Molecular Helminthology 2023
Talk Abstracts

Cannabinoid receptor signaling through CBR1 regulates macrophage and eosinophil responses in lung helminth infection

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Helminth infections are a global concern, affecting one-third of the world's population, with children being particularly vulnerable to malnutrition and impaired growth. The complex interactions between the host and parasite pose a major challenge, with the host's T-helper type 2 immune response and the helminth's immune evasion strategies constantly at war. While this dynamic immune interplay between the host and helminth is recognized, whether non-immune mechanisms influence host-helminth interaction, or the overall pathological outcome of helminth infection is less clear. We investigated the endocannabinoid system as a previously unrecognized contributor to host-helminth interaction. The lipid signaling molecules endocannabinoids (e.g., 2- AG and anandamide, AEA) are the body's natural cannabis-like molecules that regulate neural behaviors such as addiction and feeding, but also dampen inflammatory responses. Previously, we showed that infection with the parasitic helminth *Nippostrongylus brasiliensis* increased endocannabinoids, associated with reduced lung inflammation. Here, we investigated functional roles of endocannabinoid receptor signaling on immune cells using cannabinoid receptor-deficient mice (CB1R-/- and CB2R-/-). While there were no significant differences in CB2R-/- mice, *N. brasiliensis*-infected CB1R-/- mice had increased lung eosinophilia and elevated CD206- expressing M2 macrophages compared to wild-type mice. In co-cultures, CB1R-/- eosinophils and macrophages had significantly increased binding to *N. brasiliensis* larvae compared to wild-type or CB2R-/- cells. To address direct effects of endocannabinoids on immune cells, bone marrow- derived macrophages were treated with endocannabinoids. AEA treatment downregulated M2 macrophage activation in wild-type and CB2R-/- macrophages, but not in CB1R-/- macrophages. Further, CB1R-/- macrophages expressed higher levels of the eosinophil chemoattractant CCL24/eotaxin-2, which was not downregulated by AEA. Overall, our findings indicate that CB1R receptor deficiency results in increased M2 macrophages, eosinophil chemoattractant CCL24, and eosinophils, suggesting a role for CB1R signaling in *N. brasiliensis* immune evasion strategies. These findings have important implications for host-parasite interactions and therapeutic strategies for helminth infection.

Fasciola hepatica microRNAs in infected sheep sera: a route to improved diagnostic tests?

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Infection of livestock with *Fasciola hepatica* (Fasciolosis) is a pervasive threat to farming worldwide, with annual costs associated with production losses above £2 billion. Production losses are exacerbated by the predominant use of coprology-based diagnostic tools, which can only detect patent fluke infections and are poorly supportive of targeted anthelmintic treatment approaches. New biomarkers and diagnostic tests are needed. Next-generation sequencing can accelerate discovery of new biomarkers, and the subsequent development of improved diagnostic tools. This is exemplified through the idea of a liquid biopsy in which analysis of the molecular makeup of a bodily fluid sample between healthy and sick patients allows the identification of diagnostic biomarkers. Such methods are effective, mature, and constantly improving in human medical research, and have seen progression towards extracellular nucleic acid biomarkers. We are applying these ideas to ovine fasciolosis, and here we present the first analysis of the small RNA and protein profiles of sera recovered from sheep carrying *Fasciola hepatica* infections. Time series analysis of these profiles at 0 days (pre-infection) 28 days (juvenile/acute infection) and 105 days (mature/chronic infection) shows statistically significantly differentially expressed host micro (mi)RNAs as well as the presence of parasite miRNAs in the serum of infected hosts. These miRNAs show distinct profiles at each of our time points. We are currently also analysing the proteomic profiles of these samples. These datasets will enhance our understanding of RNA-based host-parasite communication in liver fluke infections, and could aid development of new liquid-biopsy based diagnostics assays capable of outperforming existing tests.

The diphyllobothriidean cestode, *Schistocephalus solidus*, modulates host innate and adaptive immunity.

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Helminths have evolved multiple strategies to dampen host immunity, including modulation of both cellular and humoral immune responses. For instance, the threespine stickleback-specific cestode, *Schistocephalus solidus,* can suppress both phagocytic activity and reactive oxygen species production in head kidney leukocytes. To better understand the effect of *S. solidus*-mediated immune modification on other immune tissues and the mechanisms behind this suppression, we used both *in vivo* and *in vitro* approaches to assess the effect of infection on both stickleback innate and adaptive immunity. In response to *S. solidus* infection, we observed that splenic melanomacrophage centers (MMCs), clusters of pigmented phagocytes exclusive to the ectotherm taxa, decreased in size compared to uninfected controls. However, not all *S. solidus* populations could mediate MMC suppression. Furthermore, MMC reduction was not observed in all host populations, suggesting that there is population-level variability in host susceptibility to *S. solidus*-mediated immunosuppression. Quantitative Trait Loci (QTL) mapping of host MMC responsiveness to *S. solidus* infection identified several loci on chromosomes 3, 14, and X associated with MMC suppression. Several B cell-related candidate genes are present in these QTL regions, suggesting B lymphocytes may also be affected by *S. solidus* infection. Supporting this finding, we observed a decrease in not only the total number IgM-expressing cells in the spleens of infected fish, but also the number of IgM^{high} cells (putative plasma cells). CD4-expressing T cells were unchanged by infection. Considering the known role for excretory-secretory (ES) products in host immune modulation, we tested the role of *S. solidus* ES products in stickleback immunosuppression using *in vitro* co-culture models. Surprisingly, *S. solidus* ES products increased stickleback splenocyte viability compared to media only controls, highlighting the complexity of helminth-host crosstalk in this system. These results indicate that helminth-mediated immunosuppression in stickleback targets both innate and adaptive immune cells in the spleen and that this suppression can vary at both the fish and worm population-level.

Heterogeneous glycosylation of proteins from *Fasciola hepatica* invasive stage reveals higher complexity in parasite-host interactions

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Fasciola hepatica is a parasitic trematode that uses glycosylated excreted-secreted (ES) and surface molecules to interact with host cells and tissues, and to evade damage caused by cellular and immune responses during host invasion. Despite the unknown glycosylation state of many of the ~100 different proteins found in the ES of the immature invasive stage of *F. hepatica* (NEJs), several are extensively used as diagnostic and vaccine targets. To develop more effective strategies against fascioliasis, information on the glycosylation profile of individual NEJs proteins is critical. In this study, we used a combination of glycan, glycopeptide, and proteomic analyses, along with bioinformatics tools, to identify the glycosylation status of individual *F. hepatica* NEJs proteins. Our results identified 123 glycoproteins in NEJs extracts, 71 of which were in the ES. We mapped 367 glycopeptides and all the 1,696 *N*-glycan forms and 37 *O*-glycan forms to their respective protein and glycosites, revealing a high degree of heterogeneity in the glycosylation of *F. hepatica* NEJs proteins (i.e., in average, 14 different glycan forms can be used to modify each glycoprotein). Unique glycan motifs, such as PC and multi-PC terminals, and xylosylated *O*-glycan cores, were found in 25 distinct NEJs glycoproteins, including cathepsin peptidases B and L, which are well-known vaccine and diagnostic targets. Furthermore, many parasite proteins carried highly truncated *N*-glycans and structures with undefined linkages that could not be assigned (i.e., $HexNAc2Hex_4dHex_1$), and the roles of which in parasite infection are largely unknown. These structures modify glycoproteins that are excreted-secreted or predicted to be membrane-bound, suggesting that they play key roles in NEJs interactions that command host invasion. Our findings demonstrate that *F. hepatica* NEJs generate great protein variability via glycosylation, and highlight that the larvae extracts are far more complex than anticipated by proteomic analysis. This data provides a foundation for improving diagnostics and vaccine development to control fascioliasis.

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

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The worldwide prevalent liver fluke *Fasciola hepatica* causes fascioliasis, a neglected tropical disease and zoonosis. Comprehensive knowledge on the parasite's cell types and cell-specific gene expression repertoire is missing to date. Such insights would be an invaluable resource for research on drug target genes, developmentally and metabolically important genes. Technological advances in transcriptomic technologies allow the analysis of a great number of cells by single-cell RNA sequencing (scRNA-seq). Here, we provide the first whole-organism cell atlas of *F. hepatica* as a basis for expression analysis of genes on a single-cell level.

In order to achieve this cell atlas, we established a workflow including a novel dissociation protocol to obtain high-quality single-cell preparations from adult *F. hepatica* worms and the enrichment of viable cells via flow cytometry. Single-cell capture was achieved by using the Chromium kit from 10x Genomics and the data subsequently sequenced. Analysis was carried out using the software package Seurat in R. Predicted clusters were validated by RNA *in situ* hybridization.

Utilizing this workflow, we successfully identified more than 17 cell clusters representing distinct cell types, including gastrodermal cells expressing cathepsins, somatic stem cells (neoblasts) expressing nanos2, and neuronal cells. By functional analysis, we detected distinct enrichment of genes connected to important biological processes, like signal transduction in neuronal cells and proliferation in the neoblast cluster. Additionally, the resolution of this dataset allowed uncovering potential lineages spanning from the central stem-cell cluster into other clusters like the vitellarium, spermatogonia and the female germline by modeling the RNA velocity. Finally, we were also able to leverage this dataset with respect to druggable targets, as we identified several tissue-specifically expressed protein kinase genes, a gene family that is described as druggable in other helminths. Notably, small-molecule inhibition of a tegumentally expressed p21-activating kinase led to rapid death of the parasite.

We present the first transcriptome for the liver fluke *F. hepatica* on a single-cell resolution. This dataset can serve as treasure trove for the discovery and study of tissue-type and cell-type specific genes in this parasite.

Filarial DAF-12 sense Δ4-dafachronic acid in host serum to resume iL3 development during infection

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Nematode parasites enter their definitive host at the developmentally arrested infectious larval stage (iL3), and the ligand-dependent nuclear receptor DAF-12 contributes to trigger their development to adulthood. Here, we characterized DAF-12 from the filarial nematodes *Brugia malayi* and *Dirofilaria immitis* and compared them with DAF-12 from the non-filarial nematodes *Haemonchus contortus* and *Caenorhabditis elegans*. Interestingly, *Dim* and *Bma*DAF-12 exhibit high sequence identity and share a striking higher sensitivity than *Hco* and *Cel*DAF-12 to the natural ligands Δ4- and Δ7-dafachronic acids (DA). Moreover, sera from different mammalian species activated specifically *Dim* and *Bma*DAF-12 while the hormone- depleted sera failed to activate the filarial DAF-12. Accordingly, hormone-depleted serum delayed the commencement of development of *D. immitis iL3* in vitro. Consistent with these observations, we show that spiking mouse charcoal stripped-serum with Δ4-DA at the concentration measured in normal mouse serum restores its capacity to activate *Dim*DAF-12. This indicates that DA present in mammalian serum activates filarial DAF-12. Finally, analysis of publicly available RNA sequencing data from *B. malayi* suggested that, at the time of infection, iL3 parasites may not actively synthetize DAF-12 ligands. Altogether, our data suggest that filarial DAF-12 have evolved to specifically sense and survive in a host environment, which provides favorable conditions to quickly resume larval development. This work sheds new light on the regulation of filarial nematodes development while entering their definitive mammalian host and may open the route to novel therapies to treat filarial infections.

Co-culture with HepG2 spheroids spurs *in vitro* growth and development of the infective stages of the helminth pathogen *Fasciola hepatica*

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The helminth parasite *Fasciola hepatica* is a significant cause of animal and human morbidity worldwide. Part of the difficulty in developing new chemotherapeutics and vaccines for the control of Fasciolosis lies in our inability to culture and propagate juvenile worms *in vitro*. *F. hepatica* juvenile can be maintained short-term (days to weeks) in simple media but not sufficiently long to perform significant biological or host-parasite studies. HepG2 is a human non-tumorigenic liver cell line with high proliferation rates and epithelial-like morphology, growing in clumps in adherent cell culture rather than a typical monolayer. Here we show that high densities of HepG2 cells in adherent culture promote the survival and growth of the infective stage of the parasite, the newly excysted juvenile (NEJ). Parasite co-culture with spheroids, three-dimensional HepG2 cell aggregates, spur NEJ growth and development *in vitro*. Parasites grown in the presence of HepG2 spheroids were observed regularly interacting with the spheroids, sometimes invading the tissue, and moving between or tangentially to them indicating the importance of tactile stimuli, and there was evidence of parasites 'grazing' on the peripheral cells of the spheroids. We investigated parasite development using antibody probes against two major NEJ proteases, FhCL1 and FhCL3, and by scanning electron microscopy (SEM). The parasites exhibited not only a rapid increase in size (length and width) and temporal expression of proteases, but also extensive development of the gut caecum, musculature, and surface sensory system. The methodology developed here mimics *in vivo* parasite-host liver interactions, greatly improving our ability to investigate and understand *F. hepatica*-host biology with future prospects for the development of new parasite control methods, such as vaccines and anthelmintic drugs.

The first genome of African Paragonimus

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The lung fluke *Paragonimus spp.* is among the most injurious of the food-borne helminths, infecting ~20 million people worldwide, with an estimated 293 million people at risk in Asia, Africa and the Americas. Most human infections are in Asia, where the intermediate crustacean hosts are commonly consumed raw or undercooked, but *Paragonimus* is also widely distributed in Africa. Only *P. uterobilateralis* and *P. africanus* are documented to infect humans in Africa, but no genome sequence is currently published for either species, limiting the ability to use bioinformatics to improve diagnostics and develop new intervention strategies for lung flukes in Africa. We have previously published an analysis of four *Paragonimus* genomes from Asia and North America, and here we present the first genome for a *Paragonimus* species from Africa, *P. africanus*. Metacercariae from freshwater crabs were collected from areas endemic for human paragonimiasis in the southwest province of Cameroon and were used to infect rats and recover adult flukes for genome sequencing (Oxford Nanopore and Illumina technology). Flukes were determined to be most similar to *P. africanus* based on detailed morphological examination, with characteristic antler-shaped testis and medium-sized eggs. The 934 Mb nuclear genome had a BUSCO completeness score of 84.5% (plus 10.6% fragmented), and a 16.6 kb mitochondrial genome was also assembled. Mitochondrial and genomic comparisons of the 5 sequenced *Paragonimus* genomes revealed the most similarity to *P. kellicotti* from North America. Comparative orthologous protein family (OPF) analysis identified 52 rapidly expanding protein families in *P. africanus*, including serpin protease inhibitors (possibly immunomodulators involved in host invasion), and stomatin tegument proteins, which have been shown to induce immunity against blood flukes. Immunohistology revealed consistent detection and localization of two previously-identified *Paragonimus* antigens (myoglobin 1 and cysteine protease 6) which had the highest sensitivity and specificity for serological diagnostics of paragonimiasis. This study fills a molecular phylogenetic gap in *Paragonimus* species from Africa and builds on an ongoing comparative genomic analysis to better understand the global diversity of this important pathogen, providing opportunities for understanding immunity and improving serodiagnosis in Africa.

Thiabendazole responses are modified by natural mutations in a cytochrome P450 gene

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In recent decades, the development of widespread anthelmintic resistance has complicated the management of nematode parasites. Resistance to the benzimidazole (BZ) drug class is nearly ubiquitous in many species, with resistance being associated with mutations in beta-tubulin. However, mutations in beta-tubulin alone do not explain all of the variation in resistant populations, indicating other genes must play a role. Here, we use genetic mapping in a panel of wild strains of the model nematode *Caenorhabditis elegans* to identify a novel region on chromosome V associated with differences in response to thiabendazole (TBZ). We used recombinant lines of two phenotypically and genetically distinct strains to further narrow the region to a few candidate genes. One of the candidate genes (*cyp-35d1*) encodes a cytochrome P450 that had previously been implicated in TBZ metabolism. TBZ binds to the nuclear hormone receptor NHR-176, which induces the expression of *cyp-35d1*. CYP-35D1 in turn binds to and metabolizes TBZ. Recombinant lines used to identify candidate genes contained only one predicted high-impact variant in *cyp-35d1*, a lysine to glutamate substitution at position 267 (K267E). Using CRISPR-Cas9 genome editing, we created strains with each allele in two parental backgrounds, as well as single gene deletions in both genetic backgrounds. We found that deletion of *cyp-35d1* conferred high levels of TBZ susceptibility, and deletion of *nhr-176* conferred even greater levels of susceptibility. Importantly, we found that glutamate confers greater susceptibility than lysine at position 267 of *cyp-35d1*. We additionally demonstrated that the K267E substitution did not decrease the fitness of animals in control conditions. Overall, we have identified and characterized the first gene independent of beta-tubulin to play a role in response to BZs, highlighting an important new aspect in the study of BZ resistance.

$TRPM_{PZQ}$ as a target for new anthelmintics

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The drug praziquantel (PZQ) has long served as the key clinical agent for treating diseases caused by parasitic flatworms. Effective against the majority of human blood, liver and lung fluke infections, as well as diseases caused by cestodes, it is recognized as one of 100 essential medications by the World Health Organization. As a cheap, safe, broadly active and well scrutinized therapy, PZQ has served as the centerpiece of mass drug administration campaigns to decrease the intensity and prevalence of schistosome infections in countries where schistosomiasis is endemic.

A target for PZQ in parasitic flatworms has finally been identified – an ion channel from the transient receptor potential melastatin family, named $TRPM_{PZQ}$ [1]. Characterization of the properties of this new target is rapidly advancing, with new data evidencing $TRPM_{PZQ}$ as the mediator of PZQ action. $TRPM_{PZQ}$ is present in all flatworms that show sensitivity to PZQ, and the basic properties of this ion channel recapitulate PZQ action on parasitic flatworms. Electrophysiological recordings of $TRPM_{PZQ}$ reveal it to be a polymodal ion channel activated by chemical and mechanical cues. Recording of native currents in adult schistosomes reveal that an endogenous current activated by PZQ matches the biophysical signature of $TRPM_{PZQ}$ recorded *in vitro*.

In other parasites, the sensitivity of different parasitic flatworms towards PZQ correlates with the sensitivity of individual $TRPM_{PZQ}$ orthologs to PZQ in these worms, and worm sensitivity to different activating analogs varies in line with the different structure-activity relationship of $TRPM_{PZQ}$ orthologs. While $TRPM_{PZQ}$ in *Fasciola* spp. is insensitive to PZQ owing to natural variation of a single binding pocket residue, engineering of new chemotypes yield potent activators of *Fasciola* $TRPM_{PZQ}$ that phenocopy the cardinal signs of PZQ action – contraction and surface damage – in this liver fluke. Similar approaches that define ligands which tolerate natural variation within the $TRPM_{PZQ}$ binding pocket could improve treatments for certain cestode and monogenean infections. $TRPM_{PZQ}$ therefore emerges as a druggable target in parasitic flatworms, and definition of the properties of this ion channel identifies various opportunities to improve and expand on the therapeutic efficacy of PZQ.

1. Rohr, C.M., et al., *Natural variation in the binding pocket of a parasitic flatworm TRPM channel resolves the basis for praziquantel sensitivity.* Proc Natl Acad Sci U S A, 2023. **120**(1): p. e2217732120.

Adjuvanted fusion protein vaccine induces a durable immune response to *Onchocerca volvulus* in mice and non-human primates

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Onchocerca volvulus, the causative agent of onchocerciasis (river blindness), currently infects an estimated 21 million people, primarily in Africa. The disease presents as intense dermatitis, which progresses to blindness if left untreated. Control of *O. volvulus* is primarily conducted through mass drug administration of ivermectin. Efforts to eliminate *O. volvulus* transmission face several challenges, including prolonged treatment periods, suboptimal responses, contraindications with other filarial coinfections, non-compliance, and age restrictions. Therefore, an effective prophylactic vaccine against *O. volvulus* is urgently needed to supplement established control methods. The current study investigates the vaccine efficacy of *Ov*-FUS-1, a bivalent fusion protein consisting of two *O. volvulus* antigens *Ov*-103 and *Ov*-RAL-2. The antigen was formulated with three different adjuvants to determine the optimal combination which resulted in consistent and durable protective immunity in two animal models. Mice and cynomolgus macaque non-human primates (NHPs) were immunized with Ov-FUS-1 formulated with Advax-2 (delta inulin with CpG oligonucleotide), alum, or AlT4™ (alum plus a TLR-4 agonist). All vaccine formulations induced high antigen-specific IgG titers in both mice and NHPs. Following immunization, both mice and NHPs were challenged with *O. volvulus* L3 contained within subcutaneous diffusion chambers. The vaccine composed of *Ov*-FUS-1/Advax-2 induced protective immunity in mice at both 4 weeks (early) and 3 months (late) post-immunization. Protective immunity could not be assessed in NHPs due to low larval survival in control animals. Serum was collected at the early and late time points to assess durability of the vaccine in mice and NHPs. Serum collected at either early or late time points from mice and NHPs immunized with *Ov*-FUS-1/Advax-2 transferred protection into naive mice against *O. volvulus*. It was concluded that *Ov*-FUS-1 can induce durable protective immunity against *O. volvulus* that is mediated by vaccine-induced humoral factors. The parallel efficacy of the vaccine in both mice and NHPs supports further clinical development and its translation for use in a phase 1 human clinical trial.

An evolutionary study of intrinsic environmental driving factors for ivermectin resistance in the parasitic model *Caenorhabditis elegans*.

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Caenorhabditis elegans (*C. elegans*) is a versatile model for understanding the effectiveness of anthelmintic drugs against parasitic nematodes. They are frequently used to uncover more drug targets and to understand current and new drugs' molecular mechanisms. As such, work with these worms has contributed mainly to identifying and characterizing molecular targets for many classes of anthelmintic drugs. Nevertheless, despite their continual use in anthelmintic studies, these worms have yet to be used to study the poorly understood selection dynamics or molecular mechanisms that underlie anthelmintic resistance evolution. Therefore, we present one of the first evolutionary experiments to look into the impact of intrinsic factors (i.e., population size and genetic diversity) on the rate of ivermectin resistance in *C. elegans*. We used a step-wise ivermectin introduction experiment to evolve resistant populations; this correlation was also predicted by mathematical modeling. Further, the difference in population size directly correlated to the rate of ivermectin resistance evolution during the experiment. The ancestor and final resistant populations were subjected to genomic, transcriptomic, and phenotypic analysis with ivermectin. The genomic resequencing is under current analysis, yet the transcriptomic data has relieved many differences in gene expression between the ancestor and resistant worm populations. Lastly, our ivermectin-resistant populations were still sensitive to monepantel, levamisole, and albendazole but resistant to moxidectin and unexpectedly to emodepside. The work done here is the first of many examples using evolution experiment guidelines to find answers for understanding which factors could influence the occurrence of anthelmintic resistance. We found that the intrinsic factor population size play a definitive role in the rate of ivermectin resistance formation in our evolutionary experiment. The findings from this study and others like it will lead to novel evolutionary-informed strategies in treating parasitic worms to minimize resistance evolution.

Comparative whole-genome analysis of African snail vector, *Biomphalaria sudanica*, reveals genomic regions associated with resistance to infection by schistosomes

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Biomphalaria sudanica is a major vector host of *Schistosoma mansoni* in the highly endemic African Great Lakes region. Current snail control methods are impractical to apply to large freshwater bodies such as Lake Victoria, and therefore schistosome transmission to humans persists. In contrast to the better-studied neotropical vector *B. glabrata*, there has been little genomic work on these African snails despite the majority of *S. mansoni* transmission occurring in sub-Saharan Africa, and the genetic basis of snail-parasite interaction is completely unknown. Identifying genes that convey natural resistance to infection in African snails may facilitate ways to leverage these immune mechanisms, disrupt the parasite's life cycle and reduce transmission to humans in approaches that are scalable to the vast transmission sites in sub-Saharan Africa.

To uncover immunogenetic pathways, we have generated three major genomic data resources for *B. sudanica*: (1) an annotated genome assembly, (2) a genome-wide scan for high intra-specific diversity among five fully sequenced inbred lines, and (3) a genome-wide association study (GWAS) using pools of infected (N=493) and uninfected (N=295) F1 offspring of wild-caught snails from Lake Victoria exposed to *S. mansoni*.

Elevated diversity among inbred lines, consistent with balancing selection, was observed in *B.sudanica* genomic regions near gene families that confer schistosome resistance in closely related *B. glabrata* (e.g. PTC1, PTC2) and also in novel regions not recognized as having immune function in *B. glabrata*. In regions of high diversity, ~23% of the genes have predicted roles in immune function, including pathogen recognition and destruction. Many of the highly diverse genomic regions appear linked, forming immune gene clusters that span across a wider genome region than has been described in *B. glabrata*. The GWAS identified several candidate genome regions associated with schistosome resistance in wild snails, many of which are positioned in the aforementioned immune gene clusters, whilst others are in novel regions that necessitated further description. Our first glimpse into the innate immune system of the major vector *B. sudanica* shows that its immune defense against schistosomes is linked to several loci, including those not previously recognized in snails, offering exciting avenues for future research that will help inform schistosomiasis control strategies aimed at predicting or manipulating vector competence.

Study of the pathogenesis of Neurocysticercosis using an animal model

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Neurocysticercosis (NCC) is an infection caused by the larval or cysticercus stage of *Taenia solium,* when it lodges in the central nervous system. It is a chronic disease, since the clinical symptoms manifest themselves months or years after the infection occurred. This means that in order to study the pathogenesis of the disease, the use of animal models is necessary. Our research group has developed an animal model of NCC using Holtzman rats, which are infected intracranially with activated *Taenia solium* oncospheres. After 2 to 3 months, the cysticercus is observed in the brain of infected rats, with similar morphological characteristics to the cysticercus that develops in the natural host. This animal model has revealed that NCC causes neuronal damage, observing axonal swelling or spheroids, neuronal death by pyroptosis or apoptosis, and alteration of axonal transport. Likewise, neuronal damage would be due to the presence of dysfunctional autophagy. Through immunohistochemistry we found that the immunoreactivity to Beclin, and LC3B of the tissue surrounding the parasite or cysticercus is higher than the tissue of a healthy brain and that the immunoreactivity to P62 is also high, being statistically significant ($p<0.05$) with respect to the control group. On the other hand, we found that autophagosomes colocalize with spheroids or axonal swelling. These findings could indicate that dysfunctional autophagy could be one of the causes of neuronal damage.

A minimal kynurenine pathway was preserved for rhodoquinone but not for de novo NAD+ biosynthesis in parasitic worms: the essential role of NAD+ rescue pathways

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The biosynthesis of rhodoquinone and NAD^+ are thought to be essential for helminth survival within the host. Under hypoxic conditions, such as those encountered by adult worms in the mammalian host gut, helminths use an alternative mitochondrial electron transport chain (ETC) in which NADH is the electron donor, fumarate is the final electron acceptor and rhodoquinone (RQ), not ubiquinone, the electron transporter. We address the biosynthesis of RQ and NAD^+ in helminths by analyzing more than one hundred helminth genomes available, performing targeted metabolomics, and using specific enzyme inhibitors. We demonstrate that rhodoquinone, the key electron transporter used by helminths under hypoxia, derives from the tryptophan catabolism even in the presence of a minimal kynurenine pathway. We show that of the kynurenine pathway genes only the kynureninase and tryptophan/indoleamine dioxygenases are essential for rhodoquinone biosynthesis. Metabolic labeling with tryptophan revealed that the lack of the formamidase and kynurenine monooxygenase genes did not preclude rhodoquinone biosynthesis in the flatworm *Mesocestoides corti*. In contrast, a minimal kynurenine pathway prevented *de novo* NAD^+ biosynthesis, as revealed by metabolic labeling in *M. corti*, which also lacks the 3-hydroxyanthranilate 3,4-dioxygenase gene. Our results indicate that most helminths depend solely on NAD^+ rescue pathways, and some lineages rely exclusively on the nicotinamide salvage pathway. Importantly, the inhibition of the NAD^+ recycling enzyme nicotinamide phosphoribosyltransferase with FK866 led cultured *M. corti* to death. Our results demonstrate that a minimal kynurenine pathway was evolutionary maintained for rhodoquinone and not for *de novo* NAD^+ biosynthesis in helminths, and shed light on the essentiality of NAD^+ rescue pathways in helminths. Importantly, we identify the essential genes needed for RQ and NAD^+ biosynthesis in diverse helminth lineages and new pharmacological targets for helminthiasis.

Role of carbon dioxide in mediating parasite-host interactions in the skin-penetrating nematode *Strongyloides stercoralis*

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Skin-penetrating nematodes are gastrointestinal parasites that infect nearly one billion people worldwide. These nematodes encounter a wide range of sensory cues throughout their life cycle. However, the specific roles of host-associated chemosensory cues in driving parasite-host interactions are poorly understood and the mechanisms underlying detection of such cues have not been investigated. We are studying the role of carbon dioxide (CO2) in promoting parasite- host interactions using the human-skin-penetrating threadworm *Strongyloides stercoralis*. Specifically, we are elucidating the role of CO2 in mediating three crucial steps of the parasite- host interaction: (a) host seeking, (b) development inside the host after skin penetration (activation), and (c) migration within hosts to complete the parasitic life cycle and establish an infection (intra-host navigation). We show that behavioral responses of *S. stercoralis* to CO2 differ across life stages. While infective larvae (iL3s) are repelled by CO2, activated iL3s are attracted and free-living adults are neutral to CO2. CO2 repulsion by iL3s may function as a dispersal cue, driving them off host feces and into the soil to host seek. Once inside the host, CO2 attraction may direct activated iL3s to the host small intestine. Using CRISPR/Cas9-mediated targeted mutagenesis, we found that the receptor guanylate cyclase GCY-9 mediates repulsion to CO2 in *S. stercoralis* iL3s. GCY-9 is also partly required to trigger activation. In addition, by using *in vivo* calcium imaging, we have found that CO2 is detected by the BAG neurons of *S. stercoralis* iL3s. Moreover, we have performed behavioral tracking of single iL3s and activated iL3s, and have identified differences in CO2-evoked movement patterns across the two life stages that reflect their distinct CO2 preferences. We are now investigating the neural mechanisms that enable *S.stercoralis* to generate life-stage-specific responses to CO2 and successfully establish infections. Our results will provide useful insights into the chemosensory mechanisms that drive host-parasite interactions in skin-penetrating nematodes.

Probing growth and development in *Fasciola hepatica*

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The liver fluke *F. hepatica* causes the disease fasciolosis which is a significant burden on livestock worldwide and is increasingly recognised as a zoonotic disease of humans in many developing nations. The acute stage of the disease occurs as the juvenile worm burrows through the liver parenchyma *en route* to the bile ducts. Triclabendazole remains the sole commercially available drug that is effective against the early juvenile stages, though drug resistance is now common such that new drugs are urgently required. As the juvenile migrates through the liver parenchyma it undergoes rapid growth and development, principally driven by its neoblast-like stem cells. Disruption of this growth/development could limit damage during infection or prevent infection altogether. We employed transcriptomic approaches to increase our understanding of the biology in this life stage and expose potential drug targets. Silencing the cell cycle regulator polo-like kinase 1 (*plk1*) *in vitro* ablated *F. hepatica* neoblast-like cells, which subsequently impeded growth and development. RNAseq of *plk1* silenced worms revealed that many cell cycle pathway members were downregulated, alongside numerous kinases (e.g. fibroblast growth factor receptors) and transcription factors which may represent druggable targets. Remarkably, we noted a simultaneous upregulation of signalling systems. In fact, of 100 manually annotated receptors and ion channels 93 were found to be upregulated, including voltage gated K^+ channels, rhodopsin GPCRs and nicotinic acetylcholine receptors. We also found significant upregulation of 23/36 neuropeptides, and KEGG pathway analysis revealed upregulation at the glutamatergic, dopaminergic, cholinergic, and serotonergic synapses. Together these data indicate that both the classical and neuropeptide signalling systems are fundamentally linked to *F. hepatica* neoblast-like cells, whereby disruption of normal growth/development causes significant transcriptional changes in neuronal signalling systems. Moreover, over 50% of the most up/downregulated genes do not have BLAST hits in model organism databases. Many of the genes identified here through transcriptomics are potential anthelmintic targets. As such, we are further investigating their links to growth/development through RNAi-mediated silencing experiments to assess their drug target candidature.

Dosage Compensation and Meioitic Silencing on the Neo-X Chromosomes of Filarial Nematodes

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Brugia malayi and *Onchocerca volvulus* are filarial nematodes consequential for public health. In each species, a different autosome fused with the X chromosome to form a neo-X chromosome. As a result, their ancestrally XX/XO systems became XX/XY, where the Y represents a degenerated version of the unattached autosomal homolog chromosome. As genes on the Y diverge or are lost, there is evolutionary pressure to: compensate for this change in gene dosage; balance expression levels between the sexes; and silence genes during meiosis. Here, we combine and reanalyze RNA-seq data sets from to reveal dosage compensation and meiotic silencing in *B. malayi* and *O. volvulus*. The presence of these mechanisms along filarial neo-X chromosomes has implications for our understanding of the unassembled Y chromosomes, transcriptional regulation, and gametogenesis in these parasitic nematodes.

Intra-Snail Stages Transcriptomics Reveals Stage-specific Gene Amplifications in *Fasciola hepatica*

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While mammalian host invasion by *Fasciola hepatica* has been extensively analyzed, little is known at the molecular level on the interaction with the intermediate host. These stages are particularly interesting since two or more cycles of asexual amplification occur within lymnaeid snails, leading to the production of hundreds of infective metacercariae. We analyzed transcriptomic data from miracidia and intra-molluscan stages seeking for clues of these interaction and developmental processes. We found evidence for the expression of 1744 novel transcripts and several isoforms of already annotated genes. Analysis of expression across the whole cycle by diverse methods resulted in five distinct groups of gene expression (egg, miracidia, intra-snail stages, invading stages and juvenile-adults). Few genes showed strict stage-specific expression, but notably most of those corresponding to miracidial and intra-snail stages are novel unannotated genes. Genes upregulated in miracidial stages include enzymes involved in neurotransmitter synthesis, energy metabolism and calcium mediated signaling, consistent with the needs of the short-lived free-living stage. Stage specific SCP/TAPS proteins and trypsin-like proteases were also detected in miracidia. Several genes associated with development and morphogenesis are characterized in early (15 dpi) intra-snail stages. Purine salvage pathway genes are upregulated in this stage, consistent with the high biosynthetic needs and the absence of a complete purine synthesis pathway in *Fasciola hepatica*. Mucins, glycan biosynthesis genes and aquaporins upregulated within late (30 dpi) intra-snail stages are interesting, considering their putative role in the following host transition. Notably, stage specific expression of distinct members of well-known protein families involved in host interaction such as cathepsin proteases, legumains, protease inhibitors, and lipid transporters can be detected both in early and late intra-snail stages. Several of these gene families are found in clusters in diverse regions of the genome. These results highlight that the parasite might have duplicated and tuned during evolution the same set of molecular mediators for the specific interaction with the intermediate and definitive hosts.

Explore Regenerative Responses In Parasitic Flatworm *Schistosoma mansoni* Following Injury

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The parasitic flatworms schistosomes can live within host for decades, how these parasites can survive in such hostile environment remains an open question. Unlike the immense regenerative capacity of free-living flatworm planarians, adult schistosomes are not capable of whole-body regeneration, but previous studies showed they can repair the tissue damage that caused by sublethal drug (Praziquantel) treatment and also heal wounds following amputation. However, the cellular and molecular mechanisms underline these regenerative responses following injury are poorly studied. Adult schistosomes have a large population of somatic stem cells called neoblasts that are responsible for tissue renewal. To investigate these neoblasts' behavior during regeneration, we applied different types of injuries (Praziquantel treatment and amputation) to adult schistosomes. We observed expanded numbers of neoblasts in worm trunks three days post-amputation. Interestingly, many of these injury-responsive neoblasts expressed *eled,* a marker of neoblasts in juvenile worms. We found these *eled*$^{+}$ neoblasts throughout the whole trunk following both Praziquantel treatment and amputation. To characterize the molecular response of various cells to injury, we performed single-cell RNAseq analysis on the amputated trunks pieces. These studies uncovered a small population of cells that are injury-specific and defined by their expression of a pair of basic helix-loop-helix (bHLH) transcription factors. Above data indicate that adult schistosomes respond to injury by altering neoblast proliferation and by the induction of specific transcription factors. We hope to define the extent to which these parasites can repair various types of damage and how this process is regulated on a molecular level.

Insect-parasitic nematodes as a model system for functional characterization of nematode ESPs

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Nematode parasites release excretory/secretory (ES) products to manipulate host biology. ES products are complex mixtures and often include small molecules, proteins, and nucleic acids, but most studies have focused on proteins. ES studies rely on release and collection of the ES products under *in vitro* conditions, where the underlying assumption that ES material collected under these conditions is relevant and similar to the ES released *in vivo*, though this is seldom directly tested. Some functional studies have evaluated individual ES components, but hundreds have been identified and few have been studied in any mechanistic detail. One hindrance for mechanistic studies is the difficulty and cost of working with some parasitic nematodes and their hosts. Using model systems is a powerful way to discover conserved biology and rapidly develop and test hypotheses. Entomopathogenic nematodes (EPNs) are closely related to important species of human-parasitic nematodes and could serve as models for studying parasitic nematode biology. We show that EPNs release a complex mixture of ES proteins, and that many of these proteins are conserved in nematode parasites of mammals. We use single-nematode transcriptional profiling to evaluate the relevance of nematodes activated *in vitro* to nematodes activated *in vivo*. Further, we assess the immunomodulatory potential of individual proteins released by EPNs during infection. In summary, our research supports the use of EPNs as a model for understanding parasitic nematode ES and provides hypotheses for the mechanistic functions of individual ES proteins.

Taenia solium exosomes interfere with PI3K-AKT-mTOR pathway and induces apoptosis in macrophages

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Abstract

Exosomes are extracellular vesicles of 30-100nm which constitutes significant part of secretome. Excretory secretory proteome plays significant role in pathogenesis and immune escapes mechanisms of complex parasites like *Taenia solium*. The cyst of *T. solium* causes infection to CNS i.e., neurocysticercosis (NCC). However, the role of exosomes in NCC pathogenesis is not understood till now. We had established that exosomes induce AKT degradation in macrophages via the autophagosomal-lysosomal pathway. The phenotype is supported by the low ROS production with impaired bacterial killing. Along with this PI3K pathway was also seen to be impaired after exosome stimulation in macrophages. We also found mTOR degradation was via the lysosomal pathway with a notable increase in the ubiquitination. Following this autophagy and apoptosis both increased with significant degradation of autophagy substrate SQSTM1. In summary, here we report that the *T. solium* exosomes modulate PI3K-AKT-mTOR pathway to induce autophagy and apoptosis in macrophages and this may exert immunosuppression via the exosomes during NCC disease. These finding helps us to understand the immune suppression induced by cyst for its survival in host.

Keywords

Taenia solium, Neurocysticercosis, exosomes, autophagy, AKT, reactive oxygen species, apoptosis

Molecular proof of hookworms and *Strongyloides* species in humans and dogs in Bangladesh

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Background: 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. This study confirmed hookworms and *Strongyloides* species from humans and dogs across northeastern Bangladesh.

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 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 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 *Ancylostoma ceylanicum* and *Strongyloides fuelleborni* in Bangladesh. Detection of the identical type of *Strongyloides stercoralis* from humans and dogs raised the question of zoonotic transmission of *Strongyloides*. Thus, the implementation of effective control measures should seriously consider this zoonotic implication.

Keywords: Soil-transmitted helminths, *Ancylostoma*, *Strongyloides*, Zoonoses, One Health

Paragonimus kellicotti extracellular vesicles released *in vitro* or present in lung cysts contain a cysteine protease that is recognized by IgG4 antibodies of infected humans

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Paragonimiasis is an important zoonotic, food-borne trematode infection that affects some 21 million people (mostly in Asia). This lung fluke infection can be efficiently treated with praziquantel, but diagnosis is challenging due to confusion with other lung diseases such as cancer or tuberculosis. Trematode parasites release extracellular vesicles (EV) in vivo that contain parasite proteins and RNA cargo that may interact with other parasites and with the host. Here we detail the composition of *Paragonimus kellicotti* EVs purified from both adult worm excretion/secretion products in vitro (EV ESP) and from lung cyst fluid of infected gerbils (EV CFP). Electron microscopy showed that most of the EVs were 30-50 nm in diameter, but small (10-20 nm) and large (90-120 nm) subpopulations of vesicles were also present. We identified 548 *P. kellicotti*-derived proteins in EV ESP by mass spectrometry. We detected 8 proteins in the EV CFP of which 7 were also present in EV ESP. A cysteine protease (MK050848, CP-6) was the most abundant protein found in EV CFP in all technical and biological replicates. Immunolocalization of CP-6 showed no staining in lung tissue of uninfected gerbils. However, strong labeling for CP-6 was observed in the tegument and suckers of adult *P. kellicotti* and in the diseased lung cyst tissue that contained worm eggs. Immunoprecipitates of *P. kellicotti* adult worm lysates with sera of infected humans identified CP-6 as a major immunoreactive antigen. Recombinant CP-6 reacts with IgG4 antibodies collected from all subjects with paragonimiasis but is not reactive in subjects with other helminth infections. A lateral flow IgG4 antibody test using CP-6 as antigen is currently under development. These results suggest that CP-6 is released within EVs by adult worms *in vivo* and provided new insights regarding interactions between *Paragonimus* worms proteins and their mammalian hosts.

Cestode tegument width is a plastic trait connected to genetic differences in host immunity

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Despite substantial progress in understanding the development and function of tissues and traits within helminths, relatively little work has addressed whether (and how) phenotypes vary across natural populations within a parasite species. This biogeographic lacuna makes it difficult to understand not only how much any particular trait can vary, but also whether variation is connected to specific environmental conditions. Although it is clear that many parasite lineages become specialized to infect only a few host species, little is known about how standing variation across populations contributes to the process of evolutionary specialization. We addressed these issues using the Pseudophyllid cestode *Schistocephalus solidus*, which uses threespine stickleback (*Gasterosteus aculeatus*) fish as a specific intermediate host. Because this parasite cannot infect any other fish hosts there is strong selection to become specialized to overcome host immunity. Likewise, the parasite imposes substantial fitness costs on stickleback that drive evolution of immunity, leading to complex patterns of resistance (or tolerance) that vary across host populations. We collected *S. solidus* across six locations in Alaska and used histology to measure variation in numerous tissues. Most tissues did not vary among populations. However, we found significantly wider teguments in cestodes from waterbodies where fish had long periods (likely thousands of years) to evolve resistance, as opposed to locations where fish and parasites had recently become established (usually less than 20 years). To test whether this difference was due to genetic or plastic differences, we performed laboratory infections where the naturally collected cestode strains were exposed to evolutionarily resistant or naïve hosts. Surprisingly, we found that tegument width was highly plastic, with wide-tegument natural strains developing significantly smaller teguments when exposed to immunologically naïve lab fish. Tegument plasticity is particularly interesting given that this organ sits at the interface of parasite-host interactions and is known to modulate host immunity. Current work in the lab is measuring how gene flow between parasite populations is associated with tegument variation and evolution, and we are further dissecting the molecular and functional relevance of tegument variation for infection outcome.

How schistosomes control their purinergic halo

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Schistosomes in the blood stream are hypothesized to control immune and hemostatic responses by regulating the nature and amount of selected host purinergic signaling molecules in their local environment. Such extracellular metabolites are collectively known as the worm's "purinergic halo", and they include adenosine triphosphate (ATP), adenosine diphosphate (ADP), and nicotinamide adenine dinucleotide (NAD). We have shown that a series of host-interactive, membrane-bound, tegumental ectonucleotidases that are all highly expressed following vertebrate host infection can degrade such proinflammatory, pro-thrombotic and immunomodulatory purinergic metabolites. For instance, the 544 amino acid *Schistosoma mansoni* tegumental ATP diphosphohydrolase SmATPDase1 and the 458 amino acid, GPI-linked pyrophosphatase/phosphodiesterase SmNPP5 can both degrade proinflammatory ATP and pro-thrombotic ADP. Both enzymes generate adenosine monophosphate (AMP), and in *S. mansoni* this can be further cleaved by the 536 amino acid, GPI-linked tegumental ectoenzyme alkaline phosphatase (SmAP) to generate adenosine. The ability of living parasites to cleave exogenous AMP and to generate adenosine is largely abolished when SmAP gene expression is suppressed following RNAi treatment targeting the gene. Working in unison, SmATPDase1, SmNPP5 and SmAP can efficiently convert proinflammatory, extracellular ATP into its anti-inflammatory degradation product, adenosine, thereby creating a more immunologically benign environment for the worms within the host vasculature. Note that while adenosine is a powerful anti-inflammatory biomolecule, it can additionally be taken in by the worms as food; since schistosomes are incapable of *de novo* purine synthesis, they are dependent on salvage of purines (like adenosine) from the external environment for their core metabolism

Finally, SmNPP5, as well as a second ectoenzyme, the 303 amino acid GPI-linked glycohydrolase SmNACE, can cleave immunomodulatory NAD. NAD catabolism by these ectoenzymes generates the essential biomolecule nicotinamide (vitamin B3) in the local vicinity of the worms from where it may be easily taken in for use in central metabolism. In sum, we envision the tegumental ectonucleotidases listed above as having a twofold role at the worm surface: first, they degrade potentially harmful host signaling molecules and second, they generate vital nutrients around the worms from where these can be conveniently imported.

Identification and characterization of mucus degrading enzymes during early whipworm infection using *in vitro* colonoid model

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Human trichuriasis is a whipworm infection caused by parasitic nematode *Trichuris trichiura*. Approximately 289 million of the world's population were estimated to be infected with whipworm particularly prevalent in developing countries. Current chemotherapeutic interventions have shown limited efficacy against *T. trichiura* highlighting necessity to develop novel anthelmintics. We sought to better understand whipworm-host interactions especially in early infection stage to facilitate drug target identification that would intervene in establishment of infection. However, understanding onset of parasitism has been limited by difficulty in experimental tracking of L1 larvae *in vivo* and *in vitro* with currently established infection models. A previous study showed that adult *T. muris* secreted 50 – 100 kDa serine protease(s) which can depolymerize murine intestinal mucin where their initial interactions likely occurs when L1 larvae penetrate the mucosal epithelium. However, mucus degrading enzymes (mucinases) have not been identified, and thus interaction between host and whipworm during early phase of infection is still elusive at a molecular level.

We employed mouse colonoids and cultured in air-liquid interface to recapitulate mucus overlaying the intestinal epithelium where L1 larvae encounter host protective barrier against invading pathogenic infection. Then, we introduced *in vitro* hatched L1 *T. muris* to apical surface of differentiated colonoids and observed mucin degradation after 48 hours of infection. In parallel, we undertook a multi-omics approach (included phylogenomics and comparative transcriptomics) to prioritize whipworm serine proteases as potential mucinases and identified a group of gene families that were exclusively conserved among *Trichuris* species and highly expressed and secreted in the early larval stage. One *T. trichiura* gene was cloned, expressed, and examined for mucinase activity. We found that the recombinant protein is ~ 100 kDa serine protease/hydrolase having active serine residue and is able to depolymerize dimeric form of recombinant MUC2 D3 protein.

In conclusion, we were able to show that not only adult but also L1 *T. muris* secrete mucinase(s) degrading mucin from *in vitro* cultured colonoids and that a *T. trichiura* ortholog of prioritized serine proteases highly transcribed in L1 larvae had a partial mucinase activity pointing to a potential mechanism essential for establishing infection using mucin degrading enzyme(s).

Fasciola hepatica Enolase, a moonlighting glycolytic enzyme on the parasite tegument that interacts with the host fibrinolytic system

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Enolase is a 47 kDa enzyme involved in the reversible conversion of D-2-phosphoglycerate (2PGA) to phosphoenolpyruvate (PEP) in glycolysis and gluconeogenesis. This is also an enzyme of potential immunomodulatory importance in the early infection and migratory stages of the trematode *Fasciola hepatica*. Studies conducted on other parasites, fungi and bacteria have shown that enolase is capable of binding plasminogen and stimulating its activation to plasmin, the main protease of the host fibrinolytic system. This secondary (so-called moonlighting) function of enolase is suggested to facilitate pathogen migration through host tissues by enhancing plasmin-mediated degradation of the extracellular matrix (ECM). The present study was performed to obtain biochemical, molecular and biological data about the *F. hepatica* enolase that may support its moonlighting role in host-parasite interaction in fasciolosis. For that purpose, we produced a functional enzyme by expressing the recombinant form of FhEnolase in an *E. coli* expression system. Recombinant protein purified using His-tag affinity purification takes on a dimeric structure of ~94 kDa, as found in other species, when analysed by size exclusion chromatography. The recombinant enolase is enzymatically functional only as a dimer, and not in its monomeric form. Immunoblotting studies of adult worm extracts indicate that the enzyme is present in the tegument and the excretory/secretory products of the parasite which supports its key role at the host-parasite interface. Confocal immunolocalization studies of the protein in newly excysted juveniles (NEJ) and adults also reveal its expression on the parasite tegument. Finally, we demonstrate by ELISA that FhEnolase is able to bind host plasminogen and enhance its conversion to plasmin in the presence of the tissue-type and urokinase-type plasminogen activators (t-PA and u-PA). Our data indicates that the moonlighting functions of the glycolytic protein enolase could allow *F. hepatica* to efficiently invade and migrate within its host, and demands further research efforts to block this function by vaccination or other means.

Wb 5, a novel biomarker for monitoring efficacy and success of mass drug administration programs for *Wuchereria bancrofti* elimination

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The success of mass drug administration (MDA) at reducing the prevalence of lymphatic filariasis (LF) in endemic countries has led to an increased need for diagnostic targets designed to monitor for recrudescence and ongoing transmission. While diagnostic targets exist for detecting *Wuchereria bancrofti* [*Wb*] and *Brugia malayi* [*Bm*] infections, additional targets may increase the sensitivity of these tests. For *Wb* at least, new biomarkers (in conjunction with Wb123) may be needed to supersede the current WHO recommendations which fail to reflect microfilariae (mf) clearance. Hence, diagnostic tools that reflect the presence/clearance of mf or early infection could be instrumental for not only MDA stopping decisions but also for monitoring. To this end, bioinformatic analyses coupled with stage-specific expression data for *Wb* and/or *Bm* resulted in the identification of 12 targets that were: 1) present in *Wb* and/or *Bm;* 2) have very little to no homology with proteins from other filariae; and 3) were enriched in the mf or L3 stages. Screening of these 12 antigens by a Luciferase Immunoprecipitation System (LIPS) assay for IgG with serum from Wb-infected (n=170) and uninfected individuals (n=60) identified a single antigen, termed Wb5, that was specific for *Wb* infections only. Recombinant Wb5 proteins were generated in multiple expression systems for use in a variety of IgG4-based immunoassays. Preliminary screening indicated a very high degree of correlation ($p<0.0001$) between the data derived from LIPS and those determined by more standard immunoassays. To assess if Wb5 could provide additional sensitivity to assays already using IgG4 antibodies to Wb123, head-to-head comparisons were performed using serum from 381 samples (231 Wb-infected; 150 controls). Using IgG4 based immunoassays at 100% specificity, Wb5 and Wb123 had individual sensitivities of 60.2% and 75.3%, respectively, while a combination resulted in 81.0% sensitivity. Moreover, kinetic studies of patients that were treated and followed up longitudinally demonstrated a sharper decline in Wb5 titers compared to Wb123, thus paving the way for Wb5 as a complementary tool to Wb123.

Prophylactic drugs that target early stages of filarial worms can support the elimination goals for onchocerciasis

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The Current Preventive Chemotherapy and Transmission Control strategy for onchocerciasis aims to interrupt transmission through annual or bi-annual mass drug administration (MDA) with ivermectin (IVM). However, by 2013, only a 31% reduction in microfilariae prevalence was achieved by MDA with IVM given for >20 years, clearly indicating that the revised WHO goal of elimination of transmission (EOT) by 2030 would not be met if it relies only on one microfilaricidal drug, IVM. Moreover, decreased susceptibility to IVM may also limit the long-term effectiveness of MDA. The current lack of macrofilaricides highlights the urgent need for developing new drugs and alternative treatment regimens. Our studies suggest that the addition of "prophylactic" drugs—that target the establishment of new early infections—to the toolbox of present microfilaricidal and/or future macrofilaricidal drugs as part of complementary treatment regimens will not only improve the chances of meeting EOT goals but may also support achieving a sustained elimination of onchocerciasis. Drug treatment studies *in vitro* against *O. volvulus* L4s and *B. pahangi* young worms (D42) support the nomination of moxidectin (MOX) and emodepside (EMO) as prophylactic potentials. MOX is microfilaricidal and EMO is currently being tested in humans by DND*i* as a macrofilaricide. We found that MOX is highly effective against *O. volvulus* L4s after 6-day treatment (IC_{50}=1.04 µM), with improved potency after 11 days (IC_{50}=0.380 µM). It is also effective against *B. pahangi* (D42) young females (IC_{50}=4.44 µM) and males (IC_{50}=5.86 µM). Remarkably, EMO is more effective at inhibiting *O. volvulus* L4 and *B. pahangi* (D42) motility than MOX; IC_{50}=0.50 nM and IC_{50}=33.0 nM (male) or IC_{50}=78.0 nM (female), respectively. Importantly, both drugs have a superior half-life and based on their PK profiles (Cmax and AUC) in humans, treatment with MOX (8 mg) and EMO (2.5 mg) results in plasma concentrations that well cover their experimental effectiveness (IC_{50}s) *in vitro* against *O. volvulus* L4 and D42 *B. pahangi* young worms' motility. Once their prophylactic efficacy is validated *in vivo*, such promising outcomes will strengthen the argument for employing EMO and MOX also for prophylaxis, and hopefully will guide their usages accordingly as they are rolled out for human use. It may also foster the development of innovative complementary elimination approaches to advance the goal of sustainable onchocerciasis EOT.

Long non-coding RNAs involved with reduced sensitivity to praziquantel in *Schistosoma mansoni*

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Schistosomiasis is a very debilitating disease, affecting more than 200 million people worldwide. Praziquantel (PZQ) is the basis of current schistosomiasis therapy. PZQ is a safe, cheap, and tolerable drug; however, the use of a single drug may contribute to resistance emergence. In fact, it has been recently shown that the major target of PZQ, a transient receptor potential melastatin ion channel (Sm.TRPMPZQ), underlies variation in PZQ responses in *Schistosoma mansoni*. However, variants underlying resistance to PZQ phenotype were only found outside the coding regions in the *Sm.TRPMPZQ* gene locus, suggesting that regulatory changes or additional factors may underlie this trait. Long non-coding RNAs (lncRNAs) are RNAs longer than 200 nt with low or no protein-coding potential involved in many biological processes in eukaryotes. We have recently shown that lncRNAs levels can be modulated by drugs in *S. mansoni* and that lncRNAs are essential for *S. mansoni* pairing-dependent adult worm homeostasis and fertility. The aim of the present work was to evaluate the involvement of lncRNAs in the development of reduced sensitivity to PZQ in *S. mansoni*, since lncRNAs are well known as implicated in the resistance to drug treatment in human cancers. We have reanalyzed two public RNA-Seq datasets looking for lncRNAs differentially expressed (DE) in laboratory strains of *S. mansoni* (PZQ-selected) whose susceptibility to PZQ was diminished across several passages through exposure to increasing sublethal doses of PZQ. The raw reads were processed and DE genes, including lncRNAs, were identified. Differential expression statistical analysis identified hundreds of lncRNAs DE in the PZQ-selected strains compared with controls, including intergenic, antisense and sense lncRNAs. We also re-analyzed public GWAS data to map loci underlying PZQ response and found 75 lncRNAs located in QTL regions, 7 of which are also differentially expressed in the PZQ-selected *S. mansoni* subpopulations. These lncRNAs are located ~450 kb away from *Sm.TRPMPZQ* on *S. mansoni* chromosome 3, representing putative regulators of *Sm.TRPMPZQ* gene. Among the DE genes, lncRNA-protein coding genes co-expression analysis identified enrichment of pathways related to ion transport and vesicles transport. This is the first step towards the functional characterization of lncRNAs possibly involved in PZQ resistance in *S. mansoni*. Selected lncRNAs will be used in RT-qPCR validations and phenotypic functional assays.

Investigating Genomic Diversity in Laboratory Schistosome Populations

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How genetically variable are laboratory schistosome populations? The parasite's life cycle can be maintained in the laboratory, and several schistosome populations are used for research. It is often assumed that labora- tory schistosome populations are genetically and phenotypically homogeneous due to repeated passage and genetic drift, but levels of genomic variation are poorly understood. We sequenced the genomes of 10 parasite genotypes from each of 4 parasite lines (SmLE and SmBRE from Brazil, SmOR from Puerto Rico, and SmEG from Egypt) maintained in our laboratory. To do this, we infected the intermediate snail host with single (mira- cidia) larvae and sequenced the clonal (cercariae) larval stage emerging. We sequenced 5 male and 5 female genotypes per population, producing 40 single genotype sequences. Using these data we asked: (i) How much variation is retained in laboratory schistosome populations? (ii) Are laboratory populations clearly distinguisha- ble and representative of field populations? (iii) Do we see evidence for laboratory contamination? Principal component analysis showed 3 distinct clusters revealing genetic differentiation between geographically distinct populations. However, admixture analyses revealed a recent contamination event between SmLE and SmBRE. In line with this, we observed a recent rapid change in shedding phenotype during life cycle mainte- nance of SmBRE. Integrating sequences from African and Brazilian field samples, we found that the laboratory populations closely reflect field populations from the same region. Finally, our laboratory populations retained a high level of heterogeneity compared with field populations. Genetic diversity was reduced 33% in Brazilian lab populations and by 63% in SmEG compared with East African field samples. These findings suggest that mo- lecular tools are needed to monitor contamination: this will help foster reproducibility across different institu- tions. The high genetic diversity retained in laboratory schistosome populations has two key implications. First, phenotypic studies of laboratory populations for traits such as drug resistance will measure average pheno- types, while individual worm based measures are likely to reveal extensive variation in phenotype within popu- lations. Second, we can exploit the genetic diversity observed within laboratory populations by using genome- wide association studies to investigate the genetic basis of biomedically important parasite traits.

CRISPR-Cas9 host signal reduction and 18S metabarcoding for parasite assemblage characterization

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Whereas sequencing-based approaches (e.g., metabarcoding) of prokaryotes and fungi are commonplace, no single method for parasite metabarcoding has been adopted. We systematically assessed published parasite metabarcoding protocols and demonstrated issues with high off-target read abundance and primer complementarity. To address the abundance of off-target host signal, we designed a novel CRISPR-Cas9 based method to enrich for reads of interest. We created guideRNAs that allow for selective targeting and digestion of vertebrate DNA while leaving helminth, protozoal, and microsporidian DNA intact for library preparation and sequencing. When applied to blood and tissue samples, our method resulted in a mean 92 % decrease in host read abundance compared to no treatment, a 61 % decrease compared to the most commonly published blocking method, and allowed for detection of hemoparasite infections that would otherwise have been missed. To address primer complementarity, we designed new 18S rRNA primers to recognize all parasites of vertebrate hosts and used *in silico* PCR to show that only our new primers (n = 4) successfully amplified all parasite clades and had the highest overall taxonomic coverage as compared to published primer sets (n = 22). Using a novel parasite mock DNA community, we further demonstrated that one of our new primer sets more closely recovered the underlying community than any other. When applied to clinical samples (n = 52) our new protocol (Vertebrate Eukaryotic endoSymbiont and Parasite Analysis, or VESPA) outperformed the “gold standard” method of microscopy with 51.3 % of identifications made by VESPA alone. VESPA identified taxa not found with microscopy, resolved a cryptic species complex not resolved by microscopy, and revealed greater prevalence and richness of parasitic organisms than microscopy.

Proof-Of-Concept Multilocus Sequence Typing Scheme to Investigate Hybridization in *Schistosoma haematobium*

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Schistosomiasis is a parasitic disease caused by blood flukes in the genus *Schistosoma* that infect human and animal hosts. Reports of ongoing hybridization between human and animal species of schistosomes in many parts of Africa suggest the existence of a species complex within the Schistosoma haematobium group, comprised of *S. haematobium*, *S. bovis*, *S. currasoni*, *S. intercalatum*, *S. guinnesis*, and *S. mattheei*.

As proof-of-concept, we developed a Nanopore multiplex amplicon sequencing (NMAS-Seq) platform based on newly identified markers to generate high-resolution data for studying schistosome genetic diversity. Using this concept, we investigated the presence of schistosome hybrids in four pastoral and non-pastoral communities in Nigeria. DNA was extracted from 119 parasite isolates obtained through urine filtration or from hatched miracidia. Multiplex nested PCR was used to amplify 12 markers, including rITS and mtCO1, and the amplicons were sequenced on the minION, and another 58 isolates were sanger sequenced. The sequence data was analyzed with Nanopolish, GATK, Geneious, and MEGA. In this study, all of the isolates had *S. bovis* alleles in at least two loci with varying levels of heterozygosity. Our multi locus sequence typing scheme detected *S. bovis* alleles in isolates that would have been tagged as pure *S. haematobium* based solely on mtCOX1 and rITS. The new markers provided additional inference on the diversity and population genetic structure within the complex group of schistosome species related to *S. haematobium*. Furthermore, we recorded a 99.9% agreement between our sanger and NMAS-Seq data with a 70% cost reduction for NMAS-Seq. We propose this multilocus typing scheme as an alternative for distinguishing between *S. haematobium*, *S. bovis*, and hybrids from both species.

Keywords: NMAS-Seq, Schistosomiasis, Hybridization, Species, Diversity, Nigeria

Spatial transcriptomics of parasites: A molecular map of the liver fluke *Fasciola hepatica*

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Fasciolosis is a food-borne trematode infection caused by the liver fluke *Fasciola hepatica* and related species. Limited therapeutic options and increasing anthelminthic resistance complicate sustainable control and highlight the need for novel anthelmintics. In order to identify new drug targets, it is first necessary to better understand the fluke's biology, including its organ function and organ-specific gene expression. Therefore, we applied Visium Spatial Gene Expression Solution (10x Genomics) on *F. hepatica*.

Cryosections of adult parasites were first imaged and subsequently permeabilized to release mRNA from the tissue. Transcripts were captured and barcoded *in situ* using oligonucleotide-coated glass slides and finally sequenced *ex situ*. Barcoding preserves positional information throughout the workflow. As a result, gene expression can be visualized spatially resolved and within the original morphological context.

By performing differential gene expression analysis, we identified gene expression profiles for eight different tissues, such as intestine, tegument and reproductive organs. We were even able to discover subsets of cells within some organs. Selected marker genes were validated by *in situ* hybridization. We then performed gene ontology (GO) enrichment analysis, which revealed characteristic biological processes and molecular functions associated with each tissue. Furthermore, we identified several drug target genes (such as ß-tubulins and calcium channels) and drug resistance genes (e.g. ABC transporters and glutathione S-transferases) with tissue type-specific expression.

Taken together, this work provides the first spatial transcriptome of a parasitic flatworm. Our dataset serves as playground for the exploration of tissue-specific gene function and opens new avenues for the discovery of new drug targets and vaccines.

Molecular Helminthology 2023
Poster Abstracts

Dysregulating the balance between cell survival and programmed cell death – a novel strategy for fasciolosis control?

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The liver fluke *Fasciola hepatica*, places a significant disease burden on ruminant livestock worldwide and is an aetiological agent of fascioliasis, a neglected tropical zoonosis. Reports of flukicide resistance are increasing, such that there is a pressing need for novel drug target discovery. A delicate balance between cell proliferation/survival and programmed cell death mediates the cellular turnover that supports the maintenance and growth of tissues. A key facet of juvenile *F. hepatica* virulence is their ability to rapidly turn over various cell and tissue types, a process driven by a population of neoblast-like stem cells. Further, neoblast-like stem cell proliferation drives juvenile growth and development, such that their dysregulation represents an appealing avenue for control. *In silico* analyses revealed that *F. hepatica* possesses the core machinery required for a functional intrinsic pathway of apoptosis, in addition to key components of major pro-survival signalling pathways known to regulate cell turnover in higher organisms. The RNAi-mediated silencing of putative apoptotic kinases led to profound reductions in juvenile growth, coupled with elevated levels of apoptosis and a temporal shift in cell proliferation levels, which ultimately proved fatal. RNAi phenotypes were more pronounced in fluke of a triclabendazole-resistant isolate. Fluorescence *in situ* hybridisation-mediated localisation revealed the ubiquitous expression of target transcripts, supporting phenotypic readouts. These data indicate that the dysregulation of cell cycle-apoptosis interplay represents a novel strategy to undermine the virulence of pathogenic juvenile *F. hepatica,* underscoring the appeal of apoptotic and pro-survival signalling pathway components as targets for novel flukicide development.

Innovative strategy for the identification of new therapeutics for onchocerciasis

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Onchocerciasis (river blindness) is a disease caused by the filarial nematode *Onchocerca volvulus*, and can manifest in severe itching, skin lesions, visual impairment, and blindness. Unfortunately, there are no vaccines or drugs available to kill the adult worms directly. Currently, control of onchocerciasis is through the mass drug administration with the microfilaricidal drug ivermectin aimed at stopping transmission. Doxycycline and other anti-*Wolbachia* antibiotics are curative as they kill adult worms indirectly, but it may take more than 18 months and cannot be administrated to children and pregnant women. To identify new macrofilaricidal drugs that act directly on adult worms, we undertook an integrated multidisciplinary study that incorporated a systematic computational search for genes essential for the survival of filarial nematodes with an experimental multifaceted in vitro screening of filarial worms with the pre-approved drugs. Three potential hits were identified, pimozide, proroxan, and clemizole that target different GPCRs with a high anthelminthic potency against *B. pahangi* (IC_{50} of 2.5, 8.5 and 12.1μM respectively).

We undertook two orthogonal approaches to expand the list of hits. Both approaches are based on an underlying target class repurposing strategy, were followed to identify potential anthelmintics, based on existing mammalian GPCR inhibitors. First, based on a chemogenic screening we identified additional 16 drugs that target GPCR, including 6 dopamine blockers, 5 histamine antagonists, and 5 adrenergic blockers. Eleven of the 16 drugs were active against adult female and male *B. pahangi* (10μM; 70% motility inhibition). The 19 drugs (including 3 parent drugs) were evaluated through viability inhibition in adult *O. ochengi* female and male worms. Four out of 19, drugs were active (100μM) against both sexes, however, 13 drugs were potent exclusively on male. Ten drugs were active on both *O. ochengi* male (100μM) and *Brugia* (10μM) adult female or male worms, but 3 of them showed efficacy against *O. ochengi* female worms. Those 19 candidates were evaluated on *O. volvulus* L4 larvae stage (10μM) where 4 drugs showed activity. Second, using optimization approach to illustrate the structure activity relationships of our lead, we synthesized 6 proroxan, 9 pimozide and 13 clemizole analogs. Out of these 28 analogs 19 were active against female and male *B. pahangi*. Interestingly, histamine and dopamine antagonist were mostly active in female while adrenergic blockers were mainly effective in male. However, 2 adrenergic blockers were found to be active in both sexes. Based on the *in vitro* testing of 47 compounds we obtained very promising results (several fold better IC_{50} values) and structure activity relationship especially for the clemizole and pimozide analogs.

Different approaches to create transgenic hookworms

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Understanding the biology of parasitic nematodes is restricted due to limited functional genomic tools that are available for their genetic manipulation. Development of the functional genomic techniques such as transgenesis, RNA interference and targeted mutagenesis is crucial for the comprehension of biological mechanisms, genetics tractability and validation of drug targets. Transgenesis as a tractable method has been reported only in a few parasitic worm species while it is widely used in *Caenorhabditis elegans*. Similarly, RNA interference and CRISPR/Cas9 methods have been tested in the limited numbers of parasitic nematodes however with rather different success. As such there is a constant need for development of new nematode model systems and associated tools for their molecular biology and genetic manipulations.

Here we focused on the development of new platforms to create transgenic human hookworm - *Ancylostoma ceylanicum*. We report our analysis of two different methods - random and targeted transgenesis. Electroporation of adult stages of worms with pre-linearized plasmid DNAs, encoding different reporter genes, was used for random integration approach. Electroporated worms were recovered and grown in a controlled media and random integration was assayed for DNA insertion, RNA transcription and expression of tagged reporter proteins. We have in this way successfully created transgenic *A. ceylanicum* expressing different cellular or secreted reporters. CRISPR/Cas9-mediated knock-in of reporter genes, in adult and eggs stages of *A. ceylanicum,* was used for targeted transgenesis*.* We explored different sets of Cas9:gRNA complexes, with either two or three overlapping guide RNAs, and multiple genome safe harbors for estimation of Cas9 efficacy (scaring) and knock-in experiments. We used different donor DNAs containing reporter genes, under the control of CMV promoter followed by signal peptides and BGH poly (A) sequence at 3' end, with short homology arms (50bp) for homologous recombination into *A. ceylanicum* genome. We evaluated different delivery methods for CRISPR/Cas9-mediated targeted mutagenesis. Neon electroporation system was more successful for A. *ceylanicum* eggs, while lipofection using RNAiMAX methodology was preferred for adult worms. Eggs were plated NGM media having OP50 or HT115 *E. coli* and developed to transgenic larvae, while adult worms were in RPMI media and assayed for protein expression by further growth in fresh starved RPMI media. Evaluation of collected samples for transgenesis was done by PCR, Sanger and NGS sequencing of amplicons and western blot analyses for expressed reporters. Our results indicate overall successful transgenesis of *A. ceylanicum* worms with a use of different and stage specific methods. Our future work will further explore use of *A. ceylanicum* as a novel and tractable model parasitic nematode system for genetic and molecular biology manipulation.

Benzimidazole inhibiting *Haemonchus contortus* tubulin dynamics by structural intradimer changes observed by *in silico* modeling

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Haemonchus contortus has multiple hosts like sheep, goats, and cattle that it can feed on, but it can also develop resistance to many classes of anthelmintics. Anthelmintic benzimidazoles (BZ) are used frequently for the treatment of *H. contortus* infections. Benzimidazoles are microtubule (MT) inhibitors and disrupt the dynamic balance between polymeric MT and dimeric tubulin. Microtubules participate in multiple cellular activities, including axonal transport, regulation of neuron ventral processes, maintaining the cellular matrix, endo- /exocytosis, and cell division.

We employed structural modeling to uncover the molecular mechanism underlying the inhibition of *H. contortus* MT polymerization by albendazole (ABZ), using the *Caenorhabditis elegans* tubulin structure (PDB: 6E88; tba-2/tbb-2) as a template to generate *H. contortus* tubulin dimers (tba-1/tbb-1). Furthermore, three tubulin dimers each with a single nucleotide polymorphism (SNP) associated with BZ-resistance (tba-1/tbb-1 F167Y, tba-1/tbb-1 E198A and tba-1/tbb-1 F200Y) were used. Tubulin dimers were modeled with ABZ and post-analyzed by calculating the binding Gibbs free energy (ΔG) with generalized born and surface area solvation (MM/GBSA).

The determined ΔG of ABZ binding to *H. contortus* tubulin dimer variants was ΔG_{WT} = -61.7 kcalmol^{-1}, ΔG_{F167Y} = -75.0 kcalmol^{-1}, ΔG_{E198A} = -73.7 kcalmol^{-1}, ΔG_{F200Y} = -25.9 kcalmol^{-1} which indicates that despite the SNPs ABZ can bind to β-tubulin. Further, it was identified that ABZ changes the secondary structure at β-sheet 4 (S4), S6, S8, S9, α-helix 7 (H7), and H8 of β- tubulin. Through changes of S8, S9, H7, and H8, the flexible movement of the intermediate domain between the α- and β-tubulin monomers could be inhibited. Also, there were changes at the intradimer loops αT5, βT7, and βT8 that could also inhibit intradimer movement. It is known that the tubulin dimer dynamic between a “straight” or “curved” state is dependent on the H6, H7, H8, S8, S9 and the βT7 loop of β-tubulin. We hypothesize that ABZ inhibits the transition of the “curved” to the “straight” dimer conformation as only the “straight” dimer can be polymerized to MT. Lastly, the introduction of the SNPs overall did not reduce the binding affinity of ABZ for tubulin. Our results indicated that E198 appears to be essential to bind the nitrogen group of the imidazole ring of ABZ. Any modification at this residue could lead to an inability for ABZ to interact with E198 and lead to resistance.

Uncovering the mechanism of action of Schistosome Paralysis Factor

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Schistosomes are parasitic flatworms that infect over 250 million people worldwide, causing the neglected tropical disease, schistosomiasis. The drug praziquantel is currently the only treatment available to combat schistosomiasis. Praziquantel is only effective on the adult life stage of the worm and there are reports of praziquantel-resistant schistosome strains emerging. Therefore, finding new treatments not only to control but to prevent infections is critical. Previous work from our lab described the purification and chemical characterization of a novel tetracyclic alkaloid named Schistosome Paralysis Factor (SPF). SPF is produced by a rotifer species that colonizes the parasite's snail intermediate host and paralyzes the schistosome's infective larvae (cercariae). Therefore, SPF is an attractive candidate molecule for preventing schistosomiasis; however, the mechanism of action of SPF remains unknown. Given that the chemical structure of SPF contains a serotonin motif, and based on serotonin's function as a neurotransmitter, we hypothesized that this alkaloid interacts with a G protein-coupled receptor(s) (GPCRs). Screening of a neurotransmitter ligand library revealed that several dopamine (but not serotonin) receptor agonists induced cercarial paralysis, although higher concentrations were needed to achieve the same paralytic effect observed with SPF. To test if the parasite's dopaminergic GPCRs are indeed the target of SPF, we cloned the most highly expressed dopamine receptor in cercariae and confirmed GPCR activation by SPF using a luciferase-based cell assay. Our work suggests that SPF may induce cercarial paralysis via interaction with a dopaminergic receptor.

Identification of an endogenous current evoked by praziquantel in schistosome neurons

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Many anthelmintic agents subvert targets that control parasite neuronal and/or muscular function [1, 2]. One good example is the drug praziquantel (PZQ), the key clinical agent for combating schistosomiasis, which causes a rapid spastic paralysis of schistosome musculature. PZQ application causes a rapid, enhanced Ca^{2+} entry and a sustained, increase in tension. However – to the best of our knowledge – endogenous currents evoked by PZQ have never been recorded in any parasitic flatworm. One possible reason for this is simply the technical challenge of measuring endogenous currents in native tissue, with little insight into what signal to look for, where exactly to look and conditions to best optimize recording of the signal.

Here, we report the identification of an endogenous current evoked by PZQ that was recorded electrophysiologically from neuronal tissue within a live, immobilized, adult schistosome. In recordings made from either the cephalic ganglia, or peripheral nerve structures, application of PZQ (10μM) caused a rapid increase in current, with resolvable single channel like fluctuations. No PZQ-evoked responses were seen in similar recordings from muscle, or tegument-derived vesicles. The biophysical signature, and pharmacology properties, of the native PZQ-evoked conductance were consistent with the properties of *Schistosoma mansoni* TRPMPZQ defined in a heterologous expression system [3]. This methodology for recording native currents from excitable cells within a living worm will enable further studies of ion channels in parasitic flatworms.

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Exploring the nature of RNA secretion by *Strongyloides ratti*

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Parasitic helminths are responsible for a range of debilitating neglected tropical diseases (NTDs). Overreliance on a limited anthelmintic armoury has exacerbated the threat of drug resistance in nematodes, highlighting an urgent need to develop novel control and monitoring strategies. Diagnostic techniques for strongyloidiasis and other soil-transmitted helminth diseases are rudimentary and predominantly dependent upon stool-based microscopy approaches, with limited sensitivity and specificity, underpinning the need to identify new biomarkers and diagnostic methods. Extracellular RNAs are of clinical interest as biomarkers in human diseases such as cancer. Helminths secrete immune modulating micro (mi)RNAs into their host environment; these could serve as diagnostic biomarkers, aiding in development of more effective diagnostic techniques which would support ongoing parasitic disease elimination efforts. We focus on the model species *Strongyloides ratti*, as a flexible system for profiling the dynamics of helminth-derived miRNAs in host biofluids. We will present data on miRNA secretion by *S. ratti*, showing detection of miRNAs in extracellular vesicles and in host samples.

Complement Regulation in Fascioliasis: Insights from Serpin Inhibition of MBL-associated serine proteases (MASPs)

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Complement response is the first-line innate host defence against invading organisms and is activated *via* Classical, Lectin and Alternative pathways. The Lectin pathway (LP) is initiated by sugar arrays on pathogens' surfaces, resulting in damage and elimination of the pathogen. Recently, we showed that the invasive stage of *Fasciola hepatica,* newly excysted juveniles (NEJs), survives in normal human serum by exclusively inactivating the complement LP. In the present study we sought to understand how serine protease inhibitors (Serpins) secreted by NEJs (rFhSrp1 and rFhSrp2), inhibit the MBL-associated serine proteases (MASP-1 and MASP-2), the key initiators of the LP. The ability of the rFhSrp1 and rFhSrp2 form complex and inhibit the rMASP-1/2 were assessed by ELISAs, pull-down, SDS-Page, biochemical assays, and Mass spectrometry (MS). rFhSrps effect on coagulation was determined by inhibition of thrombin, plasmin and Factor XIII. Similar to live *F. hepatica* NEJs, incubation of either rFhSrp1 or rFhSrp2 with normal serum leads to LP selective inhibition (>90%). Nevertheless, time-course co-incubation of these serpins with rMASPs showed rFhSrp1 high efficiency in binding and cleaving MASPs, and led to the characterization of the suicidal mechanism of inhibition between rFhSrp1 and MASP-1 and -2. Here we uncovered a novel mechanism by which rFhSrp1 inactivates MASPs to make the invading parasite refractory to killing *via* the LP. The importance of such complement regulation during *F. hepatica* infection is stressed by a redundant inhibition of MASP-1 and -2, as inactivation of MASP-1 alone would completely prevent LP response. Furthermore, inhibition of coagulation factors by rFhSrp1 highlights the close relationship between complement and coagulation regulation during fascioliasis.

An array of PCR-RFLP markers for differentiating between *Schistosoma haematobium* and *S. bovis* in field settings

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Parasitic blood flukes in the genus *Schistosoma* are known to hybridize in the laboratory. Hybridization between *S. haematobium*, a human parasite, and *S. bovis*, a cattle parasite, is of particular concern due to its potential implications for zoonotic transmission and the complex strategies required to control both human and animal pathogens. Multiple genotyping studies have identified *S. bovis* mitochondrial DNA in miracidia carrying *S. haematobium* ribosomal DNA, leading to a widespread assumption that hybridization between these species occurs commonly. By comparison, studies employing a large number of autosomal markers, including whole-genome single nucleotide variants and microsatellites, have failed to identify early generation hybrids between *S. haematobium* and *S. bovis*, and instead find that these species are strongly differentiated. We aimed to develop a simple field applicable approach, utilizing Restriction fragment length polymorphisms (RFLPs), to distinguishing S. *haematobium* and *S. bovis* in the field. We analyzed whole-genome sequence data from 167 S. haematobium and *S. bovis* samples collected across Africa and reference genome of both species to design RFLP loci for species differentiation. We identified 269,779 single nucleotide variants distinguishing the two species and generated 34 candidate autosomal RFLP markers. These markers were further tested in the laboratory using a combination of field and lab-derived *S. haematobium* and *S. bovis* samples. Our specific intention is to use these markers to distinguish *S. bovis* and *S. haematobium* cercariae shed from *Bulinus spp*. snails collected in the field. More generally, we believe that genotyping these autosomal SNPs (using RFLP or more high throughput approaches) will allow more effective differentiation of *S. haematobium* and *S. bovis* compared to markers (COX, ITS, 18s) currently in use.

Conservation of the small RNA pathways in chromosome level genomes of platyhelminthes

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Single stranded non-coding RNAs of 20 to 30 nucleotides long are key mediators in smallRNA pathways (SRP) that underlie essential biological processes. Previously, we have described the conservation and diversification of mi- and si-RNA pathway genes in all platyhelminthes, and the complete absence of the piRNA pathway in all neodermatans, while present in free-living species. We also reported the expansion of key effector genes like FL-Agos (a highly divergent Argonaute 2 of flatworms), amplified in all parasitic species, and Dicer-2, ancestrally duplicated in food borne trematodes (FBT). Recently, there has been an improvement in the quantity and quality of platyhelminthes genomes with several of them assembled at chromosome level (i.e. 14 of the 46 flatworm species in the last release of Wormbase Parasite, and other flatworm chromosome level assemblies still not deposited in the database).

In the present work, we took advantage of these resources to study the genomic conservation of key SRP differentially amplified genes. We used orthologous predictions and genomic level tBLASTn to reanalyze the small RNA pathway related genes in the new assemblies. We also focused on the expression profiles of these SRP genes analyzing their expression in the most recent single-cell and bulk RNA-seq data available.

Remarkably, the greatest argonaute class gene expansion, with more than 20 copies of FL-Agos is found in the new assembled genomes of *Trichobilharzia species*, a significant difference compared to the one copy found in the previous genome draft. Interestingly, 2 FL-Agos were conserved in Schistosomidae and other trematodes. Additionally, only one FL-Ago is consistently expressed in proliferating cells (neoblasts, stem and germline) along the life cycle of *Schistosoma mansoni* in single-cell data. It could be possible that similar expression preference occurred in other parasitic flatworms however additional single cell data from other species is needed.

Furthermore, a second notable gene expansion with at least 4 copies of dicer-2 class genes, was also found in *Trichobilharzia spp.,* a significant improvement compared to what we found in the previous draft. Interestingly, two closely placed copies of dicer-2 are consistently conserved in cestodes, monogenea and food borne trematodes, but only one copy is conserved in all the *Schistosoma species*. These results are showing a remarkably divergent evolutionary path at least for SRP within the blood flukes.

Molecular surveillance detects high prevalence of the neglected parasite *Mansonella ozzardi* in the Colombian Amazon

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Mansonellosis is an undermapped insect-transmitted disease caused by filarial nematodes that are estimated to infect hundreds of millions of people globally. Despite their prevalence, there are many outstanding questions regarding the general biology and health impacts of the responsible parasites. Historical reports suggest that the Colombian Amazon is endemic for mansonellosis and may serve as an ideal location to pursue these questions in the backdrop of other endemic and emerging pathogens. We deployed molecular and classical diagnostic approaches to survey *Mansonella* prevalence among adults belonging to indigenous communities along the Amazon River and its tributaries near Leticia, Colombia. Deployment of a loop-mediated isothermal amplification (LAMP) assay on blood samples revealed an infection prevalence of ~40% for *Mansonella ozzardi*. This assay identified significantly more infections than thin blood smear microscopy or LAMP assays performed using plasma, likely reflecting greater sensitivity and the ability to detect low microfilaremias or occult infections. *Mansonella* infection rates increased with age and were higher among males compared to females. Genomic analysis confirmed the presence of *M. ozzardi* that clusters closely with strains sequenced in neighboring countries. We successfully cryopreserved and revitalized *M. ozzardi* microfilariae, advancing the prospects of rearing infective larvae in controlled settings. These data suggest an underestimation of true mansonellosis prevalence, and we expect that these methods will help facilitate the study of mansonellosis in endemic and laboratory settings.

Advancing Parasitology Research and Sustainability: A Case Study of the Molecular Parasitology Lab in Galway, Ireland.

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The Molecular Parasitology Laboratory in Galway, Ireland is committed to conducting sustainable research practices while studying fundamental biology of parasites and developing strategies to diagnose parasitic infections and treatments. The lab is developing novel diagnostic tests and vaccines for parasites, leveraging molecular, bioinformatics, and genetic tools along with advanced technology for vaccine manufacturing. This effort benefits farmers by reducing their reliance on chemical treatments, ultimately saving money and effort. Certified by the My Green Lab organisation, the lab is reducing its carbon footprint and CO2 emissions by implementing sustainable laboratory practices.

The lab's sustainability initiatives include using energy-efficient equipment, reducing single-use plastics, and optimizing laboratory protocols to minimize waste generation. The lab is also recycling and properly disposing of hazardous waste. By adopting green lab practices, the Molecular Parasitology Laboratory in Galway is making significant contributions to sustainable scientific research and promoting a healthier planet.

This poster will discuss the challenges and opportunities of implementing sustainable practices in laboratories, including energy efficiency, water conservation, waste management, and responsible procurement. Successful case studies and best practices in laboratories will be showcased, demonstrating the feasibility and benefits of adopting sustainable practices. The Molecular Parasitology Laboratory will be used as an example to highlight its efforts in reducing energy consumption, water use, and plastic waste.

The team aims to inspire other laboratories to adopt similar sustainable practices to reduce their environmental impact and contribute to a culture of environmental responsibility in research. Ultimately, the poster hopes to promote a shift towards a more sustainable and responsible future for laboratories, contributing to the preservation of the environment for future generations. The sustainable practices implemented by the Molecular Parasitology Laboratory can serve as a model for other laboratories to follow, helping to create a more sustainable future for the scientific community and the planet.

Transcriptional profiles and functional insights into the Aspartyl Proteases Cathepsin D-like of *Schistosoma mansoni*

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Schistosomiasis mansoni is a disease caused by *Schistosoma mansoni*, a parasite that requires diverse mechanisms to enable its development in different environments and hosts. The parasite possesses ten genes with the eukaryotic aspartyl protease (PF00026) domain, here named Sm*cd*, which are involved in digesting red blood cells in the flatworms' gut, representing a gene expansion compared to the other *Schistosoma* species. Adult worm single-cell data revealed enriched expression of *Smcd1*(Smp_013040) and *Smcd2*(Smp_136730) in gut cells, while *Smcd3*(Smp_346370) is enriched in late/early vitellocytes. In schistosomula, *Smcd4.2*(Smp_132470), *Smcd4.4*(Smp_309540), and *Smcd4.5*(Smp_335890) are enriched in Meg4+cells, and *Smcd1* in cells from positional muscle and parenchymal. Publicly available RNAseq data demonstrated that only in females from mixed infections, SmCD3 shows increased expression from the (21^{st} to 38^{th} dpi), while the increased expression of Sm*cd*1 and Sm*cd*2 is observed since 21dpi, regardless of the presence of males. Experimentally, we verified that Sm*cd*1 and Sm*cd*2 expression occurs mainly in adult female worms. To functionally characterize these two targets, we knocked- down these two targets, individually and in combination, in schistosomula and adult worms, with significant reductions of transcripts in schistosomula. Reduced hemoglobin degradation was observed in *Smcd1* and *Smcd2*-knocked-down schistosomula when cultured with human erythrocytes, without affecting viability. Knocked-down schistosomula were used in experimental infections in mice. The knockdown did not affect the number of recovered worms, or the maturation of eggs retained in the final portion of the small intestine. However, phenotypic changes such as reduction in length and formation of hemozoin pigment in the digestive tract were present in adult female worms exposed to specific dsRNAs individually and in combination. Confocal microscopy analysis showed the absence of eggs in the reproductive tract, a reduction in the ovary area, and differences in the maturation of reproductive cells, with a considerable decrease in mature oocytes. Furthermore, there was a significant decrease in the number of eggs retained in the liver of the animals, indicating a decrease in oviposition. In conclusion, SmCDs are likely involved in parasite metabolism and sexual maturation, possibly participating in worm nutrition and development.

The role of nanos in germ cell regulation and regeneration in *Hymenolepis diminuta*

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Tapeworms are known to regenerate thousands of proglottids from their neck and each segment forms all male and female reproductive structures. Not much is known about how germ cells are specified and maintained in the hermaphroditic reproductive proglottids, and the role germ cells play in regeneration. Tapeworms are known to express a germ cell regulator called *nanos*; however, *nanos* is also expressed in somatic stem cells in other worms like schistosomes and acoels. Our goal is to examine the role of *nanos* in germ cell and/or stem cell regulation in the rat tapeworm *Hymenolepis diminuta*. We performed a descriptive analysis of *nanos* expression at progressive stages of reproductive development using whole mount in situ hybridization. We find that *nanos* is expressed throughout the whole neck, the genital anlagen, and gonads of both sexes. To ascertain if *nanos*+ cells in the neck bear hallmarks of germ cells, we performed double fluorescent in situ hybridization with putative germ cell markers that are known to be expressed in the gonads. All gonadal markers we examined are coexpressed with *nanos* in the neck. This suggests that *nanos* expression in the neck marks early germ cells. Interestingly, one gene (*protocadherin*α) is expressed in a subset of *nanos*+ cells indicating heterogeneity within the *nanos*+ population. Excitingly, the double positive cells are confined to the most anterior part of the neck, where regenerative ability is most pronounced. We do not know if this double positive population represents the most undifferentiated germ cells or a population of pluripotent stem cells. We are currently conducting RNA interference to silence *nanos* and *protocadherin*α and determine their effect on germ cells, the general stem cell population, and the propensity of the tapeworm to regenerate. Our study will resolve the role(s) of *nanos* and help demystify how germ cells influence tapeworm regeneration.

Wnt signaling and A-P axis patterning in the tapeworm, *Hymenolepis diminuta*

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The rat tapeworm, *Hymenolepis diminuta*, is a tractable model to study parasite stem cells, which underlie the development, regeneration, transmission, and potentially the pathology of tapeworms including those capable of infecting humans. In *H. diminuta,* the neck is the only regeneration-competent region with proglottids forming at the posterior end of the undifferentiated neck. Previous studies showed that regeneration competency is anteriorly biased along the neck. Using RNA sequencing, we uncovered polarized gene expression patterns along the neck A-P axis including many components of Wnt signaling. What is the relationship between Wnt signaling, regeneration, A-P axis patterning, and the stem cell population within the undifferentiated neck? We mined *H. diminuta* transcriptomes for putative homologs of known Wnt signaling components from *Schmidtea mediterranea* using BLAST. There are six *Wnts*, two β-*catenins*, five *frizzled* receptors, two secreted frizzled related proteins, and three *dishevelled* homologs in *H. diminuta*. Through whole mount in situ hybridization (WISH), we find there is a strong posterior enrichment of *Wnt-11* and *Wnt-11b*. *Wnt-5b* expression is at the lateral edges but is also strongly enriched at the posterior neck. *Wnt-4* is anteriorly enriched, but the Wnt inhibitor, *SFRP-1*, is expressed alongside *it*. Taken together, WISH data indicates that there is a posterior bias in Wnt signaling consistent with its conserved role in maintaining posterior identity. Similar to *S. mediterranea*, we find that Wnt signaling components are expressed in muscle cells. However, the muscle is not the only tissue harboring polarized gene expression patterns in the neck indicating an additional level of complexity in tapeworm position control gene regulation. We are currently pursuing loss-of- function experiments with both RNAi and drug inhibitors to understand the relationship between Wnt signaling, regeneration, and axis patterning within *H. diminuta*. Understanding the role Wnt signaling plays in the rat tapeworm will potentially unlock answers to the mysteries of cellular fate and polarity within helminths.

Morphological and molecular identification of gastrointestinal parasites reveals species diversity in wild capuchin monkeys (*Cebus imitator*)

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The accurate and precise identification of gastrointestinal parasites is vital to understanding their true taxonomic diversity and evolutionary relationships with their hosts. Microscopy paired with morphological identification has been the gold standard in parasitology for decades; however, recent advances in molecular methods equip researchers to identify parasites to the species and even the "strain" level. This, in turn, allows researchers to explore parasitic communities and parasite-host evolutionary dynamics with more detail than ever before. This study aims to build off these recent advances in molecular parasitology by examining gastrointestinal parasite communities in a population of wild, habituated capuchin monkeys (*Cebus imitator*) inhabiting Sector Santa Rosa of the Área de Conservación Guanacaste (ACG), Costa Rica. The main goals of this project were to 1) use traditional microscopy and morphological methods to explore the parasitic communities in our host population; 2) further expand on our morphological findings by pairing them with molecular parasite identification methods; and 3) explore which factors may affect patterns of parasitic infection and transmission in this host population. To address these goals, we developed a 'morphology-to- molecular' parasite identification pipeline in which we identified parasites in ethanol-preserved fecal samples using microscopy and then used molecular barcoding of the ITS-2 genetic marker to identify those same individual parasites genetically. These methods revealed 9 parasitic morphotypes in our samples, including the highly prevalent lungworm *Filariopsis barretoi* and the threadworm *Strongyloides sp.* Molecular barcoding assigned close genetic matches for parasites we were unable to identify using morphology alone, revealing a diversity of species likely obtained as byproducts of the capuchin diet. We also found that one of the 5 capuchin social groups studied had, on average, higher infection intensities than the others and that patterns of infection for the two most prevalent parasites, *Filariopsis* and *Strongyloides*, may be influenced by host age and sex, though patterns of infection differ between the 2 species. These results demonstrate the additional insight that molecular methods can provide in parasitology studies and highlight the importance of pairing morphological and molecular methods when possible.

Lack of Host Sex and Infection Mode Effect on *Brugia pahangi* Gene Expression

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The disproportionate infection of males with lymphatic filariasis has been long observed in human populations as well as in the laboratory models. Because of this difference in infection, typically only male gerbils are used for rearing *Brugia malayi* and *Brugia pahangi* in the laboratory. Here, we sought to determine if the sex of the vertebrate host affects the gene expression of the nematode comparing it for both *B. pahangi* recovered from both the intraperitoneal (IP) and the subcutaneous (SQ) infection models, referred to as IP worms and SQ worms, respectively. *B. pahangi* larvae were injected into the intraperitoneal cavity of 7 male and 7 female gerbils while *B. pahangi* larvae were injected into 22 male and 32 female gerbils subcutaneously. All 7 IP-injected female gerbils and 6 of the IP-injected male gerbils were successfully infected. Out of the 22 SQ-injected male gerbils and 32 SQ-injected female gerbils, 18 male gerbils and 8 female gerbils developed a successful infection. RNA was isolated and sequenced from adult male nematodes, adult female nematodes, and microfilariae from 3 gerbils from each host- sex/infection mode combination, using male/female litter mate pairs when possible for a total of 36 samples. Reads were mapped to our previously published *B. pahangi* genome. Reads were counted using gene models predicted with BRAKER using the OrthoDB v10 metazoan protein sequence database and transcriptome sequencing data, including the Illumina and ONT direct RNA sequencing reads. Putative functions were assigned to the gene models using HMMER/hmmscan, InterProScan 5.56-89.0, RNAmmer 1.2, tRNAscan-SE 2.0.3, TMHMM 2.0, RAPSearch2, BioCode, and Atrributor. As expected and consistent with previous results, life stage-specific differentially expressed genes could be identified. However, statistically significant differentially expressed genes were not identified that were associated with infection mode or host-sex. SQ worms exhibited more intersample variation than IP worms, which has likely limited our ability to identify transcriptional differences in comparisons made with SQ worms from both male and female gerbils. This transcriptional diversity of SQ worms is important to consider in future experimental design.

Development of the nervous system in embryonic *Biomphalaria glabrata,* an intermediate host of *Schistosoma mansoni*.

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The host-parasite relationship between the snail *Biomphalaria glabrata* and *Schistosoma mansoni* has been extensively studied, but less is known regarding its nervous system, especially that of embryonic and juvenile stages. Potential unique elements of the embryonic nervous system may represent targets for control of the snail intermediate host. We have traced the development of the embryonic nervous system using antibodies to select neurotransmitters, specifically serotonin and FMRFamide. Data so far indicate that the serotonergic and peptidergic nervous system starts developing just a few days after egg-laying, and a complex network of neurons and branching axons is present in the headfoot region prior to hatching at seven-eight days of age. Elements of both a central and peripheral nervous system are present and organization of the embryonic nervous system is distinct from that of the adult ganglionic nervous system. Furthermore, serotonin regulates rotational behavior of the embryos that may be important for survival. The findings of transcriptomic analyses for various stages of embryonic development that are currently underway will also be presented; it is expected that the RNAseq data will indicate the presence of additional neurotransmitters as well as molecular determinants of embryonic development.

Study of the metabolic capacity of the host gut microbiome reveals a likely dual role for intestinal bacteria in the pathophysiology of hepato-intestinal schistosomiasis

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The inflammatory granulomatous response triggered by parasite eggs trapped in host tissues underlies the pathophysiology of schistosomiasis. A finely regulated parasite egg-host immunity interplay is essential for both an efficient egg excretion and to avoid potentially fatal immunopathology. However, over the last few years, evidence has emerged of the likely contribution of a third player – the host gut microbiota – in the immunological cascade that culminates with the formation of schistosome egg-induced intestinal granulomas. In this study, we implemented shotgun metagenomic sequencing to investigate the impact of *Schistosoma mansoni* (*Sm*) infection on the gut microbial functional capacity of two mouse lines [i.e., wild type (WT) and human-microbiota-associated (HMA)] displaying vast differences in gut microbiota composition at baseline, as well as different susceptibility to infection. Comparisons of the gut microbial functional profiles between *Sm*+ and *Sm*- samples revealed infection-associated alterations in both lines, which included partially overlapping findings between WT and HMA. Consistent findings across different rodent hosts point towards a likely connection between *S. mansoni* infection and the host gut microbiome. In particular, our results suggest that *S. mansoni* and host gut bacteria might have co-evolved to limit helminth-induced tissue damage *via* enhanced production of tryptophan metabolites and butyrate, and subsequent activation of AhR (aryl hydrocarbon receptor) signaling and further butyrate-regulated pathways. These findings offer a novel and intriguing perspective of the role(s) of the host gut microbiome in schistosomiasis, as they add new knowledge to the previously raised hypothesis that the gut microbiota may contribute to *Schistosoma* egg-induced intestinal pathology. Together, data from this and previous studies hint toward a potential dual role for the gut microbiome in the pathophysiology of schistosomiasis, where intestinal bacteria may contribute to egg-associated intestinal pathology while, in turn, protect the host from excessive tissue damage.

Identification of *Brugia malayi* miRNAs involved in *Wolbachia*-Host symbiosis

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Parasitic filarial nematodes *Wucheria bancrofti*, *Brugia malayi*, and *B. timori* are the causative agents of the debilitating infectious disease lymphatic filariasis. These parasites are dependent on their endosymbiotic bacteria *Wolbachia,* which are essential for filaria survival within the insect vector and human hosts. While *Wolbachia* are a promising drug target for anti-filarial treatment, the mechanism of symbiosis between *Wolbachia* and nematode still requires further investigation. Here we focus on filarial microRNAs and their regulatory role in *Wolbachia-Brugia* symbiosis. We compared the expression of parasite miRNAs in adult female *B. malayi* worms mock-treated or doxycycline-treated for 2-3 days to target *Wolbachia*. We detected 48 *B. malayi* miRNAs that were significantly downregulated in treated worms as compared to control worms, suggesting that they could be playing a role in worm interactions with *Wolbachia*. Using a miRNA target prediction algorithm (miRanda), we identified potential *B. malayi* target genes for these miRNAs and selected those belonging to pathways most affected by anti-*Wolbachia* treatment. Particularly, the autophagy pathway, which protects bacteria in host cells and is activated by anti-*Wolbachia*; and the apoptosis pathway, as anti-*Wolbachia* treatment induces apoptosis in worm embryos. Using a dual luciferase reporter assay, we validated that bma-miR-5864 and bma-mir-86 have functional seed sites on the UTRs of CED-5 (apoptosis) and autophagy genes BEC-1 and EPG-3. These miRNAs are highly expressed in healthy adult female worms. We used miRNA-mediated interference to silence these genes and observed that inhibition of selected miRNAs significantly induced lysosomal activity, decreased *Wolbachia* loads, and induced apoptosis in developing embryos of treated worms as compared to control worms treated with a scrambled miRNA inhibitor. Our study suggests that miRNAs that are highly expressed in adult female worms downregulate in part lysosomal activity in worm cells, allowing *Wolbachia* to escape autophagy and survive in the worm host. Increasing our overall understanding of the *Wolbachia*-filaria mutualistic relationship opens exploration of symbiotic pathways as more focused drug targets for control of lymphatic filariasis.

Analysis of infrapopulation genetic diversity and sibship reconstruction in *Onchocerca volvulus* using single microfilariae whole-genome sequencing

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Analysis of the composition and genetic diversity of parasite infrapopulations is crucial to assess the infection burden, evaluate the impact of control interventions, and understand transmission dynamics. *Onchocerca volvulus* is a filarial nematode, the agent of river blindness, an important neglected tropical disease targeted for elimination. Adult worms reside in subcutaneous nodules and are only accessible by surgery and collagenase digestion of nodules. Larval parasites, microfilariae, migrate through the skin and are routinely detected to monitor and evaluate elimination programs. However, microfilariae contain limited quantities of DNA for genome sequencing, hampering population genomic studies. To resolve this challenge, we used isothermal strand displacement amplification, and successfully generated high-quality whole-genome sequencing data from 181 single microfilariae obtained from 10 infected persons from Ghana, Liberia, and the Democratic Republic of the Congo, achieving a mean coverage of 95, 100, and 74% of the nuclear, mitochondrial, and the *Wolbachia* endosymbiont genomes, respectively (minimum depth of 10x). We determined which individual microfilariae were siblings using 3.2 million SNP variants based on their identity-by-descent (IBD) allele-sharing patterns. By analyzing the relatedness among the microfilariae, we made inferences about the number of genetically distinct adult worms contributing to the infection. In addition, we differentiated the infrapopulation of a host who migrated from another region (from Nabu to Kpassa in Ghana), suggesting that fine-scale genetic epidemiology is possible using a sufficient coverage of nuclear genome data. Our approach forms a basis for genomic epidemiology in *O. volvulus* that can reveal spatiotemporal changes in transmission dynamics and genetic variation within and among hosts due to human migration or interventions.

Impact of schistosome infection on tissue microbiomes of *Biomphalaria* snails

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The microbiome plays a significant role in shaping various aspects of host biology and can affect the transmission of pathogens by vectors such as mosquitoes and snails. In a previous study, we showed that the hemolymph of *Biomphalaria* spp. snails harbor a diverse microbiome that is distinct from the water environment, and can discriminate snail species and populations. We then investigated the heterogeneity of the microbiome present in different snail organs (ovotestis, liver, gut, and stomach) bathed by the hemolymph. Sequencing the V4 16S rDNA revealed specific organ microbiomes with lower bacterial diversity than in hemolymph or in whole snail microbiomes. The phylogenetic distance between organ microbiomes was correlated with the physical distance of the organs, and the whole snail microbiome was found to be a composite of the different organ and hemolymph microbiomes. This result emphasizes the importance of sampling individual organ microbiomes to provide a complete picture of the whole snail microbiome. As *Biomphalaria* snails are schistosome parasites vectors, we then explored the potential interactions between the *B. glabrata* microbiomes and the blood fluke *Schistosoma mansoni*. We hypothesized that schistosome infection can alter the snail microbiome composition and abundance over the course of the infection. We generated two cohorts of 110 snails, exposed to the parasite or not, and sampled their hemolymph and liver during the pre-patent and patent periods. We characterized the microbiome of all samples and found that schistosome infection has little effect on the alpha-diversity (within snail) and beta-diversity (between snail) indices. This indices were relatively stable over time for both uninfected and infected cohorts. Sample type (hemolymph vs. liver) was the main driver of the differences observed. This study demonstrates the importance of sampling snail tissues for effective examination of the snail microbiomes, but reveals limited impact of schistosome infection on microbiome composition in this exciting medically-important molluscan model system.

Developing a molecular toolkit to study synthesis of Schistosome Paralysis Factor by the rotifer *Rotaria sp*.

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The rotifer *Rotaria sp.* colonizes the shell of *Schistosoma mansoni*'s molluscan intermediate host *Biomphalaria glabrata.* Previous work showed this rotifer produces a novel tetracyclic alkaloid, Schistosome Paralysis Factor (SPF), which paralyzes schistosome infective larvae and can prevent infection of a mammalian host. To elucidate the mechanisms by which the rotifer produces this compound, we are creating a molecular toolkit for studying this animal. We have developed markers to characterize diverse cell and tissue types as well as in situ hybridization techniques to analyze spatial patterns of gene expression. These markers reliably label the musculature, nervous, osmoregulatory, reproductive, glandular, and intestinal systems. To enable functional analysis, we are developing techniques to perform RNA interference (RNAi) via feeding bacterially expressed double-stranded RNAs. We will present promising preliminary results that RNAi can be used to inhibit gene expression in these animals. This work paves the way for us to uncover the mechanisms by which *Rotaria sp.* produces SPF.

An optimised *Ascaris suum* biofluid peptidomics pipeline to aid understanding of extrasynaptic transmission in nematodes

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The historical exploitation of nematode neuromusculature as a target for parasite control motivates continued drug discovery efforts focused on nematode neurobiology. Neurotransmitters have the potential to signal either at the synapse between adjacent neurons or extrasynaptically. Through exploiting the experimental tractability of adult *Ascaris suum* we have started to explore the potential for extrasynaptic neurotransmission via nematode pseudocoelomic fluid (PCF), employing LC-MS/MS pipelines to uncover the PCF peptidome (DOI: 10.1021/acschemneuro.1c00281). Here we present an optimised *Ascaris* biofluid peptidomics pipeline integrating a refined post LC-MS/MS analysis workflows (PEAKS) to enhance the sensitivity, accuracy, and confidence in peptide detection. We expanded our custom *Ascaris* peptide library beyond *A. suum* predicted mature peptides, as employed by Atkinson et al. (2021), to also include *A. suum* predicted prepropeptides and all *A. suum* predicted proteins (WBPS17; genome assemblies: ASM18702v3 and AscSuum_1.0_submitted).

Integration of the updated *A. suum* prepropeptide library into the LC-MS/MS analysis workflow has expanded the *A. suum* PCF peptidome to reveal: (i) the detection of 47 peptides at <1% False Discovery Rate (FDR) in adult females (n=6; 6 FMRFamide like peptides, 8 Neuropeptide Like proteins, 33 Antimicrobial peptides), representing a ~5 fold increase in total number of peptides (<1% FDR) detected relative to Atkinson et al. (2021), (ii) the detection of peptides that do not align with the canonical neuropeptide processing model; (iii) FLP-6 as a dominant *A. suum* PCF neuropeptide that was consistently detected with high confidence (-10logP score>200; FDR <0.01) across both male and female PCF samples and (iv) amidated versions of FLP-18 and FLP-6.

Subsequent integration of the predicted protein library facilitates: (i) a further ~10 fold increase in detected peptide numbers [406 proteins detected (<1% FDR) in female PCF; n=6] (ii) the identification of novel neuropeptide-encoding genes; (iii) the characterisation of sex-specific and abiotic stressor induced differences in *A. suum* PCF profiles.

The expansion in *Ascaris* PCF neuropeptide profiles described here will seed experiments to examine the functional relevance of specific PCF signallome components. Improved understanding of the extrasynaptic facet of nematode neurosignalling will aid in the identification and validation of novel drug targets for parasitic nematode control.

The role of p53 homologs in the parasitic flatworm *Schistosoma mansoni*

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Schistosomes are blood-dwelling parasitic flatworms that are responsible for the devastating neglected tropical disease schistosomiasis, afflicting over 200 million of the world's poorest people with debilitating chronic disease that sometimes even leads to death. One of the most remarkable features of these parasites is their incredible longevity in the hostile environment of the host's circulation. Despite being surrounded by host defense mechanisms such as the immune system and the blood clotting pathways, schistosomes an survive more than 30 years inside a human host. It is not known exactly how these parasites are capable of this resilience, but it is thought to be in part mediated by the parasite's skin-like tegument. The tegument is a syncytium that covers the entire surface of the parasite, acting is the interface between the parasite and the host and contributing to important parasite-specific processes such as nutrient uptake and immune evasion.

While studying factors that are required for normal tegument development, we identified a pair of P53 homologs in the schistosome genome. One homolog (*p53-1*) was orthologous to human TP53/63/73 as well as Smed-p53 while the other homolog (*p53-2*) was found only in other parasitic flatworms. Functional studies revealed that *p53-1* was required for the maintenance of the majority of the parasite's stem cells as well as tegument production. In contrast, *p53-2* was required for the normal response to genotoxic stress. That the parasite-specific p53 ortholog has 'tumor suppressor' like behavior but the TP53 ortholog does not suggests that the genotoxic stress response function of p53-2 evolved convergently. Our ongoing work seeks to identify the mechanisms by which these schistosome p53 homologs regulate tegument biology, stem cell biology, and the cell death pathway in these important parasites.

Mapping resistance-associated anthelmintic interactions in the model nematode *Caenorhabditis elegans*

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Parasitic nematodes infect billions of people and are mainly controlled by anthelmintic mass drug administration (MDA). While there are growing efforts to better understand mechanisms of anthelmintic resistance in human and animal populations, it is unclear how resistance mechanisms that alter susceptibility to one drug affect the interactions and efficacy of drugs used in combination. Mutations that alter drug permeability across primary nematode barriers have been identified as potential resistance mechanisms using the model nematode *Caenorhabditis elegans*. We leveraged high-throughput assays in this model system to measure altered anthelmintic susceptibility in response to genetic perturbations of potential cuticular, amphidial, and alimentary routes of drug entry. Mutations in genes associated with these tissue barriers differentially altered susceptibility to the major anthelmintic classes (macrocyclic lactones, benzimidazoles, and nicotinic acetylcholine receptor agonists) as measured by animal development. We investigated two-way anthelmintic interactions across *C. elegans* genetic backgrounds that confer resistance or hypersensitivity to one or more drugs. We observe that genetic perturbations that alter susceptibility to a single drug can shift the drug interaction landscape and lead to the appearance of novel synergistic and antagonistic interactions. This work establishes a framework for investigating combinatorial therapies in model nematodes that can potentially be translated to amenable parasite species.

The study of germinal cells in Taenia crassiceps

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One of the factors involved in the infective capacity of the flatworms is the totipotent cells called "neoblasts" in planarians and "germ" in cestodes, which have the capacity for self-renewal and differentiation and have been considered a cell population with functions like stem cells. In addition, it has been studied that the generation of newly differentiated cells depends exclusively on the proliferation and differentiation of this type of cell. The depletion of germinal cells with hydroxyurea (HU) or irradiation affects the regeneration of the parasites and when isolated germ cells are injected again into the worms, they recover their regenerative capacity.

In vitro, assays were performed to evaluate the role of germ cells, in T. crassiceps cysticerci. The viability and morphology changes were evaluated using 25 and 40mM of HU for 3 and 6 days, and then were cultured in fresh medium for 3 and 6 days to observe recovery from cisticercy in mobility and morphology, as well as if the proliferative cells replicated measured by using EdUstain as proliferation marker

Our data shown morphology changes in size, shape, and number of evaginated cysticerci in the 40 mM dose, and the mobility is less than that of the controls after three days of treatment in the cysticerci treated with HU both concentrations and on day 6 of recovery only 1-2 cysticerci have mobility in cysticerci treated with 25 mM HU. The number of proliferating cells shown a significant difference between the cysticerci treated with HU at a concentration of 40 and 25 mM and the controls and the presence of proliferating cells begins to recover after 3 and 6 days of recovery in medium without HU at the concentration of 25 mM. The proteomic analysis shows differences in the intensity of some bands, mainly at 75 and 25 kDa, and with MS-LC identifies the proteins that present a high or low expression concerning the controls and if are related to germ cells or cell proliferation processes.

Drug development for pseudophyllidean cestodes

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The drug praziquantel (PZQ) is the primary treatment for infections caused by parasitic flatworms, however, higher dosages are needed to treat certain cestodes. A target for PZQ was recently identified in schistosomes, a transient receptor potential ion channel in the melastatin subfamily ($TRPM_{PZQ}$); however, little is known about the properties of $TRPM_{PZQ}$ in other flatworms. $TRPM_{PZQ}$ orthologs were scrutinized in all currently available parasitic flatworm genomes and functionally profiled; $TRPM_{PZQ}$ is present in all parasitic flatworms, and the consensus PZQ binding site is well conserved. Three loci of variation were identified across the parasitic flatworm $TRPM_{PZQ}$ pocketome, including an acidic residue in the TRP domain. This residue acts as a gatekeeper, impacting PZQ residency within the $TRPM_{PZQ}$ ligand binding pocket. Functional profiling of trematode and cestode $TRPM_{PZQ}$ orthologs revealed differing sensitives to PZQ, matching the varied sensitivities documented clinically. In trematodes and cyclophyllidean cestodes, which display high sensitivity to PZQ, the gatekeeper TRP domain residue is an aspartic acid, allowing for nanomolar activation by PZQ. However, the presence of a glutamic acid residue, found in pseudophyllidean cestode $TRPM_{PZQ}$, was associated with lower PZQ potency. For example, functional profiling of a pseudophyllidean $TRPM_{PZQ}$ channel from *Spirometra erinaceieuropaei (Se.*$TRPM_{PZQ}$) revealed only micromolar potency toward PZQ (EC_{50} = 2.1 μM) compared with nanomolar potency of cyclophyllidean $TRPM_{PZQ}$ orthologs (for example, *Mesocestoides corti* $TRPM_{PZQ}$, EC_{50} = 120 nM). When mutating this gatekeeper glutamic acid back to an aspartic acid within the *S. erinaceieuropaei* $TRPM_{PZQ}$ backbone, higher sensitivity to PZQ was evident. Effort to identify new therapeutics that, unlike PZQ, better tolerate variation at this acidic TRP domain residue would be immensely valuable for improving treatments against pseudophyllidean cestodes – where there currently exists a poor range of effective medications.

Exploring microbial natural products as sources of novel anthelmintics using advanced phenotypic screens

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Microbial natural products have historically been successful sources of antiparasitic compounds, and the advent of new methods and technologies for natural product discovery has reinvigorated the potential to probe this chemical space. We hypothesize that microbes that share ecological relationships with nematodes are likely to have evolved pathways that produce secondary metabolites with anthelmintic properties and thus, are an excellent source of novel chemistry when screening for new antiparasitic drugs. We believe that systematically leveraging this resource is an important step to combating the limitations of our current anthelmintics and the growing concerns of resistance.

To predict *in vivo* efficacy of natural products against parasitic nematodes, we have developed an advanced phenotyping pipeline for whole organism screening. This multivariate pipeline integrates measures of motility, development, viability, and fecundity to assess the effects of microbial isolates and their extracts against both model nematodes and filarial parasitic nematodes. We have applied this pipeline to the screening of hundreds of isolates and extracts sourced from diverse ecological backgrounds including soil, plant, insect, and marine environments. In a screen of 200 bacterial isolates, ~5% inhibited growth of the model nematode *C. elegans,* and crude extracts derived from these hits exhibited a variety of effects on different filarial nematode species and life cycle stages, including macrofilaricidal effects. Among 480 fractions from 120 marine-organism extracts, we found five extracts with fractions that were highly active against both *C. elegans* and microfilariae.

To determine the compounds responsible for these effects we are relying on dereplication techniques which draw on sequencing, LC-MS/MS, and NMR data to further characterize these leads. Additionally, we are developing follow up strategies that include toxicity assays, screening throughput expansion, genetic screening for target identification, and assay extension to more nematode species and clades to fully determine the broad spectrum potential of these findings.

Genetic differences underlie variation in anthelmintic responses in *C. elegans* wild strains

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Anthelmintic drugs are our most powerful means to control parasitic nematode infections. However, resistance to these drugs threatens parasite control efforts, and little is known about the molecular and genetic mechanisms involved in resistance. Most of what we know about mechanisms of resistance comes from studies of a single strain within a single species, the laboratory-adapted strain of *Caenorhabditis elegans* called N2. However, a single genetic background cannot capture the enormous diversity present in the entire species, nor can it predict how natural populations of parasitic nematodes will respond to a drug. Here, we used genetically diverse *C.elegans* strains to perform dose-response analyses across 26 anthelmintic drugs that represent the three major anthelmintic drug classes (benzimidazoles, macrocyclic lactones, and nicotinic acetylcholine receptor agonists) in addition to seven other anthelmintic classes. First, we found that *C. elegans* strains displayed similar anthelmintic responses within drug classes and significant variation across drug classes. Second, we compared the effective concentration (EC) estimates and slope estimates of each dose-response curve of each strain to the laboratory reference strain. This enabled the identification of anthelmintics with population-wide differences to understand how genetics contribute to anthelmintic resistance. Because genetically diverse strains displayed differential susceptibilities within and across anthelmintics, we show that *C. elegans* is a useful model for screening potential nematicides. Third, we quantified the proportion of phenotypic differences attributable to genetic causes (heritability) for each drug. These results suggest drugs to prioritize in genome-wide association studies, which will enable the identification of anthelmintic resistance genes.

Transcriptomic analysis identifies potential novel targets for filarial control

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Filarial worms pose a heavy burden on the health of humans and animals worldwide. A small number of drugs are available to limit infection, however the growing threat of drug resistance makes it important to identify novel targets for filarial control. *Brugia pahangi* and *Dirofilaria immitis* are two closely related nematodes that cause filariasis in cats and dogs, respectively. The establishment of infection is dependent on the development of the infective larvae (L3) to L4 within the animal host and subsequently to adult worms. Despite decades of research, the molecular mechanisms underlying filarial nematode developmental processes are poorly understood.

Parasite transcriptomic analyses have enabled the prediction of novel drug targets. We are using *B. pahangi*, maintained in our laboratory by passage through gerbils (*Meriones unguiculatus*) and mosquitoes (*Aedes aegypti*), as a model for the dog heartworm *D. immitis*, for which no small animal model is available. RNA-seq data were generated for different life cycle stages of *B. pahangi* (larval L3-0h, L3-24h, L3-d5, L4-d10, adult males and females, microfilariae). Our differential expression analyses identified a small number of genes upregulated following infection of the mammalian host. Validation of these as possible targets and screening of potential therapeutics would benefit from an improved *in vitro* culture system. To this end, optimized development of *B. pahangi* L3 to L4 *in vitro* was achieved by addition of 75 uM ascorbic acid (AA) at day 5, demonstrating the importance of vitamin C for filarial larval molting.

Some of the developmentally regulated genes are novel to filarial nematodes and direct manipulation is required to identify their functions and help validate the potential of using our transcriptome data for identifying novel control targets. Silencing of control and target genes is being attempted by the delivery of either double stranded RNA or heterogeneous short interfering RNA for RNA interference. While providing proof of principle, this approach requires further optimisation for robust interrogation of gene function and determination of any potential phenotypes for the genes of interest.

Development of a Novel TRPM$_{PZQ}$ Activator to Target Liver Fluke

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Praziquantel (PZQ) is the mainstay for treatment of parasitic flatworm infections. After ~40 years of clinical usage, a putative target for PZQ was identified: a transient receptor potential ion channel in the melastatin subfamily (TRPM$_{PZQ}$). This channel is conserved across all profiled parasitic flatworm species, and *in vitro* potency of PZQ at each TRPM$_{PZQ}$ ortholog mirrors the known clinical sensitivity of each parasite to PZQ. Notably, *Fasciola* spp. are insensitive to PZQ mirroring the lack of activation of *Fasciola* spp. TRPM$_{PZQ}$ by PZQ *in vitro*. Unique residues in the binding pocket of TRPM$_{PZQ}$ are responsible for this insensitivity, and this insight prompted the development of molecules that could tolerate this natural variation. Given the increasing resistance of *Fasciola* spp. to triclabendazole, the discovery of new fasciolicidal targets would be timely. A target-based screen of small molecules against both *Sm*.TRPM$_{PZQ}$ and then *Fh*.TRPM$_{PZQ}$ resulted in the identification of a series of chemotypes that activate both channels. The pharmacophore most potent at *Fh*.TRPM$_{PZQ}$ was selected for further development. A library of molecules was synthesized to interrogate structure-activity relationships around the core of this molecule resulting in **S55**, a new molecule with submicromolar potency at both channels. When applied *ex vivo* to *Schistosoma mansoni*, **S55** evoked a rapid contraction with concomitant tegument damage, phenocopying PZQ. Likewise, **S55** produced an identical phenotype on triclabendazole-sensitive and triclabendazole-resistant *Fasciola hepatica*. **S55** was non-toxic in HepG2 assays and is active and well-tolerated *in vivo*, significantly reducing *Schistosoma* worm burden in infected mice. Given these results, the data evidence *Fh*.TRPM$_{PZQ}$ as a druggable target and the feasibility of designing drugs that accommodate natural variation found in different parasitic flatworm TRPM$_{PZQ}$ channels.

Leveraging the Human Druggable Genome to Uncover Therapeutic Targets in the Parasite *Schistosoma mansoni*

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Schistosomiasis is a neglected tropical disease (NTD) caused by parasitic trematodes of the genus *Schistosoma*, affecting more than 200 million of the world's poorest people, leading to ~250,000 deaths, while causing significant morbidity. Despite drug discovery efforts, there is still no vaccine and treatment relies on a single drug, praziquantel (PZQ), as it has for >40 years. While PZQ is safe, inexpensive, and reasonably effective, it has pharmaceutical and pharmacological liabilities. Therefore, it is of paramount importance to develop alternatives to PZQ. However, the natural biology of schistosomes presents a significant barrier to the development of new therapies, as the size of schistosomes makes them poor candidates for phenotypic screening, and a lack of facile tools has made identification and functional validation of potential drug targets difficult.

Our laboratory has leveraged the [Human] Druggable Genome to generate a comprehensive list of potential therapeutic targets within schistosomes, whereby we characterize candidates using a large-scale RNA interference (RNAi) pipeline to identify phenotypes affecting neuromuscular function, tissue integrity, and parasite survival. Using *in silico* criteria, we then assigned priority to potential therapeutic targets. Of particular interest to our drug discovery efforts are members the ubiquitin-mediated proteasomal degradation system (UPS). Each arm of the ubiquitin system (activating, conjugating, and ligating enzymes), deubiquitinating enzymes, chaperones, and the various subunits of the proteasome produced rapid effects detrimental to the health of adult worms in culture, as well as survival and disease pathology *in vivo*. It has been established that it is possible to develop parasite-specific proteasome inhibitors despite encoding structurally and functionally similar complexes to the mammals they infect. We are currently in the process of screening for inhibitors of two targets within the UPS, a chaperone, P97, and the proteasome itself, with the ultimate goal of developing alternative therapeutics to PZQ.

Parasite-derived Extracellular RNAs as Novel Biomarkers for *Strongyloides* Diagnosis

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Diagnosis of Strongyloidiasis and soil-transmitted helminths (STHs) is predominantly restricted to stool-based microscopy approaches. Sensitivity and specificity of these methods are typically poor. New diagnostic methods are essential to monitor the success of ongoing disease elimination efforts. Extracellular RNAs (exRNAs) have been proposed as effective biomarkers for a range of conditions in human medicine, including cancer, metabolic diseases and infections. exRNA research, in the context of parasite diagnostics is in its infancy yet has shown great potential in detecting parasite infection but has not been tested in delineating the intensity and stage of disease. Many studies have shown that microRNAs in particular are released into host tissue and circulation, potentially to facilitate host immune modulation and parasite virulence. Characterising this mode of host-parasite communication could yield a multitude of effective disease-linked biomarkers. We have exploited *Strongyloides ratti* as a model for the identification of parasite-derived miRNAs in and the dysregulation of host miRNAs in host plasma and immune tissues during infection. Ongoing experiments aim to characterise the plasma miRNome across various stages of infection and characterise the impact of parasite-derived miRNAs on host gene expression.

Identification of filarial parasite biomarker candidates in extracellular vesicles isolated from culture media and plasma

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Extracellular vesicles (EVs) are important for the interaction between parasitic helminths and their hosts. Filarial parasites survive in their hosts for many years and cause important diseases in humans such as lymphatic filariasis (LF), onchocerciasis and loiasis. EVs released by filarial parasites may carry antigen suitable as a biomarker, but reliable protocols for isolation and proteomic analysis need to be evaluated. The main objectives of these studies were to establish consistency of EV isolation in cultured microfilariae (MF) and to identify novel biomarkers in EVs isolated from host plasma. Using the ME EV isolation kit by Biosynth, EVs were isolated from cultured *B. malayi* MF. In addition, EVs were isolated from one gerbil and one cat, both infected with *B. malayi,* and sera from 10 human subjects with loiasis. Patients with loiasis were selected due to their high MF densities (between 29,120 and 81,120 MF/ml) which are not typical of other filarial species. A more in depth bioinformatics analysis was done to remove the host proteins that were identified with LC-MS to identify proteins unique to *B. malayi* or *L. loa* and to identify parasite proteins and Gene Ontology (GO) terms enriched in EVs. EVs isolated from in vitro MF culture supernatants contained more than 300 *B. malayi* proteins with high consistency across biological replicates, including the known MF excretory antigen BmR1 (AF225296). Proteins specific to *B. malayi* were identified in the gerbil and cat plasma. Enrichment analysis was completed on 56 proteins identified in both infected gerbil and cat plasma. There were significant enrichment (FDR-adjusted P values ≤ 0.05) for molecular functions GO terms including many related to hydrolase activity or binding activity (ribonucleotides, nucleotides, ions, etc.). EVs isolated from plasma from persons with loiasis contained over 20 *L. loa* proteins. Of these, 9 proteins were supported by at least 5 unique peptides and 7 with spectral counts above 10. One protein (an orthologue of BmR1) was detected all 10 samples of subjects with loiasis. This study has shown that EVs released by filarial parasites in vitro and in vivo contain parasite proteins that can be detected by highly sensitive mass spectrometry. With this, new biomarker candidates for both LF and loiasis have been identified and will be explored in future work across other filarial parasites.

Molecular Helminthology 2023
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