeria are a genus of apicomplexan parasites that infect all major livestock. Ingestion of this parasite can lead to coccidiosis, an intestinal disease whose clinical signs include haemorrhagic diarrhoea, diminished weight gain, and mortality in severe infection. The genus is comprised of more than 1,500 species, each of which are monoxenous and mostly host-specific in their lifecycle. Of particular economic importance are species which infect chickens, as coccidiosis incurs costs upwards of £10.4 billion to annual global poultry production. Despite their impact in the agriculture sector, little is known about the genetic diversity of these parasites, and how this variation contributes to the rising level of resistance to current treatment strategies. These knowledge gaps exist primarily due to the limited number of loci used in population studies, as well as the fragmented and incomplete reference sequences arising from technical limitations and the inherent repetitive structure of *Eimeria* genomes.

We developed a novel Nanopore sequencing workflow to improve upon the quality of *Eimeria* reference genomes by generating long reads capable of spanning expansive regions of low complexity. Acting as scaffolds, these large sequences augment the *de novo* assembly of *Eimeria* genomes by properly orienting shorter sequences and bridging gaps between them. We improved the contiguity and compositional quality of *E. acervulina* and *E. maxima* genomes, and generated a high-quality reference sequence for the newly characterised species *E. zaria*.

Our findings show that current *Eimeria* assemblies can be substantially improved upon with the use of Nanopore long reads. By better capturing low-complexity regions, more information is retained which allows for a more comprehensive structural comparison between *Eimeria* genomes, as well as aiding in the downstream analyses of whole genome sequencing data. This Nanopore workflow will be applied to the sequencing of field samples and archived isolates with distinctive phenotypes. By analysing these data against the improved reference sequences, we can begin to better understand the genetic diversity of these organisms, their local and regional population structures, as well as the genetic determinants of clinically relevant traits such as drug resistance and precocious development.

Fig. 1

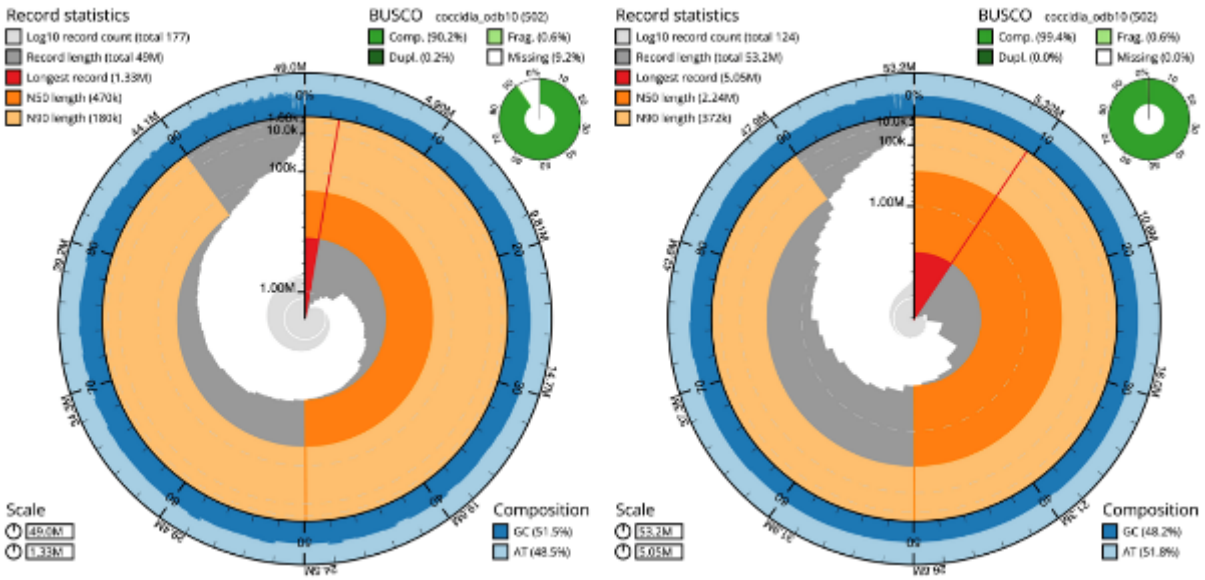
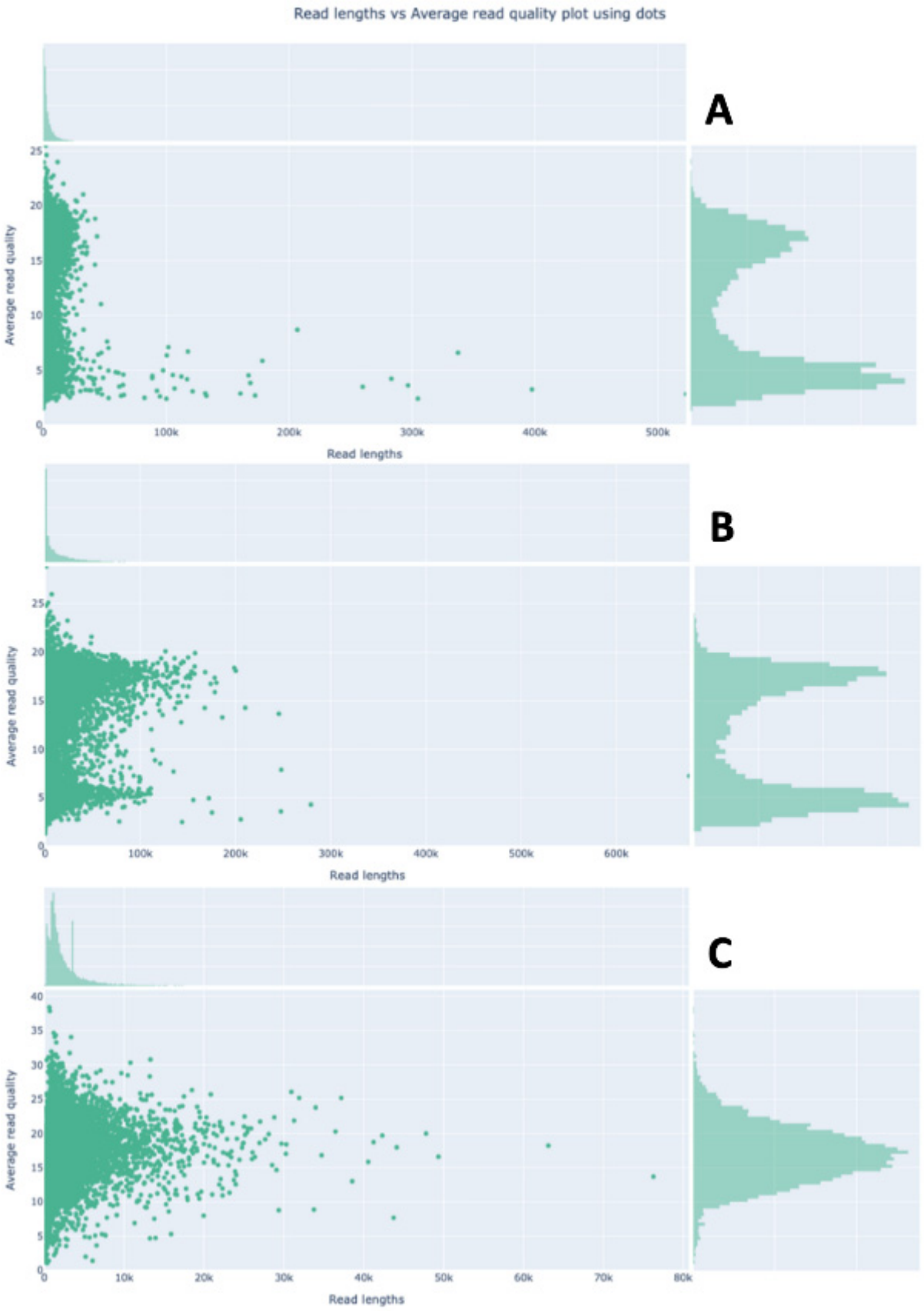


Fig. 2


Read lengths vs Average read quality plot using dots
A
Average read quality
Read lengths
0
100k
200k
300k
400k
500k
B
Average read quality
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300k
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500k
600k
C
Average read quality
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0
10k
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30k
40k
50k
60k
70k
80k

PS057.III

Structural and functional dissection of novel factors driving antigenic variation in African trypanosomes

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African trypanosomes are a model organism demonstrating extreme molecular strategies for evasion of the host immune response by periodically switching their homogeneous coat of variant-surface-glycoproteins (VSGs). *VSG* genes are co-transcribed with expression-site-associated genes (*ESAGs*) from specialized polycistronic "expression sites" (ES), however *VSG* transcripts are present at significantly higher levels. The single active *VSG* gene is transcribed within the expression-site body (ESB), a specialised sub-nuclear compartment. VSGs are generated through recombination of approximately 2,600 *VSG* genes and pseudogenes, providing a virtually unlimited diversity of VSGs to escape the immune system.

VSG-exclusion-factor-2 (VEX2), a 224 kDa large member of the Superfamily 1 (SF1) helicases, plays a crucial role for sustaining singular *VSG* expression, as its loss leads to simultaneous expression of multiple *VSGs* within individual cells. Super-resolution microscopy has revealed that VEX2 colocalizes with the ESB and interacts with VSG exclusion-1 (VEX1), a *Spliced-Leader*-(SL)-array associated protein. Together, VEX1 and VEX2 form an inter-chromosomal bridge that facilitates highly efficient *VSG* mRNA maturation. However, the mechanistic details of the function and regulation of VEX2 as well as the structural basis of its interaction with VEX1 remain poorly understood.

Firstly, we aim to investigate VEX2 substrate specificity and regulation. To that end, we recombinantly expressed and purified VEX2 full-length and its individual domains in a *Leishmania tarentolae*-based expression system (LEXSY) to pursue a biochemical and structural characterization using CryoEM. Ultimately, we plan to co-express VEX1 and VEX2 in LEXSY to better understand the basis of their interactions.

Moreover, through the determination of the VEX2 spatial proteome, using TurboID, we identified ESB2, the second known ESB-specific protein, which is uniquely expressed in the *T. brucei* mammalian-stage. Structural bioinformatics indicates that ESB2 shares structural homology to a characterized RNA nuclease family. Transcriptomic analysis of ESB2-depleted trypanosomes shows that it has a role in modulating the differential expression between *ESAG* and *VSG* genes. However, many aspects of its function and regulation remain unclear. Our goal is to further investigate the molecular basis of its transcript specificity and regulation, as well as its interactions with VEX2 and other factors.

PS060.III

Shedding light into the distribution and genetic diversity of *Hydatigera* spp. in Europe

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Introduction: *Hydatigera* is a globally distributed genus of cestodes whose life cycle involves wild and domestic felids as definitive hosts and rodents as intermediate hosts. The genus *Hydatigera* has undergone several taxonomic revisions. Recently, it was recognized as distinct from the genus *Taenia* within the family Taeniidae. This reclassification led to the transfer of the species *Taenia taeniaeformis*, *T. parva*, and *T. krepkogorski* to *Hydatigera* (Lavikainen et al., 2016; Nakao et al., 2013). Genetic studies have revealed that *Hydatigera taeniaeformis* constitutes a cryptic species complex with three clades: *H. taeniaeformis* sensu stricto, found globally in rats and mice; *H. kamiyai*, which occurs in various terrestrial and aquatic rodents across Europe; and *Hydatigera* sp., or clade C, restricted to the Mediterranean region (Galimberti et al., 2012; Lavikainen et al., 2016). Due to recent taxonomic updates, limited data exist on the recognized species, their geographic ranges and their genetic diversity.

Objectives: The aim of this study is to expand the knowledge of the geographical distribution and the genetic diversity of *Hydatigera* spp. across Europe

Materials & methods: Specimens were collected from wild and domestic felids and rodents in Germany, France, Italy, Finland, Luxembourg, and Romania. Genetic analysis of the isolates is being conducted through sequencing of the mitochondrial genes *nad1* and *cox1*. Using these genes, the phylogenetic relationships and genetic diversity of the identified *Hydatigera* species are being investigated.

Results: Preliminary findings confirm the presence of *H. kamiyai* across surveyed European countries and *Hydatigera* sp. (clade C) in the Mediterranean region. Initial intraspecific genetic analyses, among the first to be based on complete *nad1* and *cox1* gene sequences, indicate a high genetic and haplotype diversity for *H. kamiyai* in various European countries. In contrast, *Hydatigera* sp. (clade C), with its restricted geographical distribution, exhibits comparatively low genetic variability.

Conclusion: The analyses of the entire *nad1* and *cox1* sequences will provide further insights into the taxonomy, genetic diversity, and population dynamics of the cryptic species complex *Hydatigera taeniaeformis* sensu lato.

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PS063.III

Unveiling the SNARE machinery of vesicle trafficking during the egress of *Plasmodium falciparum* gametocytes from red blood cells

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Malaria is a vector-borne infectious disease caused by protozoan parasites of the genus *Plasmodium*, which are transmitted to humans through the bite of infected *Anopheles* female mosquitos. During the complex life cycle of this parasite, the egress of *Plasmodium falciparum* gametocytes from infected red blood cells (RBCs) is a critical event to ensure transmission from human to mosquito. Within RBCs, the malaria parasite is enclosed in a parasitophorous vacuole (PV). The egress of gametocytes from RBCs follows an inside-out mode, during which the PV membrane (PVM) ruptures prior to the RBC membrane (RBCM). Membrane rupture involves the exocytosis of specialized egress vesicles of the parasite, including osmiophilic bodies (OBs) containing proteins that facilitate the rupture of the PVM, as well as vesicles containing the perforin-like protein PPLP2 (referred to here as P-EVs), which are essential for erythrocyte lysis. In a previous BioID-based study, we identified 143 putative gametocyte egress vesicle proteins (GEVPs), including members of the SNARE (soluble N-ethylmaleimide sensitive factor attachment protein receptor) family, which are known to facilitate vesicle fusion in other eukaryotes. To investigate the molecular machinery of egress vesicle trafficking in *P. falciparum* in more detail, *in-sillico* analyses were performed and 25 plasmodial SNARE proteins were identified, the majority of which showed peak expression in gametocytes. Here, we investigated the expression and subcellular localization of selected gametocyte SNAREs, using tagged transgenic lines. Future studies aim at functionally characterizing the SNARE candidates using KD or inducible KO systems.

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PS066.III

Nationwide survey of borrelia prevalence and species distribution in *Ixodes ricinus* ticks in Germany

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As vector for various pathogens, in particular those causing Lyme borreliosis, *Ixodes ricinus* is the most important tick species in large parts of Europe and also Germany.

The aims of this study are to obtain a representative Germany-wide estimate of *Borrelia* prevalence in questing *I. ricinus* nymphs, to identify regional differences, and to determine the *Borrelia* species distribution. The ticks were collected at various locations throughout Germany during 2018-2020. If available, 300 nymphs per location will be examined by quantitative real-time PCR, resulting in an approximate sample size of 12,000 specimens. First results for 15 locations in the regions of Hannover, Emsland, Bremen, Uelzen and Kassel for the years 2018 and 2019 have already been published. In this north-western German region, the *Borrelia* prevalence in questing nymphs was 28.6% (300/1050) [1]. Among 48 further locations analysed so far for 2018, an overall prevalence of 32.8% (1105/3373) was determined. The preliminary data indicate possibly higher prevalences in northern Germany, e.g. in Stralsund with 31.7% (95/300) and Schwerin with 35.0% (105/300), compared to southern regions, e.g. Erlangen with 22.4% (35/156) and Schrobenhausen near Munich with 20.3% (36/177).

The *Borrelia* prevalence estimation in questing *I. ricinus* nymphs is ongoing, and additionally first results of the *Borrelia* species differentiation, including the relapsing fever spirochaete *Borrelia miyamotoi*, will be presented.

Reference:

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PS072.III

Flowing in circles: Exploring degradation and recovery of freshwater parasite communities in a mesocosm experiment

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The Ruhr region is well known for its long tradition of coal mining and its steel industry. Although the coal mines are now closed, the landscape is still clearly characterized by the past, which has also left its mark on the region's rivers: they were exposed to various anthropogenic stressors, in particular decades of sewage discharge and morphological degradation. In recent years, various restoration measures have been carried out on the affected streams, but for many communities it is not entirely clear how they develop under exposure to the stressors encountered and the subsequent recovery processes. Among these communities are eukaryotic microparasites. Here, we present how we studied the responses of parasites from the Boye River in Bottrop, Germany, to three potential stressors (increased salinity, increased temperature, and reduced flow velocity) using an outdoor mesocosm setup – the ExStream system. In this outdoor experiment, we analyzed 64 mesocosms that were constantly supplied with water from the Boye, allowing small organisms to migrate freely into and out of the mesocosms. The experimental setup underwent two phases: a stressor phase in which all stressors were applied in a full factorial design, and a subsequent recovery phase. Metabarcoding of biofilms sampled after the stressor and the recovery phase revealed a wide range of relevant parasitic taxa associated with the biofilm. Furthermore, the parasite communities differed between some of the treatments. We show that the ExStream system can help to explore the consequences of environmental degradation and recovery processes for parasites in riverine ecosystems.

PS075.III

Genetic diversity and population structure and its impact on morphometry of the liver fluke *Fasciola hepatica* in German dairy cattle

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[2]Justus Liebig University Giessen, Institute of Animal Breeding and Genetics, Giessen, Germany

The common liver fluke, *Fasciola hepatica*, is a globally distributed parasitic trematode that mainly infects ruminants and can cause significant economic losses. Knowledge of genetic diversity and population structure is crucial for understanding parasite distribution, evolution, and the spread of new alleles. This study is the first to investigate population genetics of *F. hepatica* in Germany and to link genotyping results to morphological traits in terms of fluke size.

A total of 774 adult *F. hepatica* specimens were collected from 73 bovine livers. Genetic analyses were performed by sequencing mitochondrial (*cox1*, *nad1*) as well as nuclear (ITS-1) sequences and eight nuclear microsatellite loci. Fluke lengths and widths were measured and compared with genotyping results.

The mitochondrial markers *cox1* and *nad1* revealed considerable genetic diversity, with 119 distinct haplotypes. Mean haplotype diversity (Hd) and nucleotide diversity (π) were 0.81 and 0.0041, respectively. Mitochondrial phylogenetic analysis identified two genetic clusters with no clear association to host or farm of origin. The host explained 7.40% and the farm 10.14% of the genetic variation, while the majority of variation occurred within hosts (82.46%). The nuclear ITS-1 region showed only minimal intra-species variation, as 772 out of 774 flukes exhibited an identical sequence. Microsatellite analysis revealed high polymorphism with a mean allele frequency of 19.0 per locus and high heterozygosity with a mean expected heterozygosity of 0.71. Of all successfully genotyped flukes (94.32%, 730/774), 68.49% (500/730) had a unique multilocus genotype (MLG) and 31.51% (230/730) were clones. The mean genotypic richness per farm was 0.81. Similar to the mitochondrial dataset, most of the genetic variation was found within hosts (92.87%), while the host explained 1.92% and the farm 5.21% of the variation. The number of migrants between farms was 3.5, confirming high gene flow. Population structure analysis revealed that two farms differed genetically from the others based on microsatellite loci. Fluke size differed significantly between the two mitochondrial clusters, with flukes from cluster 2 being longer and wider than those from cluster 1. Moreover, flukes from the two farms that separated in the microsatellite analysis were shorter and narrower than flukes from all other farms.

The high levels of genetic diversity and heterozygosity indicate a strong potential for adaptability, while the significant gene flow between farms facilitates the spread of new alleles, potentially harbouring adaptive traits such as drug resistance. The different population structures revealed by mitochondrial and microsatellite analyses highlight the value of comparatively investigating mitochondrial and nuclear markers. Furthermore, the identified haplo- and genotypes had an influence on fluke size, providing new insights into how genetic factors can influence phenotypic traits of *F. hepatica*.

PS078.III

An experimental tool to study exportin-1-dependent nuclear export in *Plasmodium falciparum*

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The unicellular eukaryote *Plasmodium falciparum* causes severe human malaria. All clinical symptoms occur during the blood stage of infection, when *P. falciparum* proliferates through an unusual cell cycle mode called schizogony and a multinucleated cell is formed. Although the parasite"s nuclei reside in the same cytoplasm, they multiply asynchronously and the DNA replication fork protein *Pf*PCNA1 accumulates only in those nuclei that replicate their DNA. All nuclei of a schizont appear to have access to a common pool of *Pf*PCNA1 and its nuclear accumulation occurs very fast (<5min). However, it is not known if *Pf*PCNA1 is actively transported into and out of the nucleus. In human cells, the major nuclear export receptor exportin-1 (XPO1) has been implicated in the active nuclear export of PCNA. XPO1-dependent nuclear export can be studied with the inhibitor leptomycin B (LMB), which covalently binds to a cysteine residue in the nuclear export signal (NES) binding pocket of XPO1, preventing the interaction of XPO1 with cargo proteins. In *P. falciparum* this cysteine is not conserved and the parasite is insensitive to LMB.To harness LMB as a research tool and to study a potential role of *Pf*XPO1 in *Pf*PCNA1 localization, we genetically engineered *Pf*XPO1 and mutated isoleucine 637 to cysteine (*Pf*XPO1I637C). This rendered *P. falciparum* sensitive to LMB. Our data suggest that *Pf*XPO1I637C ring stages are most sensitive to LMB, while trophozoite and schizont development is slowed down in the presence of LMB. To test if *Pf*XPO1 possesses canonical function as nuclear export receptor, we used episomally expressed synthetic and endogenous cargo proteins. While constructs with a single canonical NES fused to GFP do not appear to be functional, the *P. falciparum* homolog of the known XPO1-cargo protein signal recognition particle 54 (SRP54) shows a predominantly cytoplasmic localization. The effect of LMB-treatment on *Pf*SRP54::GFP is currently being analyzed. We also generated a *Pf*PCNA1::GFP expressing cell line in the *Pf*XPO1I637Cbackground and preliminary data hints at *Pf*XPO1 affecting the localization of *Pf*PCNA1.Together, we provide an experimental tool to study the role of *Pf*XPO1, potentially in nuclear export. Our work on the nucleocytoplasmic transport will also reveal its relevance for heterogeneous nuclear accumulation of *Pf*PCNA1. A better understanding of this fundamental biology of *P. falciparum* may also pave the way for new intervention strategies to curb malaria.

PS081.III

Biogenesis and dynamics of a membrane-devoid organelle in coccidian parasites

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The protozoan *Eimeria* is an Apicomplexan parasite with species known to infect most vertebrate species (excluding humans, dogs and cats) causing the intestinal disease coccidiosis. *Eimeria* infections has an important impact in livestock and poultry, leading to production losses and compromised animal welfare. This group of parasites present a unique, electron dense, lipid rich, membrane-less organelle called the refractile body (RB) [1]. This organelle is of unknown function and only present in the early infective stages of the animal host (sporozoites to first-generation merozoites) and then again in environmental sporulated oocysts [2]. To understand the dynamics of RBs, two transgenic population were generated, tagging to a fluorescent marker to two known refractile body proteins, sporozoite antigen seven (SO7) and transhydrogenase (NTH), respectively. These have allowed for the visualisation of the biogenesis of the organelle during oocyst sporulation, which started as a cytosolic localisation before condensing by an apparent liquid-liquid phase separation process, forming multiple condensates that coincided with the cytoplasm separating from the oocyst wall (Figure 1A). These condensates were deposited into each of the four developing sporoblasts (Figure 1B) before localising into the two final RBs in the devolved sporozoites within the sporocysts (Figure 1D). Sporozoites within the oocyst were artificially released in experimental settings and left to invade epithelial cells in vitro. As the intracellular sporozoite rounded up into trophozoites, the two RBs merged into a single one (Figure 2A). During early schizogony, when the parasite expands and the after initial nuclear division occurs, the single RB fragmented again into smaller condensates (Figure 2B). In mature schizonts when radial merozoite budding process takes place (Figure 2C), the RBs were incorporated into each merozoite as a single anterior body (figure 2D), verifying observations reported before [3]. This is the first step toward gaining new insights into these mysterious organelles. Understanding the RB dynamics and repurposing these transgenics for BioID will allow us to identify proteins within these organelles that could be potential targets for disease control.

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Fig. 1

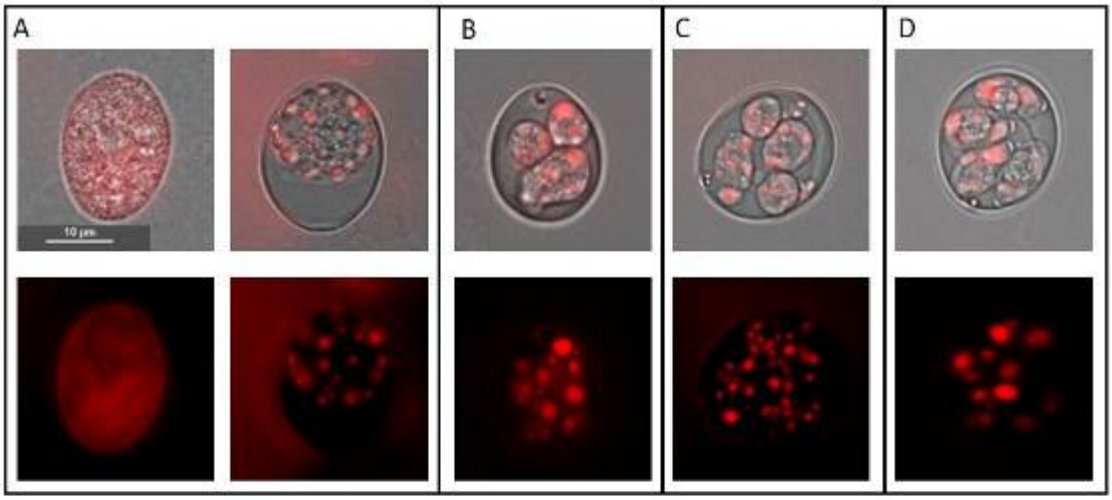

Figure 1. Biogenesis of the *Eimeria tenella* refractile body (RB) during oocysts sporulation visualised using parasites expressing fluorescently tagged SO7. (A) Early sporogony. Cytosolic RB form into multiple condensates coinciding with separation of the cytoplasm from the oocyst wall. (B/C) Sporoblast formation. RB at deposited into the four developing sporoblasts. (D) Sporozoite formation. Two RB are identified in the devolved sporozoites within matured sporocysts.

Fig. 2

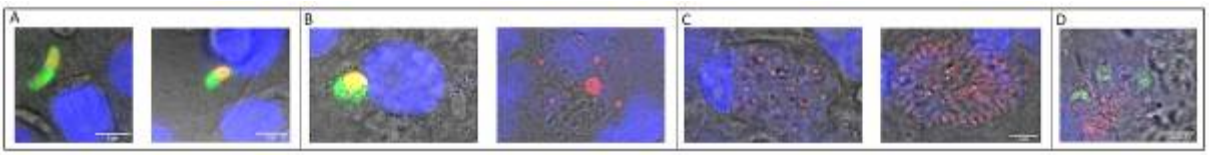

Figure 2. Dynamics of the *Eimeria tenella* refractile body (RB) during in vitro intracellular development visualised using parasites expressing fluorescently tagged SO7 and NTH. (A) Intracellular sporozoites. Two RB merge as trophozoites form. (B) Early schizonts. The single RB fragments upon parasite growth and nuclear division. (C) Mature schizonts. RBs follows the radial budding of developing merozoites. (D) Merozoite hatching. Each merozoite receives a single anterior RB.

PS084.III

Exonerating *Toxocara canis*: The dog roundworm shows no link to Alzheimer's disease in mice

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Introduction: The potential link between infections and Alzheimer"s disease (AD) has led to speculations about the role of various pathogens in triggering amyloid beta (Aβ) overproduction, potentially contributing to AD onset. The globally distributed dog roundworm *Toxocara canis* has been suggested as a possible candidate due to the neurotropism of its larvae and the chronicity of the infection. However, experimental data supporting this hypothesis are limited.

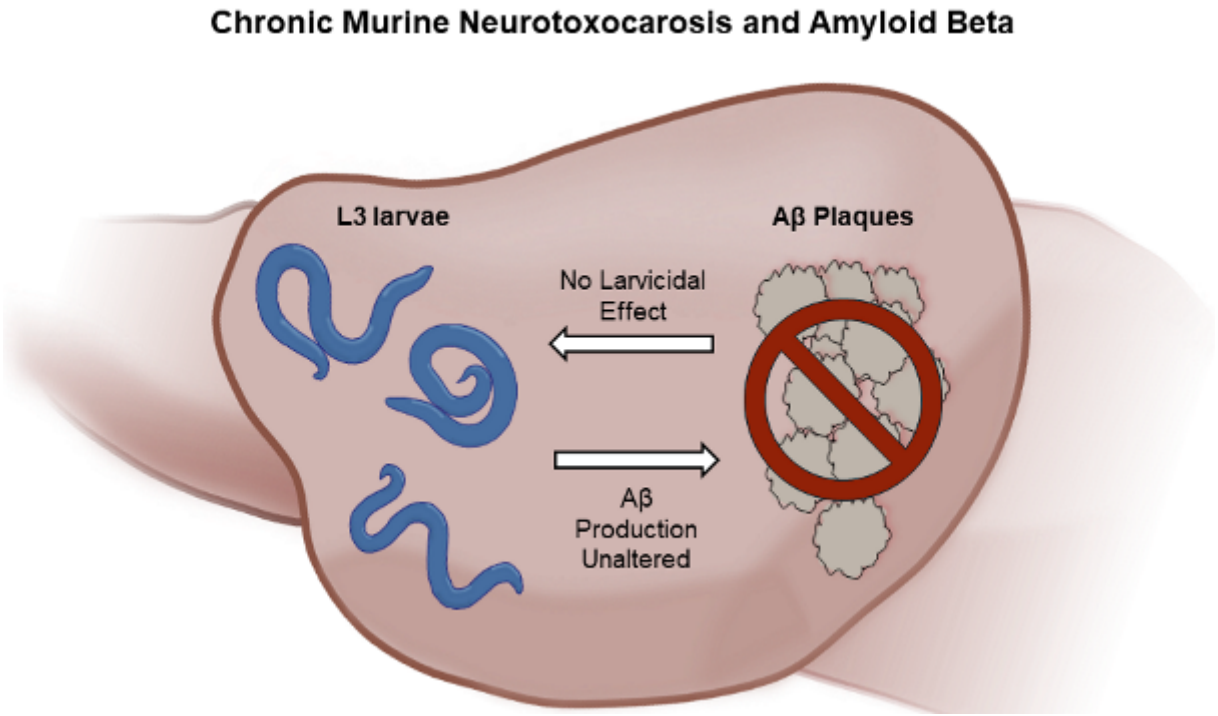
Objectives: This study aims to investigate whether chronic *T. canis* infection induces AD-like pathology in mice.

Materials & Methods: BALB/c and APP/PS1 transgenic mice, which overproduce Aβ, were infected with *T. canis* L3 larvae and monitored for larval burden, Aβ accumulation, and behavioral changes. *In vitro* tests of recombinant Aβ toxicity on the larvae were also performed.

Results: Despite the presence of *T. canis* larvae in the central nervous system 8 and 16 weeks post-infection, no significant increase in Aβ concentration or AD-related behavioral changes were observed. Interestingly, Aβ was detected on the surface and within the intestines of *T. canis* larvae, but in vitro exposure to recombinant Aβ did not affect larval viability or morphology.

Conclusions: Our findings suggest that *T. canis* infection does not trigger AD-like pathology in mice, and Aβ does not serve as an antiparasitic agent. This challenges the emerging hypothesis that chronic neurotoxocarosis infections contribute to AD development.

Fig. 1


Chronic Murine Neurotoxocarosis and Amyloid Beta
L3 larvae
Aβ Plaques
No Larvicidal Effect
Aβ Production Unaltered

PS087.III

An approach to further understand the influence of cysteine peptidases on the virulence of *E. histolytica*

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Entamoeba histolytica (*E. histolytica*) is a pathogenic amoeba and the causative agent of amebiasis. Most cases remain asymptomatic. However, about 10 % of infections lead to amoebic colitis and another 1 % of those cases lead to invasive amebiasis in which the parasite can invade different tissues in the host. One of the most common effects of invasive amebiasis is the formation of amebic liver abscesses (ALA). The formation of ALA has been researched extensively in the past years, whereas the mechanisms leading to amoebic colitis remain elusive. We aim to understand the invasion of intestinal tissue by *E. histolytica*. We have two clones available at our lab: the non-pathogenic (np) $A1^{np}$ clone and the pathogenic (p) $B2^{p}$ clone. Both clones originate from the same isolate of a patient with amoebic colitis. We strive to replace animal experiments with organoids and different cell lines, since few to none suitable, ethical animal models exist. We established human small intestine organoids, human colon organoids and Caco-2 cells to study the invasion of intestinal tissues in our lab. We use different methods which include, but are not limited to transepithelial/-endothelial resistance (TEER) measurements, immunofluorescence assays and transcriptome analyses. In particular, we are trying to understand the influence of cysteine peptidases, which are a virulence factor of *E. histolytica* during the process of invasion.

PS090.III

Impact of chemical snail control on intermediate host snail populations for urogenital schistosomiasis elimination in Pemba, Tanzania: Findings of a 3-year intervention study

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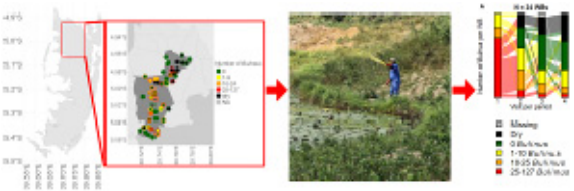
The World Health Organization (WHO) has the goal to eliminate schistosomiasis as a public health problem globally by 2030 and to interrupt transmission in selected areas. Chemical snail control is one important measure to reduce transmission and achieve local elimination. We aimed to assess the impact of several rounds of chemical snail control on the presence and number of the *Schistosoma haematobium* intermediate snail host (*Bulinus* spp.) in water bodies (WBs) on Pemba Island, Tanzania, a setting targeted for elimination of urogenital schistosomiasis.

During the three annual intervention periods of the SchistoBreak study implemented in the north of Pemba from 2020 to 2024, malacological surveys were conducted up to four times per period in WBs of hotspot implementation units (IUs). Present freshwater snail species, vegetation, and WB characteristics were recorded. If *Bulinus* was found, the snails were inspected for *S. haematobium* infection and snail control with niclosamide was conducted.

Across the three intervention periods, a total of 112 WBs were identified in 8 hotspots IUs. The spatial distribution of WBs with *Bulinus* per IU was heterogeneous, ranging from 0.0% (0/15) of WBs infested in one IU in 2022 to 80.0% (8/10) of WBs infested in one IU in 2021. *Bulinus* presence was significantly associated with lower pH values in WBs (odds ratio: 0.2, 95% confidence interval: 0.1-0.4). A total of 0.2% (6/2360) of collected *Bulinus* were shedding *S. haematobium* cerariae. Following snail control, the number of *Bulinus* decreased or remained absent in 56.7% (38/67) of visits at WBs when compared to the previous visit in 2021, 54.9% (28/51) in 2022, and 33.3% (32/96) in 2023. In a total of 43.1% (22/55) of initially infested WBs, no *Bulinus* were found in the survey round conducted a few weeks after the first application of niclosamide. However, 25.4% (14/55) of WBs showed a pattern of recurring *Bulinus* presence.

The distribution of WBs containing *Bulinus* was very heterogeneous. The percentage of *Bulinus* with patent *S. haematobium* infection in our study area was extremely low. Repeated niclosamide application reduced the number of *Bulinus* in WBs, but snails often recurred after one or multiple treatments. While chemical molluscicide can reduce snail numbers, to fully break the *S. haematobium* transmission cycle, the timely diagnosis and treatment of infected humans, access to clean water, sanitation, and health education remain of prime importance.

Fig. 1



PS093.III

Monoclonal antibody-based characterisation of *Angiostrongylus vasorum* antigens identifies prospective therapeutic protein targets conserved in a wide range of helminths of veterinary and zoonotic relevance

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Parasite proteins play crucial roles in biological processes, host-parasite interactions, evasion of host immunity, and overall viability of the parasite. In this study, we employed a comprehensive approach combining monoclonal antibodies (mAbs) with proteomic characterisation, ELISA, immunohistochemistry, immunofluorescence, protein structure analyses, and prediction of therapeutic potential to investigate the antigenic landscape of helminth parasites, and to identify potential drug targets. A panel of nine monoclonal antibodies produced against *A. vasorum* excretory-secretory products were used to identify corresponding helminth antigens, their molecular diversity, conservation across parasite species, and potential suitability as drug targets. ELISA evaluation uncovered both conserved and variable antigenic epitopes across a range of 74 different antigens from 48 parasite species. Some mAbs showed affine binding to antigens of the *Angiostrongylus* genus with stage-specific affinity, while others were species-specific for *A. vasorum,* or bound epitopes across species, genera, and classes. The results of immunohistochemistry and immunofluorescence illustrated that some mAbs strongly targeted the reproductive and/or the intestinal system, whereas others were more associated with eggs or larvae. More specifically, mAb *Av* 56/1/2/1 exhibited affine binding, indicated by OD values > 0.3, specifically to the *Angiostrongylus* genus with varying stage-specific affinity, and mAbs *Av* 5/5, 70/1, and 33/2/2 were specific at the *A.vasorum* species level. mAbs *Av* 28/1, 1/1/2, 4/3/5, 7/2, and 8/5 appeared to bind epitopes across species, genera, and classes. Potential protein targets and their range of cellular functions such as protein synthesis, metabolic processes, and cytoskeletal organisation were identified. The majority of the 34 identified proteins showed homology to known proteins in *Caenorhabditis elegans* and were associated with various drug-binding potentials. This study enhances the general understanding of helminth biology highlighting specific antigens and their relevance for parasite survival and their potential as drug targets. This suggests potential for future therapeutic exploration, though the specific functional roles of many targets remain to be characterised.

PS096.III

Prognostic factors in canine angiostrongylosis and synthesis of the existing knowledge on clinical signs

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Canine angiostrongylosis, a potentially life-threatening parasitic disease, is widespread in Europe. While reliable diagnostic tools are available, there is limited evidence on clinical parameters, leaving prognosis for affected dogs largely reliant on their clinical presentation. To address this gap, we investigated the relationship between laboratory findings and clinical presentations in *Angiostrongylus vasorum*-infected dogs presented at the University Animal Hospital of the Vetsuisse Faculty in Zurich, aiming to identify factors linked to disease severity and outcomes. In addition, serum samples from 71 naturally infected dogs, 12 experimentally infected dogs, and 16 wild red foxes were analysed for pulmonary surfactant-associated protein B (PSAPB) and chitinase 3-like protein 1 (CHI3L1) using commercial ELISA kits because quantitative proteomics had revealed that these proteins were upregulated in *A. vasorum* affected dogs in previous studies. Their prognostic value, along with other haematological parameters, was assessed. Infected dogs exhibited a variety of clinical signs, with respiratory symptoms being the most common (62.0%), followed by nonspecific signs (46.5%), bleeding disorders (42.3%), neurological symptoms (17.0%), and cardiovascular abnormalities (9.9%). A comparison with existing literature revealed higher frequencies of bleeding, neurological, and gastrointestinal symptoms in cases referred to specialist hospitals. Notably, a combination of respiratory and nonspecific signs was frequently observed in the present study. The presence of nonspecific signs, often indicative of systemic inflammatory processes, suggests a correlation with more severe disease. Blood analyses revealed that non-survivors had lower haemoglobin levels and higher eosinophil and alanine transaminase levels compared to survivors, reflecting different facets of the inflammatory response. These parameters demonstrated moderate to strong prognostic value, with area under the curve (AUC) values exceeding 0.7. Elevated PSAPB levels were detected in infected dogs, especially non-survivors, highlighting its potential as a prognostic marker (AUC 0.7). However, PSAPB"s utility as a standalone biomarker remains limited, necessitating further validation. Similarly, CHI3L1 levels were higher in infected dogs but showed limited diagnostic potential (AUC 0.6). These findings underscore the importance of integrating clinical signs, hematological markers, and serum biomarkers in the assessment of canine angiostrongylosis. Larger-scale studies are needed to refine these results and establish more robust diagnostic and prognostic tools for this disease.

PS099.III

Anopheles gambiae s.l (Diptera: Culicidae) larval habitat and its public health significance in Osun State, Nigeria

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This study reports the predominant sibling species of *An. gambiae* s.l in Osun state. Adult mosquitoes were caught quarterly in three Local Governments across the three senatorial districts of the state between 1800hr – 0600hr using the Centre for Disease Control (CDC) light trap and 0600hr – 0700hr for pyrethrum spray catch (PSC) usingWHO protocol and identified using morphological keys. Molecular analysis using polymerase chain reaction (PCR) was used for sibling species identification. CDC light trap had a total of ninety (90) catches and PSC had a relatively low number (1) of catch. Four (4) mosquito genera were identified: *Anopheles, Mansonia, Culex* and *Aedes*. *An. gambiae* s.l was the predominant mosquito species ($p < 0.05$). The CDC first quarter catch was the highest while the fourth lowest ($p > 0.05$). The outdoor catch was higher than the indoor catch ($p > 0.05$). The biting peak was highest in the first quarter outdoor catch between 02:00-03:00 and 04:00-05:00 am. Molecularly, *An. coluzii* was the predominant sibling species. The present study therefore reports *An. coluzii* as the current predominant *An. gambiae* s.l cryptic species in the state and its exophagic and exophilic biting and resting preference. Therefore the necessity to revise the current malaria vector control approach method in the state.

PS102.III

Dying to survive: deciphering how *plasmodium* orchestrates cell death pathways

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Introduction: Apoptosis has long been believed to be confined to metazoans. A form of cellular suicide it was indeed counter-intuitive and evolutionarily unfavorable in unicellular organisms until recently few findings indicated exhibition of features typical of mammalian programmed cell death in unicellular parasites including protozoa. Apoptosis plays an important role in density regulation of parasite in both host and vector preventing premature mortality before the parasite has transmitted a small part of population to another host. It also helps to remove damaged, highly antigenic, aged or genetically disadvantageous parasites from the population and saves resources for survival of the fittest.

Objectives: Despite knowing *Plasmodium* genome for more than two decades, apoptotic pathways remain uncharacterized in the parasite. The major regulators of apoptosis in metazoans; the Bcl family of proteins and caspases, are reported to be absent in *Plasmodium* suggesting different underlying mechanisms. Here, we examined the pathways of programmed cell death in *Plasmodium falciparum*.

Methods and Results: Using genome data mining, we identified several putative genes encoding proteases, nucleases and apoptosis regulators from *Plasmodium falciparum*. Metacaspases, which share homology with caspases, were reported in *Plasmodium*, however assigned different roles in the parasite. To elucidate more on the apoptotic machinery of the parasite, *Plasmodium falciparum* culture was treated with various apoptotic inducers, like Chloroquine, Artemisinin and Etoposide where the parasite presented change in morphology, damaged nuclei and mitochondria. Using a combination of cell culture based assays, fluorescence microscopy and flow cytometry, features like mitochondrial outer membrane permeabilization and *in-situ* nuclear fragmentation were detected. Drug-treated parasites also exhibited differential regulation of several putative genes we had identified, in a time-dependent fashion.

Conclusion: We identified, shortlisted and characterized ten putative genes with a role in *Plasmodium* cell death. Together, the data indicates that *P. falciparum* possesses ancient apoptosis-like cell death machinery which can be triggered by chemotherapeutic agents.

PS105.III

Evaluation of liver damage caused by the liver fluke *Fasciola hepatica* in contemporary dairy farms in Germany

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The liver fluke *Fasciola hepatica* is a globally distributed zoonotic endoparasite, primarily found in ruminants. Previous studies investigating liver damage caused by *F. hepatica* infection have mostly focused on heavily infected animals. However, as a result of treatment and prevention strategies, the prevalence and intensity of liver fluke infections decreased in recent years. The aim of this study was to assess the extent of liver damage due to *F. hepatica* infection in contemporary dairy cows with predominantly low fluke burdens, and the implications for animal health and meat inspection.

Liver, blood and faecal samples from 191 dairy cows from farms with reported *F. hepatica* infections were collected at the abattoir. Infection status was determined by the presence of adult *F. hepatica* in the bile ducts, liver fluke eggs in the faeces and macroscopic liver scoring. Livers were categorised into three groups: patent infection (presence of flukes or eggs, n=90), eliminated fluke burden (absence of flukes and eggs, but macroscopic score > 0, n=30) and parasitologically inconspicuous (absence of flukes and eggs, macroscopic score = 0, n=71). Liver damage was assessed by macroscopic as well as histopathological liver scoring and serum liver parameters.

Cows with patent infections had a mean of 9.4 flukes/liver and a mean of 0.9 eggs per gram of faeces. Macroscopically visible liver damage was more pronounced in livers with a patent infection (mean score: 1.5/3) than in livers with an eliminated fluke burden (mean score: 1.1/3) (P<0.001). There was a moderate correlation between the macroscopic liver score and fluke count in patently infected livers (Spearman`s ρ=0.56, P<0.001). Histologically, inflammation, bile duct alterations and fibrosis were present in 97.5%, 95.0% and 76.3% of patently infected livers and 100%, 100% and 72.41% of livers with an eliminated fluke burden, respectively. Even among macroscopically inconspicuous livers, inflammation was found in 92.5%, bile duct alterations in 88.1%, and fibrosis in 43.3%. Although histological scoring was significantly higher in livers with patent infections (P<0.001) and livers with eliminated fluke burdens (P=0.002) than in macroscopically inconspicuous livers, many of the observed alterations are associated with *F. hepatica* infections, suggesting the presence of prepatent, overlooked patent or long-term eliminated infections in this group. Serum liver parameters were mostly unaltered, except for slightly elevated γ-glutamyltransferase levels in patently infected animals compared to those with macroscopically inconspicuous livers (P=0.002).

The overall low level of liver damage and the mostly unaltered serum liver parameters in infected cows suggest that low fluke burdens do not crucially affect liver function. Histological analysis uncovered lasting and macroscopically undetectable damages, exposing the limitations of macroscopic assessment of infection status as used for meat inspection.

PS108.III

Identification of novel candidate detection targets for the outer wall proteins of *Giardia duodenalis* cysts

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Giardiasis is a globally prevalent waterborne zoonosis. Rapid enrichment and detection technologies for this disease are essential. Cyst wall proteins are ideal targets for the enrichment and detection of cysts in the environment, but there are few available targets with suboptimal effectiveness. In this study, *Giardia duodenalis* (*G. duodenalis*) cysts were purified from gerbils feces, and cyst outer wall proteins were biotinylated. Streptavidin magnetic beads were then used to further purify these biotinylated proteins. The purified proteins were subjected to mass spectrometry analysis, followed by GO and KEGG analyses. Among the newly identified cyst wall proteins, β-giardin and α1-giardin were predicted to be located on the outer wall of the cysts. Sequence analysis, prokaryotic expression, preparation of polyclonal antibodies, and determination of subcellular localization were conducted for β-giardin and α1-giardin. Finally, based on β-giardin immunomagnetic beads were prepared using the polyclonal antibodies and tested for their enrichment efficiency. A total of 63 new cyst wall proteins were identified. Bioinformatics analysis was used to analyze the biological functions, subcellular localization, and biological processes. β-giardin and α1-giardin proteins and polyclonal antibodies were successfully obtained. Both β-giardin and α1-giardin were found to be localized on the surface of both trophozoites and cysts of *G. duodenalis*. The enrichment efficiency of the prepared immunomagnetic beads for *G. duodenalis* cysts in feces can reach 65%, which is comparable to the enrichment efficiency of traditional *G. duodenalis* immunomagnetic beads. These results provide a proteomic framework for the cyst wall proteins of *G. duodenalis*, expanding the detection targets for *G. duodenalis* cysts. It also establishes a theoretical foundation for subsequent research on the composition and function of *G. duodenalis* cysts.

PS111.III

Membrane trafficking by retromer complex in *Trypanosoma brucei*

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Trypanosoma brucei escapes its host immune neutralization by rapidly recycling its variant surface glycoprotein (VSG) membrane coat. The entire membrane surface coat on the parasite is recycled approximately every 12 minutes (Engstler *et al.* 2004). This swift recycling through the flagellar pocket and the endosomes is facilitated by a continuous endomembrane system which is stabilized by actin-myosin cytoskeleton (Link *et al.* 2023; Link *et al.* 2024).

Electron tomographs show an interconnected endosomal system for the sorting of membrane surface molecules. Given that the rapid surface turnover must be paralleled by equally swift recycling or degradation, a robust sorting mechanism(s) must be present in *T. brucei*.

In yeast and mammalian cells, a heteropentameric endosomal retromer complex (ERC), is primarily involved in sorting and recycling of membrane receptors and transporters between the endosomes and *trans-Golgi network* (TGN) and cell surface (Seaman 2021). Vps29 subunit forms a scaffold around which the trimeric core complex is assembled.

The retromer core complex retains its characteristic arch structure in *T. brucei*. RNAi against TbVps29 is lethal and delocalizes EP1 procyclin from the endosomes, suggesting a role in the trafficking of surface proteins.

Our findings may significantly advance the general understanding of the endosomal recycling apparatus and elucidate the mechanisms of sorting in *T. brucei*.

PS114.III

Phenotypic characterization of eprinomectin resistance in *Haemonchus contortus* collected in dairy sheep farms

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Anthelmintic resistance poses a significant threat to animal welfare and productivity, particularly due to *Haemonchus contortus*, a highly pathogenic gastrointestinal nematode affecting small ruminants. The loss of efficacy of anthelmintics in *H. contortus* underscores the urgent need to extend drug resistance evaluations across worm populations. This study aims to relate clinical data on clinical EPR effectiveness in sheep dairy farms in southwestern France, with drug-susceptibility phenotypes of *H. contortus* collected in these farms.

We assessed the clinical performance of EPR by combining fecal egg count reduction test (FECRT) and HPLC analysis of serum drug concentrations in individual treated ewes in farms with previously reported therapeutic failures of EPR treatments. Additionally, we collected *H. contortus* isolates in six of these farms and characterized them phenotypically for anthelmintic drug potency, including EPR, by measuring L3 larvae motility with the WormMicroTracker® device.

Our data enabled the direct linking between the phenotypes of field *H. contortus* and treatment outcomes, including cases of clinical EPR failure. This study also lays the groundwork for providing robust diagnostics and improving treatments for helminth infections while generating valuable material for investigating ML resistance mechanisms through phenotypic and genome-wide association studies.

PS117.III

Human dendritic cells recognize *Cryptosporidium parvum* via Toll-like receptor (TLR) 2 and TLR5 and initially induce a non-protective immune response

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Introduction: In healthy individuals, a T helper 1 (Th1) immune response against the apicomplexan intestinal parasite *Cryptosporidium parvum* is protective. Vulnerability of HIV-patients to develop a life-threatening cryptosporidiosis underscores the importance of the adaptive immune response, which is initiated and shaped by dendritic cells (DC). Recently, we demonstrated that *C. parvum* induces all relevant maturation processes in human monocyte-derived DC (Mo-DC) to activate naïve T cells, including upregulation of antigen-presenting molecules, costimulatory molecules, cytokines and cell adhesion molecules. Moreover, DC displayed enhanced migratory activity and phagocytosis of *C. parvum*. The type of immune response elicited is, however, still unknown.

Objectives: Here, we aimed to characterize the molecular determinants, which human DC employ to recognize *C. parvum* stages and to identify the cytokines and surface molecules human DC express upon encounter to shape the T cell response.

Materials and methods: Immature human Mo-DC were generated from monocytic blood progenitors, co-incubated with *C. parvum* oocysts/sporozoites and analyzed by flow cytometry, ELISA or LEGENDplex™ Multi-Analyte Flow Assay.

Results: TLR2, TLR4 and TLR5 were detected on the surface of immature Mo-DC. Pharmacological activators of TLR2/1, TLR2/6, TLR4 or TLR5 induced a dose-dependent secretion of IL-6 and IL-8. Moreover, inhibitors against TLR2 and TLR5 suppressed cytokine expression previously induced by TLR-selective activators or by *C. parvum* stages, demonstrating a role of TLR2 and TLR5 in *C. parvum* recognition, while blockage of TLR4 via TAK-242 had no marked effect. Overall, *C. parvum* exposure induced the expression of MCP-1, IL-6, IL-10, IL-23 and TNF-α in human Mo-DC, thereby suggesting a biased activation of Th2 and Th17 responses but not of Th1 responses. In line, the expression of classical cytokines mediating Th1 responses (IL-12, IL-18, IFN-γ), or indicating cell destruction (IL-33) or anti-viral responses (IFN-α) was barely or not detectable. Among several surface receptors of DC with a regulatory role in T cell responses (CD137L, CD252, CD274, CD275), exclusively CD274 was upregulated upon stimulation with *C. parvum*.

Conclusions: Human Mo-DC sense *C. parvum* by TLR2 and TLR5 but not via TLR4 and early responses seem to avoid Th1 responses. Considering these findings, we attempt to provide a mechanistic view on human DC responses, including a gatekeeper function of TLR4.

PS120.III

Sex differences in the immune response to *Strongyloides ratti* infection

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A number of factors shape the immune system's ability to fight infections, including age, genetics, co-morbidities and lifestyle. Over the past decade, biological sex has also emerged as a relevant variable. Studies report that women are more resistant against viral, bacterial, and protist infections than men (Klein & Flanagan, 2016). This is also the case for helminth infections (Wesolowska, 2022) for instance *Strongyloides stercoralis* infections, which affect approx. 600 mio people worldwide (King, 2019) shows a higher incidence in men (Munisankar et al., 2022). Similarly, rodent studies using *Strongyloides ratti* showed higher parasite numbers in males (Watanabe et al., 1999). Furthermore, the murine studies indicated the potential involvement of sex hormones, as evidenced by the reduction in parasite burden following castration of male mice and the reversal of this effect by testosterone treatment (Watanabe et al., 1999). No further investigation was conducted to elucidate the responsible underlying immunological mechanisms. The immune response against *S. ratti* is already fairly well described (Linnemann & Breloer, 2024): after infectious larvae penetrate the skin, effector cells such as neutrophils attack and kill migrating larvae during the first 2 days of infection. After reaching the intestine, where parasite numbers peak at day 6 p.i., mucosal mast cells, eosinophils and basophils promote ejection from the intestine. Infection is terminated after the establishment of an adaptive type 2 immune response.

The aim of this project is to elucidate immunological sex differences in the immune response to *S. ratti*infection in mice.

Initial experiments showed no difference in the number of migrating larvae in the tissue on day 2 p.i. However, male mice had a significantly higher worm burden than female mice at day 6 p.i. Mucosal mast cell activation did not differ between sexes during the early infection phase. Also, restimulation of splenocytes revealed no sex differences in *S. ratti* antigen-specific cytokine response and T cell polarization at later timepoints. Interestingly, however, an initial screening of plasma samples showed a faster antibody response in females, reflected by significantly higher *S. ratti*-specific IgM, IgG1 and IgG2b titers at day 6 p.i. and significantly higher IgG2c titers at day 10 p.i.

Within this project, a detailed analysis of the infection kinetic, as well as the cellular, humoral immune responses will be performed.

PS123.III

Transient and heterogeneous nuclear accumulation of *Plasmodium falciparum* PCNA1 is driven by association with DNA

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Malaria is caused by a single-celled eukaryote *Plasmodium falciparum*. In the clinically relevant blood stage of infection, *P. falciparum* proliferates through schizogony, during which the nuclei multiply without cell division and the parasite develops into a multinucleated cell. Despite residing in the same cytoplasm, the nuclei multiply asynchronously, facilitating rapid parasite proliferation. Intriguingly, the DNA replication fork protein PfPCNA1 accumulates only in those nuclei that undergo S-phase. However, the molecular mechanism that drives PfPCNA1 accumulation in a subset of nuclei is unknown. To participate in DNA replication, PfPCNA1 has to enter the nuclei and, ultimately, encircle the DNA as a toroidal trimer. Hence, PfPCNA1 association with the DNA may be modulated to achieve nuclear accumulation. To test this hypothesis, we aimed to perturb the DNA association of PfPCNA1 by mutating conserved residues that likely interact with the DNA backbone and those that may participate in PfPCNA1 trimerization. Quantitative image analysis showed that these mutations abrogated the transient nuclear accumulation of PfPCNA1 during S-phase.

Furthermore, we assessed the localization of PfPCNA1 in a cell line where the essential cell cycle regulator PfCRK4 was conditionally depleted and which was unable to replicate the DNA. We found that PfCRK4-mediated origin of DNA replication firing is critical for PfPCNA1 accumulation in nuclei, corroborating that the association of PfPCNA1 with DNA is a key driver for heterogeneous nuclear accumulation. To further validate these results, we are using super-resolution STED microscopy, comparing the sub-nuclear localization of wild-type and mutant PfPCNA1. Together, our findings suggest that the mechanisms which orchestrate DNA replication also play a role in asynchronous multiplication of nuclei in *P. falciparum* schizonts.

PS126.III

Epidemiological study of gastrointestinal helminths in dromedary camels (*Camelus dromedarius*) from South Darfur, Sudan: Insights from parasitological techniques and nemabiome analysis

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Helminths pose significant socio-economic and health challenges, particularly in tropical regions, including Sudan. Dromedary camels, renowned for their resilience in arid climates, are crucial for such regions; however, their health and productivity are compromised by infections such as gastrointestinal helminths. As data on the prevalence of helminth infections in camels is limited, the present study investigates this issue in South Darfur, southwest Sudan, using parasitological techniques (Mini-FLOTAC) and molecular deep amplicon sequencing approaches targeting the internal transcribed spacer 2 (ITS-2, nemabiome). Out of 410 faecal samples examined, 71.2% were reported as helminth-infected, with 69.3% infected by gastrointestinal nematodes (GINs) and 7.8% with *Moniezia* spp. tapeworms. The Mini-FLOTAC technique identified four helminth egg types. i.e. strongyles, *Strongyloides* spp., *Trichuris* spp. and *Moniezia* spp., with strongyles being the most prevalent at 68.8%. Co-infections with two or more of these helminths were reported in 8.8% of the camels. Infections were significantly associated with region and age, with younger camels (6 years, odds ratio: 0.431, P = 0.0434), but no significant difference was observed between younger camels and those aged 3 – 6 years old (P = 0.1443). Nemabiome data revealed that *Haemonchus longistipes*, *Haemonchus contortus*, *Haemonchus placei*, *Trichostrongylus colubriformis*, *Cooperia pectinata*, *Cooperia punctata*, *Cooperia spatulata* and *Oesophagostomum radiatum* were the most abundant GINs. However, some of the amplicon sequence variants (ASVs) remained unclassified, either at the species or the genus level, to any of the aforementioned GINs. The study is among the first to perform "nemabiome" sequencing of GINs in naturally infected dromedary camels, revealing the variable nature of nematode co-infections in this animal species. It identifies a high species diversity of helminth infections in camels in South Darfur dominated by several highly pathogenic *Haemonchus* species. This emphasises the need for raising awareness among camel owners about helminth infections, treatment and control methods, including good husbandry practices. Further investigations are recommended on the seasonal patterns of infection, species identification and the effectiveness of anthelmintics.

PS129.III

Human neutrophil extracellular traps (NET) formation is induced by the zoonotic parasite *Cryptosporidium parvum*

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Introduction: *Cryptosporidium parvum* is a zoonotic enteric protozoan parasite leading to cryptosporidiosis in humans. Cryptosporidiosis is one of the major causes of mortality in children under 24 months of age and may be associated with long-term health impairment in immunosuppressed individuals. The human innate immune response to *C. parvum* infection includes reactions of polymorphonuclear neutrophils (PMN). PMN fight pathogen infections by four main mechanisms: production of reactive oxygen species (ROS), degranulation, phagocytosis and release of neutrophil extracellular traps (NETs). NETs are formed by nuclear and mitochondrial DNA containing several granule-derived enzymes. So far, human PMN responses against *C. parvum* and mechanisms of related PMN activation are not fully understood.

Material and Methods: *C. parvum*-induced NET formation was studied by scanning electron (SEM), confocal and epifluorescence microscopy. Oxidative responses and ROS production were evaluated by extracellular flux analysis (Seahorse) and luminometry. Direct interactions and NET formation in live cells were evaluated by 3D microscopy (Nanolive). The role of oxygen concentration, different neutrophil channels and pathways in *C. parvum*-induced DNA release was studied by spectrofluorimetry using chemical inhibitors of ATP purinergic receptors P2X1 and PANX1, monocarboxylate transporters 1 and 2 (MCT1, MCT2), HIF-1α, NF-κB and PI3 kinase. The role of IFN-λ in *C. parvum*-driven NETosis was studied by semi-automatic image-based NET quantification.

Results: Microscopic analyses revealed PMN activation induced by *C. parvum*. SEM showed parasite-driven pseudopod formation and release of chromatin with granular content, characteristic for NETs, which also entrapped *C. parvum* oocysts. Immunofluorescence confirmed presence of NET proteins like histones, neutrophil elastase (NE), cathelicidin (LL-37) and calprotectin (S100A8). Live cell imaging showed *C. parvum*-induced NET formation to start at 90 min of co-incubation. NET inhibition experiments suggested a role of PANX1-mediated ATP release in *C. parvum*-driven NETosis. Overall, *C. parvum* did not induce ROS formation and *C. parvum*-induced NET formation seemed independent of the oxygen concentration.

Conclusion: Overall, we here contribute with fundamental data on PMN-related activities in response to *C. parvum* stages and identified a putative role of PANX1-based purinergic signalling in *C. parvum*-induced NET formation.

PS132.III

Diagnosis of malaria and knowledge, attitudes, and practices regarding malaria in an insecticide-resistant Area: Nyabessang, South Cameroon

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Introduction: The persistent challenge of malaria transmission in endemic regions necessitates continuous monitoring of insecticide resistance and the efficacy of malaria control measures, such as long-lasting insecticidal nets (LLINs). And, before implementing a new control measure, it is important to know the level of understanding and application of the pre-existing measures by the target population. This study aimed to evaluate the susceptibility/resistance profile of *Anopheles gambiae* s.l. to various insecticides, assess the effectiveness of LLINs, the prevalence of malaria, and the level of knowledge, attitude and practice of the population towards malaria and the actual level of coverage and use of LLINs in Nyabessang, South Cameroon.

Methods: Insecticide bioassays were carried out on field strains of adult *A. gambiae* s.l. to determine susceptibility patterns, while cone bioassays measured the efficacy of different LLINs. As a control for these tests, we used the susceptible *A. gambiae* strain from Kisumu. A well-structured questionnaire was administered to household heads or their representatives aged at least 21 years to collect socio-demographic data and knowledge levels on malaria transmission and preventive measures. Identification of the parasite carriers was done by rapid diagnostic test (RDT) and direct microscopic visualization (100x) of *Plasmodium* parasites on thick blood smears and thick drops stained with Giemsa 10%.

Results: *Anopheles gambiae* s.l. exhibited full susceptibility to Pirimiphos-methyl (100% mortality) but showed resistance to bendiocarb (91.66%) and all tested pyrethroids. Pre-exposure to PBO restored susceptibility to deltamethrin, increasing mortality from 43.75% to 98.75%, while permethrin showed only partial recovery. ITNs demonstrated high efficacy against the susceptible Kisumu strain, but only the PBO-based net (Olyset Plus) was effective against the local resistant population. Socio-demographic analysis revealed that 88.79% of households owned at least one LLIN, with high usage rates among those who installed them. Knowledge gaps were identified, particularly regarding malaria vectors, breeding sites, and the causative agent of malaria. The pronounced resistance to commonly used insecticides underscores the urgent need for adaptive vector control strategies. The effectiveness of PBO-based nets suggests a potential alternative for mitigating resistant *A. gambiae* populations. The socio-demographic insights highlight areas for targeted education to improve community understanding of malaria transmission and control practices.

Conclusion: This study emphasizes the critical need for continuous monitoring of insecticide resistance and reevaluation of malaria prevention strategies. Improving community knowledge and engagement can enhance the effectiveness of existing interventions, ultimately contributing to the reduction of malaria transmission in endemic areas

PS135.III

Stressed or recovering? Effects of restoration state and microsporidian parasites on communities of their amphipod hosts

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Parasites can affect the host response to stressful conditions and can be affected by the stressors themselves, e.g. through effects on host physiology or immunity, host behaviour, and parasite transmission. We hypothesize differential responses of monoxenous and heteroxenous parasites under stressor exposure and subsequent recovery. This aspect is being investigated in the context of the Collaborative Research Centre 1439 (RESIST), and we report here the current results on monoxenous microsporidian parasites, while results on trematodes will be presented in a separate talk.

Microsporidians are intracellular parasites that use a complex spore for the transmission from one host to another. There is a large diversity of microsporidians infecting amphipods, and we are investigating their effects on the host populations under different environmental conditions. Our study systems in the Ruhr Metropolitan Area (Boye) and in Hesse (Kinzig) include stream sites with different degradation and restoration gradients, from near-natural to recently restored sites. Amphipods were collected, identified and tested for microsporidian infection by PCR and sequencing. Amphipod diversity and abundance, microsporidian diversity, and host specificity were examined and related to each other and to environmental variables. In addition, we conducted three 72 h drift experiments to elucidate the role of microsporidians on the drift behaviour of their amphipod host.

Fourteen microsporidian molecular operational taxonomic units (MOTUs), mainly generalist parasites, were detected infecting in total 6 MOTUs of *Gammarus pulex*, *Gammarus fossarum*, and *Gammarus roeselii*, adding 17 host-parasite interactions to the current knowledge of host range. We found no difference in microsporidian diversity and host specificity between restored and near-natural sites, or between urban or rural locations. Furthermore, host density did not influence microsporidian MOTU richness. High host turnover across the geographical range suggests that neither environmental conditions nor host diversity play a significant role in establishment in restored areas. Microsporidian prevalence was generally higher in drifting amphipods than in stationary ones, mainly due to differences in host size. However, for two parasites, the prevalence in drift samples was highest during the day suggesting changes in host phototaxis, probably related to the mode of transmission and site of infection of the parasite.

Host diversity and environmental parameters do not indicate the persistence and dispersal of phylogenetic host generalist microsporidians in environments that have experienced anthropogenic disturbance. However, changes in drift behaviour may have important implications for *G. pulex* population dynamics and for the dispersal of some microsporidian species. Further mechanisms such as the persistence of infective spores in the environment, or host switching will require further investigation.

PS138.III

Culturing of *Giardia lamblia* under microaerobic conditions can impact metronidazole susceptibility by inducing increased expression of antioxidant enzymes

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The microaerophilic/anaerobic protist *Giardia lamblia* is a world-wide occurring parasite of the human small intestine. It causes giardiasis which manifests as diarrhoea accompanied by other sequelae. Giardiasis is most commonly treated with either the 5-nitroimidazole metronidazole or the benzimidazole albendazole. Unfortunately, the number of refractory cases is increasing, which is probably caused, at least in part, by drug resistance. However, most attempts to isolate metronidazole-resistant *G*. *lamblia* strains from patients have failed so far because the parasites were not resistant when tested *in vitro*.

We hypothesized that this failure might be caused by drug assay conditions which are standardly anaerobic, and performed metronidazole susceptibility testing with two well studied strains, i.e. WB C6 and BRIS/87/HEPU/713 (strain 713) under microaerophilic conditions. Indeed, 713 proved to be less susceptible to metronidazole under microaerophilic conditions as compared to anaerobic conditions, and residual growth was even noted at concentrations of metronidazole similar to those in the serum of treated patients (i.e. about 100 µM). Further experiments showed that 713 also grows much faster under microaerobic conditions than WB C6. Reduced susceptibility to metronidazole under microaerobic conditions was also observed in a clinical isolate from a refractory giardiasis case.

Two-dimensional gel electrophoresis showed that microaerobic growth was accompanied by the upregulation of superoxide reductase, a pyridoxamine 5'-phosphate oxidase putative domain-containing protein, and a TlpA-like protein in 713 but not in WB C6. All three proteins are known, or can be predicted to have antioxidant functions. Indeed, overexpression of pyridoxamine 5'-phosphate oxidase in WB C6 from a plasmid carrying the respective gene behind the arginine deiminase promoter significantly improved growth of the transfected cell line under microaerobic conditions. Moreover, similarly overexpressed superoxide reductase conferred significant protection against metronidazole.

Our results suggest that oxygen concentrations can affect the outcomes of metronidazole treatment against *G*. *lamblia*.

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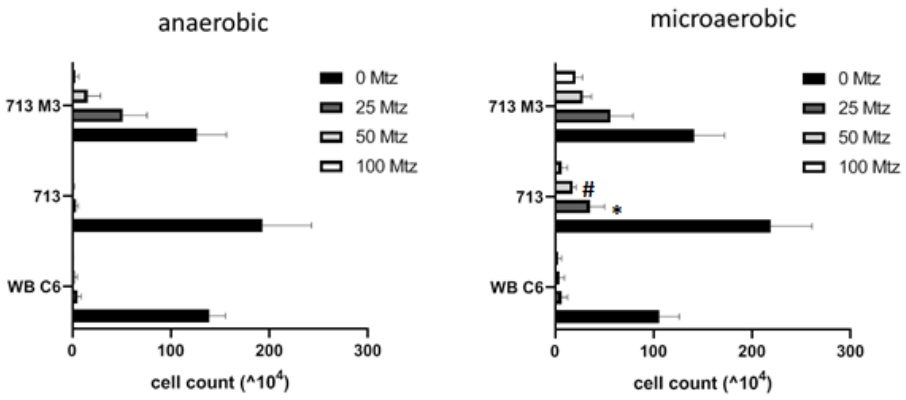
Fig. 1


anaerobic
microaerobic
0 Mtz
25 Mtz
50 Mtz
100 Mtz
713 M3
713
WB C6
0
100
200
300
cell count (^10^4)
#
*

PS141.III

Unveiling anthelmintic modes of action using SydLab™: A microfluidic high-content screening approach in *Caenorhabditis elegans*

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Anthelmintic resistance poses a significant global challenge in controlling parasitic nematodes affecting human and animal health. This study leverages SydLab™, an innovative microfluidic robotic platform, to evaluate the phenotypic effects of eight anthelmintic compounds—albendazole, ivermectin, milbemycin oxime, emodepside, levamisole, tribendimidine, monepantel, and closantel—on the model organism *Caenorhabditis elegans*. Using both wild-type N2 and *hsp-6::gfp* transgenic strains, we systematically assessed the dose-dependent impacts of these compounds on developmental growth, reproduction, motility, and morphology.

Our findings reveal distinct compound-specific phenotypic profiles, with emodepside, ivermectin, and monepantel inducing severe larval arrest and neuromuscular paralysis, while levamisole and albendazole exhibited moderate effects on development and reproduction. Morphological analysis confirmed particular drug-induced phenotypes, such as coiled and paralyzed forms, indicative of specific modes of action. The oxidative stress-sensitive *hsp-6::gfp* strain demonstrated heightened sensitivity to mitochondrial stress-inducing compounds like emodepside and ivermectin, offering insights into strain-specific mechanistic effects. Despite these differences, overall phenotypic responses remained conserved across strains.

SydLab™ was validated against classical methods, including larval development and migration assays, demonstrating its high sensitivity and reproducibility in detecting dose-dependent phenotypes. The platform"s ability to integrate continuous data acquisition and advanced image analysis provides a transformative tool for high-content drug screening. This comprehensive approach enables precise phenotypic characterization, enhancing the understanding of anthelmintic mechanisms and resistance.

Despites its low throughput, this study underscores the potential of SydLab™ in accelerating the phenotypic characterization of next-generation anthelmintics by combining advanced microfluidics, machine learning, and model organisms. By extending its application to parasitic species, SydLab™ could further drive breakthroughs in combating drug-resistant parasitic infections, addressing a critical global health challenge.

PS144.III

Fasciola hepatica kinome: A roadmap towards anthelmintic compounds

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Introduction: *Fasciola hepatica*, the common liver fluke, causes fascioliasis, a significant parasitic disease in humans and livestock. Control measures heavily depend on triclabendazole (TCBZ), but rising resistance necessitates novel therapeutics. Protein kinases (PKs) are increasingly recognized as essential targets in parasites due to their critical roles in survival and development, as we have shown in first studies for *F. hepatica* [1,2]. However, knowledge of PKs in this parasite is still in its infancy and a better understanding of *F. hepatica* kinome is needed as a basis for future research.

Objectives: Our study aimed to provide a comprehensive analysis of the *F. hepatica* kinome to identify potential protein kinase targets for novel therapeutic interventions and to identify PK inhibitors with anti-*Fasciola* activity.

Methods: We utilized a bioinformatics pipeline to predict, curate, and classify PKs in *F. hepatica*. To prioritize targets, we focused on PKs expressed in neoblasts and muscle cells, crucial for parasite survival. Based on available omics data and literature, eleven PK inhibitors targeting those selected kinases were evaluated against different *F. hepatica* life stages *in vitro*.

Results: Our bioinformatics analysis generated a comprehensive kinome dataset for *F. hepatica*, comprising 241 eukaryotic PKs and 4 atypical PKs. The multi-RTK inhibitor vandetanib, the PKCβ inhibitor ruboxistaurin, the PAK-4 inhibitor LCH-77499, and two PIM inhibitors showed potent activity against immature and adult flukes. The reduction of motility and vitality at 25 µM and 50 µM was comparable to TCBZ after 24 hours. Additionally, molecular docking calculations targeting *F. hepatica* PIM kinase with a library of 85,000 compounds optimized for lead-likeness are ongoing to identify new potential inhibitors. Top candidates from this screening will undergo *in vitro* testing to assess their ability to inhibit parasite survival.

Conclusion: This study emphasizes the potential of PKs as drug targets for *F. hepatica* and provides a robust kinome dataset for deeper investigations into kinase functions in the parasite"s biology. These findings pave the way for novel anthelmintic drug discovery efforts to combat fascioliasis.

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PS147.III

Molecular and genomic characterization of pathogens in filarial lymphedema wounds in Ghana: Antibiotic resistance and fungal diversity

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Lymphatic filariasis (LF) patients presenting with symptomatic lymphedema in Ghana often experience recurrent wounds. These wounds significantly impair quality of life and contribute to a cycle of poverty and disability as they are most often than not complicated by microbial colonization leading to increased inflammation, delayed wound healing, and the potential for systemic infections. A molecular characterization study of filarial wounds identified *Staphylococcus aureus* as a prevalent Gram-positive bacterium associated with these wounds. Antibiotic susceptibility testing (AST) of the *S. aureus* isolated revealed that 100% were resistant to at least one antibiotic, and 33.3% were multi-drug resistant (MDR). All isolates were methicillin-resistant *S. aureus* (MRSA), posing significant public health risks. The isolates also carried various virulence genes, indicating the need for continuous monitoring and effective control measures to mitigate antibiotic resistance in LF patients. Furthermore, the assessment of fungal microbiome of the filarial wounds was investigated, revealing the presence of 24 distinct fungal species, primarily from genera such as Cladosporium, Aspergillus, and Candida. Many wounds exhibited polymicrobial infections, highlighting the complex nature of managing LF-related wounds and the potential contribution of not only bacterial but fungi to chronic wound pathogenesis. These findings emphasize the importance of comprehensive treatment approaches that address both bacterial and fungal infections to reduce the risk of antimicrobial resistance and improve patient outcomes in LF-endemic regions

PS150.III

Five years of West Nile virus surveillance in Southern Switzerland: A comprehensive overview

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In recent decades, human outbreaks caused by arboviruses have increased in Europe and have also appeared in new geographical areas. Southern Switzerland borders northern Italy, where West Nile virus (WNV) is endemic and has been spreading since 2008. Autochthonous human or animal cases of West Nile disease has never been reported in Switzerland, despite the virus was found in mosquitoes since 2022 thanks to the regular flavivirus surveillance. We present here the results of the entomological surveillance carried out in mosquitoes of the *Culex* genus for the past five years (2020-2024) in Canton Ticino, a Swiss canton south of the Alps.

Mosquitoes were captured using gravid traps (Bioquipe, USA) coupled with honey-baited FTA cards. Traps were left in the field throughout the season and monitored every fortnight for battery changes and collection of mosquitoes and FTA cards. The sampling period started from the beginning of July (in 2020, 2021 and 2022) or from the beginning of June (in 2023 and 2024) until the end of September/beginning of October mainly in natural or peri-urban areas characterized by the presence of permanent and stagnant water. The number of sites has expanded over the years, starting with five sites in 2020 to 13 sites in 2024. Both FTA cards and *Culex* spp., were sorted according to sampling date and site and pooled up to 50 specimens for subsequent analysis.

In 2020, a pool of *Culex pipiens* was found positive for Usutu virus (USUV), while in 2021 all samples were negative. In 2022, WNV (lineage 2) was detected for the first time in Switzerland starting from August, in several locations (8 out of 12 sites) [1]. WNV was identified both in FTA cards and in several pools of *Culex* spp. (21/99 captures). In 2023, WNV was identified for the second consecutive year in 5 out of 13 sites (8/124 captures), while USUV was identified at 7/13 sites (21/124 captures). Finally, in 2024, 4/13 USUV-positive sites were identified and no WNV was found in any sample.

With these data, the epidemiological situation has clearly changed since 2022. Extreme weather conditions throughout the year, but especially the months from May to October, with high temperatures for long periods, created ideal conditions for the spread of the pathogen. Canton Ticino presents all the key factors that sustain the WNV cycle: reservoir birds, the vector, natural habitats with permanent water, and a mild climate. However, the critical element that determines whether WNV spreads is the presence of prolonged high temperatures, particularly from the onset of summer. Given the potential for significant shifts in an area's epidemiological landscape due to climate change, it is crucial to maintain ongoing surveillance of this disease to safeguard both human and animal health.

Cazzin S, Liechti N, Jandrasits D, Flacio E, Beuret C, Engler O, Guidi V. First Detection of West Nile Virus Lineage 2 in Mosquitoes in Switzerland, 2022. Pathogens. 2023 Dec 7;12(12):1424.

PS153.III

The first record of feather mites (acari: astigmata) infesting mallards and their association with fungus species

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Feather mites (Acari: Astigmata) have intricate relationships with their hosts and the environment, making them common ectoparasites of birds. This study emphasizes the relationship between feather mite species and their fungi. For the present investigation, the mallard ducks, *Anas platyrhynchos* were collected from various water bodies of Sindh province and were examined for the infestation of feather mites. The feather mites collected from the mallard ducks were prepared for the permanent microscopic mount and identified as *Freyana anatina* with higher intensity of females and heteromorph males from 13 birds, indicating a new geographic record from Pakistan. The feathers of ducks were used to culture the fungus in the laboratory using standard conditions that took 6 – 11 days to grow the feather fungus in SDA medium. Notably, our research indicated the symbiotic association of *Freyana anatina* with the feather fungus, *Chaetomium globosum* (Sordariales: Chaetomiaceae), which may have an impact on the morpho-texture of the duck feathers. The study highlights the need for further research on the ecological dynamics between feather mites, their avian hosts, and associated microbial communities, particularly fungal species, which could have implications for understanding avian health and parasite-fungal interactions. This is a new study in the region, hence making the first records for the feather mites and their association with the host bird, as well as the myco-flora of Pakistan.

PS156.III

Polymerase chain reactions on nucleic acids isolated from *in situ* collected small intestinal mucosal swabs may represent alternative tests to diagnose *Echinococcus multilocularis* infection in its definitive hosts

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The cestode *Echinococcus multilocularis* parasitizes the small intestines of carnivores such as foxes (*Vulpes vulpes*) and raccoon dogs (*Nyctereutes procyonoides*) as definitive hosts and represents an important zoonotic threat. This study aimed to determine whether *E. multilocularis* DNA detection methods by real-time PCR involving easy-to-collect sample types, such as swabs could represent alternatives to traditional parasitological techniques. Three analytes from foxes and raccoon dogs were tested. i. Swabs taken immediately during necropsy (*in-situ*) from the small intestinal mucosa (FastMucSwab), ii. swabs from scraped-off small intestinal mucosa (IsolMucSwab), also used for the sedimentation-counting-technique (SCT) and for comparative reasons iii. feces from the *Ampulla recti*. Only minor differences in *E. multilocularis* DNA detection, independent of sample type, were observed. High levels of concordance between PCR results were noted in the comparison of results on FastMucSwabs, IsolMucSwabs or feces. The agreement between FastMucSwab and IsolMucSwab was excellent (Kappa 0.86 [95% CI: 0.79-0.92]). If inconclusive PCR results were excluded, PCR on FastMucSwabs had a diagnostic sensitivity of 92.7% (95% CI: 83.0–97.3%) and a specificity of 91.5% (95% CI: 88.4–93.9%) relative to SCT. Compared with SCT, Feces real-time PCR had a diagnostic sensitivity of 82.8% (95% CI: 63.5–93.5%) and a specificity of 90.9% (95% CI: 81.4–95.9%). Segmental swabbing had no diagnostic advantages. Overall, there was evidence that the DNA detection methods used here had a higher diagnostic sensitivity than SCT did. Results suggest that simple alternative methods, such as PCR on FastMucSwabs represent an efficient tool for performing larger-scale epidemiological studies.

PS159.III

The burden of malaria in Mauritania : An epidemiological analysis

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This retrospective study aims to assess the epidemiologic profile of malaria in Mauritania between 2021 and 2022. A total of 100,665 cases of malaria were recorded during this period. The incidence rates for uncomplicated and severe malaria were 86.2 and 13.8 per 1000 person-years, respectively. Although malaria affects all age groups, children 5 years and older are most affected. The disease was observed in all regions of the country, with particularly high incidence in the wilayas of Hodh El Charghi and Hodh El Gharbi. These results indicate that malaria remains a public health problem in Mauritania, although a decrease in cases is observed compared to the statistics presented in previous research.

Keywords: Malaria, Epidemiology, Mauritania

PS162.III

Triclabendazole resistance in Swiss *Fasciola hepatica*

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Fasciola hepatica is a trematode of Fasciolidae family responsible for a parasitic liver disease called fasciolosis affecting humans and livestock [1]. In Europe and other developed regions, fasciolosis imposes substantial economic losses in cattle farming due to reduced meat and milk production as well as reproductive failure [2]. In contrast, in underdeveloped countries, it is classified as a neglected tropical disease by the World Health Organization [3]. As no vaccine is currently available, control relies exclusively on drug treatment, particularly Triclabendazole (TCBZ) [4]. However, the intensive use of TCBZ over the past four decades has led to widespread treatment failures and the emergence of drug resistance globally [5]. Understanding the mechanisms of resistance and screening for new therapeutic options are therefore critical priorities.

Recent studies have identified a 3 Mpb genetic locus associated with resistance in a British *F. hepatica* isolate [6]. Our project aims to determine the occurrence of drug resistance and pinpoint the genetic basis and mechanisms of resistance in Swiss *F. hepatica* isolates. In parallel, we aim to identify potential new drug targets. For this, we are isolating adult specimens from infected bovine livers sourced from slaughterhouse and assessing their resistance status towards TCBZ. Our goal is to collect sufficient resistant and sensitive populations for genomic population analyses, focusing on low-heterozygosity loci as candidates for resistance-associated regions. We established the infection of the intermediate host in laboratory-reared *Galba truncatula* by exposure to *F. hepatica* miracidia. The resulting metacercariae are used to obtain juveniles needed for in-vitro drug assays. Through imaging and motility scoring, we are determining IC50s and MIC for TCBZ and its active metabolite TCBZ-SO. Additionally, sensitive newly excysted juveniles obtained from commercial metacercariae (RidgewayResearch) are being used to screen novel compounds for anti-parasitic activity.

Understanding the mechanisms and genomic traits underlying resistance will enable improved surveillance, the development of diagnostic tests, and optimized treatment strategies in the presence of resistance. From a research perspective, identifying resistance-associated genetic variants will provide valuable insight into the molecular targets of TCBZ and facilitate the discovery of new, more effective treatments.

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PS165.III

Circulation of *Dirofilaria immitis* DNA in mosquitos of Emilia-Romagna region (Italy): An alternative way to complete the epidemiological data set of heartworm parasite diffusion in our country

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Dirofilaria immitis, the agent of canine and feline heartworm disease, is a widespread mosquito-borne helminth. The distribution of *D. immitis* in Europe is expanding from traditionally endemic areas to areas that were, until recently, considered free of infection (Fuehrer et al., 2021). Essential prerequisites for heartworm transmission include the presence of competent mosquito vectors, a climate that provides adequate temperature and humidity to support the mosquito population and that also allow maturation of ingested microfilariae into infective, third-stage larvae (L3) within the vector. The present study is aimed at updating current knowledge of the composition of competent vector species in the northern region of Emilia Romagna, a traditionally endemic area for *D. immitis* and *D. repens*.

Mosquitoes were collected in 2022 by the "Istituto Zooprofilattico della Lombardia ed Emilia-Romagna (IZSLER)" as part of the regional surveillance plan for West Nile Virus (WNV). The capture zones included peri-urban and rural areas and mosquitos were captured with CDC-CO2 traps. Mosquitoes were stored at -20°C and then sorted by capture zone and species identified following the dichotomous keys (Becker et al. 2020). DNA from female mosquitoes was extracted and analyzed for the presence of *D. immitis* and *D. repens* (Sulesco et al. 2016). From the positive pools, RNA was also extracted and analyzed for the presence of WNV (Eiden et al., 2010).

The majority of captured mosquitos belonged to *Culex pipiens* species followed by *Aedes caspius, Aedes vexans*, *Aedes albopictus*. Of these 8248 and 15746 female mosquitos (about 30% of each species) have been randomly selected and used to create pool for DNA extraction. A total of 140 pools (~ 20 mosquitos/each) in 2022 and 133 in 2023 have been analyzed. DNA of *D. immitis* was identified in 14 pools of 2022 captures and in 15 pools of 2023. None of the pools was positive for *D. repens* neither for WNV. In 2022, about 85% of the positive pools belonged to *Ae. caspius* species (11/13) and the other three pools to *Ae. vexans*. In 2023, 73% of the positive pools belonged to *Ae. caspius*, followed by *Ae. vexans* and *Ae. albopictus* (both 13.3%). A significant overlap emerged from the same traps positioned in Ferrara and Bologna province, which tested positive for *D. immitis* in both 2022 and 2023.

These data highlight how, despite the abundance of *C. pipiens* captured, the most receptive species for *D. immitis* appear to be *Ae. caspius* and *Ae. vexans*. Furthermore, the geographical data highlights how the areas of the province of Ferrara and Bologna are the main geographical reservoirs of the parasite. Further in-depth studies should be carried out by using other types of traps and also focusing on urban areas.

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PS168.III

Empowering high school students in one health research: A case study on parasite surveillance in European hares using galaxy bioinformatics

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Introduction: Whole genome sequencing (WGS) has transformed molecular epidemiology, by eliminating the reliance on time-consuming molecular and phenotypic tests. Application of WGS to host-parasite interactions remains underexplored, largely due to bioinformatic limitations. The Galaxy platform (https://usegalaxy.org) is user-friendly, cloud-based solution, helping researchers with limited bioinformatic expertise to engage in WGS data analysis. Herein, we demonstrate how a community program successfully introduced One Health (OH) concepts to high school students through a hands-on project focusing on surveillance of parasitic disease of hares from Europe (*Lepus* spp.). This case study, highlights the intersection of ecology, epidemiology, and bioinformatics. Using Galaxy, students explored the biodiversity and epidemiology of parasites affecting hares – species vital to ecosystem health, yet experiencing sharp population declines.

Objectives: a) engage students in real-world scientific research, emphasizing the importance of data analysis; b) promote OH by showcasing how multidisciplinary and transdisciplinary scientific collaborations can address pressing challenges; c) evaluate the performance of Galaxy"s bioinformatic tools in identifying diverse parasite DNA sequences within hare WGS data. At the conclusion of the sessions, students were encouraged to create scientific posters to disseminate their research findings, fostering their skills in science communication.

Materials and Methods: DNA sequences from *Babesia* sp.*, Besnoitia* sp.*, Encephalitozoon cuniculi, Echinococcus* spp.*, Cryptosporidium parvum, Leishmania* spp.*, Neospora caninum, Sarcoptes scabiei, Theileria parva and Toxoplasma gondii* were screened in 66 hares from 5 species, across 12 European countries. Parasite DNA sequences were retrieved from NCBI Gene Bank and used as queries for an alignment with bowtie2.

Results: *T. gondii* and *Echinococcus spp.* were the most frequently detected parasites (58%), followed by *T. parva (52%)*, *C. parvum (45%), B. bovis (36%), B. besnoiti (16%) and N. caninum (3%).* Five Spanish and one Italian hare (total 11%) tested positive for *L. infantum*. A likely false-positive result occurred in a hare (*L. timidus)* from Switzerland. No DNA of *E. cuniculi* was found.

Conclusions: From this initiative, students developed a deeper understanding of the role of research in addressing critical environmental challenges and recognized the importance of scientific communication for knowledge transfer. Galaxy bioinformatic tools demonstrated promise to detect host-parasite interactions. Yet, further refinement is necessary to optimize DNA template size and source (e.g. WGS, partial or full sequences, 16S rRNA, COX, ITS, Cyt b), and evaluate the software´s ability to detect overlapping DNA regions from different organisms. Screening should be based on parasite´s tropism for the host tissues used as source of genomic DNA.

PS171.III

Functional characterization of *Schistosoma mansoni* serpin SmSPI: Insights into host-parasite interactions

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Introduction: *Schistosoma mansoni* is a hematophagous parasite residing in the circulatory system of mammalian hosts for decades. Its survival is facilitated by antihemostatic, anti-inflammatory, and immunomodulatory molecules that manipulate host defense mechanisms. Protease inhibitors, particularly serine protease inhibitors (serpins), play a pivotal role in these processes. SmSPI, a promising serpin, exhibits marked upregulation in adults and eggs, i.e. stages directly interacting with the host blood.

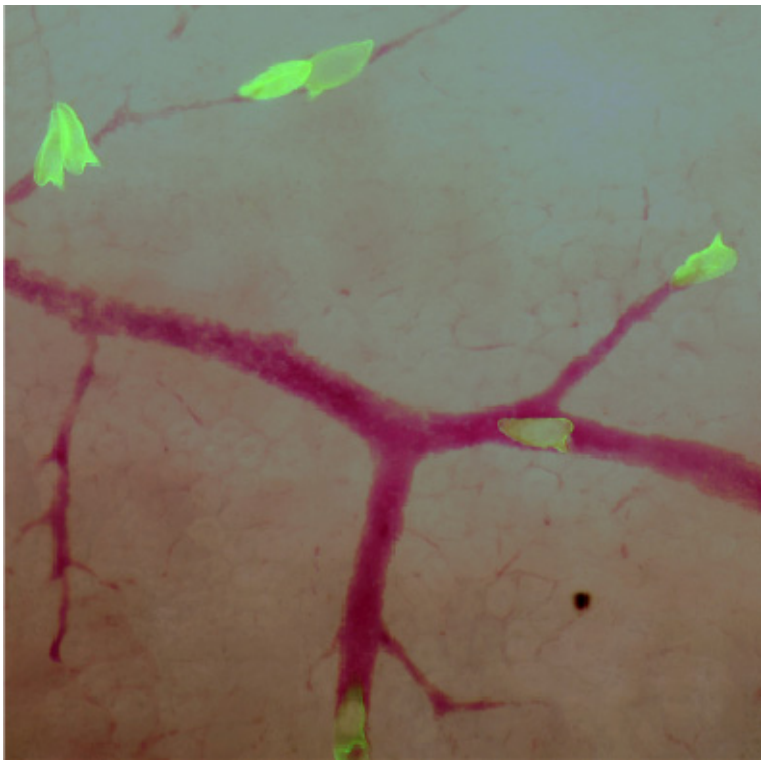
Objectives: This study aimed to characterize the inhibitory activities of SmSPI against key host serine proteases, determine its localization within host and parasite tissues, and evaluate its functional roles in host-parasite interactions during *S. mansoni* infection.

Materials & Methods: SmSPI was recombinantly expressed in HEK293 cells, subsequently purified, and utilized to generate specific antibodies in mice. Immunohistochemical analysis was performed to localize SmSPI within parasite and host tissues, providing insights into potential host-parasite interactions. Its inhibitory specificity was characterized using fluorogenic assays targeting key serine proteases involved in host digestion and hemostasis. Furthermore, RNA interference was applied *in vitro* to assess the functional impact of SmSPI knockdown on parasite viability/phenotype.

Results: Recombinant SmSPI inhibited chymotrypsin, pancreatic elastase, urokinase, and cathepsin G. Immunohistochemical analysis revealed SmSPI localization in *S. mansoni* adults, predominantly in the male tegument as well as in subshell envelope of eggs and developing miracidia. SmSPI was also identified in cholangiocytes within the liver of infected mice and in bile indicating its excretion into the intestine through the biliary system. Finally, preliminary *in vitro* silencing experiments showed morphological changes in eggs laid by dsSmSPI-treated adults.

Conclusion: This study highlights the functional significance of SmSPI in *S. mansoni*. The recombinant SmSPI exhibited inhibitory activity against key host serine proteases, suggesting its potential role in modulating host hemostasis, immune responses and digestion. Localization studies further revealed SmSPI to be exposed to host tissues on multiple levels, suggesting its involvement in host-parasite interactions. Additionally, *in vitro* silencing experiments indicated that SmSPI may play a crucial role in parasite viability, particularly in the eggs.

Fig. 1



PS177.III

Occurrence, diversity and risk factors of parasitic gastrointestinal nematodes in smallholder dairy calves from Nandi county, Kenya

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Gastrointestinal nematodes (GIN) are of major importance in dairy farming, particularly in smallholder systems, because of their impact on calf health and productivity. Most of these infections occur as coinfections. While several studies have investigated GIN infections in cattle, many have relied on microscopy that may not accurately identify mixed infections or species level. To address this limitation, molecular techniques, such as deep amplicon sequencing, have emerged as powerful tools for characterizing GIN communities and identifying mixed infections. In this article, we present results for a cross-sectional study on 289 smallholder dairy farms with 532 calves, in Kenya from September to December 2023. Fresh fecal samples were collected and Fecal Egg Count (FEC). Identification of nematode species was conducted by deep amplicon sequencing of Internal Transcribed spacer-2 rDNA locus of first stage larvae. Generalized mixed-effects models were used to identify risk factors associated with FEC, Heart girth as an indicator of weight and coinfections. Nemabiome analysis revealed nine GIN species, with *Cooperia punctata* , *Haemonchus placei ,* and *Haemonchus contortus* being the most prevalent. A high proportion (69.5%) of infections were coinfections. Sex and age were significantly associated with both FEC and heart girth. *H. placei* and *C. punctata* were significantly associated with FEC while *O. ostertagi* and *T. colubriformis* were associated with Hearth girth. Management and breed were significantly associated with coinfections. The result of this study shows that deep amplicon sequencing can accurately identify GIN species of dairy calves, particularly in cases of coinfections.

PS180.III

Effects of the novel bumped kinase inhibitor BKI-1708 against *Toxoplasma gondii*, *Neospora caninum* and *Besnoitia besnoiti*: Differences and similarities

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Apicomplexans are major morbidity-causing pathogens distributed worldwide. Among the compound classes being currently developed against these parasites, bumped kinase inhibitors (BKIs) – optimized to target the apicomplexan calcium-dependent protein kinase 1 (CDPK1) – have proven to be safe and active *in vitro* and *in vivo*. The structure of BKI-1708 is based on the same central scaffold as BKI-1748, which has exhibited promising *in vitro* and *in vivo* efficacy against both *N. caninum* and *T. gondii*. When applied *in vitro* concomitantly to infection, BKI-1708 displayed IC50 values of 120nM for *T. gondii* and 480nM for *N. caninum* and did not affect host cell viability at concentrations up to 25µM. BKI-1708 was highly effective against *T. gondii* and *N. caninum* in experimentally infected mice by reducing parasite burden and vertical transmission. *In vitro* treatment with 2,5µM BKI-1708 induces the formation of multinucleated complexes (MNCs) - characterized by continued nuclear division and enclosing intracellular zoites lacking the outer plasma membrane, unable to finalize cytokinesis. MNC formation upon BKI treatment was reported in *T. gondii*, *N. caninum* and *B. besnoiti*. BKI-treatment has a parasitostatic effect, inhibiting proliferation under drug pressure but not clearing the infection, allowing parasites to regain infectivity. Major differences after drug removal can be noted, with *B. besnoiti* reconverting to infective tachyzoites more rapidly compared to *T. gondii* and *N. caninum*. Moreover, differences in antigen expression were observed between the three species. IFA demonstrated the presence of cyst wall components in treated *T. gondii*, but not in *N. caninum* or *B. besnoiti*. Proteomic analysis of BKI-1708 induced MNCs in *T. gondii*, *N. caninum* and *B. besnoiti* revealed differentially regulated proteins compared to untreated parasites, again with distinct differences between the three species. Further studies on the effects of BKI treatment can provide insights into the differences in stage conversion between closely related apicomplexans.

PS183.III

Tubulin tyrosination-detyrosination in *Trypanosoma brucei:* Phenotypes and crosstalk with other tubulin modifications

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The unicellular parasite Trypanosoma brucei possesses a distinctive, highly ordered microtubule-based cytoskeleton. This cytoskeleton is best characterized by its robust tolerance to mechanical and chemical stress and its fundamental role in the parasite's helical shape and drill-like movement[1]. However, the regulation of the trypanosome cytoskeleton is poorly understood. Our research focuses on the specific function of tubulin post-translational modifications (PTMs) on microtubule dynamics and structure, collectively referred to as the tubulin code[2].

By generating specific knockouts that delete genes encoding PTM-catalysing enzymes, we were able to modulate or ablate individual PTMs. Our results show that tubulin hypo-tyrosination, achieved by deletion of the tubulin tyrosine ligase, results in an aberrant microtubule organization at the posterior pole of the parasite cytoskeleton. Tubulin hyper-tyrosination, caused by deletion the tubulin tyrosine carboxypeptidase, has only a limited effect on the overall structure of the microtubule cytoskeleton, but significantly affects cellular motility by altering the balance between propulsive and tumbling motion. Nevertheless, both tyrosination states have no significant effect on cell growth, and mitotic spindle formation and function appear normal. However, we observe a crosstalk between the tubulin tyrosination state and its polyglutamylation state. This correlation has previously been postulated in *in vitro* studies, and our findings provide the first *in vivo* validation of this crosstalk[3]. The regulatory mechanism underlying this cross-regulation appears to be independent of direct enzyme-to-enzyme interactions. Rather, it relies solely on the presence of the respective other modification, specifically polyglutamylation of the C-terminal tails of α- and β-tubulin.

These results demonstrate the existence of a network of interactions between different tubulin PTMs suggesting complex levels of regulation of the trypanosome cytoskeleton.

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PS186.III

Tubulin polyglutamylation and acetylation in *Trypanosoma brucei*

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Tubulin posttranslational modifications (PTMs) are essential for regulating microtubule function and dynamics in eukaryotic cells[1]. In the parasitic protozoan *Trypanosoma brucei*, tubulin PTMs play a critical role in maintaining cytoskeletal integrity and supporting essential processes such as cell division and motility[2,3]. While some tubulin PTMs have been characterized in *T. brucei*, the functional significance of polyglutamylation patterns and α-tubulin acetylation remains unclear[4].

To address this, we used a knockout approach to target three uncharacterized proteins that catalyze some of these PTMs. We generated knockout mutants of the polyglutamylases TTLL4C and TTLL6B and the α-tubulin N-acetyltransferase (ATAT). We analyzed the mutant cell lines using Western blotting and mass spectrometry to identify TTLL4C as an initiase for α-tubulin polyglutamylation, and TTLL6B as an elongase for β-tubulin. In addition, we found that TTLL4C-mediated glutamylation positively regulates α-tubulin detyrosination, and we identified the role of ATAT as the sole α-tubulin N-acetyltransferase in *T. brucei*.

To study the cellular phenotypes, we analyzed the morphology of the mutant cell lines by immunofluorescence microscopy. Similar to our previous studies on TTLL13, *ttll4c-/-* cells displayed a blunt posterior cell pole, whereas *ttll6b-/-* cells were narrow and elongated. ATAT-deficient cells were larger overall and showed a severe growth defect. Loss of tubulin acetylation also impaired motility. Wild-type cells typically exhibit both "running" and "tumbling" behaviors, allowing for adaptive movement. In contrast, *atat-/-* cells are predominantly in an "tumbling-only" state, indicating a shift in motility dynamics.

These findings reveal distinct roles for TTLL4C, TTLL6B, and ATAT in modulating cytoskeletal organization, cell morphology and growth, and highlight the complex interplay between tubulin modifications and their cellular functions in *T. brucei*.

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PS189.III

Towards a neuroethological model for trematode larvae

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Despite their economic and health relevance, parasitic flatworms' neurobiology remains an underexplored frontier. Here, we apply a systems neuroscience approach to the swimming larvae (cercariae) of *Cryptocotyle lingua*, the causative agent of black spot disease, which impacts cod farming. Its streamlined nervous system offers an attractive platform for investigating the molecular and functional basis of behavior in trematodes.

Using transcriptomics, we identified the molecular toolkit of the cercarial nervous system, including genes upregulated in the larvae. Notably, we observed the absence of nitric oxide synthase but the presence and upregulation of putative nitric oxide receptors. We also identified upregulated genes involved in aminergic pathways, TRPA channels, and synaptic vesicle trafficking, highlighting adaptations related to larval dispersal and host-finding behavior. These findings were validated through *in situ* hybridization and antibody staining.

At the cellular resolution, we characterized the neuronal network responsible for cercarial locomotion (swimming). Using TEM and *in situ* hybridization with neuronal markers, we characterized a minimal network of 20 coupled neurons, including some with unique features and a potential unpaired pacemaker. RNA sequencing of the cercarial tails, where neuronal nuclei predominate, revealed upregulated genes encoding neuronal machinery.

In the ongoing phase, we are mapping these genes—primarily representing receptor pathways—onto the existing neuronal network. Integrating deep learning-based behavior quantification with pharmacological experiments, we aim to elucidate the functional relevance of these pathways. This work establishes a foundation for applying neuroethological principles to trematodes, bridging basic neuroscience and parasitology with translational potential.

Fig. 1

PS192.III

L. mexicana v-ATPase knockout mutants are sensitive to changes in external pH, osmolality and temperature

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Introduction: *Leishmania* are the causative agents of the neglected tropical disease Leishmaniasis. Parasites have two main life forms in two different hosts: promastigotes in the gut of sand flies and amastigotes in the macrophages of the mammalian host. In this digenetic life cycle, parasites must adapt to different conditions, including changes in pH and temperature. A CRISPR/Cas9 knockout (KO) screen of *Leishmania mexicana* membrane transporters revealed that the vacuolar-type H^+ ATPase (v-ATPase), a multi-subunit proton pump, is indispensable for survival of intracellular amastigotes(1). v-ATPase KO promastigotes were viable *in vitro*, with normal growth rates at neutral pH, but their growth was adversely affected in acidic pH(1).

Aims: To understand the vital role of the v-ATPase in the parasites" adaptation to different environments, we tested (i) the effect of different environmental conditions on the growth of v-ATPase KO parasites and (ii) the morphology of v-ATPase KO mutants.

Results: In addition to acidic pH (5.5), v-ATPase KO promastigotes showed growth defects when grown in alkaline conditions (pH 8.45), hyperosmotic stress and at elevated temperatures (34°C). These growth defects were rescued by reintroduction of the deleted gene (add-back, AB). We also discovered that, independent of the pH of cell culture media, v-ATPase KO mutants displayed enlarged vacuoles upon reaching stationary phase of growth, and when grown at 34°C or hyperosmotic conditions. These vacuoles did not occur in the control or AB cells. Transmission electron microscopy imaging revealed that some of these vacuoles contained partially digested organelles, and some vacuoles were double membrane enclosed, a feature of autophagosomes. Molecular markers of autolysosomes, the autophagosome marker ATG8 and lysosome marker Cysteine Peptidase A (CPA) were tagged with mNeonGreen, in two separate cell lines, followed by deletion of the v-ATPase subunit E. Imaging of these cells revealed that the enlarged vacuoles were associated with both ATG8 and CPA signal, suggesting they are autolysosomes.

Endogenous tagging and fluorescence imaging showed that v-ATPase subunits have strong signals near the flagellar pocket, and in addition diffuse signals throughout the cytoplasm.

Conclusion: These results show that the v-ATPase is essential for *Leishmania* both in acidic and alkaline pH and at elevated temperature. The presence of enlarged autolysosomes in v-ATPase KO mutants suggests that the cells are halted at the final stage of autophagy, most likely due to a reduction in lysosomal enzyme activity, caused by pH dysregulation. Our results identify v-ATPase as an important stress regulator in *L. mexicana*, which is essential for the completion of the parasite"s life cycle.

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PS195.III

Evaluation of the diagnostic performance of a new ELISA for the detection of anti-trichinella antibodies

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Background: Trichinosis is a zoonotic parasitic disease which is spread worldwide and caused by nematodes of the genus *Trichinella*. Human infection occurs following consumption of raw or undercooked meat containing infective larvae. The disease is a major public health concern, particularly in regions with traditional dietary practices involving raw meat or inadequate food safety regulations. The infection proceeds in two distinct phases: an enteral phase, marked by gastrointestinal symptoms, and a parenteral phase, characterized by the migration and encapsulation of larvae in skeletal muscles. Clinical manifestations range from mild, nonspecific symptoms such as myalgia and fever to severe, potentially life-threatening complications, including myocarditis and encephalitis. Diagnosis primarily relies on serological assays, supplemented by muscle biopsy in cases of diagnostic uncertainty. The aim of this study is to evaluate the performance of the EUROIMMUN Anti-Trichinella ELISA (IgG) for the detection of *Trichinella*-specific antibodies in human sera.

Methods: The Anti-Trichinella ELISA (IgG) is based on somatic *Trichinella spiralis* lysate. Assay performance was examined using serum samples from 50 patients with clinically confirmed trichinosis and a total of 220 samples from subjects without laboratory or clinical evidence of trichinosis (200 healthy blood donors, 10 patients with schistosomiasis, 10 patients with echinococcosis). Borderline results were excluded from the determination of test sensitivity and specificity and the confidence interval (CI) was specified as 95%. Additionally, a cross-reactivity panel was tested, comprising 42 serologically pre-characterized samples from patients tested positive for another parasitic disease, including ascariasis (n=10), toxocariasis (n=10), filariasis (n=8), and strongyloidiasis (n=14).

Results: Of the 50 patients with confirmed trichinosis, 47 were positive using the Anti-Trichinella ELISA (IgG), one was borderline, and two were negative. This corresponds to a sensitivity of 95.9% (CI 95%: 86.0-99.5%). Of the 220 samples without proof of trichinosis, 215 were negative, three samples were classified as positive and two samples as borderline, which equates to a specificity of 98.6% (CI 95%: 96.0-99.7%). The somatic *Trichinella spiralis* lysate showed no cross-reactivity with anti-*Ascaris*-positive or anti-*Toxocara*-positive sera. However, 2 out of 14 samples from patients with strongyloidiasis (14.3%) and 7 out of 8 samples from patients with filariasis (87.5%) were found positive.

Conclusion: The results of this study demonstrate that the Anti-Trichinella ELISA (IgG) detects anti-*Trichinella* antibodies with a high sensitivity and specificity. However, as cross-reactions with antibodies against other parasites (especially filarial nematodes) cannot be excluded, the patient"s anamnesis should be considered when interpreting serological results.

PS198.III

The coarser filter gets the worm – Environmental monitoring of *Trichobilharzia* using environmental DNA

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Introduction: *Trichobilharzia* is a genus of avian schistosomes parasitising freshwater birds and is the causative agent of human cercarial dermatitis (CD, swimmer"s itch). Similarly to schistosomiasis, CD is caused by infection with cercariae during water-contact activities, such as bathing and open-water swimming. Although avian schistosomes cannot complete their life cycle in humans, CD can cause severe discomfort to those affected and economic losses due to bathing site closures. Traditional monitoring of *Trichobilharzia* relies on collecting and examining freshwater snail intermediate hosts. This method requires trained personnel and is labour-intensive. Infected snails are challenging to find and identify and do not always shed cercariae, resulting in limited sensitivity. Here, we field-validate environmental DNA (eDNA) monitoring approach using a new *Trichobilharzia* qPCR assay and identify the most suitable filters for eDNA collection.

Methods: From May to September 2024, snails and eDNA were sampled thrice at eight localities with CD history in Czechia. At each locality, snails were collected for shedding examination, and eDNA was collected in triplicate using three filter types (Sterivex 0.45 µm, Sylphium 0.45 µm, and Sylphium 5 µm filters). Extracted eDNA was then tested for *Trichobilharzia* spp. DNA using a novel genus-specific TaqMan qPCR assay.

Results: *Trichobilharzia* eDNA was detected at all localities at least once and proved more sensitive than malacological monitoring. One locality was identified as a persistent *Trichobilharzia* hotspot, with infected snails and eDNA detected during all three visits. Two additional sites hosted infected snails on single occasions. Sterivex filters exhibited the lowest positivity rate for *Trichobilharzia* eDNA by qPCR (8/72), whereas Sylphium 0.45 µm and 5 µm filters were positive in 22/72 and 33/72 instances, respectively. Environmental DNA was detected at all sites where shedding snails were present only by Sylphium 5 µm filters, with Sylphium 0.45 µm and Sterivex filters negative at one site where shedding was observed. The Sylphium 5 µm and 0.45 µm filters filtered significantly more water (mean volumes filtered 2751 ml and 1822 ml, respectively) before clogging than the Sterivex filters (mean volume 601 ml). Moreover, the Sylphium 5 µm filters reached the 5-litre filtering cutoff threshold 19 times, indicating good resistance to clogging, whereas the Sterivex filters were prone to early clogging.

Conclusions: We conducted a field validation of a new qPCR assay and identified the optimal filtering strategy for monitoring *Trichobilharzia* eDNA. Sylphium 5 µm filters proved to be the most suitable filter type due to their resistance to clogging and high eDNA capture rates. This study offers a novel tool for the environmental monitoring of *Trichobilharzia* spp., and the insights from this study may be beneficial in eDNA surveillance of other waterborne parasites, such as *Schistosoma* spp.

PS201.III

Food-borne parasites of wildlife and one health in the Arctic

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Over a decade of research on food borne parasites in the Canadian Arctic has resolved some fundamental ecological and epidemiological questions, while raising new questions and needs to address the significance and impact of zoonotic parasites at the shifting human/wildlife interface. The coccidian *Toxoplasma gondii* is arguably the most ubiquitous parasite, infecting many vertebrate hosts and ranging from the equator to the poles. The ultimate source of *T. gondii* is the intestines of wild and domestic felids, whereas the pathogenicity of *T. gondii* lies primarily in vertebrate intermediate hosts, where it can cross the placenta and affect mammalian fetuses (sometimes triggering abortion), and reactivate in immunocompromised hosts. Unique among coccidians, *T. gondii* can transmit from intermediate host to intermediate host through carnivory. Therefore, while cats are the ultimate source of environmental contamination, the importance of food borne routes of exposure is increasingly recognized. This is almost certainly the case in the Canadian Arctic, where there is high seroexposure to *T. gondii* in wildlife and people, but felids are few and far between above treeline. Over a decade of research in the Canadian Arctic reveals that lynx are a potential source of environmental contamination with *T. gondii*, but are far more commonly infected as intermediate hosts; terrestrial carnivores are good sentinels of circulating levels of *T. gondii,* which are higher in the eastern vs western Canadian Arctic; and exposure to *T. gondii* in the eastern Canadian Arctic is primarily linked to consumption of migratory and aquatic wildlife, rather than terrestrial herbivores like caribou. These findings in wildlife are congruent with human seroepidemiological studies and have significance for exposure of Indigenous harvesters. Concomitant investigations for *T. gondii* also detected high prevalence and diversity in wildlife of other coccidian parasites in the genus *Sarcocystis*, which also transmit through carnivore/herbivore predation cycles and for which the human health significance is entirely unknown, revealing evidence of Palearctic species in the Nearctic and a potentially new species that may cycle in marine environments. Finally, food borne nematodes in the genus *Trichinella* spp. have high prevalence in Arctic wildlife, with zoonotic significance for Indigenous harvesters and visiting hunters alike, including large scale, cross border outbreaks, cryptic diversity, and further enigmas of transmission between terrestrial and aquatic ecosystems. This work highlights the need to better understand transmission of food-borne parasites in the Arctic, the most rapidly warming region of the globe, and enabling One Health approaches to detection and management of these parasites that are sustainable and successful.

PS204.III

Genotyping L1 larvae of metastrongylid nematodes in wolves: A major case of pseudoparasitism shows potentially new species of lungworms

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Introduction: The grey wolves were historically hunted to extinction in Czechia but are returning since 2014[1]. Their migration potential allows them to spread parasites of medical or veterinary importance into new regions. For example, *Angiostrongylus vasorum* - clinically important parasite in dogs[2]. As wolves are protected species non-invasive samples are needed for large studies. This complicates the detection of L1 larvae because of rarely fresh samples, free living nematodes or pseudoparasitism[3].

The aim of the study was to assess the diversity of lungworms in wolves in Czechia (Šumava mountains), using the combined sieving and molecular approach, focusing on detection of *A. vasorum*.

Materials & methods: A total of 357 samples, collected between 2020 and 2023 were analysed using modified sieving method[4]. The DNA was extracted by NucleoSpin Tissue XS kit (Machery-Nagel) and the samples were genotyped using conventional and nested PCR, targeting ITS2 and COI respectively. Phylogenetic trees were constructed to assess the relationship between detected species.

Results: PCR product and sequence corresponding to lungworms were obtained in 23.5 % (84/357) of the positive samples, with 2 mixed infections. Surprisingly, only 4.8 % (4/84) of the positive samples represented parasites of wolves (*C. vulpis*), *A. vasorum* was not detected. The rest (95.2 %; 80/84) were lungworms of prey, representing 5 other genera. The phylogenetic analysis showed high diversity of detected lungworms, hinting at several undescribed species.

Conclusion: Our data show that vast majority of L1 larvae detected in faeces of free-ranging wolves belongs to prey, highlighting the need to use genotyping for species determination in L1 larvae of lungworms. This phenomenon has been described in wolves only once, questioning previous reports based on morphology of metastrongylid L1 larvae alone. We also show high diversity of lungworm species infecting ruminants with several potentially new species. Further studies should focus on the diversity of lungworms of wild ruminants in Europe and the notion that wolves could also play role in eco-epidemiology of lungworm infections in their prey.

Fig.1 Abundance of detected lungworm species

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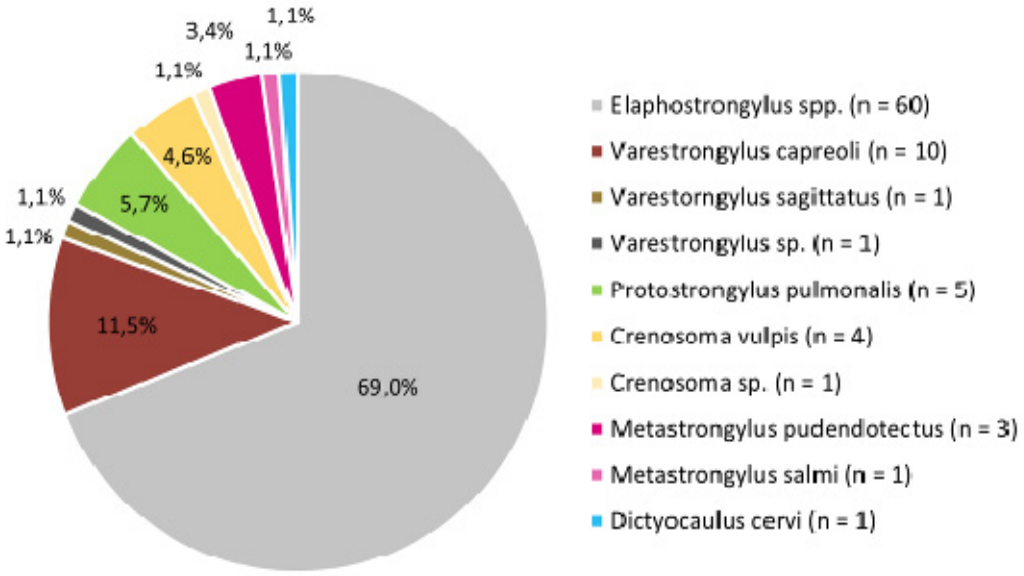
Fig. 1


1,1%
3,4%
1,1%
1,1%
4,6%
1,1%
5,7%
1,1%
11,5%
69,0%
Elaphostrongylus spp. (n = 60)
Varestrongylus capreoli (n = 10)
Varestorngylus sagittatus (n = 1)
Varestrongylus sp. (n = 1)
Protostrongylus pulmonalis (n = 5)
Crenosoma vulpis (n = 4)
Crenosoma sp. (n = 1)
Metastrongylus pudendotectus (n = 3)
Metastrongylus salmi (n = 1)
Dictyocaulus cervi (n = 1)

PS207.III

Helminthicidal activity of juniper *(Juniperus communis)* extract confirmed using the planarian *(Schmidtea lugubris)* laboratory model: An anthelmintic from ancient Egypt

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Planarians are free-living flatworms and their use as convenient laboratory models for schistosomes was first proposed by Collins & Newmark (2013). These authors suggested that, among other uses, the planarian model might be used to test candidate anthelmintics by posing the question, "Can planarians be used to identify new therapeutics?"

In this study we used a basic form of the planarian laboratory model to test the helminthicidal properties of the aromatic water extract (hydrolate) of Juniper berry *(Juniperus communis)*. Anecdotal mention of the use of Juniper fruits (berries) as an anthelmintic dates back to to Ancient Egypt where it is reportedly mentioned in a medical context in a papyrus of 1500 BC as a cure for tapeworm infection (El-Juhany, 2021). Interestingly, this period of Ancient Egypt falls within the Eighteenth Dynasty (New Kingdom) which, interestingly, included the pharaoh Tutankhamun.

Five planarians of the species *Schmidtea (Dugesia) lugubris*, obtained from a biological supplier (Blades Biological UK), were placed into each of ten small, 5cm diameter, glass petri dishes each containing 10ml of juniper berry aromatic water (hydrolate) obtained from Fragrant Earth Organics Ltd. UK. Planarians used were in the length range 7-10mm. A group of fifty *S. lugubris*, in the same size range were retained in spring water, in the same type of petri dishes, as untreated controls. Survival of planarians in both treated and control groups was monitored every ten minutes with the aid of a dissecting microscope. Planarian death was recorded when an individual failed to respond to mechanical stimulation in the form of three light contacts with a fine stainless-steel needle. Dead individuals were removed from the petri dish.

Maximum survival time of planarians in the juniper treated group was two hours and forty minutes, with most mortality occurring post one hour of exposure. The rate of mortality in the treated group was time dependent, the survival curve being inverse sigmoidal in form. No mortality of planarians was observed in the untreated control group.

The results of this study seem to indicate the presence of a helminthicidal property of juniper extract that may warrant further investigation. It is possible that such an anthelmintic property might eventually prove particularly useful as a plant-based remedy in the treatment of some neglected tropical diseases of the intestinal tract caused by helminths such as echinostomes which cause the disease Echinostomiasis.

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PS210.III

In vitro drug screening cascade for _E. granulosus:_ Screening of the MMV pandemic response box

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E. granulosus causes the severe zoonotic disease cystic echinococcosis (CE). It is predominantly affecting sheep and cattle, but also humans as accidental intermediate hosts. The disease results in organ damage due to pressure exerted by expanding cysts. Treatment strategies depend on cyst size and location and include surgery, the PAIR method (puncture, aspiration, injection, re-aspiration), drug therapy with albendazole, and the watch-and-wait approach for inactive or calcified cysts. However, these options often have limitations in efficacy and safety, with potential toxicity during prolonged use. Therefore, there is an urgent need for innovative and effective therapies.

We recently adapted an *in vitro* screening cascade, originally developed for *E. multilocularis,* to assess *E. granulosus (*Kaethner *et al*., 2023, https://doi.org/10.1371/journal.pntd.0011343). This innovative cascade enables a comprehensive evaluation of potential therapeutic compounds against *E. granulosus* by incorporating multiple assays, including: (i) metacestode vesicle damage by damage marker (phosphoglucoisomerase) assay, (ii) metacestode vesicle viability assay, (iii) germinal layer (GL) cell viability assay, and (iv) protoscolex (PSC) motility assay. This cascade offers a robust platform for testing drug efficacy and advancing therapeutic development against *E. granulosus*.

In this study, we plan to screen 400 compounds from the open-source Medicines for Malaria Venture (MMV) Pandemic Response Box. The initial focus was placed on the PSC stage of *E. granulosus*. PSCs are clinically relevant as they can induce secondary CE through dissemination post-surgery or PAIR. Preliminary results identified 10 active compounds from 150 tested, all reducing PSC motility to 40% or less after 24 hours at a concentration of 10µM. Notable compounds included Clofazimine (MMV687800, 4.6% motility); and MMV1579844 (1.02% motility), both outperforming the current standard drug against PSCs, Praziquantel (8.64% motility).

Metacestode cultures derived from sheep GL or activated PSCs were established and maintained in co-culture with rat hepatoma cells, enabling large-scale metacestode production. This system allows now to further evaluate PSC-active compounds within the broader screening cascade.

This research underscores the value of robust *in vitro* tools for assessing drug efficacy against CE. The findings provide a solid basis for the continued investigation and development of promising treatment candidates, with the potential to address unmet needs in CE therapy.

PS213.III

Role of IL-9 signaling in the filaria-mediated protection against diet-induced insulin resistance and adipose tissue inflammation

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Introduction: Obesity causes the death of 2.8 million people worldwide every year. Despite the established role of inflammation in the pathogenesis of obesity and type-2 diabetes and our previous data supporting a protective role of helminth infections, the mechanisms remain unclear. In the present project, we studied the role of the type-2 cytokine IL-9, which has multiple roles in helminth infections, allergy and autoimmune diseases, but has not been investigated so far for its role in obesity and type-2 diabetes. Based on the type-2 dependent helminth-mediated protection observed during diet-induced insulin resistance and the central role of IL-9 in driving protective immunity against helminths, we hypothesized that helminths might act via IL-9 to induce insulin sensitivity during diet-induced insulin resistance. Our findings in animal models as well as human data suggest that the protective effect of helminth infection on insulin resistance and obesity might be partly mediated by IL-9 signalling.

Methods: Serum samples were obtained from 159 participants of which 83 were infected with the filarial nematodes *Onchocerca volvulus* (48.2%) and/or *Mansonella perstans* (54.2%) and/or *Loa loa* (47%) from the Littoral regions of Cameroon. In this study cohort, 85 participants were classified as non-diabetic and 74 as diabetic subjects. Animal studies were done by using IL-9R(receptor) knock out (KO) mice that are deficient in IL-9 signalling and recombinant IL-9 (rIL-9) treatment to high fat diet (HFD)-induced obese mice.

Results: IL-9 levels were significantly lower in the uninfected diabetes subjects compared to their corresponding uninfected non-diabetic controls. However, serum levels of IL-9 were comparable between subjects with and without diabetes in the infected group. We also observed decreased IL-9 levels in HFD-induced obese mice. On the other hand, rIL-9 treatment rescued insulin insensitivity and inflammation following HFD. IL-9R KO mice fed HFD exhibited weight gain, impaired glucose and insulin tolerance, defective insulin signalling, increased adipocyte size and decreased energy expenditure. In line with the human data, we also found that IL-9 is important for the beneficial effects mediated by infection with the rodent filarial nematode *Litomosoides sigmodontis* on diet-induced glucose tolerance and inflammation in HFD mice.

Conclusion: Our findings in animal models as well as human data suggest that the protective effect of helminth infection on insulin resistance and obesity might be mediated by IL-9 signaling.

PS216.III

Three cases of schistosomosis in Brazilian tanagers (*Ramphocelus bresilia* linnaeus, 1766) in a German zoo

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Schistosomosis is a major neglected tropical disease that affects around 250 million people worldwide each year and puts a further 700 million people at risk of infection. Schistosomatidae (blood flukes) infect mammals, including humans as the final host, while birds also experience widespread infections, with high species diversity even in temperate zones such as Central Europe. A meta-analysis estimates a 38% prevalence of schistosomosis in European aquatic birds. The indirect life cycle of the blood flukes involves freshwater snails as intermediate hosts, which release cercariae into water that infect final hosts percutaneously. In birds, nasal and visceral forms of disease can occur. In the human body, schistosomes of aquatic birds cannot complete their life cycle, but can cause cercarial dermatitis (swimmers itch), characterized by severe inflammatory reactions with itching and maculopapular eruptions. With rising summer temperatures, the importance of this disease increases.

Here, three cases of schistosomosis in Brazilian tanagers (*Ramphocelus bresilia* L., 1766) from Zoo Berlin are reported. Their enclosure included an indoor and an outdoor area, was temporarily shared with a Socorro dove and did not allow direct contact with aquatic birds. A water basin was present in both areas. The birds hatched in two different German zoos in different years and were relocated to Zoo Berlin. Thus, the infection definitely occurred in Germany and probably at Zoo Berlin. The animals from the same aviary died acutely at the age of 4, 8 and 9 years and without any other obvious previous symptoms. Pathohistological examination revealed hematogenous spread of schistosome eggs, particularly to the liver, lungs, intestines and kidneys. Relevant co-infections were excluded by bacterial, parasitological and virological examinations. No adult blood flukes were found in the routine pathological examination, whereby the vascular network of the Vena porta was not specifically sampled. For species identification, the eggs from the organs were analyzed by PCR and sequencing of 18S, cytochrome oxidase I and ITS-1/58S/ITS-2 regions.

A precise determination of the species was not possible based on the available sequences, as no sufficient sequence matches were found in GenBank. It is possible that this is a previously undescribed species, or at least one for which no sequences have been deposited in GenBank. Nevertheless, it has been determined that it belongs to the subfamily Gigantobilharziinae, for which there are previous reports of infection in songbirds (Passeri).

The infection route has not yet been clarified and potentially involved intermediate hosts could not be identified in order to interrupt the development cycle. Tanagers have not yet been described as hosts of Schistosomatidae. In zoos with many different host species in a small space, transmission from reservoir hosts to exotic species should be considered.

PS219.III

A quantitative mass spectrometry approach identified novel proteins involved in macrophage infection by *Leishmania* parasites

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Leishmania are single-celled kinetoplastid parasites that live intracellularly in phagocytic cells of their host. Transcriptomic analyses have already provided insights into host defense mechanisms and parasite responses however these analyses do not accurately reflect the cellular protein composition due to posttranscriptional gene regulation in kinetoplastids. Here, we conducted a systematic comparative *in vitro* analysis of the infection process using a quantitative mass spectrometry (MS) approach. Murine bone marrow-derived macrophages (BMDMs) were infected with 3 *Leishmania* species (*L. mexicana, L. major,* and *L. infantum*). Whole cell protein extracts from parasites and BMDMs were collected at 7 postinfection timepoints and analyzed by MS. We quantified the expression of 2000 murine proteins and 1500, 1000, and 1400 proteins after infection with *L. mexicana, L. major*, and *L. infantum*, respectively. The number of differentially expressed proteins detected during infection varied by species. Our study revealed numerous proteins putatively involved in the infection process that were not detected in previously published transcriptomic analyses. We also plan to develop a web-based interactive platform for the community to conveniently access detailed data on protein enrichment, lists of all detected IDs for each species, and statistical analyses. We are currently investigating several candidate proteins of unknown function that are essential for the infection process. The aim of this study was to identify additional crucial virulence factors and elucidate their functions during infection of vertebrate hosts.

PS222.III

Prevalence of dhps K540E and A581G mutations in *Plasmodium falciparum* isolates among asymptomatic parasitaemic pregnant women attending antenatal care booking in Nchelenge district Northern Zambia

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Background: Interventions to reduce the burden of malaria in pregnancy in sub-Saharan Africa are inadequate. Malaria infection during pregnancy is responsible for adverse birth outcomes. To protect against adverse pregnancy outcomes in malaria-endemic areas, the WHO recommends providing intermittent preventive treatment with sulfadoxine-pyrimethamine (IPTp-SP) to pregnant women at each scheduled antenatal (ANC) visit as directly observed therapy from the second trimester to delivery with at least one month between doses. However, the loss of parasite sensitivity to SP has compromised the efficacy of IPTp-SP. Studies have revealed that resistance to SP is associated with single nucleotide polymorphisms in the dihydrofolate reductase and dihydropteroate synthase (dhps) genes of Plasmodium falciparum including in position 540 and 581 of the dhps gene. Mutations in codon 540 and 581 are proxy measures for the presence of all five key mutations that are markers of resistance to SP. The current study is part of the ASPIRE trial (Registration: NCT04189744) which is designed to reduce the dual burden of malaria and curable sexually transmitted and reproductive tract infections in pregnancy. The objective of this study was to estimate the prevalence of the K540E and A581G mutations in samples of 200 malaria positive women.

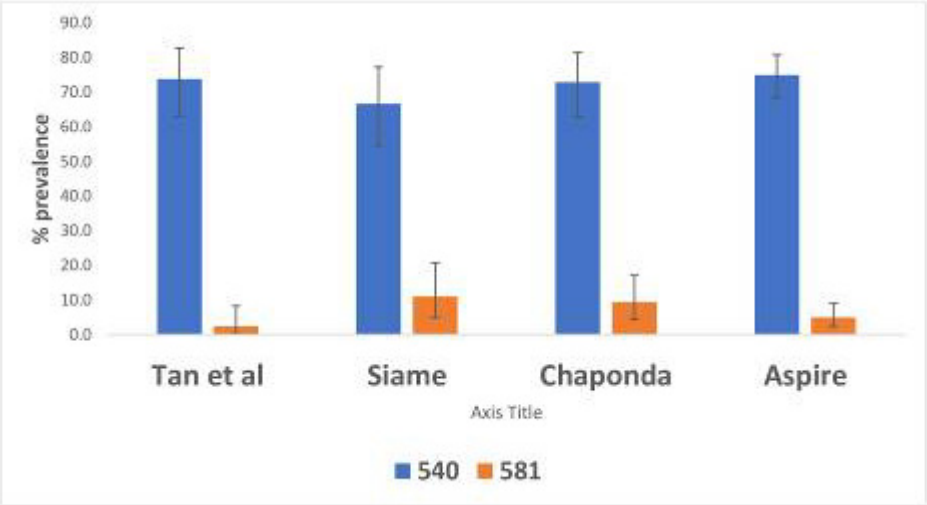
Methods: The ASPIRE trail was conducted in four health facilities of Nchelenge District, a holoendemic area with a malaria prevalence estimated at 50% throughout the year. A total of 5,436 pregnant women were recruited at their first antenatal visit from November 2019 to August 2022. Dried blood spots samples were collected from all the participants on whatmann 3mm filter paper. Plasmodium falciparum DNA was isolated using the Chelex DNA extraction method and detected using SYBR green on the ABS 7500 fast real-time polymerase chain reaction (PCR) platform. The mutations were detected using PCR restriction fragment length polymorphism method.

Results: Out of 5,436 samples, 2888 (53.1%, 95% CI= 51.9-55.5) tested positive for malaria by PCR. From those that were positive, 200 samples were randomly selected for determination of P. falciparum dhps mutations associated with drug resistance. The K540E and the A581G resistance markers were found in74.8% (95% CI=61.2-74.1) and 5.03% (95% CI=1.2-8.1) of the samples respectively.

Conclusion: The data suggest a high prevalence of P. falciparum K540E and low prevalence of A581G mutation which is associated with resistance to SP among pregnant women, which may explain reduced efficacy of IPTp treatment in Zambia. More efficacious antimalarials are urgently needed to address malaria in pregnancy.

Keywords: IPTp-SP; sulfadoxine-pyrimethamine resistance; malaria; mutation; Pfdhps.

Fig. 1


90.0
80.0
70.0
60.0
50.0
40.0
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% prevalence
Tan et al
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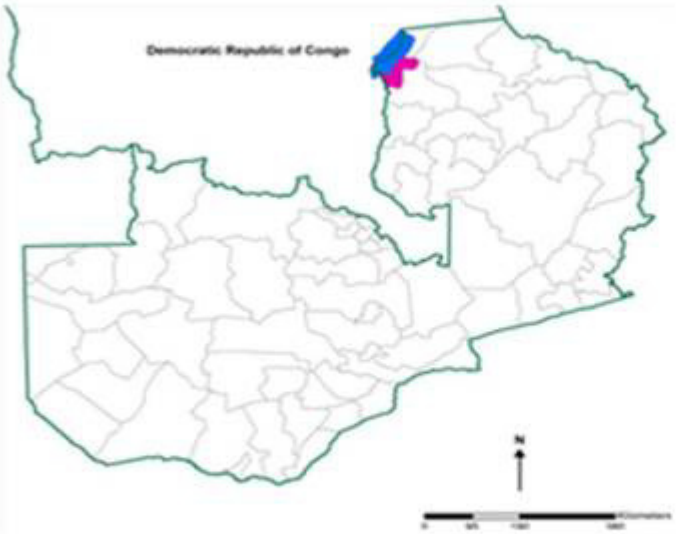
540
581

Fig. 2


Democratic Republic of Congo
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PS225.III

Eosinophils and macrophages undergo metabolic changes in the course of *Litomosoides sigmodontis* filarial infection

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Introduction: Filarial infections trigger a characteristic type 2 immune response in their host, with a hallmark increase in eosinophil cell numbers. Eosinophils are primary effector cells that mediate protection against filarial infection via diverse effector mechanisms, including the release of cytotoxic granule proteins, reactive oxygen species (ROS), cytokines and extracellular DNA traps (ETosis). Additionally, macrophages mediate immunity to filarial helminth infection by sustaining eosinophilia, producing nitric oxide, inducing granuloma formation and releasing cytokines. These immune responses are bioenergetically expensive and are tightly connected with the cellular metabolism. To ensure that enough energy is produced to supply cellular demands, energy can be obtained through several metabolic pathways. Although energy production is an essential step in the fight against invading pathogens, the immune metabolism in relation to filarial infections is mainly unknown. Thus, we investigated eosinophil and macrophage metabolic requirements and effector mechanisms on different life-cycle stages of the rodent filarial nematode *Litomosoides sigmodontis*.

Methods: Eosinophils and macrophages were either isolated from the pleural cavity or generated from the bone marrow of naïve and *L. sigmodontis*-infected female BALB/c mice at (1) 35 days post-infection (dpi), when adult worms are present in the pleural cavity, but microfilariae offspring (MF) are not produced yet, and (2) at 63 days post-infection, when MF are found in the peripheral blood. Immunological parameters, such as cytokine responses and metabolic pathways were analysed ex vivo.

Results: Pleural cavity eosinophils and macrophages presented similar metabolic requirements and different cytokine profiles through the course of *L. sigmodontis* infection compared to naïve controls. In vitro eosinophils and macrophages (from the pleural cavity and bone marrow-derived) from 35 dpi mice, obtained energy mainly via oxidative phosphorylation. In contrast, when MF were present at 63 dpi, maximal mitochondrial respiration was reduced and IL-4 and IL-6 cytokine release were increased in pleural cavity eosinophils. At 63 dpi, bone marrow-derived macrophages also showed a similar decrease in mitochondrial respiration, but with an associated increase in TNF and IL-6. A similar trend was seen in pleural cavity macrophages as well.

Conclusions: The results of our study indicate that immune responses during *L. sigmodontis* infection are highly dynamic and that the metabolic requirements are dependent on the filarial life-cycle stage, showing a metabolic shift in the presence of MF characterized by a reduction of mitochondrial respiration and increased IL-6 levels.

PS228.III

Leishmania infantum infection in co-cultures of hepatic organoids and immune cells

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Introduction: Visceral leishmaniasis is a neglected tropical disease caused by *Leishmania* parasites, resulting in systemic infection. After invading the host, the parasite is engulfed by phagocytic cells such as macrophages, neutrophil granulocytes and monocytes eventually spreading to the spleen and liver. While the liver can partially eliminate the pathogen, the release of chemokines triggers the recruitment of additional immune cells. This amplifies the infection and ultimately causes liver damage. However, the precise pathophysiological effects of *Leishmania* infection on hepatocytes - the central functional cells of the liver - and their interactions with immune cells remain largely unexplored.

Objective: Characterization of *Leishmania infantum* infection in co-cultures of hepatic organoids and immune cells

Methods: Three-dimensional hepatocyte organoids were first generated from primary murine hepatocytes. These organoids were then infected with *L. infantum* to study their immune response through cytokine analysis. Various co-culture systems involving monocytes/macrophages were tested, using either infected hepatocyte organoids or infected macrophages as the source of infection. The immune response was assessed by ELISA, multiplex cytokine assays, and RT-PCR.

Results: The hepatocyte organoids exhibited a low immune response following infection with *L. infantum*. However, when co-cultured with monocytes, there was a distinct infection-specific upregulation of cytokines, including CCL3, CCL2, TNF, IFN-γ and IL-10, which mirrored responses observed in murine models. Notably, the production of CCL3 and TNF was exclusive to the co-cultures with monocytes. Furthermore, infection-dependent changes in the gene expression were observed in the organoids co-cultured with infected macrophages, with *Nos2* being upregulated and *Arg1* downregulated, indicative of mechanisms involved in the elimination of *Leishmania* parasites.

Conclusion: Co-cultivating of hepatocyte organoids with monocytes allows for the simulation of infection-specific immune responses, highlighting the potential of this system to reduce animal testing in accordance with the 3R principle. Furthermore, the established methodology provides a versatile platform for future studies using human-derived cell sources, offering valuable insights into the immune mechanisms underlying visceral leishmaniasis in humans.

PS231.III

Leveraging blood-feeding to identify active compounds against human and agricultural nematodes: A comparative approach

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Human hookworm infections are caused primarily by the blood-feeding helminths *Necator americanus* and *Ancylostoma duodenale*, leading to iron-deficiency anemia in children and pregnant women. Concerns have been raised about the rise of genetic drug resistance against the current treatment, albendazole, due to its repetitive and widespread use in mass drug administration programs. Together with albendazole"s low cure rate for hookworms, there is a clear need for discovery of new drugs and alternative treatments.

The blood-feeding and heme detoxification pathways of hookworms are essential for their development and survival. To date, they have been overlooked as a promising drug targets in hookworm drug discovery. We have recently developed a screening assay based on blood feeding.

Utilizing a fluorescence based drug-screening assay in the rodent parasite model *Nippostrongylus brasiliensis*, we screened 400 compounds from the MMV Pathogen box library in the presence and absence of blood. We identified compounds that reduced the viability of L3 stage *N. brasiliensis* in a blood-dependent manner at 100 µM: MMV007920, MMV688845, MMV001493, MMV011511, MMV085071, MMV689255, and MMV026490. These hits were further validated against the human hookworm *Necator americanus* (L3) and the nematode of livestock *Haemonchus contortus* (L4) to reveal that some of the hits were translatable to two parasites of human and agricultural importance. This comparative and blood-based approach could be used to identify pan-anthelmintics in a medium to high-throughput screening setup, which may accelerate drug discovery and development against neglected tropical diseases.

PS234.III

First molecular insights into *Echinococcus canadensis* (G7) in pigs in Guatemala

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[2]Universidad de San Carlos de Guatemala, Facultad de Medicina Veterinaria y Zootecnia, Guatemala, Guatemala
[3]Universidad Galileo, Facultad de Biología Química y Farmacia, Guatemala, Guatemala
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Cystic echinococcosis (CE), caused by *Echinococcus granulosus* sensu lato, is a zoonotic parasitic disease with a complex life cycle involving canids as definitive hosts and ungulates as intermediate hosts. While CE is relatively well-documented in North and South America, recent information on its occurrence in Central America is lacking, including in Guatemala, where the most recent data are from nearly 80 years ago. This study sought to address this gap by initiating the generation of updated data on the prevalence, genetic diversity, and epidemiology of *Echinococcus* spp. in Guatemala. It specifically focused on backyard pigs, an important livestock animal, to establish baseline information for further investigations. Research was conducted at the municipal slaughterhouse of Quetzaltenango from March to August 2022. Post-mortem examinations of 117 backyard pigs revealed a high prevalence of 38.46% for CE, with male pigs showing higher infection rates (43.6%) compared to females (33.9%). A total of 1,140 cysts were extracted, predominantly from the liver, with only two cysts found in the lungs. Male pigs not only harbored more cysts on average but also had a higher proportion of fertile cysts. Molecular analysis of 32 randomly selected hydatid cysts confirmed the presence of *E. canadensis* G7 in all cases. Complete mitochondrial *cox1* gene sequences were obtained from 28 samples isolated from 20 pigs, revealing nine distinct haplotypes. Haplotype network analysis, incorporating globally documented *E. canadensis* G7 haplotypes from GenBank, demonstrated considerable genetic diversity among the Guatemalan haplotypes, suggesting a long-standing endemic presence of *E. canadensis* G7 in the region. While haplotype Ht01 was globally widespread and also found in Guatemala, the remaining eight haplotypes were unique to the country. Notably, haplotypes Ht01 and Ht02 occurred with equal frequency (10 each), contradicting the notion of a recent introduction of Ht01 followed by local evolution and spread. Infections with multiple haplotypes were observed in four of the eight pigs from which two cysts were analyzed, indicating substantial infection pressure in the region. The study also identified a minimum prevalence of 4.27% for *Taenia hydatigena*. These findings underscore the role of backyard farming practices and inadequate sanitation in sustaining the transmission cycles of these cestodes. Practices such as feeding dogs with contaminated offal perpetuate the parasite life cycle, posing a significant zoonotic risk. This study represents an important first step in understanding the local transmission dynamics of *Echinococcus* spp. and highlights the urgent need for improved hygiene practices, public awareness campaigns, and further research in Guatemala and Central America.

PS237.III

HNF4 as a key regulator of gut biology in the liver fluke *F. hepatica*

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Fasciolosis, a food-borne trematode infection caused by the liver fluke *Fasciola hepatica* and related species, poses a threat to human and animal health worldwide. Limited therapeutic options and increasing anthelminthic resistance complicate sustainable control and highlight the need for novel anthelmintics. The development of new anthelmintic strategies requires a deeper understanding of fluke biology, particularly regarding organ function and organ-specific gene expression.

Our ongoing work focuses on genes with prominent expression in the parasite gut - an organ vital for parasite survival. The transcription factor HNF4 is a highly conserved regulator of metabolic homeostasis and cellular differentiation of endodermal organs such as the liver, gut, pancreas, but its role for liver fluke biology is unknown. In our work, we identified an orthologue of human and schistosome HNF4 in the liver fluke proteome. With help of our spatial and single-cell transcriptomes, as well as *in situ* hybridization, we localized *hnf4* expression to the parasite"s gastrodermis. Functional studies utilizing RNA interference (RNAi) and RNA sequencing in immature parasites revealed that HNF4 was crucial for the maintenance of gut-associated gene expression, particularly genes encoding proteases such as cathepsins and legumain. RNAi-mediated knockdown of *hnf4* led to a significant reduction in worm viability *in vitro* and caused structural disruption of the intestine. Treatment with a commercial small-molecule inhibitor of HNF4 had similar effects, however, our transcriptomics data indicated that the inhibitor was not HNF4-specific.

These findings highlight the critical role of HNF4 in maintaining the gut function and overall viability of *F. hepatica*. Further investigations of the molecular mechanisms by which this nuclear receptor regulates gut biology may provide new avenues for drug development, targeting key pathways essential for fluke survival.

PS240.III

The power of immune signatures & predictive models – Early assessment of antibodies decline in chagas patients following treatment using a serological multiplex immunoassay

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Introduction: Chagas disease, caused by *Trypanosoma cruzi*, remains a significant global health concern, with its spread extending beyond endemic regions due to migration. The efficacy of the existing anti-parasitic drugs, nifurtimox and benznidazole, remain insufficiently investigated in adult patients. The progress in treatment evaluation and drug development is further hindered by the absence of reliable markers for parasitological cure. Moreover, traditional serological methods often fail to provide timely insights into treatment success due to the prolonged persistence of antibodies post-therapy. A novel serological multiplex immunoassay (MultiCruzi) is capable of simultaneously detecting multiple anti-*T. cruzi* antibodies to early assess the humoral immunity changes following treatment.

Objectives: The aims of this study are to explore the potential of the MuliCruzi test in evaluating the decline in specific antibodies as an early indicator of treatment efficacy in Chagas patients and, to validate the performance of this serological multiplex immunoassay in monitoring therapy outcomes and improving disease management strategies.

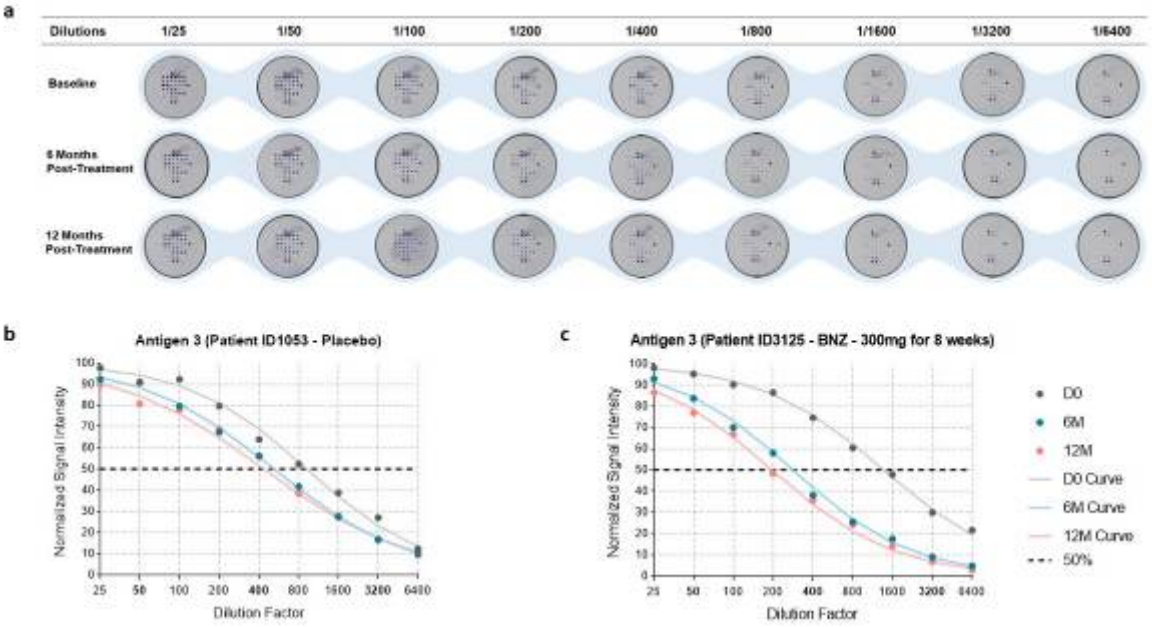
Materials & Methods: A retrospective analysis of the phase 2 randomized controlled BENDITA trial (ClinicalTrials.gov: NCT03378661) that includes adult patients in the indeterminate chronic stage of Chagas disease, treated with different benznidazole regimens and combinations. Serum samples collected at baseline, 6 and 12 months post-treatment were tested with MultiCruzi to quantify antibody levels against T. cruzi antigens. Advanced statistical analytical methods were used to determine trends in antibody decline and correlate these trends with the response to anti-parasitic treatment.

Results: Patients who responded to treatment exhibited a significant and measurable decline in antibody levels within 6 months following treatment in sharp contrast to data obtained with conventional and recombinant *T. cruzi* ELISA tests. The data obtained using the multiplex immunoassay and analyzed with advanced statistical methods suggest that early antibody decline correlates with therapeutic success and can serve as a marker for treatment efficacy.

Conclusion: MultiCruzi shows promise as an efficient tool for early assessment of treatment efficacy in Chagas disease. By enabling timely monitoring of immune responses, this approach could significantly enhance therapeutic decision-making and disease management. Further validation in larger cohorts is warranted to establish its role in clinical practice.

References: Saade, U., de Boer, J., Scandale, I. *et al.* Early assessment of antibodies decline in Chagas patients following treatment using a serological multiplex immunoassay. *Nat Commun* 15, 10530 (2024). https://doi.org/10.1038/s41467-024-54910-x

Fig. 1


a
Dilutions
1/25
1/50
1/100
1/200
1/400
1/800
1/1600
1/3200
1/6400
Baseline
6 Months Post-Treatment
12 Months Post-Treatment
b
Antigen 3 (Patient ID1053 - Placebo)
Normalized Signal Intensity
Dilution Factor
c
Antigen 3 (Patient ID3125 - BNZ - 300mg for 8 weeks)
Normalized Signal Intensity
Dilution Factor
D0
6M
12M
D0 Curve
6M Curve
12M Curve
50%

PS243.III

A serological test based on mutated recombinant *Fasciola hepatica* cathepsin L protease for the diagnosis of equine fasciolosis

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Background: *Fasciola hepatica* is a common trematode parasite of livestock in many regions, causing significant economic losses and affecting animal welfare. Horses rarely develop patent liver fluke infection, even though liver damage can affect animal health and welfare.

Objectives: Optimization of a reported enzyme-linked immunosorbent assay (ELISA) for the antibody-detection in horses with equine fasciolosis based on recombinant parasite cathepsin L proteases with bioengineered diagnostic epitope mutation. Study design: Descriptive, cross-sectional, retrospective cohort, interrupted time series study.

Methods: Epitopes from different homologues of *F. hepatica* cathepsin L proteases were modified, and canonical and mutated versions were recombinantly produced. Best performing candidate was evaluated with 175 serum samples from slaughtered horses in Ireland. Samples from 7 horses with suspected liver fluke infection were followed-up for 1 year after treatment with triclabendazole. A cohort of 368 samples from Swiss horses were tested for prevalence data.

Results: A test based on an epitope-mutated antigen showed a sensitivity and specificity of 65% and 97.4%, respectively. Follow-up of horses with a suspected *F. hepatica* infection showed, in addition to improved biochemical liver values, a drop in antibody titers, which fall below the test threshold after approx. 6 to 9 months. This group allowed to define thresholds to distinguish between ambiguous/borderline results and clear positives. The prevalence of equine fasciolosis in Swiss horses was between 3.5 and 5.7%, depending on the applied diagnostic threshold. Main limitations: This study describes the development and evaluation of a diagnostic test with limited available well-characterized positive samples. The correlation of clinical data to parasite infestation has to be interpreted with care, as they are biased by the criteria for inclusion.

Conclusions: The results indicate that *F. hepatica* might be a neglectable infection in Swiss horses. Reliable diagnostic tests are useful to detect cases, especially in horses with liver disease of unknown origin/reason, or which are co-grazed with ruminants in endemic regions.

PS246.III

Hemoparasite circulation in common kestrel (*Falco tinnunculus*) in Apulia, South of Italy

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Infections with hemoparasites in birds are mainly caused by parasites belonging to the genera *Plasmodium*, *Haemoproteus*, and *Leucocytozoon*. These parasites often lead to asymptomatic infections but can sometimes cause diseases that can be fatal. This study tested 33 common kestrels (*Falco tinnunculus*) hosted at the Apulian Wildlife Rescue Center (Italy) from 2021 to 2023 for hemoparasites. The data collected allowed for some evaluations of the characteristics of the infection, depending on the physiological status, age, and especially the cause of admission.

Blood or liver samples of spontaneously dead kestrels were used for the investigations, carried out by nested PCR, according to Hellgren et al. (2004) (1). The nucleotide sequencing of the gathered amplicons was performed using the Sanger method. The assembled sequences have been compared by BLAST with those in GenBank (Sayers et al., 2021) (2) to identify the infective parasite species.

The positivity rate assessed in the sampled birds was 58%; specifically, 55% of the common kestrels were positive for *Haemoproteus* spp., and 3% for *Parahaemoproteus* spp., while no birds showed positivity for *Leucocytozoon* spp. and *Plasmodium* spp. The geographical distribution of positive and negative kestrels is shown in Figure 1.

The study confirms the presence of *Haemoproteus* sp. and *Paraemoproteus* spp. in common kestrels from the Apulia region. Seasonality seems to play a significant role, with higher parasite prevalence in spring and summer, potentially linked to breeding cycles and vector activity. Stress and captivity may also exacerbate parasitemia, increasing health risks. The role of the recovery center as a reservoir for hemoparasites highlights the need for targeted vector management and further research. (This study was partially funded by grants of the Minister of Health, Ricerca Corrente IZSPB 01/2021)

Figure 1. Spatial distribution of the investigated common kestrels. Negative birds are in blue, birds positive to Haemoproteus spp. are in red, and birds positive to Parahaemoproteus spp. are in yellow.

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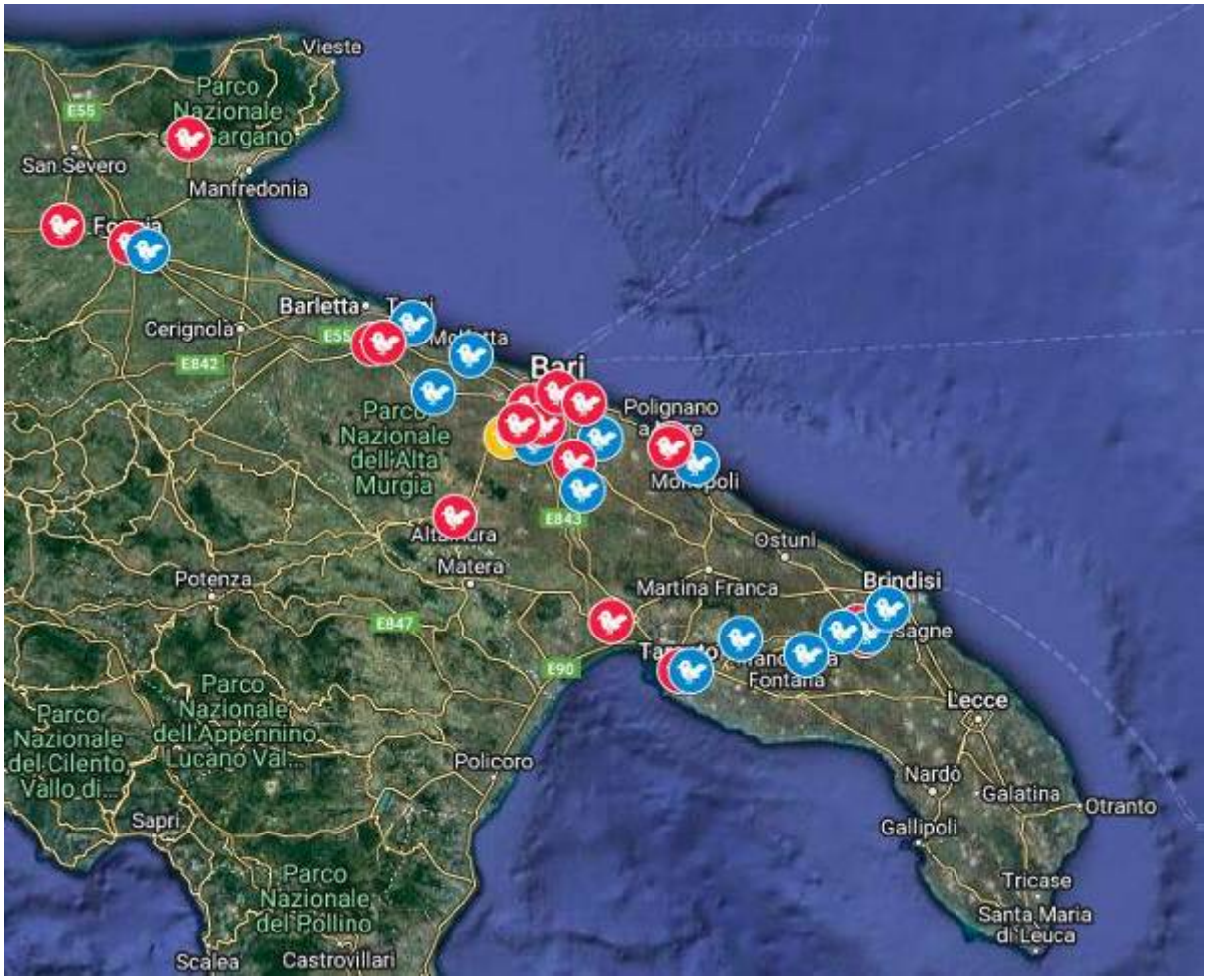
Fig. 1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Potenza
Martina Franca
Brindisi
Lecce
Parco Nazionale del Cilento Vallo di...
Parco Nazionale dell'Appennino Lucano Val...
Policoro
Nardò
Galatina
Otranto
Gallipoli
Sapri
Parco Nazionale del Pollino
Tricase
Santa Maria di Leuca
Scalea
Castrovillari
E55
E842
E843
E847
E90

PS249.III

Continuous endosomes form functional subdomains and orchestrate rapid membrane trafficking in *Trypanosoma brucei*

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Trypanosoma brucei is an extracellular human and livestock pathogen that follows an effective strategy to evade the host immune system. This strategy relies on stochastical changes in the expression of variant surface glycoproteins (VSGs) that cover the entire cell surface of the parasite. Remarkably, trypanosomes recycle one cell surface equivalent within just 12 min and internalize surface bound antibodies within 2 min. These fast-paced dynamics of membrane flow challenge the conventional notion of distinct compartments for early, late, and recycling endosomes.

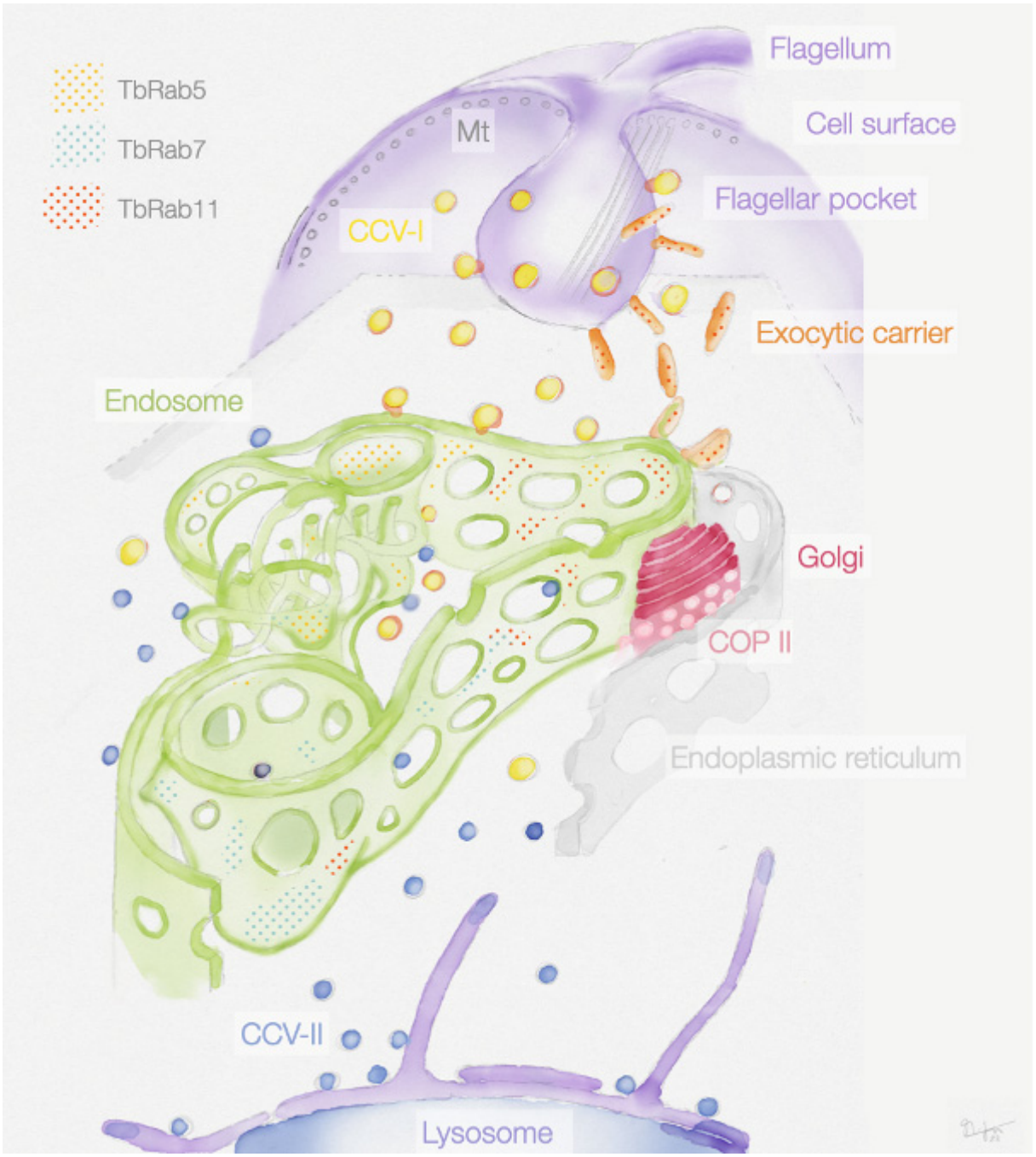
To understand the structural basis for this remarkably efficient membrane traffic, we employed state-of-the-art light and electron microscopy techniques to explore the three-dimensional architecture of the endosomal system. The results demonstrate that the endosomal system in trypanosomes consists of an intricately interconnected structure. Instead of being compartmentalized, it constitutes a continuous membrane system, with specific endosomal functions organized into membrane subdomains enriched with classical markers for early, late, and recycling endosomes. These subdomains can partially overlap or be interspersed with regions lacking endosomal markers. This continuous endosome permits rapid membrane flow through facilitated diffusion that isn't hindered by numerous fission and fusion events.

Considering the significant forces generated by flagellar movements and continuous swimming, it is reasonable to hypothesize that the endosomal system has a supportive framework. Thus, we analysed the actomyosin system of *T. brucei* using various imaging techniques. The results support the idea that the actomyosin system plays a pivotal role in post-endocytic membrane trafficking and may be essential for maintaining the intricate endosomal membrane morphology in *T. brucei*.

After the characterization of the endosomal apparatus, our obvious next steps include the analysis of the ultrastructural morphology of the lysosome as well as the functional description of the retrograde cargo transport.

Figure 1. Schematic representation of the endosomal system in *T. brucei*. The endosome is marked by the presence of small GTPases of the Rab family: Rab5A (yellow dots), Rab7 (cyan dots), and Rab11 (red dots). Class I clathrin-coated vesicles (CCV-I), class II clathrin-coated vesicles (CCV-II), vesicles coated with coat protein (COP) II.

Fig. 1


TbRab5
TbRab7
TbRab11
Flagellum
Mt
Cell surface
Flagellar pocket
CCV-I
Exocytic carrier
Endosome
Golgi
COP II
Endoplasmic reticulum
CCV-II
Lysosome

PS252.III

Three-dimensional cellular architecture of the sigmoid filament and a new parabasal filament in *Trichomonas vaginalis*

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Trichomonas vaginalis is a parasite protozoan that causes human trichomoniasis, a sexually transmitted infection (STI) that affects more than 156 million people worldwide. *T. vaginalis* contains an uncommon and complex cytoskeleton constituting the mastigont system, formed by several fibers and proteinaceous structures associated with basal bodies. Among these structures is the pelta-axostylar complex made of microtubules and striated filaments such as the costa and the parabasal filaments. In addition, some structures are poorly known and studied, such as the sigmoid filament and the X-filament. Here, we have isolated the *Trichomonas vaginalis* cytoskeleton and used UHR-SEM (ultra-high resolution scanning electron microscopy), tomography, immunofluorescence, immunolabeling, and backscattered electrons on SEM, negative staining to model the three-dimensional architecture and possible function of the sigmoid. In addition, although the structural organization of trichomonad cytoskeletons has been analyzed using several techniques, observation using a new generation of scanning electron microscopes with a resolution of below 1 nm has allowed more detailed visualization of the three-dimensional organization of the mastigont system. In this study, we have investigated the cytoskeleton of *T. vaginalis* using a diverse range of scanning probe microscopy techniques, which were complemented by electron tomography and Fast-Fourier methods. This multi-modal approach has allowed us to characterize an unknown parabasal filament and reveal the ultrastructure of other striated fibers that have not been published before. Here, we show the differences in origin, striation pattern, size, localization, and additional details of the PBs, thus improving the knowledge of the cell biology of this parasite. In conclusion, our study contributes to a better understanding of the cell biology of *T. vaginalis,* which will permit new therapies.

Figure 1. General view of *T. vaginalis* in high-resolution SEM (a) and transmission electron microscopy (TEM) of the whole cell (b) and the isolated cytoskeleton (c). AF, anterior flagella; RF, recurrent flagellum; Ax, axostyle; H, hydrogenosomes; N, nucleus; G, Golgi; BB and asteriscs, basal bodies of the flagella; C, costa; R, basal body of the recurrent flagellum; P, pelta; S, sigmoidal filament; and Parabasal filaments (Pf) numbered from 1 to 4.

Figure 2. Ultra-high-resolution SEM of the isolated cytoskeleton of *T. vaginalis* in low (a) and higher magnification (b). The four striated fibers are colored orange (PF1), green (PF2), blue (PF3), and purple (PF4). Notice a remnant of a probable Golgi. PF1-PF3 run on the cis-Golgi face, whereas PF2-PF4 run on the trans-Golgi's face. The costa (C) is colored in yellow. Notice that PF1 is the longest fiber accompanying the axostyle (Ax). Flagella (F), Golgi (G), Pelta (P).

Fig. 1

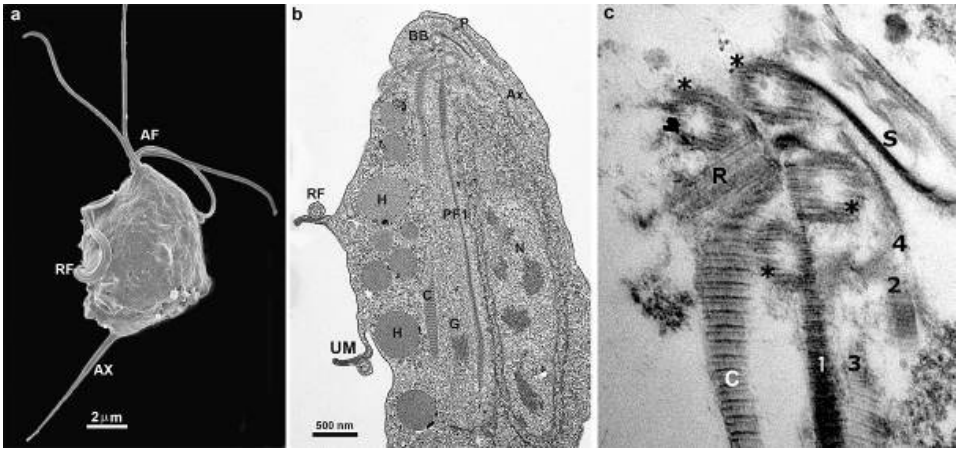
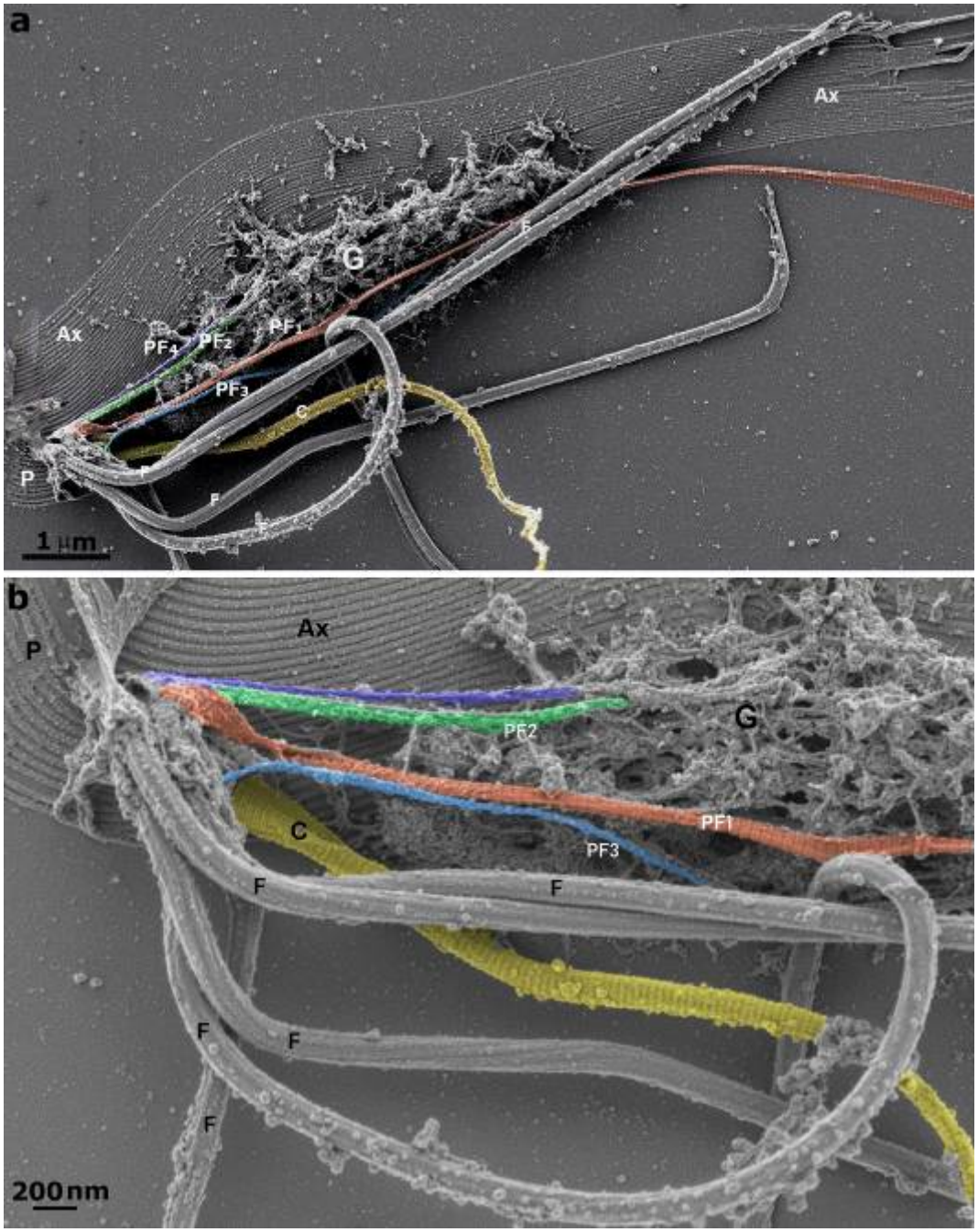


Fig. 2


a
Ax
G
F
Ax
PF4
PF2
PF1
PF3
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1 µm
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PF1
C
PF3
F
F
F
F
F
200nm

PS255.III

Modelling host: Parasitic nematode interactions with ovine 'mini-gut' organoids

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Teladorsagia circumcincta is one of the most predominant gastrointestinal (GI) nematodes of sheep in temperate regions. Reported resistance to anthelmintics is increasing and therefore research into new control strategies (e.g. vaccination) is vital. One area of interest for identification of potential vaccine candidates are extracellular vesicles (EVs). Extracellular vesicles are lipid membrane-enclosed packages which contain effector proteins and immune modulators and play important roles in establishing helminth infections. However, there are challenges in studying these interactions between the host and GI nematodes due to the lack of accessibility of the infection site and the need to rely on infection models which have ethical implications. Recently, ovine gastrointestinal organoids have been developed which allow host-parasitic interactions to be studied in a physiologically-relevant and host-specific *in vitro* cell culture system. The overall aim of the project is to use ovine abomasum organoids to identify and characterise active components of *T. circumcincta* EVs at different infective life stages. The separation and characterisation of EVs from adult and larval stage 4 excretory/secretory products has been achieved. Protein characterisation of these EVs has revealed a consistency with proteins found in other nematodes (e.g. M13 metallopeptidases, actin) which further supports the presence of EVs. To progress understanding of these proteins on the host, the uptake of EVs by organoids must be confirmed. Further investigations are underway to look at the interactions and potential implications of these EVs at the host epithelial cell interface using species- and tissue-specific ovine abomasal organoids.

PS258.III

Characterisation of clusters of parasitism-associated genes in diverse parasitic nematodes

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In recent years, there has been a significant increase in the availability of highly contiguous genome assemblies for parasitic nematodes. This increase in data enables a deeper exploration of genome architecture, particularly concerning gene localization, which was previously difficult due to genome assembly fragmentation. Here, we have investigated gene arrangement related to colocalised clusters of genes associated with parasitism. Analysis in a diverse range of evolutionarily distinct parasitic nematodes, covering 22 species across three different clades of Nematoda has uncovered the widespread presence of clusters of parasitism-associated genes, similar to those previously identified in *Strongyloides* species. Gene clusters size varied, containing between 4 and 42 genes covering between 2 - 200 Kb of the genome. The clusters exhibit enrichment for several gene families including genes that encode peptidases and CAP domain proteins. The widespread presence of these clusters suggests potential evolutionary advantages for parasites colocalising parasitism-associated genes. Interestingly, 56% of clusters identified across species include a combination of between 2 and 15 gene families, indicating a complex evolutionary history, rather than just tandem duplications. In conclusion, the co-localisation of parasitism-associated genes is prevalent not just restricted to *Strongyloides* species. Though variance has been found in the number and size of clusters between species. Characterization of these gene clusters provides a foundation for further research into their benefits for parasitic nematodes during infection and the regulatory mechanisms of parasitic traits.

PS261.III

VSGseq2: An updated pipeline for analysis of *Trypanosoma brucei* VSGseq data

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Trypanosoma brucei displays an extensive capacity for immune evasion, through the expression of distinct variant surface glycoprotein (VSG) coats. VSG-encoding genes account for ~10% of the T. brucei genome, and this capacity for antigenic diversity can be expanded by the creation of mosaic VSG genes formed from the extensive VSG repertoire. Each parasite expresses just one VSG at a time, but many VSGs can be expressed simultaneously across the parasite population. VSGSeq is an amplicon sequencing approach to survey the population-wide diversity of expressed $VSGs_1$. Here, we present "vsgseq2", an updated bioinformatics pipeline that improves the reproducibility, scalability and efficiency of VSGseq analysis.

To evaluate vsgseq2, we applied the analysis pipeline to the comparison of chronic murine and bovine infections with T. brucei. In the field, Trypanosoma brucei infections are sustained long-term in livestock hosts, whilst most experimental VSG surface antigen diversity studies have focused on mouse infections in the first waves of parasitaemia.

Despite their much lower parasitaemia, we found that parasites from cattle infections expressed a more diverse range of VSGs later in infections than those from mice. Moreover, in the chronic phase, we rarely found "dominant" VSGs in either mice or cows, with the most highly expressed VSG comprising less than 1% of overall VSG expression. Interestingly, the expression of similar VSGs with modified C-terminal domains was a feature of both mouse and cow infections. To assess the impact of perturbed recombination mechanisms on VSG expression, we analysed mouse infections using RAD51 and BRCA2 null mutants of pleomorphic T. brucei AnTa1.1. Both mutants could establish chronic infections but the diversity of expressed VSG was lower than with wild-type parasites. Analysis is underway relating the changes observed in VSG expression to changes in the genome using long-read gDNA sequencing of clones derived across infections. Overall, these analyses extend our understanding of VSG expression diversity from short-term rodent infections to a chronic bovine setting relevant to infections in the field

Mugnier MR, Cross GAM, Papavasiliou FN. The in vivo dynamics of antigenic variation in Trypanosoma brucei. Science. (2015);347(6229):1470-3. doi: 10.1126/science.aaa4502. PubMed PMID: WOS:00035213640003

PS264.III

Quantifying two-dimensional colony growth patterns of trypanosoma social motility

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In vitro, colonies of the flagellated parasite Trypanosoma brucei form characteristic flower like patterns [1]. The underlying cause of this complex emergent behavior called social motility remains a topic of intense debate in the scientific community. Numerous hypotheses involving physical and chemical mechanisms at the cellular and environmental levels have been proposed.

In order to study these mechanisms, it is crucial to develop quantitative methods to assess and measure social motility patterns beyond qualitative image comparisons. Here, we present two scale free metrics designed to quantify the growth of 2D colony patterns. While initially developed for yeast colonies, we have adapted and modified these metrics for the Trypanosoma system [2].

The first metric analyses the radial density distribution of a colony, producing a "frequency spectrum". The second metric calculates a scaled count of pair angles between colony points. Together with additional statistical analysis tools like Fourier analysis, these metrics enable the mapping of complex 2D pattern development to simplified 1D or scalar quantities.

Since these metrics are scale-free, they can be applied to any type of 2D image data, regardless of resolution or origin, pertaining to colony patterns. Our intention is to utilize these metrics to validate mechanistic computational models of social motility against real image data.

[1] T. Krüger et al., "Single-cell motile behaviour of Trypanosoma brucei in thin-layered fluid collectives", The European Physical Journal E 44 (2021)

[2] A. Kuhn et al., "Quantification of *Trypanosoma brucei* social motility indicates different colony growth phases", Roy Soc Interface (2024)

PS267.III

CRISPR/CAS-mediated integration of a reporter-gene into a predicted genomic safe harbor in Schistosoma mansoni: A new approach for functional genomics in trematodes

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[2]George Washington University, Department of Microbiology, Immunology and Tropical Medicine, Washington DC, United States
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Schistosoma mansoni, the cause of schistosomiasis, is a trematode characterized by a complex life cycle. For investigating the function of a gene of interest (GOI) in model organisms, knock-out (KO) and knock-in (KI) models are common. However, in the post-genomic era of schistosome research, no established protocol exists for generating KO strains or for the stable transformation of this and other platyhelminth parasites. Until now, RNA interference (RNAi) has been used as the most suitable method for functional gene characterization. However, RNAi efficiency varies, and it can lead to ectopic effects. Over the past decade, CRISPR/Cas-based genome editing has proven to be a powerful tool for gene characterization in various non-parasitic species. To make this technique also accessible for trematode research, we aimed to establish a protocol for editing a genomic safe harbor site (GSH) of *S. mansoni*. GSHs represent distinct sites in the genome that allow the integration of new genetic material without negatively affecting genome integrity or gene expression. Therefore, GSHs should allow constitutive reporter-gene expression throughout the life cycle. By bioinformatics, we predicted four potential GSHs in the genome of *S. mansoni* and selected one, GSH1, for pilot editing experiments (1). For editing GSH1, a 5'C6-PEG10-modified construct encoding an eGFP reporter-gene placed under the control of a strong and constitutively active promoter of *S. mansoni* was used as donor repair template. CRISPR/Cas-guided microhomology arms-mediated integration of the transgene was performed by electroporation of eggs. For comparison, we generated ribonucleoprotein complexes (RNPs) of both Cas9 and Cas12a and found significant differences in editing efficiencies between both enzymes. By PCR and sequencing, we next confirmed the integration of the reporter-gene into the GSH1 using both RNPs. The detection of eGFP signals in eggs and developing miracidia confirmed the feasibility of GSH1 for KI transgenesis approaches. Finally, we successfully reintroduced transgenic larvae into the life cycle and obtained transgenic cercariae as well as green-fluorescing worms. This is the first demonstration of germline transgenesis in schistosomes with reporter-gene activity at GSH1.

Our results provide proof of concept for a novel genome-editing approach in *S. mansoni* by demonstrating that both Cas9 and Cas12a nucleases are powerful tools for KI experiments. This approach fills an existing technical gap in *S. mansoni* research and opens new perspectives for functional genomics in schistosomes. Furthermore, the presented approach may serve as a template for similar research activities in trematodes and further helminths.

(1) Ittiprasert W, Moescheid MF, *et al*, Grevelding CG, Grunau C, Brindley PJ. Targeted insertion and reporter transgene activity at a gene safe harbor of the human blood fluke, *Schistosoma mansoni*. *Cell Rep Methods*. 2023 Jul 24;3(7):100535.

PS270.III

Parasite airlines: Mapping the distribution and transmission of avian blood parasites in migratory birds

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Bird migration poses significant costs, such as physiological challenges and the exposure to different diseases, transmission vectors and pathogens. While some parasites are widely distributed, transmission is not inevitably assured and depends on the presence of competent arthropod vectors and parasite compatibility with native bird species. Therefore, distinguishing between parasite distribution and transmission areas is crucial for monitoring and assessing potential infection risks to local bird species. In this study, blood samples from 456 reed-living birds of the genera *Acrocephalus, Locustella*, and *Emberiza* collected in the nature reserve "Die Reit" in Hamburg (Germany) were screened, targeting haemosporidian parasites. Transmission areas were established based on information provided by resident and juvenile birds, as well as on findings in competent vectors. Our results stated that long-distance migratory birds of the genus *Acrocephalus* showed a higher prevalence and diversity of haemosporidians compared to partial migratory birds like *Emberiza schoeniclus*, suggesting greater tolerance. Notably, an age-dependent difference in parasite prevalence was observed in *Acrocephalus spp*., but not in *E. schoeniclus*. Haemosporidian parasites were not detected in the long-distance migrants *Locustella naevia* and *L. luscinioides*, indicating a possible evolutionary adaptation to resist these parasites. Transmission areas were proposed for nine haemosporidian lineages, showing three distinct types: either with limited transmission in Europe or Africa, or active transmission in both regions. In conclusion, future research should focus on the investigation of various migratory bird species, considering factors like bird age to differentiate between distribution and transmission areas of parasites and the consequences of migration to native populations.

Fig. 1

PS273.III

Correlation of a hematological score with antibody titers and C-reactive protein in dogs with *Babesia canis* infections in Germany (2018-2024)

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[2]Justus Liebig University Giessen, Department of Veterinary Clinical Sciences, Clinical Pathology, and Clinical Pathophysiology, Giessen, Germany
[3]University of Veterinary Medicine Hannover Foundation (TiHo), Institute for Parasitology, Centre for Infection Medicine, Hannover, Germany
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Objectives: Infections with *Babesia canis* are of rising importance in Germany. The aim of our retrospective study was to analyze hematological parameters in dogs infected with *B. canis* with known status regarding stays abroad.

Materials & Methods: Dogs tested PCR-positive for *B. canis* and negative for *Anaplasma phagocytophilum* from January 2018 to November 2024 were included, if data on hematocrit, leukocytes, and platelets were available. Hematological scoring (HS) was performed by addition of points for mild (+1), moderate (+2), and marked (+3) anemia, thrombocytopenia, and leukopenia as well as pancytopenia (+3) and leukocytosis (+1). C-reactive protein (CRP) and *Babesia* ELISA titers were included, if available. $P \leq 0.05$ was considered significant in Mann-Whitney-U-testing. Spearman"s rank correlation was used.

Results: 167 dogs were included (stays abroad n=72 [43.1%], no stays abroad n=95 [56.9%]). A higher HS was seen in dogs without (median=7) compared to dogs with stays abroad (median=4) ($P<0.001$). Higher CRP was seen in dogs without (n=50, median=119.0 mg/l) compared to dogs with stays abroad (n=30, median=50.4 mg/l) ($P<0.001$), and higher antibody titers in dogs with (n=39, positive n=24) compared to dogs without stays abroad (n=49, positive n=7) ($P<0.001$). A negative correlation of the CRP was demonstrated with titers (?=-0.463, $P<0.001$) and a positive correlation with HS (?=0.474, $P<0.001$). The titers correlated negatively with HS (?=-0.579, $P<0.001$).

Conclusions: Autochthonous infections with *B. canis* in Germany are seen in significant numbers. Dogs without stays abroad had lower antibody titers and more severe hematological abnormalities. This may be explained by infection in an immunologically naïve group of dogs in Germany.

PS276.III

Optimizing fluralaner-based management strategies for the poultry red mite *Dermanyssus gallinae* (acari : dermanyssidae): A cohort-based population model

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[2]École Nationale de Médecine Vétérinaire, Institution de la Recherche et de l'Enseignement Supérieur Agricoles, Université de la Manouba, Sidi Thabet, 2020, Tunis, Tunisia
[3]École Nationale d'Ingénieurs de Tunis, Université Tunis El-Manar, Tunis, 1068, Tunisia, Tunis, Tunisia

Introduction: This project addresses the challenge of controlling Dermanyssus gallinae infestations in poultry farms, focusing on mitigating their significant economic impact on productivity and their health implications for both poultry and farm workers. It is centered on optimizing the use of Exzolt, a Fluralaner-based systemic acaricide, to develop cost-effective and sustainable strategies balancing treatment frequency and dosage while ensuring effective parasite control.

Objectives: The ultimate aim is to optimize treatment strategies in terms of dose and administration schedule to minimize the impact of these parasites on poultry farming costs.

Materials & methods: The cost optimization problem related to dose and scheduling is reformulated in terms of dose and the population threshold tolerated by a laying hen, where the carrying capacity is a characteristic of the building. A cohort-based model is developed that couples the pharmacokinetics of Exzolt in the plasma of laying hens with the dynamics of the mites. The model also integrates the impact of temperature choice. This enables the simulation of various treatment administration strategies while quantifying costs. Analyzing the effect of each parameter, dose and threshold, enabled us to solve the optimization problem based on a case study from the literature.

Results: The study reveals that administering a reduced dose of Exzolt at high frequencies, in conjunction with a customized infestation tolerance threshold for each farm, provides a cost-effective solution for mite control. Specifically, this approach offers significant savings, with up to a 14.15% reduction in treatment costs in a case study involving moderate infestation levels. The findings emphasize that, in many cases, maintaining mite populations below acceptable limits is more economical than complete eradication.

Conclusion: This research contributes to advancing parasite epidemiology and control strategies in poultry farming, with a focus on optimizing management practices to enhance both animal health and farm profitability.

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E.C. Tucci, A.P. Prado, and R.P. Araújo. Development of dermanyssus gallinae (acari : Dermanyssidae) at different temperatures. Veterinary Parasitology, 155(1) :127–132, 2008.

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Fig. 1

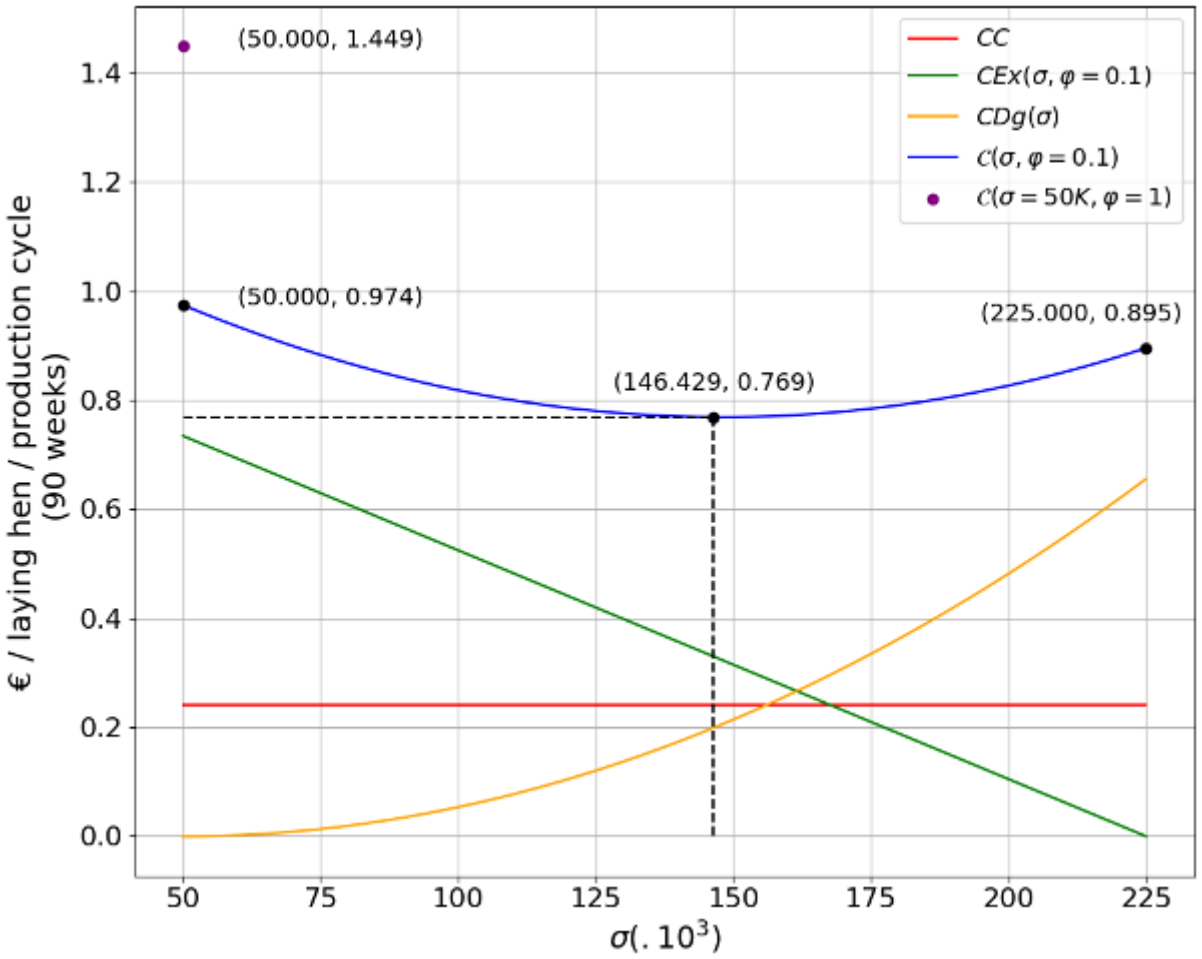
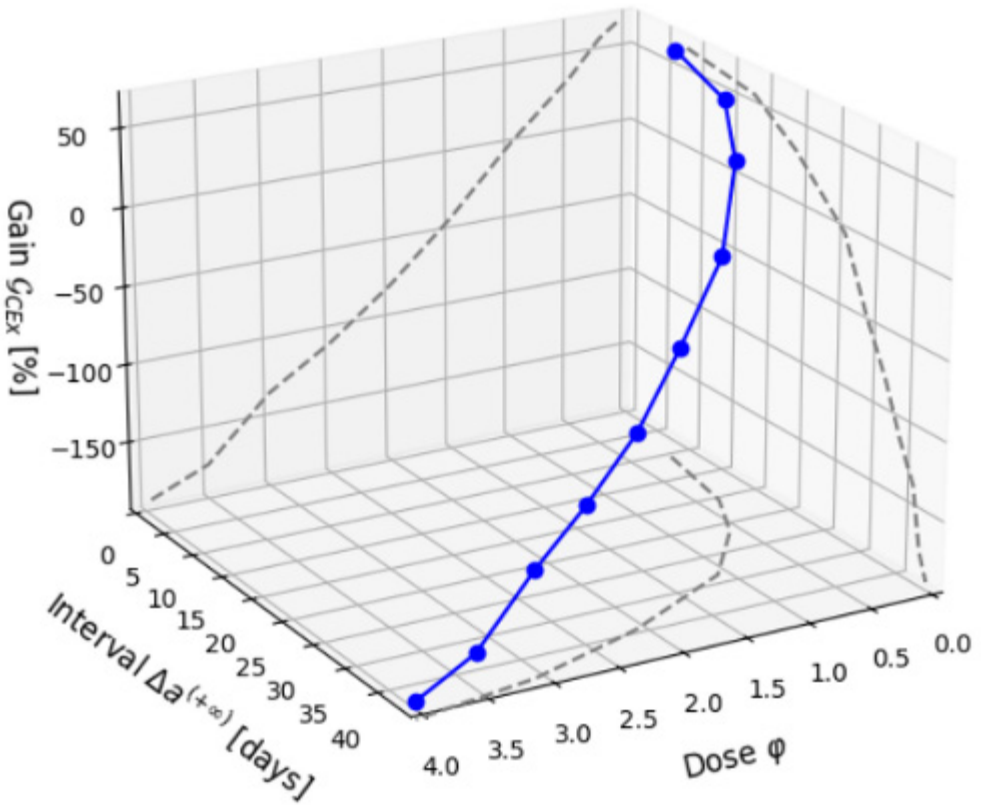


Fig. 2


Gain ζ_{CEx} [%]
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PS279.III

In silico vaccine discovery against protozoan parasites and its application to _Toxoplasma gondii_

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Introduction: Vaccine discovery against eukaryotic parasites is not trivial and very few exist. One promising approach is reverse vaccinology, designed to identify vaccine candidates given thousands of protein sequences encoded by a genome. Reverse vaccinology, nonetheless, is simply an overarching concept with no standardized guidebook on how to implement the approach. Previously, we produced the Vacceed bioinformatics pipeline for identification of vaccine candidates. We improved upon machine learning as the final decision-making process to identify parasite proteins that induce a protective response in an animal model. Subsequently, we combined Vacceed with metrics on epitope types for additional *in silico* discovery. This approach integrates a parasite"s biology, a host's immune system defenses, and importantly, bioinformatics programs needed to predict vaccine candidates.

Objective: Developability of proteins as vaccines is often affected by a protein"s solubility and insoluble proteins and those prone to aggregation typically may be discarded from the development pipeline. We extend the existing Vacceed/*in silico* vaccine discovery workflow to include the physicochemical properties of proteins. In order to provide confidence in the ranking of vaccine candidates we describe the use of multicriteria decision making (MCDM), aggregate ranking and conformal prediction of prediction intervals.

Materials & methods: Physicochemical properties of proteins were predicted and incorporated into the existing *in-silico* workflow. All *T. gondii* proteins were re-analysed by the *in-silico* workflow and ranked by multicriteria decision making and aggregate ranking to generate a consensus rank. Levels of uncertainty in the consensus rankings was assessed by conformal interval prediction in association with a machine learning model.

Results: Several of the top ranked proteins were novel, uncharacterized membrane transporters, which by their very nature contain membrane associated domains. Such an observation would normally raise questions concerning the solubility of these proteins, despite their characteristics of antigenicity and exposure to the immune system indicating they would be viable candidates. Including metrics for solubility into the workflow has therefore provided a method to balance these contrasting considerations in the decision-making process. MCDM methods automated decision making while prediction intervals varied significantly across the 8000+ proteins of *T. gondii*. Highly ranked proteins (eg the top 100) typically generated low prediction intervals, indicating higher levels of confidence in their ranks.

Conclusions: Reverse vaccinology is maturing into a useable state of the art technology, spanning bioinformatics, machine learning, immuno-informatics and developability criteria. The inclusion of solubility characteristics assists in the workflow decision making, while conformal prediction helps in understanding ranking confidence.

PS282.III

Dissecting the flagellar pocket collar biogenesis in *Trypanosoma brucei*: Discovery of novel structures and mechanistic interplay

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[2]Université de Bordeaux, Laboratoire de Microbiologie Fondamentale et Pathogénicité (MFP), Bordeaux, France
[3]Bordeaux INP, Microbiologie Fondamentale et Pathogénicité, Bordeaux, France
[4]Medical University of Vienna, Max Perutz Labs, Vienna Biocenter, Vienna, Austria

Trypanosomes are flagellated protist parasites that are cosmopolitan in distribution on the planet. Their distribution varies based on human, animal, and vector biology, climate, and environmental factors. They are responsible for numerous diseases, including human and animal trypanosomiases. Trypanosomes share cytoskeleton structures that are required for correct cell function and survival. One important structure is an invagination of the plasma membrane called the flagellar pocket (FP), which is formed by a complex organization of cytoskeletal proteins orientated around the flagellar pocket collar (FPC). In this study, we investigate flagellar FP and FPC biogenesis in *Trypanosoma brucei*, focusing on the formation of a specialized microtubule quartet (MtQ) and proteins that are associated with the FPC. By employing Ultrastructure Expansion microscopy, we delineate the emergence of the new MtQ (nMtQ) alongside flagellar growth and refine the understanding of its growth. Additionally, the study elucidates the localization patterns of key proteins BILBO1 and MORN1 during FPC (flagellar pocket collar) and HC (hook complex) biogenesis. Importantly, our study unveils the transient nature of a new structure, named the FPC-interconnecting fibre, shedding light on its potential role in FPC assembly. Furthermore, the study explores the interplay between BILBO1 and neighbouring proteins, providing insights into their collective contribution to FPC biogenesis and paving the way for further investigations into the functional significance of these proteins.

PS285.III

Characterization of the secreted *Theileria annulata* effector protein Ta9

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In tropical theileriosis of cattle, the major driver of pathology is the transformation of host macrophages by the intracellular apicomplexan parasite *Theileria annulata*. In *Theileria*-transformed leukocytes several oncogene-associated signaling pathways, including Activator Protein 1 (AP-1) and NF-kB, are constitutively activated in a parasite-dependent manner. The proto-oncogene haematopoetic cell kinase (Hck) of the Src family of non-receptor tyrosine kinases is constitutively active in *T. parva*-transformed B cells and contributes to AP-1-driven transcription (1). Ta9 is a *T. annulata* secreted protein that we have shown capable of stimulating AP-1-driven transcription (2) raising the possibility that Ta9 might do so by augmenting Hck signaling. Using two independent anti-Hck antibodies, we now demonstrate co-localization of Ta9 with the active form of Hck in/on cytoplasmic vesicle-like structures. These vesicle-like structures were confirmed to be mitochondria. Expression of full-length GFP-tagged Ta9 in bovine macrophages or mouse fibroblasts (3T3 cells) results in the appearance of numerous spike-like membrane protrusions ("hairy phenotype") and dorsal ruffles. In addition, Ta9 upregulates Hck transcripts (RNA-seq and qRT-PCR) and protein levels (IFA) in bovine macrophages. These results are consistent with Ta9 potentially binding to and activating Hck and treatment of *T. annulata*-infected macrophages with a selective Hck inhibitor (A419219) negatively impacted on the parasite-dependent transformed phenotype, as estimated by cell proliferation and soft agar colony formation assays. The mitochondrial localization of Hck in macrophages is novel and therefore requires further exploration.

Keywords: *Theileria annulata*, leukocyte transformation, Hck kinase signaling, proto-oncogene

1. *Baumgartner et al. 2003,* Blood.
2. *Unlu et al. 2018,* PLOS ONE.

Fig. 1

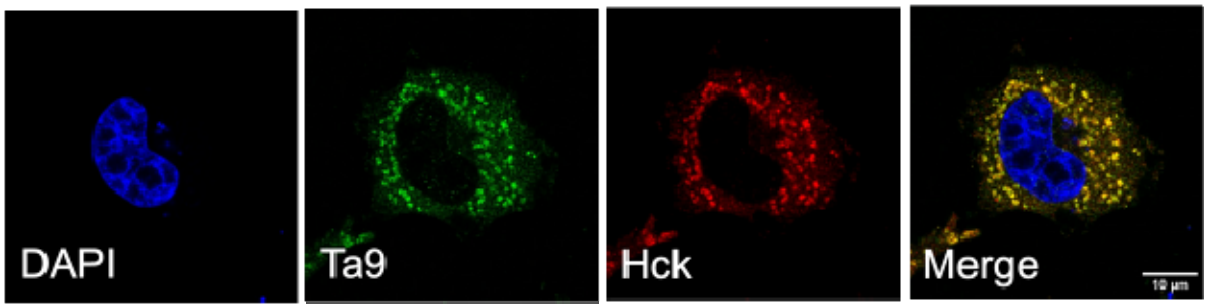
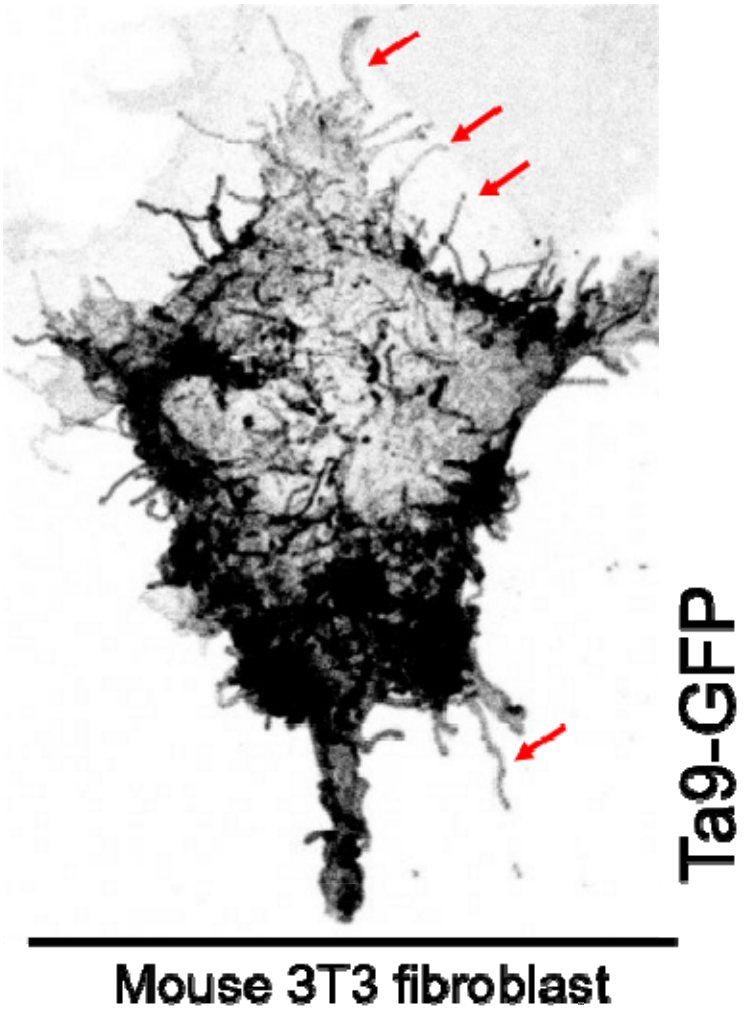


Fig. 2



PS288.III

Ocurrence of *Toxoplasma gondii* antibodies in an isolated population of free-ranging dolphin (*Inia geoffrensis*) from Amazon, Brazil

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Toxoplasma gondii is a protozoan parasite that infects a large spectrum of warm-blooded animals, including aquatic mammals. Its presence in these animals indicate contamination of the aquatic environment by oocysts of this parasite. Investigation of infectious diseases in wild dolphins is of primary importance to understand their current health status and to guide proper management plans for populations. The present study aimed to evaluate the occurrence of *T. gondii* antibodies in an isolated free-ranging population of *Inia geoffrensis* from the Brazilian Amazon. Blood samples were collected from 32 dolphins (28 males and 4 females) in 2019 and 2020 at the Balbina Hydroelectric Dam reservoir, State of Amazonas. Antibodies were detected, by the modified agglutination test (MAT ≥ 25), in 34.37%, 11 of the 32 tested samples, with titres of 25 in 10 individuals and 50 in one. This study suggests that Amazon River dolphins, inhabiting the Balbina reservoir, were infected by *T. gondii*, however, no evidence of clinical signs or mortalities by this coccidia were detected in the animals so far.

Notes

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ABSTRACTS

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