Parasitic Helminths: New Perspectives in Biology and Infection

Hotel Bratsera, Hydra, Greece

Abstracts for Poster Session 1

4 September 2023

POSTER SESSION 1: MONDAY 4 SEPTEMBER 6:00 – 8:00 PM

1	**Kelsilandia AGUIAR MARTINS**	Royal Veterinary College - University of London	Genetic analysis of *Schistosoma mansoni* pre-and post-treatment from a high morbidity (FibroSchot) hotspot within Lake Albert, Uganda.
2	**Annia ALBA**	University of Perpignan	How the environment influences the transmission of zoonotic diseases: linking abiotic and biotic factors to the vectorial capacity of an intermediate host snail
3	**Georgia BALDWIN**	University of Manchester	Ym1 as a Regulator of IL-17
4	**Luke BECKER**	Malaghan Institute of Medical Reasearch	Methods for the evaluation of the viability and infectivity potential of reanimated cryopreserved hookworm larvae used for human therapy.
5	**Ilaria BELLINI**	Sapienza University of Rome	Exploring pathogenicity and tumorigenic potential of the nematode *Anisakis* using human intestinal organoids and extracellular vesicles
6	**Sarah BUDDENBORG**	Wellcome Sanger Institute	Optimisation of single cell, nuclei, and spatial RNA-seq in nematodes
7	**Geraldine BUITRAGO**	University of Strathclyde	The parasitic worm product ES-62 protects the osteoimmunology axis in a mouse model of obesity-accelerated ageing.
8	**Alice COSTAIN**	University of Manchester	Tissue damage and microbiota modifications provoke intestinal Type 2 immunity during *Schistosoma mansoni* infection
9	**Padraic FALLON**	Trinity College Dublin	Retinoic acid-related orphan receptor alpha (RORα) is required for generation of Th2 cells during *Nippostrongylus brasiliensis* infection
10	**Peter FISCHER**	Washington University School of Medicine	A *Paragonimus kellicotti* cysteine protease recognized by IgG4 antibodies of infected humans is found in extracellular vesicles produced by the parasites in vitro and in the lung cysts
11	**Sandra GAVA**	Oswaldo Cruz Foundation, Fiocruz	The effect of Aspartyl Proteases Cathepsin D-like knockdown in *Schistosoma mansoni* schistosomula and adult worms

12	**Richard GRENCIS**	University of Manchester	Neutrophil modulation of immunity during chronic intestinal helminth infection: Haptoglobin regulation of B cell responses
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14	**Kelly HAYES**	University of Manchester	Genetic manipulation of the parasitic nematode *Trichuris muris*
15	**Malcolm KENNEDY**	University of Glasgow	What mediators might the p43 immunomodulatory proteins of *Trichuris spp.* deliver to the infection site?
16	**Xeusong LI**	Justus-Liebig-University, Giessen	Identification of GPCR-neuropeptide interactions and their functional analyses in *Schistosoma mansoni*
17	**Maria Cristina LOADER**	St George's University London	Frequency of soil-transmitted infections in tuberculosis patients in the Peruvian Amazonian city of Iquitos and impact on markers of lung tissue destruction in TB-helminth co-infection

1

Genetic analysis of *Schistosoma mansoni* pre- and post- treatment from a high morbidity (FibroSchot) hotspot within Lake Albert, Uganda.

KELSILANDIA AGUIAR MARTINS[1], EDRIDAH TUKAHEBWA[2], MOSES ADRIKO[2], MARTIN WALKER[1], JAMES A. COTTON[3], MATT BERRIMAN[3], SHONA WILSON[4], JOANNE P. WEBSTER[1]

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Schistosomiasis is a major neglected tropical disease of profound medical importance, infecting approximately 240 million people, 90% living in sub-Saharan Africa. Severe intestinal schistosomiasis is associated with periportal fibrosis and portal hypertension, which cause severe morbidity and even mortality without appropriate disease management. The mainstay of current control and elimination efforts is based on mass drug treatment programs, using praziquantel, of at-risk population groups as school children. Despite community treatment coverage of > 80% in Uganda, the prevalence and associated morbidity of schistosomiasis remains very high among communities on the shores of Lake Albert. In an effort to both understand the aetiology, and mitigate against the morbidity, the FibroScHot consortium is currently conducting a clinical trial in this area aimed to determine the effect of increased frequency of praziquantel to school children. To evaluate the genetic basis co-related with the treatment effectiveness, we used microsatellite markers from 13 previously published *S. mansoni* microsatellite loci on miracidia hatched from individual school-children during baseline and different timepoints after praziquantel treatment. We discuss our results in terms of their theoretical and applied implications.

2

How the environment influences the transmission of zoonotic diseases: linking abiotic and biotic factors to the vectorial capacity of an intermediate host snail

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The environment is a major component of host-parasite interactions that can influence the ecological fitness, immune reactivity/virulence of hosts/parasites and the ultimate compatibility and transmissibility of the infection. The impact of fasciolosis on livestock and as a zoonosis worldwide has been on the rise, related among other factors, to different environmental perturbations including biological invasions. For instance, the flash expansion of *Pseudosuccinea columella* snails from North America to other world regions (reaching Europe and spreading in France) boosted *Fasciola* transmission in the invaded areas through successful parasite spill-backs. Noteworthy, although *P. columella* is one of the main intermediate hosts of *F. hepatica* with most individuals being susceptible (S) to infection, a few field-occurring populations (in Cuba) are strictly resistant (R; 0% prevalence) to the parasite at the expenses of a fitness cost. By profiting from this exceptional model, we aim at answering how abiotic and biotic (in the field and within the snails) factors associate with the susceptibility/resistance and invasive capacity of *P. columella* and how the immune competence of the snail varies depending on the environment. Ecological characterization (*in situ* measurement of ecological factors), eDNA (from water and sediment) and snail sampling in contrasted environments from Cuba (highly contrasted sites where resistant and/or susceptible *P. columella* occurs), France (introduced susceptible *P. columella* in natural habitats) and Zimbawe (invasive susceptible *P. columella* in an artificial lake) were carried out. Microsatellite markers and 16S metabarcoding analyses were used to describe the population genetics of *P. columella* and the trematode fauna, food resources and microbial communities to snail population/each site, and were associated with the ecological factors to depict differential ecological patterns. RNAseq analyses were used to assess the immune competence of the snails in the different environmental settings. Field derived-laboratory populations from Cuba (R and S) and France (S) were stablished up to the F2. Experimental infections with *F. hepatica* were carried out and parasitological indexes (prevalence, redial intensity and cercarial shedding) and life traits parameters (survival, fecundity and fertility) were measured for assessing vectorial competence and invasive potential (= vector capacity) of the susceptible population from France compared to the susceptible/invasive and resistant/non-invasive from Cuba. Transcriptomic analysis at different time points of exposure as wells as shifts in microbial communities from field to lab conditions, and following the exposure were carried out, compared among populations/phenotypes and associated with the ecological and infective traits. From this, we gauge the contribution of the environment in shaping the differential patterns of resistance, infection and/or invasion of *P. columella.*

3

Ym1 as a Regulator of IL-17

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Ym1 is a key component of the host response to helminth infection. In addition, it is substantially upregulated in many inflammatory settings, being implicated in IL-17 induction and tissue remodelling. Nevertheless, the exact functions and mechanisms of action of Ym1 remain to be defined. Therefore, we generated a Ym1 knockout (KO) mouse line to enable us to assess the different aspects of Ym1 biology. During infection with lung migrating nematode *Nippostrongylus brasiliensis*, Ym1 KO mice display a trend towards reduction in IL-17 producing γδ T cells in the lungs, when compared with their wild-type counterparts. This, alongside a reduction of total bronchoalveolar lavage (BAL) cells in infected Ym1 KO mice, supports a role of Ym1 in the initial IL-17-dependent immune response. As most of our current understanding of Ym1 induction of IL-17 stems from type-2 immunity models, we employed exogenous LPS delivery as an additional model to understand the interactions of Ym1, γδ T cells and IL-17 production. Mice treated intraperitoneally with LPS together with Ym1 showed increased recruitment of neutrophils and monocytes to the peritoneal cavity, when compared with those that received LPS alone. Furthermore, Ym1 was found to enhance IL-17 production by γδ T cells when peritoneal exudate cells were stimulated *in vitro.* Importantly, Ym1 fails to enhance IL-17 production, *in vitro*, on purified γδ T cells, suggesting it acts through a yet unidentified player. Thus, our results suggest that Ym1 promotes myeloid cell migration into inflamed tissues via indirect enhancement of IL-17 production by γδ T cells.

4

Methods for the evaluation of the viability and infectivity potential of reanimated cryopreserved hookworm larvae used for human therapy.

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With the increased interest in experimental human hookworm infection comes the need for better techniques to evaluate viability and infectivity potential of hookworm. We assessed several methods for evaluating viability of reanimated cryopreserved hookworm larvae. These include thermal stimulation, dye exclusion and lipid energy reserves. We found that thermal stimulation is a simple but effective method for assessing larval motility on a small scale. The fluorescent viability dye (SYTOX™ Green) provides a rapid and scalable system for quantifying non-viable larvae and can be useful screening worms against various treatments including shelf life, temperature, drug titration, storage media and methods of decontamination. The lipid dye was useful in indicating development and growth during pre- and post-manipulation. In conclusion, these assays have the potential for semi-automated medium throughput and could be incorporated as part of quality assurance to produce larvae used in clinical studies.

5

Exploring pathogenicity and tumorigenic potential of the nematode *Anisakis* using human intestinal organoids and extracellular vesicles

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Anisakiasis is an epidemiologically and medically underestimated accidental fish-borne zoonosis. Its chronic form can potentially lead to erosive ulcers, granuloma formation and chronic inflammation at the human gastro-intestinal tract, features known to be involved in the onset of a carcinogenic microenvironment. In this regard, case reports of gastric or intestinal tumors in co-occurrence with anisakiasis are increasing from endemic countries. Therefore, investigations on the tumorigenic potential of *Anisakis* need to be addressed. Our work centers on the study of *Anisakis*-human interactions through a comparative transcriptomic approach using: i) the human intestinal organoids (HIO), a bi or three-dimensional cutting-edge model exhibiting the architecture and functionality of the organ of origin; and ii) a newly discovered *Anisakis* messenger of pathogenicity, the extracellular vesicles (EVs). EVs are nano-scaled particles involved in cell-cell and inter-species communication, having a new role in host-pathogen interface. EVs content is characterized by lipids, proteins, nucleic acids such as miRNAs, which are involved in complex regulatory networks by modulating host's genes expression. Analysis of data revealed top 100 abundant transcripts in the HIO and 7 differentially expressed genes in HIO treated with *Anisakis* EVs. Among them, transcripts showing potential link to cancer processes have been detected. In particular, a downregulation of *EPHB2* and a upregulation of *NUPR1* emerged and, interestingly, these alterations have been associated to colorectal cancer in the literature. In addition, qRT-PCR on inflammatory products (*Il33, Il8, Il1β*) showed a decreasing trend in *Il33* gene expression, an increasing trend in *Il1β* and no alteration in *Il8*, a dynamic previous described as involved in helminths infection chronicity. This project represents the first attempt to investigate *Anisakis* tumorigenic potential using HIO and EVs, revealing interesting outcomes that we hope will open new ways for the study of anisakiasis and its potential consequences in humans.

6

Optimisation of single cell, nuclei, and spatial RNA-seq in nematodes

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Single cell/nuclei RNA-seq (sc/snRNA-seq) and spatial transcriptomics (spRNA-seq) are increasingly used to study gene activity in all the cells of a sample, identify and differentiate cell types, and map cellular locations within tissues. Several challenges must be overcome to apply these techniques in nematodes, such as limitations of sample collection and preparation, efficient cuticle breakdown, and large and small debris removal. We have systematically tested and optimised single nuclei and single cell isolation procedures with consideration to cost, availability of reagents/equipment, and type of starting material (fresh, flash-frozen, fixed) while limiting dissociation-induced artefacts. We focused on documenting key quality control steps and best practices for optimal recovery of viable cells/nuclei. To test these protocols and techniques, we used different developmental stages of the ruminant gastrointestinal nematode *Haemonchus contortus*. In parallel, we have tested two spatial transcriptomics approaches to be integrated with the sc/snRNA-seq data. To support users accessing and analysing sc/snRNA-seq and spRNA-seq data, we have also developed publicly accessible simple and easy to use analytical pipelines that can automate data processing from raw data through to cell clustering and trajectories. These data and pipelines will provide standards for sc/snRNA-seq and spRNA-seq that can be applied to future studies with nematodes and other helminths.

7

The parasitic worm product ES-62 protects the osteoimmunology axis in a mouse model of obesity-accelerated ageing.

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Significant increases in human lifespan over the past decades have not been accompanied by similar improvements in healthspan. Reflecting this, the widespread adoption of a high calorie 'Western' style diet (HCD) has seen a rising incidence in metabolic ageing-associated co-morbidities, including T2 diabetes, osteoporosis, obesity, stroke, and cardiovascular disease. Previously, we demonstrated that ES-62, derived from the secretome of the filarial worm *Acanthocheilonema viteae,* and its drug-like synthetic small molecule analogues (SMAs) can improve the healthspan of C567BL/6 mice undergoing obesity-accelerated ageing, positively impacting a number of metabolic, inflammatory, and gut microbiome parameters, and also increasing median lifespan in male animals. Intriguingly, with respect to the latter, ES-62 also demonstrates a male-specific protective effect against the HCD-accelerated ageing-associated loss of trabecular bone that is essential for the haematopoiesis-supporting bone marrow niche. We are now able to further report on the impact and potential mechanisms of ES-62 in the context of the bone marrow niche. In our most recent studies, we provide evidence of protection against age-related cellular senescence in cells of haematopoietic and mesenchymal lineage after exposure to ES-62. Treatment with ES-62 limits obesity-accelerated ageing-related decline in trabecular bone architecture and protects against the associated BM myeloid/lymphoid bias in male, but not female, mice. Reflecting this, ES-62 maintained B cell populations in male BM, and this was associated with increases in IL-10-producing Bregs in the spleen and mesenteric lymph nodes. Sexual dimorphism relating to mesenchymal cell lineages was also apparent, with ES-62 reducing BM adipocyte numbers only in male HCD-fed mice. ES-62-based SMAs recapitulated the key findings, attesting to the translational potential of these molecules in maintaining osteoimmunological homeostasis in an ageing cohort.

8

Tissue damage and microbiota modifications provoke intestinal Type 2 immunity during *Schistosoma mansoni* infection

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During mammalian infections with *Schistosoma mansoni,* hundreds of parasite eggs rupture across the intestinal wall and into the lumen. Although this destructive process is central to schistosomiasis-associated pathology, there are limited studies exploring the impact of egg transit on the intestinal environment, including barrier integrity, the microbiota, and mucosal immunity. Herein, we provide a high-resolution image of the intestinal interface during murine schistosomiasis, using a combination of egg-producing vs non egg-producing, and high vs low dose infections. We reveal a significant increase in intestinal permeability during patent infections, with evidence for systemic responses to gut bacteria, and infection intensity altering the kinetics of intestinal 'leakiness'. Due to the vigorous anti-parasitic response elicited by schistosome infection, it has proven notoriously challenging to extract live immune cells from schistosome-infected murine intestine, with researchers often using the mesenteric lymph nodes (MLNs) as a proxy for intestinal responses. Here, we show egg-producing infections to induce Type 2 dominated immune responses in the colonic lamina propria and MLN, as evidenced by increased Th2 cell cytokine production, transcription factor expression, and enhanced proportions of alternatively activated macrophages and DCs. We highlight discrepancies between colonic and MLN responses, and show the dramatic Type 2 shift to coincide with alterations in microbiota composition. Finally, through the use of germ free mice and faecal transplants, we provide evidence that the schistosome infection-associated microbiota can promote the emergence of unique MLN immune populations, and can impact the outcome of unrelated distal inflammation. In ongoing work, we aim to further dissect the immune environment during schistosomiasis and determine which bacterial species (and/or their products) contribute to schistosome-mediated immunomodulation.

9

Retinoic acid-related orphan receptor alpha (RORα) is required for generation of Th2 cells during *Nippostrongylus brasiliensis* infection

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Nippostrongylus brasiliensis elicits a potent type 2 innate and adaptive immune response that leads to worm expulsion after primary infection and renders mice resistant to secondary infection. The innate and adaptive type 2 response that mediates rejection involves respective roles for ILC2 and Th2 cells. The transcription factor RORα is expressed by ILC2 with ILC2 deficient ($Rora^{fl/fl}$Il7raCre) mice demonstrating essential roles for ILC2 in the generation of a functional type 2 response to lead to worm rejection. As IL-7Ra is expressed on ILC2 and lymphocytes, there are potential roles for both Rora-expressing ILC2 and CD4 cells in these mice. Using Rora-YFP reporter mice, *N. brasiliensis* infection increased Rora+ILC2 and led to the expansion of Rora-expressing $GATA3^{+}$CD4 T (Th2) cells in the lung indicating a role for Rora in Th2 cellular development. We therefore generated mice with conditional and inducible cell-specific deletion of RORα in ILC2 and CD4 cells. While CD4-specific Rora-deficient ($Rora^{fl/fl}$CD4Cre) had significantly reduced frequency of Th2 cells, but not ILC2, in the lungs following *N. brasiliensis* infection, they had intact expulsion of worms following primary and secondary infection. Tamoxifen mediated selective deletion of ILC2 ($Rora^{fl/fl}$Id2CreER^{T2} mice) or CD4 ($Rora^{fl/fl}$Cd4CreER^{T2} mice) cells during primary infection confirmed functional roles for Rora-expressing ILC2, but not CD4 cells, in N*. brasiliensis* infection. This study demonstrates a new role for RORα in the generation of Th2 cellular development during pulmonary inflammation following nematode infection that could be relevant to the range of inflammatory lung diseases in which RORα is implicated.

10

A *Paragonimus kellicotti* cysteine protease recognized by IgG4 antibodies of infected humans is found in extracellular vesicles produced by the parasites *in vitro* and in the lung cysts

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Paragonimiasis is a foodborne trematode infection that affects about 23 million people and leads frequently to chronic cough with fever and hemoptysis. This lung fluke infection can be efficiently treated with praziquantel, but diagnosis is often delayed because of confusion with other lung diseases such as lung cancer or tuberculosis. North American paragonimiasis is caused by *Paragonimus kellicotti*, and represents an excellent model for other *Paragonimus* species. We used mass spectrometry to identify targets present in extracellular vesicles produced by *P. kellicotti* for antibody and antigen detection assays. We maintained adult worms *in vitro* or in gerbils where they induced lung cysts. Proteomic analysis of the purified vesicles produced *in vitro* or in the lung cyst fluid detected 548 and 8 proteins, respectively. A cysteine protease (Cp-6, MK050848) was the most abundant protein in the cyst and frequently detected after *in vitro* culture. Immunohistology studies using antibodies against recombinant Cp-6 showed localization in tegument and the suckers of adult *P. kellicotti.* Analysis of the lung cyst tissue showed also strong labeling for Cp-6, often in the vicinity of eggs. Analysis of immunoprecipitates of *P. kellicotti* adult worm antigens using sera of infected humans identified CP-6 as a major immunoreactive antigen. IgG4 antibodies reactive with recombinant Cp-6 were found in all 17 subjects tested with proven paragonimiasis but not in 23 subjects with other helminth infections. A lateral flow IgG4 antibody test using Cp-6 as antigen is currently under development. Our study identified CP-6 as promising target for both antibody and antigen detection assays to improve diagnosis of human paragonimiasis.

11

The effect of Aspartyl Proteases Cathepsin D-like Knockdown in *Schistosoma mansoni* schistosomula and adult worms

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Schistosomiasis mansoni is caused by *Schistosoma mansoni*, a parasite that requires diverse mechanisms to develop in different environments and hosts. The parasite possesses ten genes with the eukaryotic aspartyl protease (PF00026) domain, here named *Smcd*, which are involved in digesting red blood cells in the flatworms' gut, representing a gene expansion. 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 *Smcd3* shows increased expression (21st to 38th dpi) only in females from mixed infections, while *Smcd1* and *Smcd2* expression increases since 21 dpi, regardless of the presence of males. Experimentally, we verified that *Smcd1* and *Smcd2* expression occurs mainly in adult female worms. We knocked-down these two targets, individually and in combination, in schistosomula and adult worms for functional characterization, with significant reductions (~99.9%) 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 mouse infections. 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, recovered adult female worms presented reduced length and hemozoin pigment formation in the digestive tract. Confocal microscopy analysis showed the absence of eggs in the reproductive tract, reduced ovary area, and altered maturation of reproductive cells, with a considerable decrease in mature oocytes. Furthermore, there was a significant decrease in the number of eggs retained in mice livers, indicating a decrease in oviposition. In conclusion, SmCDs are likely involved in parasite metabolism and sexual maturation, possibly participating in worm nutrition and development.

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Neutrophil modulation of immunity during chronic intestinal helminth infection: Haptoglobin regulation of B cell responses

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Chronic infection by mouse whipworm *Trichuris muris* infection presents with low numbers of parasites, driven by a Type 1 cytokine immune response, regulated by interleukin 10. We initially defined the molecular profile of secreted intestinal mucus at the site of chronic *Trichuris* infection. A striking observation was the presence of raised haptoglobin (Hp) levels in mucus following infection. Hp is an acute phase protein and binds free haemoglobin restricting oxidative damage. Hp protein levels were also elevated in the *T. muris* infected intestinal tissue, as was the expression of *Hp* mRNA. Upregulation of *Hp* mRNA was localised to intestinal neutrophils, which showed a distinct transcriptional profile to those from non-infected mice or bone marrow derived neutrophils. Low level infection of *Hp* null mice did not alter worm numbers, but displayed marked alteration of antibody responses to *T. muris*. Infected *Hp* null mice displayed a reversal of the parasite specific antibody profile seen in WT mice (characterized by a strong IgG2a/c; low IgG1 and absent serum IgE responses) to a strong IgG1 response, low/absent IgG2a/c response and elevated serum IgE, typical of that seen in WT mice resistant to *T. muris.* This was also associated with elevated levels of IL-5 and IL-13 in intestinal tissue. Low level infection of mice with conditional *Hp* deletion in neutrophils presented with markedly altered total serum immunoglobulin levels, antigen specific isotype responses, and alteration of B cell populations within the draining lymph node. Taken together the data suggest a novel modulatory role for neutrophil derived Hp in Type 2 and B cell responses during chronic intestinal helminth infection.

13

ONCHOCERCIASIS-ASSOCIATED EPILEPSY: THE PATHOPHYSIOLOGICAL MECHANISM

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Introduction: Epidemiological evidence suggests that *Onchocerca volvulus* is causing epilepsy (onchocerciasis-associated epilepsy (OAE)), including nodding syndrome. Currently the pathogenesis of OAE remains to be determined.

Aim: To investigate whether the pathogenesis of OAE is directly or indirectly linked to *O. volvulus or* to another pathogen transmitted by blackflies *(Simulium)*.

Methods: Critical literature review combined with: (1) an exploratory proteomic study of the *O. volvulus* secretome, and of blood and cerebrospinal fluid (CSF) of OAE patients and (2) a metagenomic study of *O. volvulus* adult worms and blackflies. Worms were obtained in onchocerciasis endemic regions from Ghana and Cameroon, blackflies in Cameroon and human samples in South Sudan.

Results*:* Higher *O. volvulus* microfilariae (mf) densities in children increase the risk to develop epilepsy later in life. Before the implementation of mass treatment with ivermectin, mf were detected in cerebrospinal fluid (CSF) by several researchers (Hisette, Mazoti and Duke). More recently, neither *O. volvulus* mf nor DNA were detected in CSF or brain tissue; however, these samples were obtained years after seizure onset reducing the chances of identifying the original cause. It is possible that infection induced inflammation could increase the BBB permeability and allow mf to enter the brain. Dying mf will release their microbiome, e.g. *Wolbachia*, which may cause an inflammatory reaction inducing seizures. On the other hand, all *O. volvulus* stages secrete excretory/secretory proteins (ESPs) necessary for their migration through the host tissues. These ESPs might also help mf cross the BBB. The blackfly metagenomic study did not reveal any potential human pathogenetic virus but the metagenomic study of the worm identified a novel rhabdovirus. So far a proteomic study of 9 CSF samples of persons with OAE did not reveal *O. volvulus* proteins and the secretome of the adult worms is being described.

Conclusion: Most likely *O. volvulus* is able to directly or indirectly induce epilepsy. We need to increase our knowledge about the *O. volvulus* worm, its ESPs, and its microbiome. Further research is needed to investigate whether the novel rhabdovirus could play a role in the pathogenesis of OAE.

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Genetic Manipulation of the parasitic nematode *Trichuris muris*

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Trichuris muris is a well-characterised gastrointestinal nematode of mice that serves as an excellent animal model of the prevalent human whipworm, *T. trichiura*. This parasite infects approximately 500 million people worldwide and cause significant morbidity and loss of life years and is relatively refractory to anthelmintics used for other common soil transmitted helminths of man. Following definition of *Trichuris spp.* genomes the ability to carry out genetic manipulation of this worm would provide a step change in our ability to define the biology of these important parasites. We trialled lentiviral DNA delivery to successfully introduce genetic constructs encoding fluorescent genes under the control of various promoters (both mammalian, and nematode) into L1 stages of *T. muris.* These were then orally gavaged into a susceptible mouse and allowed to progress to adult stages. Adult worms were removed and eggs collected, screened for presence of the plasmid by PCR and allowed to embryonate. Once embryonated, eggs were then used to infect a mouse. In this way, we have maintained germ-line transmission of ectopic DNA within this parasite detectable for at least 4 generations of the worm, confirmed by PCR and sequencing. Although possible to transgenerationally introduce foreign DNA into the *Trichuris* genome, we were unable to see expression of fluorescent reporter proteins or mRNA from any lentiviral construct. Hypothesising an endogenous gene silencing of exogenous DNA in the worm, we flanked the transgenes with insulators but again detected no gene product. We have shown transgenesis of *T. muris* is possible and are currently further refining the constructs to maintain transgene expression. The development of a robust genetic manipulation method will enable us to further our understanding of *Trichuris* worms and define the genes that are essential for parasite growth and survival.

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What mediators might the p43 immunomodulatory proteins of *Trichuris spp.* deliver to the infection site?

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The p43 protein of *Trichuris muris* contributes more than 95% of the protein secreted by adult worms, is nonimmunogenic in infection in mice, has IL-13 and glycan-binding activities that likely underpin chronic intestinal infection, and is demonstrably immunomodulatory. Using rapid competitive fluorescence-based methods, we previously found that p43 binds fatty acids and a range of other lipids, such as retinol, and some pharmacologically-active lipids and their precursors.

We now report on an attempt to identify with what ligands the parasite itself loads p43 as opposed to what we can make it bind in our fluorescence-based analyses. Preliminary untargeted metabolomic analyses were performed on solvent extracts generated from p43 protein that had been purified by affinity chromatography from the secretions of adult worms cultured in a simple, defined, lipid-free medium. Ion signals were detected that could be attributable to the fatty acids, arachidonic acid, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). Subsequent profiling of eicosanoids and related bioactive lipid mediators indicated that 5,6 epoxyeicosatrienoic acid (5,6-EET) and 15-hydroxyeicosatetraenoic acid (15-HETE) may be present in the p43 extracts, although further analyses are required to determine whether these lipids were generated through enzymatic routes. Adults of *Trichuris* species are known to release large quantities of prostaglandin E_2 (PGE_2) that can modify porcine, murine and human macrophage activation. Interestingly, we were unable to detect PGE_2 associated with p43. Thus, while the pH of the infection site may mean that PGE_2 is water-soluble and does not partition into a carrier or delivery protein, the parasite does load p43 with polyunsaturated fatty acid precursors of inflammatory and immune mediator lipids. Whether they originally derive from the host or are synthesised or modified by the parasite itself, and whether they influence the infection in the parasite's favour, remains to be established.

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Identification of GPCR-neuropeptide interactions and their functional analyses in *Schistosoma mansoni*

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Schistosomes are parasitic flatworms that cause schistosomiasis. Standard treatment relies on a single drug, praziquantel. Due their proven druggability, G protein-coupled receptors (GPCRs) represent promising targets for new anthelmintics. GPCRs can be activated by neuropeptides, important messenger molecules that act as neurotransmitters or neuromodulators. Our previous comparative transcriptomics analyses of paired and unpaired *Schistosoma mansoni* and their gonads revealed 59 differentially regulated GPCR genes putatively involved in neuronal processes. Furthermore, 23 of 27 *S. mansoni* neuropeptide precursor (NPP) genes of *S. mansoni* exhibited higher transcript levels in males (paired or unpaired) and unpaired females. Goal of this study was to characterize one rhodopsin-like orphan GPCR (*Sm*GPCR20). The previous RNAseq study showed its preferential expression in males and unpaired females, which we confirmed by qRT-PCR analyses. Next, we employed the MALAR-Y2H system and identified specific interactions with *Sm*NPP26 and *Sm*NPP40. Whole mount *in situ* hybridization localized transcript of these genes in the head region and along the worm body, also around the ootype and the uterus in females. Particularly, the spot-like signal patterns suggested expression mainly in neuronal cells. Phenotype analyses following RNAi against these molecules (double and triple knockdowns) indicated a substantial decline in egg production compared with a control group, especially in the combination *Sm*GPCR20/*Sm*NPP26. Correspondingly, CLSM analyses revealed morphologic changes in the female gonads, and qRT-PCR analyses showed a significant decrease of transcript levels of egg production-associated genes, again significantly for *Sm*GPCR20/*Sm*NPP26. Following *Sm*GPCR20/*Sm*NPP26 RNAi, dsRNA-treated females failed to reach the size of control females. The obtained results suggest that *Sm*NPP26 and *Sm*NPP40 are ligands of *Sm*GPCR20. Of these molecules, especially *Sm*GPCR20 and *Sm*NPP26 appear to be involved in processes controlling growth and sexual maturation of female *S. mansoni* following pairing. In conclusion, GPCRs and neuropeptides may play important roles for male-female interaction and the control of reproduction.

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Frequency of soil-transmitted infections in tuberculosis patients in the Peruvian Amazonian city of Iquitos and impact on markers of lung tissue destruction in TB-helminth co-infection

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Approximately 1/4 of the world's population are infected by soil-transmitted helminths (STHs), which may cause anaemia and can impair school performance and growth in children. Tuberculosis (TB) also infects roughly 1/4 of the world's population and is often considered in isolation despite many TB patients carrying other infections. STHs thrive where there is poverty and hardship, much like TB, and previous studies have shown an association between the two. Co-infected individuals appear to have more lung damage compared to those with TB alone, possibly due to the impact of STH infection on the host immune response to TB. We recruited 61 adults with culture-confirmed active pulmonary TB and 51 healthy controls in Iquitos, a city in the Peruvian Amazon only accessible by boat or plane. Three consecutive stool samples were obtained for copro-parasitological examination (formol-ether concentrate) and blood plasma taken for analysis of cytokines and matrix metalloproteinases (MMPs) via ELISA or Luminex multiplex assay. Prevalence of soil-transmitted helminth and other gastrointestinal parasite infections will be illustrated. *Strongyloides* was the most frequently detected STH followed by *Trichuris*, Hookworm and *Ascaris* in both healthy controls and TB-positive participants. Being diagnosed with any STH was associated with TB-positivity (p=0.02) and *Strongyloides* and hookworm were also independently associated with TB-positivity (p<0.05 & p=0.01). Multivariable logistic regression, controlling for age, sex and socioeconomic status, showed significantly increased odds of TB-positivity in the presence of *Ascaris* compared to STH-negative healthy controls (OR 4.1 95% CI 1.06-20.83) and *Strongyloides* and hookworm species approached significance. TB was associated with STH infection and there were increasing odds of TB positivity with increasing numbers of STH infection diagnosed [Table 2.]. The effect of STH infection on MMPs was explored by looking at effect of multiplicity of STH infection on MMP concentrations through 6 multivariable linear regression models, summarised in Table 3. Mono-infection with a single STH was significantly associated with MMP-10 and MMP-13, and two or more STHs was significantly associated with all MMPs tested [Table 3.]. These findings provide a possible mechanism for the increased tissue destruction observed in TB and soil transmitted helminth co-infection.