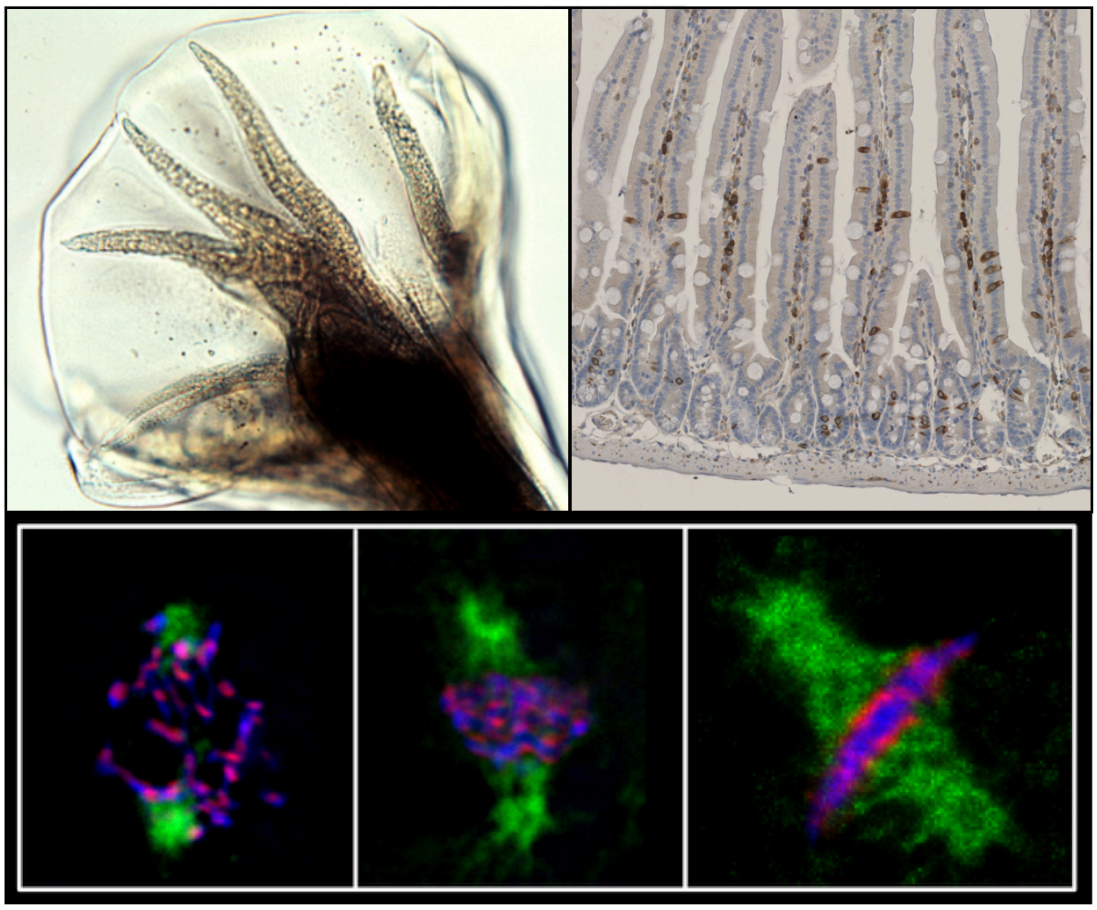
Parasitic Helminths - New Perspectives in Biology and Infection



1 - 6 September 2019

Bratsera Hotel,

Hydra, Greece

COVER IMAGES :

Top Left: Posterior end (bursa) of *Ancylostoma* adult hookworm (R.E.Davis, University of Colorado School of Medicine).

Top Right: Intestinal epithelial tuft cells (stained for Dclk1 transcription factor) and goblet cells (visible as unstained vacuolar cells) in mouse small intestine following infection with the *Nippostrongylus brasiliensis* (F.Varyani, R.M.Maizels, F.Gerbe, P.Jay, University of Glasgow and INSERM Montpellier)

Lower Panels: Immunohistochemical staining of microtubules and CENP-A (histone H3 variant at centromeres) in a two-cell *Ascaris* embryo in progression from prophase to metaphase. Note that CENP-A extends along the length of the holocentric chromosomes and is localized asymmetrically to the centrosome side of the metaphase chromosomes (R.E.Davis, University of Colorado School of Medicine)

ORGANISERS, 2019

Amy Buck (University of Edinburgh, UK)
Jim Collins (University of Texas Southwestern Medical Center, USA)
Richard E Davis (University of Colorado, School of Medicine, USA)
Kleoniki Gounaris (Imperial College, UK)
Rick Maizels (University of Glasgow, UK)
Murray Selkirk (Imperial College, UK)

Dates of Next Meeting : 30 August - 4 September 2020

Parasitic Helminths - New Perspectives in Biology and Infection 1-6 September 2019, Hydra, Greece

	Sunday 1 September	Monday 2 September	Tuesday 3 September	Wednesday 4 September	Thursday 5 September	Friday 6 September
	ARRIVE	**Session 1** **Genomics**	**Session 4** **Th2 Immunity**	**Session 7** **Small RNA Symposium I**	**Session 10** **Immunomodulators**	DEPART
9:00		**Elodie Ghedin**	**P'ng Loke**	Dick Davis	**Nicola Harris**	
9:20				**Luisa Cochella**		
9:40		Jose Tort	Maria Yazdanbakhsh		Francesco Vacca	
10:00		Sarah Buddenborg	Andrew MacDonald	Alison Ricafrente	Sheila Donnelly	
10:20		Rebecca Cole	Pedro Gazzinelli-Guimaraes	Vicky Hunt	Allison Bancroft	
10:40-11:10			**Coffee Break**			
		Session 2 **Immune Interactions**	**Session 5** (11:00 start) **Nematodes Galore**	**Session 8** **Small RNA Symposium II**	**Session 11** **Worms in Nature**	
11:10		Claire Drurey	David Mangelsdorf	**Amy Buck**	Amy Pederson	
11:30		Henry McSorley	James Lok		Anthony Page	
11:50		Minka Breloer	Ferdinand Ngale Njume	Peter Taylor	Navonil Banerjee	
12:10		Matthew Taylor	Mericien Venzon	**Peter Sarkies**	Michael Povelones	
12:30		John Miles	Gustavo Salinas		Phil Newmark	
12:50-16:00			Andy Fraser **Afternoon Break**			
		Session 3 **Development**	**Session 6** **Molecular Advances**	**Session 9** **Molecular Biology**	**Session 12** **Helminths and People**	
16:00		**William Sullivan**	**Karl Hoffmann**	Carmen Lidia Diaz Soria	**Alison Elliott**	
16:20	**Registration Opens at Bratsera Hotel**			Alex Dulovic		
16:40		Alicia Costabile	Steve Ward Mark Taylor	Jana Hagen	Clarissa Prazeres da Costa	
17:00		George Wendt	Marc Hübner	Rosie Spencer	Tom Nutman	
17:20		Poster Pitches, 20 x 2 minutes	Ron Hokke	Poster Pitches, 20 x 2 minutes	Warwick Grant	
17:40			Mark Robinson		Alex Loukas	
18:00		**Poster Session 1**	*End of Session*	**Poster Session 2**	*End of Session*	
18:45	Pre-lecture Drinks		**Vlychos Taverna Dinner** (Boat leaves 19:00)			
19:45	**Keynote Lecture: Alejandro Sánchez Alvarado**				**Bratsera Farewell Dinner** (20:30)	
21:00	**Welcome Dinner Bratsera Hotel**	*End of Session*		*End of Session*		

NOTES

Sunday 1 September

Chair: **Jim Collins**, University of Texas Southwestern Medical Center	
19:45	**Keynote Lecture: Pluripotency beyond embryogenesis** **Alejandro Sánchez Alvarado,** Stowers Institute for Medical Research, Missouri
21:00	**Welcome Reception and Dinner, Bratsera Hotel**

Monday 2 September

09:00 - 10:40 Session 1: Genomics

Chair: **Amy Buck**, University of Edinburgh		
09:00	**Elodie Ghedin,** New York University	**Sex-biased glycosylation profiles and immunomodulatory glycoprotein discovery in the filarial worm *Brugia malayi***
09:40	**Jose Tort,** Universidad de la República, Montevideo	**Novel insights into the evolution of trematodes in the family Fasciolidae**
10:00	**Sarah Buddenborg,** Wellcome Sanger Institute	**Sexual revolution in platyhelminths: the case of schistosomes**
10:20	**Rebecca Cole,** University of Bristol	**Genetic variation in the parasitic nematode *Strongyloides ratti***

11:10 – 12:50 Session 2: Immune Interactions

Chair: **Eileen Devaney,** University of Glasgow		
11:10	**Claire Drurey,** University of Glasgow	**Investigation of the host-parasite Interface at the small intestine epithelium**
11:30	**Henry McSorley,** University of Edinburgh	**IL-33 modulators from *Heligmosomoides polygyrus***
11:50	**Minka Breloer**, Bernhard Nocht Institue for Tropical Medicine, Hamburg	**IL-33 improves expulsion of the parasitic nematode *Strongyloides ratti* from the intestine by rapid mucosal mast cell activation**
12:10	**Matthew Taylor,** University of Edinburgh	**PD-L2 downregulates GATA-3 in Th2 cells during filarial infection resulting in Th2 cell-intrinsic dysfunction and plasticity**
12:30	**John Miles**, James Cook University, Cairns	**Strong changes in peripheral blood immune signatures after experimental hookworm infection**

16:00 - 17:20 Session 3: Development

Chair: **Niki Gounaris,** Imperial College London		
16:00	**William Sullivan,** University of California Santa Cruz	**The role of *Wolbachia* somatic cell-to-cell transfer in filarial nematode symbiosis**
16:40	**Alicia Costábile,** Universidad de la República, Montevideo	**Transcriptomic characterization of the proliferative cells of the cestode *Mesocestoides corti* by depletion and purification**
17:00	**George Wendt,** University of Texas Southwestern Medical Center	**Leveraging single-cell RNA sequencing to understand stem cell biology in the parasitic flatworm *Schistosoma mansoni***

Poster Session Monday 2 September 2019 18:00-20:00 (following Poster Pitches at 17:20)

Chair: Niki Gounaris, Imperial College London			
1	**Sasisekhar Bennuru**	National Institutes of Health	Onchocerciasis RDT: Integrating multiple biomarkers that push the detection sensitivities of infection
2	**Zwi Bentwich**	Ben Gurion University of the Negev	Control of neglected tropical diseases should start from early childhood- success of an Improved comprehensive model
3	**Rui Chen**	University of Texas Southwestern Medical Center	Identify somatic nursing machinery of the male-induced development of the female schistosome reproductive system
4	**Maude Dagenais**	McGill University, Montreal	Vesicle-based secretion in schistosomes: Profiling of *Schistosoma mansoni* exosome surface glycans
5	**Maria Adelaida Duque Correa**	Wellcome Sanger Institute	Initial host intestinal epithelia interactions with whipworms unravelled
6	**Moses Egesa**	MRC Uganda Virus Research Unit	*Schistosoma mansoni* schistosomula antigens induce Th1/Pro- inflammatory cytokine responses
7	**Alexandra Ehrens**	University Hospital Bonn	Microfilariae trigger eosinophil extracellular traps in a Dectin-1-dependent manner
8	**Tom Gasan**	Leiden University Medical Center	Deducing the protective mechanism of Omega 1
9	**Steffen Hahnel**	Bayer Animal Health GmbH, Monheim	Evaluation of derivative-specific pharmacokinetics and potencies of anthelmintic drug classes by assessing nematode motility and pharyngeal pumping
10	**Andy Hinck**	University of Pittsburgh	Structure-function studies of the *H. polygyrus* TGF-β mimic TGM
11	**Min Hu**	Huazhong Agricultural University	The circRNAs expression profiles of different developmental stages of parasitic nematode, *Haemonchus contortus*
12	**Malcolm Kennedy**	University of Glasgow	The major pseudocoelomic proteins of the giant kidney worm, *Dioctophyme renale*, and what they can tell us about other Clade 1 nematodes
13	**Paula Licona Limón**	Universidad Nacional Autónoma México	Role of IL-9 in basophil differentiation and function
14	**Maibritt Mardahl**	Aarhus University	Immunomodulation of human cytokine responses by the porcine whip worm, *Trichuris suis*, and the porcine nodular worm, *Oesophagostomum dentatum*
15	**Kirsty McCann**	La Trobe University, Melbourne	Orthologues of the immunodominant antigens of *Onchocerca volvulus* in the rodent onchocerciasis model *Cercopithifilaria johnstoni*.
16	**Palak Mehta**	Malaghan Institute of Medical Research	The gastrointestinal helminth *Heligmosomoides polygyrus* suppresses inflammation in a model of atopic dermatitis
17	**Tapoka Mkandawire**	Wellcome Sanger Institute	Understanding the molecular basis of hatching in *Trichuris* species
18	**Peter Nejsum**	Aarhus University	Immunosuppressive effects of helminth antigens on TLR-mediated inflammatory responses in macrophages
19	**Gyaviira Nkurunungi**	MRC Uganda Virus Research Unit	Helminth-allergy associations in rural and urban Uganda: insights from carbohydrate-specific IgE studies
20	**Gilda Padalino**	Aberystwyth University	*In silico* methods and drug reposition for the identification of new anti-schistosomal drugs

Tuesday 3 September

09:00 - 10:40 Session 4: Type 2 Immunity

Chair: **Richard Grencis,** University of Manchester		
09:00	**P'ng Loke,** New York University	**Heterogeneity of type-2 immune responses during helminth infections**
09:40	**Maria Yazdanbakhsh,** Leiden University Medical Center	**Characterization of type-2 immune responses during helminth infection by mass cytometry**
10:00	**Andrew MacDonald,** University of Manchester	**Environmental control of pulmonary macrophage responsiveness during type-2 inflammation**
10:20	**Pedro Gazzinelli-Guimares,** National Institutes of Health	**Allergen pre-sensitization drives an eosinophil-dependent lung-specific arrest of helminth development**

11:00 - 13:00 Session 5: Nematodes Galore

Chair: **Murray Selkirk,** Imperial College London		
11:00	**David Mangelsdorf,** University of Texas Southwestern Medical Center	**Targeting the DAF-12 nuclear receptor pathway in parasitic nematodes**
11:20	**James Lok,** University of Pennsylvania	**Genetic and phenotypic analyses of CRISPR/Cas9-induced mutations in *Ss-daf-16*, which encodes a forkhead transcription factor in the parasitic nematode *Strongyloides stercoralis***
11:40	**Fredinand Ngale Njume**, Université Libre de Bruxelles	**A hypodermally expressed putative GM2 activator protein is essential for cuticular integrity in *Caenorhabditis elegans***
12:00	**Mericien Venzon,** New York University	**Gut microbe metabolism influences *Trichuris* hatching and fecundity**
12:20	**Gustavo Salinas,** Universidad de la República, Montevideo	**Nematode complex I and II subunit gene duplications provide increased fitness to hypoxia**
12:40	**Andrew Fraser,** University of Toronto	**How rhodoquinone is made and how to find drugs that block rhodoquinone synthesis**

16:00 – 18:00 Session 6: Molecular Advances

Chair: **Alex Loukas**, James Cook University		
16:00	**Karl Hoffmann**, Aberystwyth University	**The development of epigenetic modulators as next-generation anti-schistosomals**
16:40	**Steve Ward** and **Mark Taylor,** Liverpool School of Tropical Medicine	**A-WOL: A decade of drug discovery and development of safe macrofilaricides for onchocerciasis and lymphatic filariasis**
17:00	**Marc Hübner,** University Hospital Bonn	**Efficacy of the novel macrofilaricidal drug candidates ABBV-4083 and oxfendazole**
17:20	**Ron Hokke,** Leiden University Medical Center	**Glycan targets for intervention and control of schistosomiasis**
17:40	**Mark Robinson,** Queen's University Belfast	**Spilling your guts: extracellular vesicles contribute to haemoglobin digestion by *Fasciola hepatica***

Wednesday 4 September

09:00 - 10:40 Session 7: Small RNA Symposium I

Chair: **Dick Davis,** University of Colorado		
09:00	**Dick Davis,** University of Colorado	**Small RNAs and their functions in helminths**
09:20	**Luisa Cochella,** Institute for Molecular Pathology, Vienna	**Roles of miRNAs in development: lessons from *C. elegans***
10:00	**Alison Ricafrente,** University of Technology Sydney	**Understanding how the miRNAs of *Fasciola hepatica* contribute to the host-parasite relationship**
10:20	**Vicky Hunt**, University of Bath	**Small RNA pathways with a role in parasitism in *Strongyloides* nematodes**

11:10- 13:00 Session 8: Small RNA Symposium II

Chair: **José Tort,** Universidad de la República, Montevideo		
11:10	**Amy Buck,** University of Edinburgh	**RNA in cross-species communication**
11:50	**Peter Taylor,** Imperial College London	**The role of small RNA in parasite-host communication during *Trichinella spiralis* infection**
12:10	**Peter Sarkies,** MRC London Institute for Medical Sciences, Imperial College London	**Small RNAs in epigenetics and evolution**
12:50	**Poster Pitches 21-24** (2 minutes each)	

16:00 – 17:20 Session 9: Molecular Biology

Chair: **Jim Collins**, University of Texas Southwestern Medical Center		
16:00	**Carmen Lidia Diaz Soria,** Wellcome Sanger Institute	**Cell type transcriptomic analysis of larval stage of *Schistosoma mansoni***
16:20	**Alex Dulovic,** Max Planck Institute for Developmental Biology	**Successful development of RNAi within the model parasitic nematode *Strongyloides ratti***
16:40	**Jana Hagen,** Imperial College London	**Genetic manipulation of *Nippostrongylus brasiliensis* by lentiviral transduction**
17:00	**Rosie Spencer,** University of Aberdeen	**New insights into nematode spliced leader *trans*-splicing**

Poster Session Wednesday 4 September 18:00-20:00 (Poster Pitches 12:50-13:00 and 17:20-18:00)

Chair: Niki Gounaris, Imperial College London			
21	**Santiago Fontenla**	Universidad De La República, Uruguay	Role of small RNAs in *Fasciola hepatica* development and the interaction with the host
22	**Yi Mu**	QIMR Berghofer Medical Research Institute	Serum parasite-derived microRNAs as novel biomarkers for the detection of *Schistosoma japonicum* infection: lessons learned from a setting of low infection intensity
23	**Kyriaki Neophytou**	University of Edinburgh	Elucidating the function of an extracellular Argonaute secreted from a gastrointestinal nematode
24	**Linh Thuy Nguyen**	Charles University, Hradec Králové	Comparative analysis of microRNA levels in *Haemonchus contortus* strains with different drug-susceptibility
Poster Pitches 21-24 at 12:50, Poster Pitches 25-40 at 17:20			
25	**Gabriela Paludo**	Universidade Federal do Rio Grande do Sul	Identification of developmental genes and pathways involved with cestode strobilation
26	**Gordana Panic**	Imperial College London	Metabolic signatures of urinary schistosomiasis and praziquantel pharmacokinetics in pre-school and school aged children from rural Côte d'Ivoire
27	**Wendy Sue Phillips**	Elanco Animal Health, Illinois	Differential gene expression of *Dirofilaria immitis*
28	**Shannon Quek**	Liverpool School of Tropical Medicine	Biochemical pathways supporting *Wolbachia* population growth and regulation in *Brugia malayi*
29	**Estelle Remion**	Museum National d'Histoire Naturalle, Paris	Microfilariae and lung pathology
30	**Faye Rodgers**	Wellcome Sanger Institute	Exploring helminth gene expression data using WormBase ParaSite
31	**Danielle Smyth**	University of Glasgow	Can parasite TGF-β superfamily mimics bind and signal via Activin or BMP receptors?
32	**Sivap Sungpradit**	Mahidol University	*Spirometra erinaceieuropaei* in a free-roaming cat from the lower portion of Thailand's northern region
33	**Nermina Vejzagic**	Technical University of Munich	A novel cell-free method to culture *Schistosoma mansoni* from cercariae to juvenile worm stages for in vitro drug testing
34	**Mark Viney**	University of Liverpool	Immune networks in wild mice, *Mus musculus domesticus*
35	**Jiří Vorel**	Masaryk University	Omics analyses and functional proteins of *Eudiplozoon nipponicum* (Monogenea)
36	**Holly Webster**	University of Glasgow	IL-10 is key in driving tissue-based Th2 responses to helminth infection in the small intestine
37	**Ruby White**	University of Edinburgh	Host modulation by helminth parasite *Heligmosomoides polygyrus bakeri* extracellular vesicles
38	**Shi Yan**	University of Veterinary Medicine Vienna	Mass spectrometric characterisation of novel glyco-epitopes in free-living and parasitic nematodes
39	**Bibek Yumnan**	Malaghan Institute of Medical Research	In vitro development of *Nippostrongylus brasiliensis* larvae on nutrient agar plates
40	**Francis Zeukeng**	University of Yaounde	Prevalence of malaria and soil-transmitted helminth infections and their association with undernutrition in schoolchildren residing in Mfou health district in Cameroon

Thursday 5 September

09:00 - 10:40 Session 10: Immunomodulators

Chair: **Maria Yazdanbakhsh**, Leiden University Medical Center		
09:00	**Nicola Harris,** Monash University, Melbourne	**Hookworms evade host immunity by secreting a deoxyribonuclease II to degrade neutrophil extracellular traps**
09:40	**Francesco Vacca,** University of Edinburgh	***Heligmosomoides polygyrus* secretes a suppressor of ST2**
10:00	**Sheila Donnelly,** University of Technology, Sydney	**Structure/biological activity relationships in the *Fasciola hepatica*-secreted immunomodulatory 68-mer peptide FhHDM-1**
10:20	**Allison Bancroft,** University of Manchester	**The major secreted protein of the whipworm parasite tethers to matrix and inhibits interleukin-13 function**

11:10 – 12:50 Session 11: Worms in Nature

Chair: **Irma Schabussova**, Medical University of Vienna		
11:10	**Amy Pederson,** University of Edinburgh	**The ecology of helminth infection and immunity in a wild rodent model**
11:30	**Anthony Page,** University of Glasgow	**The golden death bacillus *Chryseobacterium nematophagum;* a novel matrix digesting pathogen of nematodes.**
11:50	**Navonil Banerjee**, University of California Los Angeles	**Deciphering the responses of skin-penetrating nematodes to carbon dioxide.**
12:10	**Michael Povelones,** University of Pennsylvania	**Man's best friend: Activation of mosquito immunity blocks the development of transmission-stage *Dirofilaria immitis* and *Brugia malayi***
12:30	**Phil Newmark,** University of Wisconsin-Madison	**A rotifer-derived paralytic compound prevents transmission of schistosomiasis to a mammalian host**

16:00 – 18:00 Session 12: Helminths and People

Chair: **Rick Maizels**, University of Glasgow		
16:00	**Alison Elliott,** MRC Uganda Virus Research Institute	**Immunomodulation studies in human populations … gang aft agley!**
16:40	**Clarissa Prazeres da Costa,** Technical University Munich	**Modulation of allergy and vaccine responses through maternal *S. mansoni* infection**
17:00	**Tom Nutman,** National Institute of Allergy and Infectious Diseases, NIH	**Neurotoxic autoantibodies induced by *O. volvulus* infection underlies the nodding syndrome, a rare epidemic form of epilepsy associated with onchocerciasis**
17:20	**Warwick Grant,** La Trobe University, Melbourne	**Population genomics and molecular phylogenetic relationships of *Simulium* vectors of onchocerciasis in Ghana: large interbreeding sympatric populations and large transmission zones**
17:40	**Alex Loukas,** James Cook University, Cairns	**A phase 1b clinical trial to assess safety and efficacy of an attenuated hookworm larvae vaccine**
20:30	**Bratsera Farewell Dinner**	

ABSTRACTS

KEYNOTE LECTURE

Pluripotency beyond embryogenesis

Alejandro Sánchez Alvarado, Ph.D.
Scientific Director, Stowers Institute for Medical Research
Investigator, Howard Hughes Medical Institute
Kansas City, Missouri, USA

Member, National Academy of Sciences
Fellow, American Academy of Arts and Sciences
Fellow, Marine Biological Laboratory, Woods Hole, MA, USA

Plurtipotent stem cells are generally associated with embryonic stages of animal development. In mice, for example, embryonic stem cells normally exist for a few hours after which they are no longer maintained in the embryo. The quality of these stem cells determines the fitness of the resulting progeny and tissues, including the germline, which ultimately will determine the survival and perpetuation of the species. As such, when we think about natural selection, we conceive of this process operating at the level of either individuals or communities of individuals. In some organisms, however, it is normal for pluri- if not totipotent cells to persist beyond embryogenesis and carry out both tissue homeostatic and regenerative functions in the adult. The persistence of pluripotent stem cells beyond embryogenesis is a common feature of plants but it is also known to occur in many animal species. One such group of animals are planarians, members of the phylum Platyhelminthes, which are known to possess abundant numbers of adult pluripotent stem cells known as neoblasts. Unlike most other cells in animal body plans, stem cells self-renew, form clones and perpetuate themselves throughout the life of the animal. In planarians, which can reproduce asexually, these pluripotent stem cells perpetuate themselves across generations as well. Hence, competition among these pluripotent cell clones is likely to be constant, essentially transforming neoblasts into units of natural selection. Of the thousands of species known thus far, the planarian Schmidtea mediterranea has become a fruitful research organism in which to explore and dissect the functions, behaviors and properties of these adult pluripotent stem cells. Their embryonic origin, diversity in adults, prospective isolation and variable genomic output will be discussed in the context of both tissue homeostasis and regeneration.

Sex-biased glycosylation profiles and immunomodulatory glycoprotein discovery in the filarial worm *Brugia malayi*

Elodie Ghedin

Center for Genomics and Systems Biology, New York University, New York, USA

There is increasing evidence for immunomodulatory glycans expressed by a diversity of parasitic worms. In this study, we integrated transcriptome, glycome, proteome, and immune data to characterize the glycomic profile of the filarial worm *Brugia malayi*. We identified clear sex-specific expression of glycosylation enzymes and show sex-specific display of glycan epitopes with a significant overrepresentation of terminal alpha-N-acetylgalactosamine (GalNAc)—including the known immunomodulatory glycan epitope Tn antigen—in female worms. Females also up-regulate O-glycosylation while male worms favor N-glycosylation. Males preferentially display Fuc-α6GlcNAc, while females express core α1-2 fucose, previously associated with egg-sperm biocommunication. Further investigation of this differential glycan display by lectin affinity chromatography coupled to mass spectrometry led to the purification of sex-specific glycoprotein fractions. We identified a subset of secreted proteins displaying both fucose- and galactose-containing epitopes and show differential effects of the purified glycofractions on naïve human PBMCs *in vitro* suggesting that male and female worms may act on the host immune system in a radically different way. Recombinant expression of the identified subset and subsequent immune evaluation on PBMCs validate further our glyco-centric approach to immunomodulatory glycoprotein discovery in parasitic worms.

Novel insights into the evolution of trematodes in the family Fasciolidae

Santiago Fontenla[1], Young-Jun Choi[2,] Alicia Costabile[1], Makedonka Mitreva[2], **José Tort**[1]

[1]*Departamento De Genética, Facultad De Medicina, Universidad de la República, Uruguay*
[2] *McDonnell Genome Institute at Washington University in St. Louis, USA*

Within the Fasciolidae species the comparison of the intestinal fluke *Fasciolopsis buski* and the liver flukes *Fasciola gigantica* and *Fasciola hepatica* offers a good opportunity to evaluate the adaptations to diverse environments and the invasion process. We approached this problem from the comparative genomics perspective, analyzing the three Fasciolidae species. The *Fasciola* spp. genomes are the largest within trematodes (~1135Mb), being 1.5 times bigger than *F. buski* (748Mb), the second largest. The accumulation of certain families of transposable elements (TE) seems responsible for the genome expansion of *Fasciolas* compared to other trematodes. Interestingly, this expansion was associated to a significant increment in gene sizes caused by the accumulation of TEs at the intronic regions, not affecting the CDS lengths. A more detailed comparison of the gene complement of Fasciolidae and other trematodes highlighted several interesting gene family amplifications, particularly in proteases and transporters. While several cathepsins can be found in the Fasciolinae a reduced set is present in *F. buski*. Interestingly distinct cathepsin gene subfamilies were amplified in the *Fasciolas*, *Opisthorchiidae* and *Schistosomas,* paralleling diverse invasion mechanisms. A similar phenomenon was detected with the endopeptidases (legumains) that participate in the maturation of the cathepsin proenzyme, and with other proteins involved in the host-parasite interaction like the CAP domain superfamily. Some lipid transporters like the NPC2 and FABPs are amplified in Fasciolinae in relation to *F. buski*, a feature shared with the Opisthorchidae, suggesting parallel common adaptations to the rich lipidic environment of the liver. Other gene families such as the HSP70, aquaporins and cystatins were amplified in all three Fasciolidae species, in relation to other trematodes. An extended analysis of diverse metabolic pathways that might differ in the context of the intestine or the liver is warranted to gain further insights into family evolution.

Sexual revolution in platyhelminths: the case of schistosomes

Sarah Buddenborg, Alan Tracey, Gabriel Rinaldi, Matt Berriman

Wellcome Sanger Institute, UK

Schistosomes are dioecious (separate sexes), a remarkable feature among platyhelminths. They evolved a ZW-chromosomal sexual determination system in which females are heterogametic (ZW) and males are homogametic (ZZ). The recent sequencing and assembly of *Schistosoma mansoni* sex chromosomes gives us the opportunity to address questions about evolution of the Z and W chromosomes and the specific mechanisms underlying sex determination. Our analysis of W and Z orthologs among *Schistosoma* species, *Trichobilharzia regenti,* and the hermaphroditic platyhelminth *Echinostoma caproni*, has refined previously characterized evolutionary strata providing relevant clues for the evolutionary origins of sex chromosomes in the family Schistosomatidae. Structurally, *S. mansoni* W and Z chromosomes exhibit features of early evolving sex chromosomes, providing opportunities to use this species as a model organism for studying the evolutionary origins of metazoan sex determination. The Z chromosome contains a non-recombining Z-specific region (ZSR) flanked by two pseudoautosomal regions (PAR) that undergo recombination along their entire length. The W chromosome is largely heterochromatic, with a large proportion of repetitive DNA, and contains PARs homologous to those on Z as well as a non-recombining W-specific region (WSR). Although all protein-coding genes in the WSR have a homolog within the ZSR (termed gametologues), we have identified several W-specific non-coding RNAs (ncRNAs) with sex- and stage-specific expression. Whole-mount *in situ* hybridization, RNAi-mediated gene silencing, and single-parasite RNA-seq was employed to reveal regulatory mechanisms underlying dioecy during *S. mansoni* development. Understanding how female schistosomes are genetically determined and develop may lead to the discovery of novel targets for control of this neglected tropical disease pathogen.

Genetic variation in the parasitic nematode *Strongyloides ratti*.

Rebecca Cole, Nancy Holroyd, Alan Tracey, Matthew Berriman, Mark Viney

University of Bristol and Wellcome Sanger Institute, UK

We have investigated the population genetics of the nematode *Strongyloides ratti* by sequencing the whole genomes of more than 80 individual worms, sampled non-destructively from three wild rat populations in the southern UK. We find that these parasites genetically cluster into two distinct groups (with a range of intermediate genotypes), but these groups do not match sampling site. The life history of *Strongyloides* spp. is dominated by repeated cycles of mitotic parthenogenesis, with rare facultative sexual reproduction, and so these genetic clusters may represent clonal descendants from a single individual, with intermediate genotypes arising from occasional sexual reproduction between these clusters. In contrast to this whole-genome pattern, some genomic regions have allele frequencies that are correlated with sampling site. These genomic regions may be under sampling-site specific selection. We have also investigated genes specifically expressed in the parasitic phase of this life cycle among these more than 80 genomes. This work represents the first whole genome analysis of a parasitic nematode on this scale, and is illuminating new population genetic patterns in a species with an unusual mode of reproduction.

Investigation of the host-parasite interface at the small intestine epithelium

Claire Drurey[1], Gillian Coakley[1], Dennis Hoving[1], Janice Murray[2], Rick Maizels[1]

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Heligmosomoides polygyrus is an intestinal parasite that utilises multiple immunomodulatory mechanisms to establish chronic infections in mice. The production of a mixture of proteins termed *H. polygyrus* excretory-secretory products (HES) is known to manipulate a range of immune cell types to promote regulatory T cells and tolerogenic myeloid cells. However, little is known of interactions with the intestinal epithelium, the cell layer in closest contact with adult parasites, and which is known to play an active part in stimulating anti-helminth immunity. We have investigated this question at two levels. First, we carried out RNAseq on HES-and IL-4/13 exposed small intestinal organoids to identify pathways that are regulated by type 2 immune responses and targeted by *H. polygyrus;* HES inhibits expression of key IL-4/13-regulated epithelial genes in this system. HES components also bind to intestinal epithelial cells, and we have screened HES proteins for binding to key populations within the intestinal environment to identify specific host-interactive products. We have identified a single protein, VAL-4, that as a recombinant binds both Paneth cells and Goblet cells, and co-precipitation studies identify candidate host ligands responsible for this staining. VAL proteins form the most abundant products within HES and are related to the *Ancylostoma* secreted proteins (ASPs) identified in hookworm species. Investigating the interaction between the helminth and its intestinal epithelial environment both in terms of overall pathways and specific proteins should identify how *H. polygyrus* modifies the intestinal environment to enable its own survival.

IL-33 modulators from *Heligmosomoides polygyrus*

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Infection with the murine parasite *Heligmosomoides polygyrus,* or administration of its secreted products, suppresses allergic responses in mouse models of asthma. We set out to define the molecular mechanisms by which *H. polygyrus* achieves this, with a view to developing new treatments for human asthma. We found that the inhibition of responses by *H. polygyrus* products was mediated by inhibition of IL-33 release, and through fractionation of these products we identified a single *H. polygyrus* protein with IL-33 suppressive activity: we named this protein the Alarmin Release Inhibitor (HpARI). Recombinant HpARI binds with high affinity and specificity to the active form of IL-33, and has secondary binding affinity for genomic DNA. This dual binding ability effectively tethers IL-33 within necrotic epithelial cells, and blocks IL-33/ST2 interactions. In an *Alternaria* allergen mouse model of asthma, HpARI is effective at suppressing IL-33 release, ILC2 activation, and recall responses to allergen, leading to reduced pathology in the lung and abrogated airway hyperresponsiveness. Furthermore, using a human lung explant system, we can show that the IL-33-suppressive effects of HpARI translate to human IL-33 release, and thus has potential as a novel therapeutic in allergic disease. HpARI can suppress parasite-specific type 2 immune responses, and HpARI administration delays ejection of *Nippostrongylus brasiliensis*. The HpARI protein consists of 3 consecutive atypical CCP domains, similarly to the TGF-β mimic HpTGM (5 CCP domains) and the ST2-suppressor HpBARI (2 CCP domains). CCP proteins are highly represented in *H. polygyrus* secretions, and we hypothesise that this family contains further immunomodulators. We have characterised the function of each of the CCP domains of HpARI, and have created a range of mutants and truncations of HpARI with either IL-33-inhibiting or unexpected IL-33-amplifying activity, and are using these to further characterise the effects of the IL-33 pathway in a range of allergic and inflammatory models.

IL-33 improves expulsion of the parasitic nematode *Strongyloides ratti* from the intestine by rapid mucosal mast cell activation

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Strongyloides ratti is a rodent specific parasitic nematode that displays tissue migrating and intestinal life stages. Larvae penetrate the skin of their hosts and migrate within 2 days via skin, head and lung to the mouth. They are swallowed, reach the intestine and moult to adults by day 5. Immune competent mice terminate the infection in the context of a type II immune response within 2-4 weeks. IL-33 is a tissue-derived nuclear cytokine that was shown to promote type II immune responses during allergy and helminth infection, specifically enhancing lung eosinophilia and ILC2 expansion during *Strongyloides venezuelensis* infection. Here we analyse the role of IL-33 during the early immune response to the closely related *S. ratti.* Administration of IL-33, either intranasal or intraperitoneal, reduced intestinal parasite burden day 6 in the context of increased mucosal mast cell activation. Neutralization of endogenous IL-33 by administration of the helminth-derived IL-33 inhibitor HpARI reciprocally increased intestinal parasite burden. The IL-33-mediated reduction of intestinal parasite burden was strictly dependent on the presence of mast cells and functional IL-9 receptor signaling but independent of basophils, eosinophils, or granulocytes. Of note, also IL-33 administration after the tissue migration phase, on days 4 and 5 post infection, significantly reduced day 6 intestinal parasite burden. Moreover, despite the reduced intestinal parasite burden, numbers of migrating larvae in skin, lung and head day 2 p.i. were significantly increased in IL-33-treated mice. Thus IL-33 did not improve host defense during *S. ratti* tissue and lung migration but acted directly in the intestine by inducing IL-9-dependent mast cell degranulation. In summary our data show that IL-33 can play contradictory tissue-specific roles during helminth infection. The source(s) of IL-33 as well as the IL-33 responsive cells in the different tissues are currently investigated.

PD-L2 downregulates GATA-3 in Th2 cells during filarial infection resulting in Th2 cell-intrinsic dysfunction and plasticity

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T cell-intrinsic regulation, such as anergy and exhaustion, is central to immune regulation. However, knowledge on the intrinsic fate and function of Th2 cells in chronicity is lacking. We show that infection with the filarial nematode *Litomosoides sigmodontis* causes Th2 cells to down-regulate GATA-3 in a PD-L2 dependent manner. The Th2 cells subsequently diverge from a classical GATA-3^{+}IL-4^{+}IL-5^{+}IL-13^{+} Th2 effector phenotype, both differentiating towards a novel intrinsically dysfunctional GATA-3^{-}Egr2^{+}cMaf^{+}Blimp-1^{+}IL-4^{lo}IL-5^{lo}IL-13^{lo}IL-21^{+} Th2 phenotype and repolarising towards T-bet^{+}IFN-γ^{+} Th1 cells. GATA-3 downregulation coincides with an increased recruitment of PD-L2^{+} F4/80^{lo}Ly6C^{+} monocytes to the infection site, and is independent of B cell derived PD-L2, suggesting that monocytes are the PD-L2^{+} cell type regulating Th2 cell function. The dysfunctional Th2 cells had a divergent transcriptional profile to classical Th2 cells, and retained their unresponsive phenotype upon transfer to naïve recipients, indicating a distinct and stable state of Th2 cell differentiation. Th2 cell dysfunction is transcriptionally distinct from exhaustion, but shares similarities with T cell anergy and tolerance. The dysfunctional Th2 cells produced IL-21, and IL-21R blockade increased resistance and humoral immunity to *L. sigmodontis*, indicating a novel regulatory role for IL-21 during filariasis. Together, this suggests that the PD-1/PD-L2 pathway is an important regulator of GATA-3 and Th2 plasticity, and drives a novel form of Th2 cell tolerance that inhibits protective immunity to filarial helminths via IL-21. This divergence towards alternate Th2 phenotypes during chronicity has broad implications for the outcomes and treatment of chronic Type 2-related infections and diseases.

Strong changes in peripheral blood immune signatures after experimental hookworm infection

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Animal models of helminth infection have shown a critical role for T cells in governing resistance/susceptibility to infection. In humans, much less is known about the nature of the T cell response to helminth infection. This has not been helped by confounding variables such as burden of infection, numbers of previous infections and extraneous infections. We have completed a placebo trial where hookworm naïve volunteers were infected with a defined number of *N. americanus* parasites and age/sex matched no parasite controls. We analysed blood at day 0 and week 8 using a novel unbiased template-switch anchored NGS methodology for quantitating human T cell receptors (TCRs). This method allows us to identify and track millions of clonotypes over time entering and leaving the system. Our analysis showed an influx of new clonotypes entering the blood post infection and, surprisingly a pronounced restructuring of the pre-existing T cell repertoire. It is interesting that a small number of helminths in the gut can cause such a dramatic restructuring of the T cell repertoire in the blood. It is simultaneous alarming on the immunological consequences of this “aftershock”. To conclude, this is the first study to quantify T cell repertoire changes after a controlled hookworm infection in humans.

The role of *Wolbachia* somatic cell-to-cell transfer in filarial nematode symbiosis

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Wolbachia, an obligate bacterial endosymbiont of filarial nematodes, is vertically transmitted from one generation to the next through the maternal germline. Rather than undergoing a strict germline-to-germline transmission as observed in other endosymbionts, *Wolbachia* undergoes a circuitous germline-to-soma-to-germline transmission route. This requires that *Wolbachia* undergo cell-to-cell transmission and maneuver through distinct nematode cell-types. Here we discuss our in vitro and in vivo studies examining symbiont-host interactions enabling *Wolbachia* to effectively navigate filarial nematode somatic and germline tissues.

Transcriptomic characterization of the proliferative cells of the cestode *Mesocestoides corti* by depletion and purification

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In free living Platyhelminthes, somatic differentiated cells are not able to divide, and the cells involved in tissue renewal, homeostasis and regeneration are undifferentiated pluripotent stem cells, called neoblasts. Cellular and molecular studies in *Schistosoma mansoni* and in the cestode *Echinococcus multilocularis* have shown that in parasitic flatworms this proliferative capacity relies on similar cells that are named neoblast-like or germinative cells respectively. Aiming at generating tools to study development in the model cestode *Mesocestoides corti,* we localized and characterized the proliferative cells and optimized a FACS protocol to isolate them. Now we advance in the characterization of these proliferative cells using two complementary transcriptomic approaches. We compared the transcriptomics of normal and irradiated worms (depleted of proliferative cells) and also the transcriptomic profile of purified proliferative cells vs differentiated cells. These are the first transcriptomic analyses obtained for purified proliferative cells in cestodes. Our results show that several molecular markers of pluripotent cells of free living and trematodes are also preferentially expressed in *M. corti* proliferative cells, confirming a basic common molecular signature of these cells. Despite this, a comparison of the "neoblast" like data available in the diverse species highlight several marker genes that are not shared, suggesting that despite their morphological similarity, flatworm proliferative cells are diverse at the molecular level. These comparative results allow defining similarities and differences among flatworm pluripotent cells.

Leveraging single-cell RNA sequencing to understand stem cell biology in the parasitic flatworm *Schistosoma mansoni*

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Parasitic flatworms of the genus *Schistosoma* (schistosomes) are the causative agent of the devastating neglected tropical disease schistosomiasis. Despite the importance of these pathogens, very little is understood about their incredible biology. One remarkable trait of the schistosome is that it can thrive inside of the vasculature of their host for decades, constantly battered by blood flow and surrounded by the host's immune system. This combined with the ordinary wear-and-tear experienced by any long-lived animal implies that the parasites have some way to renew and maintain their various tissues. Our group has previously characterized a population of stem cells that resides within the parasite and found that they are involved in tissue homeostasis in adult schistosomes. While attempting to identify genes that regulate these stem cells, we discovered that a homolog of the tumor suppressor *p53* is required for maintenance of most, but not all, stem cells in the animals, suggesting the parasites have a heterogenous population of stem cells. We performed single-cell RNA sequencing which enabled us to identify the subpopulation of stem cells that remains following *p53* loss-of-function. Next, we performed a small-scale RNAi screen to determine the function of heterogeneity in schistosome stem cells. From this screen we identified several genes that appear to be important for maintaining the identity and the differentiation potential of the parasite's stem cells. This work furthers our understanding of how these deadly parasites can survive and thrive in their hosts for decades on end and it highlights the utility of single-cell RNA sequencing in the stud

Heterogeneity of Type-2 immune responses during helminth infections

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Our goal is to understand the heterogeneity of type 2 immune responses during helminth infections. Although we have co-evolved with helminths and most infections are asymptomatic, these parasites can cause pathology in some individuals that either mount a response that is too strong or too weak during infection. An appropriately regulated type-2 response is critical in maintaining the balance between expelling enough parasites, and tolerating the remaining parasites without excessive collateral tissue damage, in order to maintain the fitness of the host. Helminth infections may be therapeutically beneficial for improving symptoms of inflammatory bowel diseases and metabolic syndrome, but this may also benefit only specific subsets of individuals. The mechanisms underlying the heterogeneity of type-2 responses between individuals still remains poorly understood. We believe that characterizing these mechanisms will enable us to develop strategies to utilize helminth infections as treatment for specific inflammatory conditions (e.g. autoimmune diseases and metabolic conditions) for the right individuals, as well as designing better approaches towards limiting pathology that is caused by helminth infections. Our studies on macrophage biology and translational research studies on the microbiota are converging towards the goal of understanding factors that regulate variation of type-2 immune responses. Using a combination of mouse models, as well as field and clinical studies, we will describe some of our efforts to characterize heterogeneity of immune responses.

Characterization of type-2 immune responses during helminth infection by mass cytometry

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Helminths can have a profound role in shaping immune profiles by inducing strong type 2 and regulatory responses, however, the degree of heterogeneity of such cells has not been studied before. Mass cytometry was used to profile these cells in Europeans not exposed to helminths, and in Indonesians who were infected with soil-transmitted helminths. To assign immune alteration to carrying helminth infection, the profiling of Indonesians was performed before and 1 year after deworming. Very distinct signatures were found in Europeans and Indonesians, showing expanded frequencies of Th2 cells, in particular $CD161^+$ cells, and ILC2s, in helminth-infected Indonesians, which was confirmed functionally through analysis of cytokine producing cells. Besides ILC2s and $CD4^+$ T cells, type 2 cytokines were also produced by $CD8^+$ T cells and γδ T cells in Indonesians; this was considerably less in Europeans. The regulatory T cells were also expanded in Indonesians, but only those expressing CTLA4, and some co-expressed CD38, HLA-DR, ICOS or CD161. Whereas IL-10 production could not capture the difference that was seen in regulatory T cells, $CD11c^+$ B cells were found to be the main IL-10 producers among B cells in Indonesians, a subset which was almost absent in Europeans. A number of the distinct immune profiles were driven by being infected with helminths as they reverted following clearance of helminth infections. The detailed insight into the human type 2 and regulatory networks could provide possibilities to design interventions more precisely to target these cells during disease process.

Environmental control of pulmonary macrophage responsiveness during type-2 inflammation

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Refined control of macrophage (MΦ) activation is necessary to prevent inflammatory disease, particularly at barrier sites such as the lung. However, the main mechanisms that regulate pulmonary MΦs during type-2 inflammation, which underpins helminth infection and allergic disease, are poorly understood. We have discovered that murine airway MΦs are considerably less able to respond to the type-2 cytokine IL-4 *in vivo* than lung tissue or peritoneal cavity MΦs. This phenotype was apparent following administration of IL-4:anti-IL-4 complex, during infection with *Nippostrongylus brasiliensis,* or following exposure to house dust mite. We have shown that MΦ responsiveness to IL-4 is controlled by the lung environment, though independent of surfactant protein D, mucin 5B or the host microbiota. Notably, compared to cavity MΦs, airway MΦs displayed severely dysregulated metabolism. Further, upon removal from the lung, these MΦs regained IL-4 responsiveness in a process that depended on glycolysis. These novel data reveal central roles for the lung environment and glycolysis in regulation of MΦ responsiveness during type-2 inflammation *in vivo*.

Allergen pre-sensitization drives an eosinophil-dependent lung-specific arrest of helminth development

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This study investigates the relationship between helminth infection and allergic sensitization by assessing the influence of pre-existing allergic sensitization on the outcome of helminth infections rather than the more traditional approach whereby the helminth infection precedes the onset of allergy (hygiene hypothesis). Here we used a murine model of house dust mite (HDM)-induced allergic asthma inflammation followed by *Ascaris* infection to demonstrate that allergic sensitization to environmental aeroallergens drives an eosinophil-rich pulmonary type-2-immune response (Th2 cells, M2 macrophages, ILC2s, IL-33, IL-4, IL-13 and mucus) that directly hinders larval development and reduces the parasite burden in the lungs by up to 70%. This effect is dependent on the presence of eosinophils, as eosinophil-deficient mice were unable to limit parasite development or numbers. RNA-seq analyses of *Ascaris* larvae from allergen pre-sensitizes mice compared to larvae from non-allergic mice demonstrated marked alterations between the 2 sets of larvae with 1,330 genes being significantly differentially expressed. Using L3 and L4 larval stage-specific signature mRNA profiles, it could be shown that the *Ascaris* larvae recovered in the airways of non-allergic mice showed a transcriptomic-signature of a larvae transitioning between the L3-lung stage and that felt to represent the L4-intestinal stage; in marked contrast however, the larval stages recovered in the airways of HDM-pre-sensitized mice had a molecular signature of L3-liver stage. Our data suggest that HDM-allergen sensitization drives a response that mimics a primary *Ascaris* infection, such that lung-specific type-2 mediated eosinophil-dependent helminth larval killing in the tissue occurs. This study provides a window through which the mechanisms underlying tissue-specific responses that can drive a protective response against the early stages of helminth parasites. It also helps identify at a molecular level potential genes product crucial for larval development that could be used as potential targets for the prevention of chronic infections in the host.

Targeting the DAF-12 nuclear receptor pathway in parasitic nematodes

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The ability to regulate nutrient metabolism in both fed and fasted states is a physiologic process that coincided with the evolution of nuclear receptors in all multi-cellular organisms. In mammals, these receptors have evolved to respond to dietary lipid metabolites of cholesterol and fatty acids and thereby govern nutrient storage and utilization. In the free-living nematode, *Caenorhabditis elegans*, we have shown the existence of an orthologous pathway that is mediated by the nuclear receptor, DAF-12, and functions as crucial regulator of the nematode's life-cycle. This discovery led to the finding that the DAF-12 signaling pathway is conserved in parasitic species and functions as the key regulator of the infectious stage. One such parasite is *Strongyloides stercoralis*, which infects over 100 million people, is practically treatable by only one anthelmintic drug, and often develops into a lethal hyperinfection. We have shown that *S. stercoralis* may be particularly sensitive to DAF-12 based pharmacology and current efforts are focused on this strategy as a novel anthelmintic approach.

Genetic and phenotypic analyses of CRISPR/Cas9-induced mutations in *Ss-daf-16*, which encodes a forkhead transcription factor in the parasitic nematode *Strongyloides stercoralis*

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The purpose of the study was to interrogate the function of the forkhead transcription factor Ss-DAF-16 in the morphogenesis, invasiveness and viability of infective third-stage larvae (iL3) of *Strongyloides stercoralis*. We used CRISPR/Cas9 to create disrupting insertional mutations in the fifth exon of Ss-daf-16. Plasmid encoded CRISPR/Cas9 elements, including the Cas9 endonuclease, gene-specific and control guide RNAs (gRNAs) and a repair template containing multiple stop codons, were microinjected along with a fluorescent marker transgene into the syncytial gonads of parental free-living *S. stercoralis* females. These females were mated with wild type free-living males and their progeny assayed for presence of the transgene cocktail by fluorescence microscopy, for the insertional mutation by PCR and for any morphological or behavioral phenotypes by visual inspection by stereomicroscopy. The insertional mutation was detected by PCR in 30% of F1 larvae expressing the co-transformation GFP marker indicating transformation with the CRISPR/Cas9 elements. A significant proportion of these PCR-positive/GFP-positive individuals exhibited abnormalities in pharyngeal and intestinal morphology, incomplete molting and ecdysis, impaired motility, delayed or blocked development or inability of infective third-stage (iL3) to penetrate isolated murine skin. A similar proportion of GFP+ individuals that did not have the insertional mutation also exhibited one or more of these phenotypes. These phenotypes coincided with random insertions or deletions at the target site' which were not subject to incorporation of the repair template via homology directed repair. No such phenotypes occurred in F1 progeny of females transformed with CRISPR/Cas9 elements including a control gRNA lacking homology within the *S. stercoralis.* These results combine with previous observations to support that Ss-DAF-16 is required for the pharyngeal and intestinal remodeling that is essential for the transformation of soil-dwelling pre-infective larvae of *S. stercoralis* into infective larval stages capable of invading a mammalian host.

A hypodermally expressed putative GM2 activator protein is essential for cuticular integrity in *Caenorhabditis elegans*

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Human onchocerciasis is the world's second cause of infectious blindness caused by the parasitic nematode *Onchocerca volvulus*. As with other helminths, *O. volvulus* is known to be a master immuno-modulator and surface proteins/excretory secretory products (ESPs) are thought to be key mediators in the immunomodulatory process. Functional, molecular and immunological characterization of these ESPs will provide new insights into underlying mechanisms. Because *O. volvulus* does not propagate *in vitro*, we herein utilized the tractable *C. elegans* model for functional characterization of a previously identified *O. volvulus* ESP of the GM2AP superfamily (OvGM2AP). We uncover that the *C. elegans* orthologous gene (C34E7.4) has 2 other paralogous partners which are all expressed predominantly in the hypodermis, with paralogous forms confined to specialized hypodermal cells such as the seam cells, socket cells and UV1 cells of the vulva. Translational expression of C34E7.4 indicates the presence of the protein at the surface coat of the cuticle. CRISPR mediated knock-out of C34E7.4 resulted in dauers which are sensitive to 1% SDS. The survival of the mutant dauer in contaminated liquid cultures was also observed to be reduced compared to the wild type (N2). To further assess the integrity of the cuticle, we measured the resistance of the mutant worms (PHX 712) to hypotonocity and uncovered that PHX 712 could not survive in deionized water for 30 mins unlike N2. Furthermore, PHX 712 was rescued by translational expression of C34E7.4, confirming the responsibility of the gene for the phenotype. Expression of C34E7.4 under the control of a hypodermal promoter *amgo-1* successfully rescued the phenotype in PHX 712 worms, strongly supporting a hypodermal involvement in the phenotype. We previously showed that OvGM2AP is a lipid binding protein and are currently analyzing the surface lipids of N2 and mutant worms to ascertain the involvement of lipids in the SDS-sensitive phenotype. Transmission electron microscopy of N2 and PHX 712 dauer is in progress to identify possible phenotypic defects on the cuticle. Lastly, since the intestine of adult *Onchocerca* is almost non-functional, entry of drugs is mainly by the transcuticular route. We therefore intend to assess the cuticular permeability of N2 and PHX 712 worms to cuticle-insensitive drugs as well as sub-optimal doses of Ivermectin. Because previous studies on the expression profile of molting worms showed an upregulation of C34E7.4 and paralogues, we postulate based on evidence gathered so far, that the gene is involved in biogenesis of the cuticle. Its presence at the surface coat of the parasite warrants further immunological characterization for possible roles in immunomodulation.

Gut microbe metabolism influences *Trichuris* hatching and fecundity

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Helminths are parasitic worms that often inhabit the intestine and affect about 1.5 billion people worldwide. While standard chemotherapy has been shown to be effective against most species, it is less than 50% effective against the whipworm—*Trichuris trichiura*. While the interrelationships between microbial communities in the gut and the host immune system have been greatly studied, little is known about the effect of microbes on helminth infection. Therefore, a better understanding of vital microbial contributions at key whipworm life cycle stages presents the possibility of identifying new therapeutic targets. The aim of this study was to identify microbiota-derived factors that are essential for *Trichuris* development using the whipworm species that infects mice*, Trichuris muris*, as a model. Both members of the phyla Nematoda, we hypothesized that essential requirements for development in *C. elegans* are likely to be conserved in *T. muris*. First, we used the Keio *E. coli* mutant library to identify five candidate genes that are essential for *C. elegans* fertility. Next, we sought to investigate the effect of these mutants on two separate life cycle stages of *T. muris*: hatching and sexual maturation. We developed an *in vitr*o assay to examine the effect of these *E. coli* mutants on hatching. We then monocolonized germ-free mice with each mutant strain prior to acute *T. muris* infection to examine their contributions to *T. muris* development *in vivo*. Our preliminary results indicate that *E. coli* mutants deficient in the fatty acid synthesis pathway fail to support whipworm hatching, while mutants lacking key ethanolamine degradation enzymes decrease fecundity. These findings establish a novel system for investigating the role of specific microbial genes and pathways in supporting the helminth life cycle and identify key metabolic pathways as necessary for *T. muris* infection.

Nematode complex I and II subunit gene duplications provide increased fitness to hypoxia

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Helminths use an alternative mitochondrial electron transport chain (ETC) under hypoxic conditions, such as those found in the gastrointestinal tract. In this alternative ETC fumarate is the final electron acceptor and rhodoquinone (RQ) serves as an electron carrier. RQ receives electrons from NADH through complex I and reduces fumarate through complex II. In this latter reaction complex II functions in opposite direction to the conventional ETC (*i.e.* as fumarate reductase instead of succinate dehydrogenase). Studies in *Ascaris suum* indicate that this is possible due to changes in complex II, involving alternative SDHA and SDHD subunits derived from duplicated genes. We analyzed helminth genomes and found that distinct lineages have different gene duplications of complex II subunits. Similarly, we found lineage specific duplications in genes encoding complex I subunits that interact with quinones. Phylogenetic analyses indicate that there were independent evolutionary events associated with these alternative ETCs. The free-living nematode *C. elegans* uses RQ and has two genes encoding SDHA (*sdha-1* and *sdha-2*) and two genes encoding NDUF2, a quinone interacting subunit of complex I (*gas-1*, formerly *nduf-2.1* and *nduf-2.2*). While *sdha-1* and *gas-1* are essential genes, we found that the knock out strains in *sdha-2* and *nduf-2.2* are not essential, even in hypoxia. Interestingly, *sdha-1* and *sdha-2* as well as *gas-1* and *nduf-2.2* showed inverted expression profiles during the life cycle. *sdha-2* and *nduf-2.2* expression increased in the early embryo and in dauer larvae, stages where there is lower oxygen tension. Importantly, we found that *sdha-2* and *nduf-2.2* KO mutant strain progeny is affected in hypoxia. Our results indicate that different complex I and II subunit gene duplications provide increased fitness to hypoxia in worms.

How Rhodoquinone is made and how to find drugs that block Rhodoquinone synthesis

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Parasitic helminths infect over a billion humans. To survive in the low oxygen environment of their hosts, these parasites use unusual anaerobic metabolism. This requires Rhodoquinone (RQ), an electron carrier that is made by very few animal species and, crucially, it is not present in any parasitic hosts. RQ synthesis is thus an ideal target for anthelmintics but little is known about how RQ is made and no drugs are known to block RQ synthesis. I hope this talk will give a good sense of the progress we've made in understanding how RQ is made in helminths and the novel assay we've developed that can drive the development of a new class of anthelmintic drugs. Studying RQ synthesis directly in parasites is hard. However, the non-parasitic nematode *C.elegans* makes RQ and can use RQ-dependent metabolic pathways — *C.elegans* can thus be used as a model to understand how RQ is made and for drug screens to find compounds that block RQ synthesis or RQ use. I'll present our recent work where we show that *C.elegans* makes RQ from precursors that derive from tryptophan via the kynurenine pathway. Worms without a functional kynurenine pathway cannot make RQ and we show that these RQ-deficient worms cannot survive in hypoxic conditions. I will also present unpublished data showing the key RQ-dependent metabolic pathways that *C.elegans* uses in hypoxic conditions which maps out potential targets for drugs that could disrupt this RQ-dependent anaerobic metabolism. Finally, I'll present some initial results of a screen for drugs that interfere with RQ synthesis or use. We established a robust assay for drugs that block RQ-dependent metabolism using an image-based assay for worm movement. We screened a panel of ~3000 FDA approved drugs and have 50 strong hits. I'll set out how we combine this drug screen with genetic screens to identify the targets of each drug and our future steps.

The development of epigenetic modulators as next-generation anti-schistosomals

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As a component of an integrative strategy for pursuing schistosomiasis elimination, the identification and lead progression of new anti-schistosomal drugs is vital. Our interest in epigenetic processes shaping schistosome development has identified an important role for histone modifying enzymes. Here, we summarise our approaches used to identify histone methyltransferases (HMTs) as druggable targets and illustrate the optimisation of GPV56 as an anti-SmHMT drug lead. Analysis of the *Schistosoma mansoni* genome for the presence of epigenetic proteins involved in histone methylation and demethylation revealed 27 histone methyltransferases (SmHMTs) and 14 histone demethylases (SmHDMs). One SmHMT was selected for further studies due to its similarity to an essential gene product in *Caenorhabditis elegans* and its inclusion as a TDR drug target. Molecular modelling of this SmHMT and *in silico* docking of a compound library (4000 compounds) led to the selection of the GPV series. This series contained more selective SmHMT docking characteristics when compared to the human homolog and was progressed for *in vitro* anti-schistosomal screening. Iterative *in silico* docking followed by *in vitro* screening led to GPV56, a lead compound displaying potent (schistosomula EC_{50} = 400 nM, adult worm EC_{50} = 50 nM) and selective activity (selectivity indices, SI: 24 to 190) when compared to HepG2 cells. Medicinal chemistry optimisation of GPV56 led to the creation of 20 derivatives, some representing entirely new chemical entities. Dose response titrations of these derivatives identified examples displaying greater anti-schistosomal potency (e.g. schistosomula EC_{50} = 380 nM; adult worm EC_{50} = 2.6 nM), increased selectivity (SI: 225 for adult worms) and anti-fecundity activity. ADME (Caco-2 cell permeability, plasma protein binding, microsomal metabolic stability, semi-thermodynamic solubility), genotoxicity (micronucleus assays) and DMPK trials are currently ongoing. This portfolio of preclinical data will be used to further progress GPV56 as a next generation anti-schistosomal for *in vivo* investigations.

A-WOL: A decade of drug discovery and development of safe macrofilaricides for onchocerciasis and lymphatic filariasis

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A-WOL aims to discover and develop new drugs against onchocerciasis (river blindness) and lymphatic filariasis (elephantiasis). A-WOL is working to develop novel approaches that are capable of killing adult worms within a 7-day treatment period by targeting Wolbachia, a mutualist bacterium essential for the worms' survival. The consortium has identified a candidate molecule, TylAMac, which is the first next-generation anti-Wolbachia drug to be designed specifically as a macrofilaricidal agent. The lead molecule is undergoing formal preclinical evaluation in partnership with AbbVie with successful phase I trials. A-WOL has screened the human pharmacopeia, focused anti-infective and large diversity libraries to identify 6 novel anti-Wolbachia chemotypes with suitable drug-like qualities. The first industrial scale screening of 1.3 million compounds in partnership with AstraZeneca delivered 20,000 hits and a further 10 novel chemotypes as promising new leads. Several of these lead candidates show rapid killing of Wolbachia which can deliver even shorter treatment periods. A-WOL has also identified two repurposing molecules, high-dose rifampicin and fusidic acid, which significantly reduce treatment times compared to the current gold standard, doxycycline. A radical improvement to targeting Wolbachia occurs via a drug synergy between a common anthelmintic drug (albendazole) and different classes of antibiotics (tetracyclines and rifampicin). Using the drugs in combination reduced the length of treatment required from several weeks to 7 days, opening up the opportunity to scale-up this approach at the community level.

Efficacy of the novel macrofilaricidal drug candidates ABBV-4083 and oxfendazole

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ABBV-4083 and oxfendazole are two novel drug candidates that are currently considered for phase 2 clinical trials for the treatment of onchocerciasis and lymphatic filariasis. ABBV-4083 is a tylosin analog, which targets the *Wolbachia* endosymbionts of filariae, whereas oxfendazole directly acts on filarial tubulin. *In vitro*, oxfendazole was active against pre-adult worms of *Onchocerca volvulus* and *O. gutturosa* adult worms and had a marginal efficacy against *Onchocerca lienalis* microfilariae. Accordingly, oxfendazole lacked a microfilaricidal efficacy in mice injected with *L. sigmodontis* microfilariae, but mediated a complete clearance of the *L. sigmodontis* adult worm burden after 5 days of oral treatments with oxfendazole (2x12.5, 2x25mg/kg). Oral oxfendazole treatments (10 days, 2x5mg/kg) of patently *L. sigmodontis*-infected jirds reduced the peripheral microfilaremia by >99% within 3 weeks after treatment start and completely inhibited the embryogenesis of remaining female adult worms 16 weeks after treatment start. These results indicate that oxfendazole has an excellent macrofilaricidal efficacy following oral administration, but lacks a strong microfilaricidal efficacy, which reduces the risk of associated severe adverse events. Oral treatment of *L. sigmodontis*-infected mice with ABBV-4083 reduced the *Wolbachia* in female adult worms as soon as three days after treatment start with continued declining *Wolbachia* levels throughout a 3-week washout phase. Two weeks of ABBV-4083 treatment in patently *L. sigmodontis*-infected jirds led to a >99.9% *Wolbachia* depletion in female adult worms, the inhibition of filarial embryogenesis and clearance of peripheral microfilaremia. Combination of ABBV-4083 with albendazole allowed lower doses of ABBV-4083 and shorter treatment regimens of 7 days, leading to an improved *Wolbachia* depletion and maintained clearance of peripheral microfilaremia compared to treatments with ABBV-4083 or albendazole alone. Thus, ABBV-4083 represents a promising anti-*Wolbachia* candidate that is efficacious in animal models of onchocerciasis and was recently tested in a phase 1 clinical trial.

Glycan targets for intervention and control of schistosomiasis

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Helminths express an abundance of proteins and lipids with complex and antigenic glycosylation patterns. In particular schistosomes have been extensively studied with respect to structural and functional glycomics and the exploration of glycan-based intervention tools. We previously applied mass spectrometric (MS) approaches to determine expression profiles of protein- and lipid-linked glycans during the schistosome life cycle. Striking shifts and switches in the expression of putative functional glycan motifs during during worm and egg development were identified and we have generated a microarray covering the entire glycome of *S. mansoni*. This microarray was used to interrogate longitudinally *S. mansoni*, S. *haematobium* and *S. japonicum* infection sera from experimentally infected animals, as well as from human cohorts, with respect to serum IgM, IgG and IgE against each glycan motif. The dynamics and intensity of the antibodies were analysed in relation to infection duration, intensity and treatment and the diagnostic potential of glycan-specific antibody responses will be highlighted. Overall, the most intense IgM and IgG responses are against a range of highly fucosylated glycans associated with cercarial and egg glycoproteins and glycolipids, but IgE responses were restricted to glycoprotein N-glycan core modifications only. On the basis of these initial cohort screenings for anti-glycan responses and antigen identification we reconstructed a number of highly fucosylated schistosome glycan antigens synthetically and through glycan-engineered expression in plants. In addition to their exploration as diagnostic targets, the produced glycans antigens were tested as potential prophylactic vaccine in rodent models of schistosome infection, and their protective potential will be presented on the basis of recent data. Observations in schistosomiasis will be discussed in relation to the glycans and glycan-induced immune responses in other helminthiases. It is concluded that parasite glycans are attractive targets for novel therapeutics and control tools of helminth infections.

Spilling your guts: extracellular vesicles contribute to haemoglobin digestion by *Fasciola hepatica*

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The liver fluke, *Fasciola hepatica*, is an obligate blood-feeder. Using their muscular pharynx, adult parasites draw blood into their gut where haemoglobin is digested, by cathepsin L peptidases, to supply the amino acids needed for the production of huge amounts of eggs. We have previously shown that the low pH microenvironment of the fluke gut lumen is required for auto-activation of cathepsin L zymogens. Furthermore, partial denaturation of haemoglobin at this pH was sufficient to relax the structure of the molecule and make it susceptible to proteolysis. Our recent data suggest that parasite-derived extracellular vesicles (EVs) carrying inactive cathepsin L zymogens may also contribute to the digestion process. Immunolocalisation studies, using markers identified following proteomics analysis of *F. hepatica* EVs, indicate that the vesicles are produced by the gastrodermal cells that line the fluke gut. This observation is supported by comparative proteomics analysis of isolated *F. hepatica* EVs and laser-microdissected gut caeca. Whilst EVs were stable at physiological pH, incubation in acidic conditions led to their lysis and release of cargo molecules. One of the most dominant cargo molecules was found to be the inactive 37 kDa cathepsin L zymogen which could be rapidly activated at low pH to yield the active 24 kDa mature enzyme that was capable of digesting host haemoglobin. Thus, our studies suggest that a sub-population of EVs are released *en masse* from the *F. hepatica* gastrodermal cells into the gut lumen where they mix with ingested host blood. There, the acidic conditions cause them to lyse and release their cathepsin L cargo (which rapidly undergoes pH-dependent auto-activation) resulting in efficient degradation of haemoglobin. Our findings suggest a novel functional role for *F. hepatica* EVs and highlight the importance of pH in the acquisition of nutrients by the parasite.

Small RNAs and their Functions in Helminths

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Diverse repertoires of small RNAs function in transcriptional and post-transcriptional gene regulation in a breadth of biological processes. Small RNAs and their protein effectors, Argonautes, can be classified into 3 major categories: miRNAs/siRNAs and Argonautes (Ago-clade), piRNAs and PIWI Argonautes (PIWI-clade), and worm-specific Argonautes and their secondary siRNAs (WAGO-clade). miRNAs predominantly function in post-transcriptional gene regulation modulating mRNA stability and translation. piRNAs predominantly function in genome defense repressing mobile elements in the germline. siRNAs play diverse roles including defense against viral infection but also in a variety of molecular and cellular processes. Our understanding of small RNA pathways, in general, and in nematodes comes from extensive and pioneering studies in *C. elegans*. *C. elegans* has a diverse and complex set of small RNAs, Argonautes, and small RNA pathways. *C. elegans* has 27 Argonautes that associate with miRNAs, PIWI-like small RNAs (21U-RNAs), and a spectrum of siRNAs (including 26G- and 22G-RNAs). A major expansion of *C. elegans* Argonautes (known as WAGOs) is associated with the complex secondary 22G-siRNAs that are amplified through RNA-dependent RNA polymerases. 22G-RNAs are the most abundant small RNAs in *C. elegans* and in other nematodes. Nematodes are exceptionally diverse having adapted to virtually all environments including a spectrum of parasitic hosts and lifestyles. Differences in Argonautes and small RNA pathways has been observed in nematodes, particularly the loss of piRNAs and PIWI proteins, suggesting fluidity and flexibility in nematode small RNA pathways. Notably, nematode small RNAs function in cell-to-cell and cross-species communication and can lead to epigenetic changes that can be transgenerational. Flatworms are also a diverse assemblage of organisms including free-living forms (Turbellaria) and parasitic forms including monogenes (ecto-parasites), flukes (trematodes), and tapeworms. Free-living flatworms have piRNA, miRNA, and siRNA pathways. However, parasitic flatworms have lost the piRNA pathway and appear to have an expansion of flatworm Argonautes (Fl-Ago). Small RNAs play diverse roles in flatworms including cross-species RNA communication. Small RNA pathways in helminths, their contribution to biological processes, and their role in host-parasite interactions and transgenerational inheritance are the subjects of this Mini-Symposium.

Roles of miRNAs in development: lessons from *C. elegans*

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A major goal in biology is to understand how the information encoded in one genome unfolds into a vast diversity of cell types that make up an animal. During this fascinating process, asymmetries in gene expression in the developing embryo are introduced and propagated by a variety of transcriptional and post-transcriptional regulators. A class of RNA-based, post-transcriptional repressors called microRNAs, is essential for animal development. Indeed, lack of the enzymes that produce miRNAs causes severe embryogenesis defects. Despite their collective importance and their general conservation, loss of most individual miRNAs has little or no effect on embryogenesis, and the functions of most miRNAs remain unknown. We recently found that except for a few miRNAs, most are expressed with very high cell-type specificity in *C. elegans*, suggesting that they have cell-specific functions. Here we propose a framework that reconciles these observations, based on two hypotheses: (1) A few broad and abundant embryonic miRNAs are necessary and sufficient to account for the collective need for miRNAs. (2). Most embryonic miRNAs are highly cell-type specific and may play roles at later stages in the differentiation and/or function of these specific cells; this would impact the development and evolution of animal complexity. We explore these hypotheses using a variety of approaches, including a new miRNA profiling method. We use *C. elegans* as our main model system as it allows a comprehensive view that bridges from whole-organism to single-cell level. Our goal is to reveal the roles of members of both miRNA classes: the early and essential ones, and the later, cell-specific ones - the latter, in the context of neuronal and muscle-cell diversification.

Understanding how the miRNAs of *Fasciola hepatica* contribute to the host-parasite relationship

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Investigations into the possible mechanisms utilised by parasites to modulate host immune responses, to support their long-term survival, has identified an emerging role for miRNAs. Utilising a non-biased pipeline to interrogate the recently completed genome of *Fasciola hepatica* we have expanded the current panel of miRNAs characterised for this parasite. Among these, we have identified a panel of miRNAs that are specific to *F. hepatica*, notably distinct from the miRNAs characterised for either *Schistosoma japonicum* or *S.mansoni*. To determine functionality for these miRNAs, we measured their differential expression during the different life stages (newly excysted juvenile (NEJ), 21 day old, adult) of Fasciola with miRNASeq. These profiles were then paired with the transcriptome of the same worms to create an interactome allowing the identification of novel targets and pathways associated with parasite development and pathogenicity. Target prediction software also supported the possibility that some of these novel helminth miRNAs acted to regulate host immune responses. We hypothesised that the miRNAs predominantly expressed by the NEJs and packaged within their exosomes are the main protagonist in the regulation of host immune responses during the early phase of infection as the juvenile parasites migrate through the peritoneal cavity to reach the liver. Indeed, one of these miRNAs was detected within the peritoneal macrophages of infected mice. Furthermore, this miRNA functioned to prevent the activation of murine macrophages by inflammatory ligands. These results confirm a role for parasite miRNAs in the regulation of host immune responses and identify new miRNAs and the pathways they regulate as potential targets for parasite control.

Small RNA pathways with a role in parasitism in *Strongyloides* nematodes

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Small RNAs (sRNAs) have two possible roles in parasitism, they may regulate the expression of endogenous genes involved in parasitism or they may be secreted into the host to target and repress the expression of host genes. Using comparative analyses of genetically identical parasitic and free-living life cycle stages of *Strongyloides* nematodes we have identified sRNA pathways with a putative role in parasitism. We have identified a class of microRNAs (miRNAs) with a conserved seed region that are highly expressed in parasitic, compared with free-living, *S. ratti* nematodes and are likely to target and repress expression of endogenous nematode genes. A subset of genes encoding worm specific Argonaute proteins (WAGOs), an important protein involved in sRNA pathways, and a class of 21-22U nucleotide small interfering RNAs (siRNAs) are both highly upregulated in the parasitic life cycle stage in *Strongyloides* spp. We hypothesise that these WAGOs and siRNAs represent a novel sRNA pathway involved in parasitism. We have also identified miRNAs and siRNAs that are secreted in exosome-like vesicles by the parasitic life cycle stage and are likely to target and repress host gene expression. To understand the conservation and diversification of these sRNA pathways across nematodes more generally, we have compared orthologous genes of several diverse nematodes and predicted which sRNA pathways are highly conserved or clade specific.

RNA in communication

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RNA is a dynamic molecule that underpins biological complexity through diverse mechanisms of gene regulation. In many organisms, RNA transmits information between cells as an important mechanism of cell-to-cell communication and antiviral defence. In some living systems RNA can also transmit information between different species. I will give an overview of our understanding of the mechanisms of this form of communication, and its functional importance, in gastrointestinal nematode infections of mammals. I will focus on the molecular mechanisms involved in RNA transmission and show that specific RNA sequences are selected for export by an extracellular Argonaute protein in Clade V nematodes. I will compare properties of the nematode extracellular Argonaute protein with our unpublished data showing transmission of RNA between cells by mammalian Argonaute proteins. Our work suggests RNA-binding proteins are key to RNA mobility and function and represent a new player in parasite-host communication.

The role of small RNA in parasite-host communication during *Trichinella spiralis* infection

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Extracellular small RNAs (18-30 nt) have been characterised in a variety of systems, including parasitic nematode infection. Secreted small RNAs are often encapsulated in extracellular vesicles, protecting them from degradation by extracellular nucleases. The function of extracellular small RNAs is largely unclear, but there is great interest in the possibility that they could contribute to host-parasite communication. *Trichinella spiralis* is an excellent model to study this process, as adult worms live largely in an extracellular environment in the small intestine, but muscle stage larvae live intracellularly within terminally differentiated skeletal muscle cells. Infection leads to morphological shifts in muscle cells including partial reversion of differentiation, although the molecular mechanisms underpinning this phenomenon are unknown. We are investigating whether secreted small RNAs could contribute to remodelling of skeletal muscle and completion of the parasite life cycle. We show that both larval and adult *T. spiralis* secrete extracellular vesicles and small RNAs, but our data suggest that whilst adult worms use these extracellular vesicles to deliver miRNAs, muscle stage larvae secrete miRNAs without encapsulation, demonstrated by the sensitivity to RNase digest of muscle stage larvae secreted RNA. Small RNAs enriched in secreted products include several with homology to host miRNAs known to play a crucial role in muscle development and disease, such as miR-31. We show that *T. spiralis* miR-31 is able to target host mRNAs, with targeted genes including those linked to muscular dystrophy, muscle development and cell cycle regulation. Our data suggest a novel secretory mechanism for small RNAs and raise the possibility of a role in reprogramming host muscle cells during *T. spiralis* infection.

Small RNAs in epigenetics and evolution

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Epigenetic gene regulation refers to stable changes in gene expression state in the absence of DNA sequence changes, which can be propagated through cell division. It is becoming clear that some epigenetic changes can be inherited transgenerationally. One such example of transgenerational epigenetic inheritance is small RNAs in the nematode C. elegans, which can lead to transgene silencing that is inherited over hundreds of generations. We are interested in the possibility that changes in epigenetic states (epimutations) due to altered small RNA levels could be propagated longitudinally through populations, thus acting as the raw material for evolutionary processes such as selection and drift. To investigate this possibility we used laboratory evolution of C. elegans over hundreds of generations and mapped the rate, spectrum and stability of epimutations. We find that epimutations arise rapidly, but have a short half-life of only 3-5 generations. Our results have key importance in understanding the contribution of non-genetic changes to evolution.

Cell type transcriptomic analysis of larval stage of *Schistosoma mansoni*

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The transcriptome of cell types and their spatial positions in the organism strongly determine their biology. So far, data-driven efforts to characterise populations in *Schistosoma mansoni* have relied on bulk RNAseq and single cell experiments with low cell numbers for specific cell populations. Thus, we do not yet have a complete understanding of the different gene markers that characterise the numerous populations in *S. mansoni*. In this study, we have generated a single-cell transcriptomic map from 2-day old schistosomula that comprises 2,144 cells. We have cells representing three distinct muscle clusters. Furthermore, there are three distinct clusters of the nervous system, two tegument clusters, two parenchymal/gut clusters and one cluster of stem cells, as well as a cluster of cells with no apparent known marker genes. We have performed extensive validation of cell markers for all these clusters by *in situ* hybridisation in 2-day old schistosomula as well as in adults. This has allowed us to provide an expression map with spatial resolution for novel and poorly characterised cell populations at different parasite stages. Overall, our study enables us to identify relatively rare populations representing ~1% of the dataset, thus highlighting the importance of massively parallel single cell platforms such as 10X Chromium for capturing the heterogeneity of cell populations in *S. mansoni*. The full dataset is intended to be the foundation for an atlas of *Schistosoma mansoni* biology.

Successful development of RNAi within the model parasitic nematode *Strongyloides ratti*

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Studies of gene function within the important model parasitic species *Strongyloides ratti* have long been stymied by a lack of appropriate genetic tools. While CRISPR/Cas9 has recently been successfully reported for *S. stercoralis*, it did not work as effectively in *S. ratti*. To counter this we recently succeeded in establishing RNAi in *S. ratti*. By firstly determining if *S. ratti* had the requisite proteins within its genome, we then developed a protocol for RNAi by soaking, by systematically varying the soaking medium, duration of soaking, addition of pharyngeal-pump-inducing compounds, the age of larvae and the soaking temperature. By using siRNAs, we bypass the need for dsRNA processing which *Strongyloides* appears to be refractory to. This approach was successful, with three separate genes so far knocked down with minimal off-target effects detected. This approach also has the added benefit of allowing analysis of gene function at different stages of the life cycle that occur outside a host, without the need of host passage, which means we do not have to maintain multiple mutant lines in a substantial amount of laboratory animals which is both laborious and expensive. We then used this technique to study the important gene *daf-12*, which has long been proposed to have role in infective larvae development within *Strongyloides* based upon the external application of dafachronic acid and gene expression studies. We found that suppression of *daf-12* severely impairs the formation of infective larvae and redirects development towards free-living larvae. We also found that *daf-12* functions in other processes (e.g. fat metabolism or temperature stress tolerance) are conserved in *S. ratti*, compared with *C. elegans*. Our experiments also confirm that the previously reported pharmacological effects of dafachronic acid within Strongyloides acted through DAF-12. Inhibitory molecules with optimized pharmacological properties can now be developed based on the DAF-12 structure.

Genetic manipulation of *Nippostrongylus brasiliensis* by lentiviral transduction

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Methods for genetic manipulation of animal-parasitic nematodes are extremely limited, and most attempts to reliably trigger the RNAi pathway using exogenous dsRNA have failed or been inconsistent. The major blockade to RNAi in most parasitic nematodes is likely to be effective delivery of sufficient dsRNA to initiate the response. Moreover, in order to test the role of defined genes on development and survival in the definitive host, sustained silencing must be initiated in infective or pre-infective larvae. We have demonstrated lentiviral delivery of a functional expression cassette to *Nippostrongylus brasiliensis* infective larvae following virus entry via the intestinal tract, with CMV promoter-driven reporter gene expression recorded for up to 5 days of *in vitro* culture. To improve functionality of the expression cassette, we are testing *C. elegans* promoters found in CpG-rich regions associated with protection from epigenetic silencing. Thus far, expression of the reporter gene mCherry under the control of the *C. elegans hlh-11* promoter resulted in a 10-fold increase of mCherry transcripts and improved detection of reporter gene expression by confocal microscopy. Furthermore, we demonstrated that following lentiviral delivery of a miRNA-adapted shRNA expression cassette, shRNAmirs are processed into mature miRNAs. Preliminary investigations into transcriptional silencing resulted in partial knockdown of selected targets at 72 hr post virus exposure. However, since silencing initiated by shRNAmirs would be limited to immediately accessible tissues, we are currently testing dsRNA constructs in order to engage with endogenous small RNA pathways, with the aim of systemic silencing throughout the animal and establishing robust protocols for gene silencing in parasitic nematodes.

New insights into nematode spliced leader *trans*-splicing

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Nematode infections cause a significant worldwide health and economic burden, costing over £1 billion a year in crop protection alone. Resistance to current anthelmintics is spreading globally, creating a demand for novel, broad spectrum anthelmintic targets. In this work, we are investigating spliced leader (SL) *trans*-splicing and its key RNA and protein components as potential anthelmintic targets, using *Caenorhabditis elegans* as a model system. SL *trans*-splicing is an essential process in nematode gene expression and while it is known that SL *trans*-splicing is dependent upon two functionally distinct ribonucleoprotein complexes, the mechanism by which SL *trans*-splicing is achieved is poorly understood. To better understand these complexes and their protein components, we have developed a novel reporter gene assay to monitor SL1 *trans*-splicing. We have used this assay to show that loss of key nematode-specific protein and RNA components impairs SL1 *trans*-splicing. Our recent unpublished data has expanded and refined our understanding of the ribonucleoprotein complexes involved in SL1 *trans*-splicing and led to the identification of new key players involved in SL1 *trans*-splicing. Importantly, we have identified a new, highly conserved, nematode-specific protein, SNA-3, that is an essential component of the SL1 ribonucleoprotein complex. SNA-3 is a promising drug target, since bacterial and plant homologues act as N-glycosidases. Further exploration of the function of SNA-3, and the SL associated ribonucleoprotein complexes, will provide new opportunities for the development of new anthelmintics.

Hookworms evade host immunity by secreting a deoxyribonuclease II to degrade neutrophil extracellular traps

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Hookworms are skin-penetrating parasites infecting about 2 billion people, principally in the south hemisphere. The skin has recently been shown to be an important bulwark against parasite establishment in a memory setting. However, the initial interaction between host and parasite in the skin during a primary infection is still poorly characterized. Here, we present the fate of the larvae from their skin penetration to their migration to the lungs using intravital microscopy. We observe that neutrophils are rapidly recruited to the site of infection, adhere to the larvae and deploy Extracellular traps around the larvae. Surprisingly however, neutrophils are not sufficient to cause parasite killing. We further show that the parasite adjusts its development to the neutrophils presence by an evasion demarche: on one hand, the parasite delays its exsheathment to benefit from an additional layer of cuticle protection; on the other, in response to bleach induced by the neutrophils, the parasite secretes specific Excretory-Secretory (ES) products with anti-neutrophil activity. We further identified that the larvae were able to evade killing by secreting a deoxyribonuclease (DNAse II) that degraded the DNA backbone of the NETs. Altogether, this study highlights that targeting both the nematode's sensing mechanism and its anti-neutrophil products could enable parasite killing early during its migration and thus block its transmission.

***Heligmosomoides polygyrus* secretes a suppressor of ST2**

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Type 2 immune responses are triggered during helminth infection, and optimal inflammatory responses are required for helminth expulsion, healing and repair responses. Contrarily, in diseases such as allergic asthma, type 2 inflammation is detrimental, causing inflammation and pathology. To evade expulsion helminths can modulate the host immune system through the release of immunomodulatory products which suppress initiation of type 2 responses and inducing an anti-inflammatory environment. A strong inducer of type 2 immunity is the alarmin IL-33, which is released upon necrotic cell death of epithelial cells, and acts on its receptor, ST2, which is expressed on a range of pro-allergic immune cells. In vivo administration of the excretory/secretory products of the intestinal murine nematode *Heligmosomoides polygyrus* (HES) have been shown to suppress asthmatic immune responses and expression of ST2. Through fractionation and mass spectrometry a single protein was identified which suppresses ST2 detection by flow cytometry. *H. polygyrus* Binds Alarmin Receptor and Inhibits (HpBARI) is a recombinant protein that binds directly to ST2 with high affinity, blocking IL-33 interaction with its receptor and suppressing IL-33-dependent immune responses. *In vitro,* HpBARI ablated bone marrow ILC2 responses to IL-33, while *in vivo*, HpBARI suppressed eosinophilia and ILC2 activation 24h after *Alternaria* administration and abrogated ST2 detection. HpBARI consists of 2 atypical consecutive CCP domains, similarly to the other *H. polygyrus*-derived immunomodulators HpARI (IL-33-suppressor, 3 CCP domains) and HpTGM (TGF-β mimic, 5 CCP domains). To dissect the activity of the CCP modules of HpBARI, each of them will be expressed independently and tested for ST2 binding and suppression. In conclusion, HpBARI is a further *H. polygyrus*-derived suppressor of the IL-33 pathway, further increasing our knowledge of the sophisticated immunomodulatory capabilities of this parasite, and the importance of IL-33 in type 2 immune responses.

Structure/biological activity relationships in the *Fasciola hepatica*-secreted immunomodulatory 68-mer peptide FhHDM-1

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During the pre-hepatic stage of infection with *Fasciola hepatica*, when the juvenile worms excyst and migrate through the intestine and peritoneal cavity, inflammatory changes are not observed in the intestinal wall and pathological findings are rare. Microarray analysis of peritoneal macrophages during infection of mice with *F. hepatica* revealed that as early as 8 hours after infection, all genetic markers of M1 macrophage activation, including TNF, IL-6 and IL-12, were significantly inhibited. In determining the mechanisms used by *F. hepatica*, we identified a novel protein, FhHDM-1 within the secretions of the parasite, with potent immune modulating ability. FhHDM-1 specifically interacts with the surface of macrophages, is endocytosed and trafficked to the lysosome where, through an interaction with vacuolar ATPase, it alters the pH. This influences the response of these cells to a range of inflammatory ligands, and significantly inhibits the activation of an M1 phenotype. Although possessing a unique amino acid sequence, FhHDM-1 shares structural similarity with the human cathelidin LL-37, displaying a predominant α-helix with an amphipathic region at the C-terminus. The biological activity of FhHDM-1 is dependent on this structure, as destruction of the helix or disruption of the amphipathic region, resulted in the loss of immune modulatory activity. To better understand how the unique structure of FhHDM-1 is critical to cell penetration, trafficking and biological activity, a series of derivative peptides were rationally designed around the various modules within the structure. These were then assessed for their ability to interact with macrophages, their intracellular localisation and their biological activity. Through this analysis we determined the minimum amino acid sequence required for activity and gained insight into the mechanism by which this peptide regulates the activation of inflammatory macrophages.

The major secreted protein of the whipworm parasite tethers to matrix and inhibits interleukin-13 function

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Infection by soil transmitted parasitic helminths e.g. *Trichuris spp* are ubiquitous in man and studies of mouse whipworm (*T. muris*) show that resistance is dependent on the action of interleukin (IL-13). Mechanisms determining persistence of chronic infection, however, are poorly understood. We show that p43, the single most abundant protein in *T. muris* excretions/secretions has a novel structure, binds to interleukin 13 and can inhibit its activity *in vitro* and *in vivo*. Tethering of p43 to matrix glycosaminoglycans may facilitate this process. Thus p43 may be a major immunomodulatory molecule that can enhance parasite survival. We are co-crystallising p43 with IL-13 and heparan sulphate to define functionally important binding sites. We have generated recombinant p43 homologue from human whipworm (*T. trichiura*) and will present data on its function. Exploiting the biology of p43 and homologues may open up new approaches to modulating IL-13 function and control of *Trichuris* infection.

The ecology of helminth infection and immunity in a wild rodent model

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Despite great concern about the current global health threat of infectious diseases in humans and domestic animals, we still don't have a clear understanding about how ecological heterogeneity determines infection burdens, disease, transmission, or how to successfully control infections in variable populations. Most drug treatments are selected using data from laboratory animal systems such as inbred mice. Our over-reliance on highly controlled, laboratory models may underlie some of our failures to adequately manage disease burdens in real-world settings, where individuals compete for food, mates and space; endure seasonal and spatial environmental variability; and are exposed to a vast array of parasites and pathogens. These sources of variation affect the dynamics of infection and yet this variation is missing in most laboratory studies. We have established a hybrid wild/laboratory rodent model system in order to investigate the causes and consequences of this ecological heterogeneity for host-parasite interactions. Specifically, this work focuses on *Heligomosoides polygyrus* and its natural host, wood mice (*Apodemus sylvaticus*) and addresses the following questions: (i) what determines susceptibility and resistance to *H. polygyrus* in the wild? and (ii) how does nutritional availability and coinfection with other gastrointestinal parasites impact *H. polygyrus* infection and immunity. From a series of field and controlled laboratory infection and coinfection experiments, we find that nutritional availability and coinfection with the coccidian *Eimeria hungaryensis* significantly impact *H. polygyrus* worm burdens, egg shedding, H. polygyrus specific IgG1, the efficacy of anthelmintic treatment, and the success of post-treatment reinfection. Our results highlight how pairing both the lab and natural setting provides a unique and powerful opportunity to understand the causes and consequences of ecological heterogeneity on infection, immunity and disease control.

The golden death bacillus *Chryseobacterium nematophagum;* a novel matrix digesting pathogen of nematodes.

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A highly virulent nematode killing bacterial pathogen has recently been isolated from rotten fruit in association with the free-living nematodes. These bacteria belong to the *Chryseobacterium* genus (Golden bacteria) and represent a new species which we have named *Chryseobacterium nematophagum*. *Caenorhabditis elegans* are attracted to and eat these bacteria, however very rapidly the bacilli degrade the anterior pharyngeal lining, enter the body cavity and digest the nematodes from the inside out. Importantly, these bacteria will also infect and kill bacteriverous, free-living (L1-L3) stages of all tested parasitic nematodes; including the important veterinary Trichostrongylids such as *Haemonchus contortus* and *Ostertagia ostertagi*. The bacteria exhibit potent collagen and chitin digesting properties and genome sequencing has identified novel metalloprotease, collagenase and chitinase enzymes that potentially represent virulence factors. The microbiological characterization, nematode-infecting properties and the bioinformatic characterization of this new nematode pathogen will be discussed.

Deciphering the responses of skin-penetrating nematodes to carbon dioxide.

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Skin-penetrating nematodes are gastrointestinal parasites that infect nearly one billion people worldwide. They respond robustly to a wide range of sensory cues. 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 (CO_2) in promoting parasite-host interactions using the human-skin-penetrating threadworm *Strongyloides stercoralis*. Specifically, we are elucidating the role of CO_2 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 to CO_2 differ across life stages. While infective larvae (iL3s) are repelled by CO_2, activated iL3s are attracted and free-living adults are neutral to CO_2. CO_2 is also critical for triggering activation. CO_2 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, CO_2 attraction may direct activated iL3s to the host small intestine. We are now using CRISPR/Cas9-mediated targeted mutagenesis to identify genes required for CO_2 response in parasitic nematodes. To date, we have found that the receptor guanylate cyclase GCY-9 mediates repulsion to CO_2 in *S. stercoralis* iL3s. GCY-9 is also involved in promoting activation. Additionally, by using *in vivo* calcium imaging, we have found that the *S. stercoralis* BAG neurons detect CO_2. We are now investigating the neural mechanisms that enable *S. stercoralis* to generate life-stage-specific responses to CO_2 and successfully establish infections. Our results will provide useful insights into the chemosensory mechanisms that drive host-parasite interactions.

Man's best friend: Activation of mosquito immunity blocks the development of transmission-stage *Dirofilaria immitis* and *Brugia malayi*

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Mosquito-borne helminth infections are responsible for a large worldwide disease burden in both humans and animals. Accordingly, development of novel strategies to reduce disease transmission by targeting these pathogens in the mosquito vector are of paramount importance. We found that a strain of *Aedes aegypti* that is refractory to infection by *Dirofilaria immitis*, the agent of heartworm disease, mounts a stronger immune response during infection than does a susceptible strain. Moreover, using a newly developed method to quantitate transmission-stage larvae capable of emerging from individual mosquitoes, we excitingly found that activation of the Toll immune signaling pathway in the susceptible strain through genetic manipulation arrests larvae development within the Malpighian tubules, thereby strongly blocking the number of larvae that develop to the transmission-stage. Notably, this strategy also blocks the development of transmission stage *Brugia malayi*, an agent of Lymphatic filariasis. Our data show that the mosquito immune response plays a pivotal role in restricting the development of transmission-stage filarial nematodes and suggest that to successfully complete their lifecycle, these pathogens must overcome or evade the mosquito immune response. Furthermore, despite their different sites of development in the mosquito, *D. immitis* is an informative model for dissecting the molecular interactions between mosquitoes and *B. malayi*. Novel strategies are needed to bolster our current control efforts. Our work opens the door to genetically engineering mosquitoes with enhanced immunity to help reduce disease transmission.

A rotifer-derived paralytic compound prevents transmission of schistosmiasis to a mammalian host

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Schistosomes are parasitic flatworms that infect over 200 million of the world's poorest people, causing the major neglected tropical disease, schistosomiasis. A single drug, praziquantel, is currently used to treat schistosome infection. Limitations of this drug in mass drug administration programs, the development of human/livestock hybrid schistosomes, and the emergence of schistosomiasis in non-tropical areas all indicate the urgent need for new strategies to prevent infection. It has been known for several decades that rotifers colonizing the schistosome's snail intermediate host produce a water-soluble factor that paralyzes cercariae, the life-cycle stage infecting humans. In spite of its potential for preventing infection, the nature of this factor has remained obscure. We report the purification and chemical characterization of Schistosome Paralysis Factor (SPF), a novel tetracyclic alkaloid produced by the rotifer Rotaria rotatoria. We show that this compound paralyzes schistosome cercariae and prevents infection, and does so more effectively than analogous compounds. This chemical interaction between four species (rotifer, snail, schistosome, and the mammalian host) has important implications for understanding the motility of cercariae and may ultimately lead to new approaches for preventing schistosome infection.

Immunomodulation studies in human populations … gang aft agley!

Alison Elliott

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There is evidence of geographical variation in vaccine response for a number of important licenced and investigational vaccines. Responses tend to be lower in low-income and tropical countries. Live vaccines may be affected more than inert vaccines. Animal models suggest that immunomodulation by parasites has a role, but the extent to which parasitic infections contribute to differences between human populations, and whether interventions against parasites can improve vaccine responses and efficacy is not known. Approaches to addressing this will be discussed.

Modulation of allergy and vaccine responses through maternal *S. mansoni* infection

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Chronic infection with the parasitic helminth *Schistosoma mansoni* is characterized by a modified Th2 response coupled to immunosuppression. This protects the host against overwhelming inflammatory responses against the parasite, but has spillover effects to bystander antigens, such as allergens. There is recent evidence that schistosomiasis during pregnancy similarly influences offspring allergic responses, as well as to vaccines. We have shown that allergic airway inflammation (AAI) in adult murine offspring from schistosome-infected mothers is strongly modified by the phase of maternal infection, and suppressed when pregnancy was initiated during late chronic stages. Further, this was associated with changes at the fetomaternal interface, specifically in terms of an infection-phase-specific shifts in placental transcriptional profile as well altered cytokine production to schistosome antigens. We have since further investigated the potential effect of this maternal infection within the immune cell compartments of these offspring, and in our *in vivo* model begun exploring the early sensitization stages of allergic response in these offspring, as well as other vaccination models. In line with epidemiological trends, we have found differential T and B cell responses to vaccination of offspring exposed to transmaternal schistosomiasis, with these changes relying heavily on vaccine vector and the mode of antigen delivery. Through altered vaccine strategies, we have been able to further modify these antigen-specific responses, and our continued work investigates the mechanisms underlying this. Our studies will help to understand the effects of the maternal immune status during pregnancy on immune predisposition in later life, and mechanisms for fine tuning immune responses.

Neurotoxic autoantibodies induced by *O. volvulus* infection underlies the nodding syndrome, a rare epidemic form of epilepsy associated with onchocerciasis

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Nodding syndrome (NS) is a childhood-onset (5-15 years) epileptic encephalopathy identified in epidemics in Northern Uganda and South Sudan. There is an epidemiological association with *Onchocerca volvulus* (Ov) the parasitic worm that causes onchocerciasis (river blindness), but the parasite itself is not neuroinvasive. Having hypothesized that NS may be an autoimmune-mediated disease, we used protein chip methodology to identify autoantibodies that were more abundant in patients with NS to uninfected age- and location-matched controls. Leiomodin-1 (Lmod-1) autoantibodies were found in both the sera and cerebrospinal fluid of patients with NS. Lmod-1 was found to be expressed in mature and developing human neurons in vitro and was localized in mouse brain to the CA3 region of the hippocampus, Purkinje cells in the cerebellum, and cortical neurons, anatomical structures that also appear to be affected in patients with NS based on autopsy and MRI findings. Antibodies targeting leiomodin-1 were neurotoxic in vitro, and leiomodin-1 antibodies purified from patients with NS were cross-reactive with *Ov* tropomyosin. Human Lmod-1 overlaps structurally to both Ov tropomyosin and Ov tropomodulin. Lmod-1 autoantibodies were found to only be neurotoxic to newly formed neurons, and these Lmod-1 expressing neuroprogenitor cells appear primarily to be found at high levels in brain tissue from children (but not infants or adults). This study provides additional evidence supporting the concept that NS is an autoimmune epileptic disorder caused by molecular mimicry with *O. volvulus* antigens and provides insights into the underlying pathogenesis of this devastating disorder. It also suggests that patients with NS may benefit from immunomodulatory therapies providing they can cross the blood-brain barrier.

Population genomics and molecular phylogenetic relationships of *Simulium* vectors of onchocerciasis in Ghana: large interbreeding sympatric populations and large transmission zones

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Onchocerca volvulus, the causative agent of onchocerciasis, is transmitted by anthropophilic flies of the genus *Simulium*. Taxonomic identification of African *Simulium* vectors is challenging, relying on a combination of cytogenetics derived from banding patterns of polytene chromosomes in the salivary glands of aquatic larvae and/or on a suite of overlapping morphometric characters. Several attempts have been made to construct molecular phylogenies of the *Simulium* vectors of *O. volvulus* but in general there has been poor concordance between the molecular phylogenies and the cytogenetic/morphometric phylogenies. The low level of polymorphism observed in the mitochondrial barcode amplicons that have been used is the most likely cause of the poor resolution achieved to date using molecular methods. We show here that relatively low depth whole genome sequencing of single flies yields sufficient data to reconstruct the entire mitochondrial genome of individuals at >50X depth, and a nuclear genome that is >90% complete by CEGMA and BUSCO criteria. We have constructed molecular phylogenies using (a) conventional, relatively short barcode amplicons (b) a single amplicon spanning >3kb of the mitochondrial genome and (c) whole mitochondrial genomes of >60 flies reconstructed from low-depth whole genome sequences, and (d) partial CEGMA and BUSCO genes. The flies were collected from 3 river basins across a >500km east-west transect in central Ghana (the "transition zone" between forest and savannah) during the dry season.

A phase 1b clinical trial to assess safety and efficacy of an attenuated hookworm larvae vaccine

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Control of human hookworm infection through mass drug administration is impeded by high rates of reinfection, leading to the proposal for a human hookworm vaccine. We hypothesised that percutaneous exposure to *Necator americanus* larvae (L_3) attenuated with ultraviolet C (UVC) light would result in protective immunity. We conducted a phase 1b clinical trial using an attenuated L_3 vaccine delivered via dermal application to healthy human volunteers. Immune response and protective efficacy were secondary endpoints. Fifteen healthy, hookworm-naive volunteers were randomised (1:2) to receive placebo (tabasco sauce) or vaccine (50 attenuated L_3) via dermal application. L_3 were attenuated by exposure to 700mJ of UVC. Two cycles of placebo or vaccine were administered 6 weeks apart, followed 6 weeks later by challenge with 30 unattenuated L_3. Adverse events (AEs), including dermal reactions, were recorded. Faecal samples were collected from each subject 10-11 weeks post challenge and larval culture was performed by modified Harada-Mori. Faecal hookworm DNA intensity was estimated by qPCR. No serious AEs occurred. Following challenge, dermal erythema at day 3 measured a median of 20 mm vs 32.5 mm (p=0.007) and persisted for a median of 2.0 days vs 5.5 days (p=0.007) in controls and vaccinated subjects respectively. A significant difference in the median peripheral eosinophil count was observed at day 38, 0.49 vs 1.44 (p=0.008) which persisted until day 71 post challenge, 1.13 vs 2.32 ($10^{\wedge 9}$), (p=0.014), in controls and vaccinated respectively. Significantly fewer larvae were recovered per gram of cultured faeces from vaccinated than control subjects (0.78 vs 10.16; p=0.014). Vaccinated subjects had lower but non-significant faecal hookworm DNA content than control groups (cycle time 18.67 vs 16.630; p=0.87). Dermal application of attenuated L_3 was well tolerated. Vaccinated subjects had more robust dermal reactions and peripheral eosinophilia than control subjects, consistent with immune sensitisation following vaccination. Lower faecal hookworm DNA intensity and recovery of significantly fewer larvae are clinically meaningful outcomes that warrant further investigation.

Poster Session 1 Monday 2 September 2019

1. RDT Onchocerciasis: Integrating multiple biomarkers that push the detection sensitivities of infection

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As efforts shift from control to elimination of *Onchocerca volvulus (Ov)*, additional tools to identify those contributing to ongoing *Ov* transmission in low transmission settings will be needed to get beyond the ~80% sensitivity of the IgG4 Ov16 assays (ELISAs, RDTs). By isotype-specific screening of protein arrays developed from ~400 *O. volvulus* proteins, we identified several biomarkers. Screening the shortlisted proteins using luciferase immunoprecipitation assays (LIPS) resulted in the identification of 2, OVOC10469 and OVOC3261, as being the most promising candidates. Using rOVOC10469 and rOVOC3261 as the basis for standard IgG4 immunoassays (e.g. ELISAs), we were able to show by the use of well characterized sera from microfilariae (mf) positive patients with Ov (n=245) and appropriate controls (other filariae [n=93] or healthy controls [n=24] that each antigen by itself had sensitivities that ranged from 53% (for OVOC10469) to 78% (for OVOC3261) with specificities >99%. Moreover, each of these newly-identified biomarkers were able to detect some (but not all) of the Ov mf-positive Ov16-negative individuals. Thus, when used in combination with Ov16, the sensitivity for truly Ov-infected individuals was close to 94%. The kinetics of appearance of these IgG4 responses based on experimentally infected non-human primates show that the IgG4 responses to OVOC10469 and OVOC3261 are patency driven (i.e. when mf appear in the skin). The IgG4 responses to both OVOC10469 and OVOC3261 (as well as to Ov16) drop significantly following cure of onchocerciasis. Our data suggest that by using IgG4-based immunoassays for OVOC3261 (or OVOC10469) with the currently available Ov-16-based assays will improve sensitivities of the assay. Indeed, testing of a lateral flow prototype developed using rOVOC3261 in combination with Ov16 resulted in ~94% sensitivity, thus paving the way for improved case detection and more thorough transmission assessment and certification for the onchocerciasis elimination program.

2. Control of neglected tropical diseases should start from early childhood - Success of an improved comprehensive model

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Infection with any one of the neglected tropical diseases (NTD) is a major cause in preventing children from reaching their developmental potential and gain the knowledge and skills they need for success. Soil transmitted helminths (STH), and schistosomiasis account for several health impairments, but also have a direct effect on cognitive capabilities and skill learning. That is why their control is so critical especially in young children, and why it is essential to have a comprehensive program that will include behavioral change together with improved water sanitation and hygiene (WASH) and mass drug administration (MDA). During the past nine years we have developed a comprehensive integrated model intervention that combines all these elements and has been successfully implemented in Ethiopia where it has achieved a significant and sustained decrease in prevalence of both schistosomiasis and STH in several sites where it has been applied. Since children are at the highest risk to contract these diseases, we launched an innovative pilot program in 2017, training educators, parents and community leaders to playfully create healthy hygiene habits for young children. Schools, educators and community leaders were trained in early childhood education methods, focusing on innovative ways to transmit messages and reinforce behaviors and habits in young children and support health and hygiene. The results of this intervention demonstrate: 1) Sustained reduction in prevalence of STH and Schistosomiasis with a marked change in behavior among the children participating in the intervention. 2) The processes through which behavior change influences knowledge and practices of children. These results lend support to the importance of a comprehensive approach and to the focus on children in the control of helminth infections. The insight gained from these studies, may lead to expansion of programs and their universal application in the ultimate control of NTDs

3. Identifying somatic nursing machinery of the male-induced development of the female schistosome reproductive system

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Schistosomes are blood dwelling parasitic flatworms that effect 200 million people in the developing world. The resulted neglected tropical disease schistosomiasis causes serious chronic disabilities due to their enormous egg output in their hosts. Understanding the basic reproductive biology of these worms will input important new insights into druggable targets. Of our particular interest is an observation that female worms rely on continuous pairing with male worms to develop and maintain a mature reproductive system and to sustain egg production. This process is sperm-transfer dispensable and completely reversible depending on the presence of physical contact from male parasites. It has been suggested that mechanosensory during physical pairing might take a role as the stimulation, which should be heavily conducted by the somatic system. Transcriptome comparison between pre-and post-pairing female parasites robustly fished out three top up-regulated neuronal/muscle *Male Induced Genes (Migs)*. A novel female schistosome single cell transcriptome atlas indicated their destinated neuron population to be ciliated. Immunostaining and transmission electron microscopy uncovered the existence of a large number of ciliated cells near worm surface as well as inner organs. *In situ* hybridization of all *Migs* in both sexes showed consistent expression patterns in many relatively-peripheral puncta cells. However, each *Mig* showed no overlapping with each other: locating from the worm trunk, reproductive-organ-adjacent tissues to the head/brain. This implicates that there are distinct subgroups of this unknown cell type marked by different *Migs*. Ongoing experiments including RNA interference of *Migs* and co-immunostaining with neuron and muscle molecular markers will further characterize the function and cellular roles of *Migs*.

4. Vesicle-based secretion in schistosomes: Profiling of *Schistosoma mansoni* exosome surface glycans

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There is increasing evidence of the release of extracellular vesicles (EVs) in parasitic diseases, with roles both in parasite-parasite inter-communication as well as in parasite-host interactions. EVs are small spherical particles enclosed by a phospholipid bilayer, that contain a mixture of macromolecules, including proteins and miRNAs and are released by many cell types. EVs are known to transfer molecules from one cell to another via membrane vesicle trafficking, thus explaining the broad array of functional activities attributable to them. There are many subclasses of EVs, with current research interest focusing principally on exosomes. We have described a protocol for the isolation of adult *Schistosoma mansoni*-derived exosomes by differential centrifugation, followed by sedimentation on sucrose density gradients. We have shown that *S. mansoni* releases exosome in vitro and characterized their protein and miRNA content. Using quantitative PCR, we have been able to detect some of the most abundant *S. mansoni* exosomal miRNAs in infected mouse serum, suggesting the release of these vesicles *in vivo*. Here, using lectin microarrays, we have identified several lectins that exhibit strong association with *S.m.* EVs, including SNA-II, DSA, LEL, Calsepa, NPA, GNA, HHA, WGA, SNA-I, PHA-E, RCA-I and CAA. These results indicate the presence of several glycan structures on these vesicles. We are currently working to profile glycans at the surface of *S.m.* exosomes and to identify the exosomal proteins which are glycosylated, which is of great interest as glycoproteins secreted by helminth parasites are immunogenic and represent appealing components of vaccine preparations. A better understanding of the vesicles secreted by the parasite could lead to the identification of novel biomarkers for the development of superior diagnostic tools as well as new targets for the potential prevention and therapy of schistosomiasis.

5. Initial host intestinal epithelia interactions with whipworms unravelled

María A. Duque-Correa[1], Catherine Sharpe[2], Faye Rodgers[1], David Goulding[1], Hayley Bennett[1], Adam J. Reid[1], Catherine McCarthy[1], Magda Lotkowska[1], Nancy Holroyd[1], Kelly S. Hayes[2], Allison J. Bancroft[2], Claudia M. Carneiro[3], Tobias Starborg[2], Gordon Dougan[1], David J. Thornton[2], Richard K. Grencis[2], Matthew Berriman[1]

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Whipworms live preferentially in the cecum of their hosts where they tunnel through intestinal epithelial cells (IECs). Despite extensive research, the early whipworm interactions with host IECs triggering parasite establishment remain unclear. Here, we investigated novel interactions of whipworms with host IECs through the first events of infection. Imaging caecum of *Trichuris muris*-infected mice (a mouse model of *T. trichiura* infection in humans) during the first three days post infection has revealed whipworm larvae (L1) infecting the epithelium at the base of the crypts. These images suggest a close interaction between L1 larvae and host goblet cells. To further understand this critical early colonisation event, we are using intestinal organoids as a replacement model of murine infections. We have established an *in vitro* model of *T. muris* L1 larvae infection of caecal murine organoids grown in transwells displaying all IECs populations organized in structures resembling intestinal crypts and producing mucus. Imaging of these infections showed L1 larvae creating syncytial tunnels and actively infecting IECs. Transcriptomic analysis of these cultures suggest L1 larvae dampens IECs inflammatory responses as a mechanism of establishment in the intestinal epithelium. To further analyze the transcriptomic responses of both host and parasite during early infection, we are performing laser microdissection experiments of L1 larvae and infected IECs in transwells. Moreover, we are measuring IECs cytokine production in response to L1 larvae infection. Infections of organoids derived from mice deficient in mucus production and lacking mature goblet cells are planned. These experiments will allow us to further investigate our results of transcriptomic and mucus degradation studies showing L1 larvae expression of proteases involved in the degradation of mucus that potentially supports parasite survival during early stages of infection.

6. *Schistosoma mansoni* schistosomula antigens induce Th1/proinflammatory cytokine responses

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Larvae of *Schistosoma* (schistosomula) are highly susceptible to host immune responses and are attractive prophylactic vaccine targets, although cellular immune responses against schistosomula antigens in endemic human populations are not well characterized. We collected blood and stool from 54 *Schistosoma mansoni*-infected Ugandans, isolated peripheral blood mononuclear cells and stimulated them for 24 hours with schistosome adult worm and soluble egg antigens (AWA and SEA), along with schistosomula recombinant proteins rSmKK7, Lymphocyte Antigen 6 isoforms (rSmLy6A and rSmLy6B), tetraspanin isoforms (rSmTSP6 and rSmTSP7). Cytokines, chemokines and growth factors were measured in the culture supernatants using a multiplex luminex assay, and infection intensity was determined before and at one year after praziquantel (PZQ) treatment using the Kato Katz method. Cellular responses were grouped and the relationship between groups of correlated cellular responses and infection intensity before and after PZQ treatment investigated. AWA and SEA induced mainly Th2 responses. In contrast, rSmLy6B, rSmTSP6 and rSmTSP7 induced Th1/pro-inflammatory responses. While recombinant antigens rSmKK7 and rSmLy6A did not induce a Th1/pro-inflammatory response, they had an association with pre-treatment infection intensity after adjusting for age and sex. Testing more schistosomula antigens using this approach could provide immune-epidemiology identifiers necessary for prioritizing next generation schistosomiasis vaccine candidates.

7. Microfilariae trigger eosinophil extracellular traps in a Dectin-1-dependent manner

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During primary filarial infection, eosinophils mediate protection against adult worms and microfilariae, but not against infective third-stage larvae. A recently described defense mechanism by eosinophils is the production of extracellular DNA traps (EETosis), a form of cell death where intracellular DNA is explosively released. The results of the present study demonstrate by scanning electron microscopy, confocal microscopy and quantitative fluorescence DNA assay that microfilariae, but not third-stage larvae, trigger DNA release by eosinophils. These eosinophil DNA nets, consisting of nuclear and mitochondrial DNA, inhibit microfilariae motility in a DNA- and cell contact-dependent manner *in vitro*. Eosinophils isolated from the gut of naïve and gut and pleura of filariae-infected-animals demonstrate an enhanced microfilariae motility reduction compared to bone-marrow-derived eosinophils, whereas gut- and pleura-derived eosinophils primed during *Litomosoides sigmodontis* infection and gut-derived eosinophils from naïve animals showed comparable DNA-trap-dependent microfilarial motility reduction. *In vitro* assays further reveal that microfilariae-induced EETosis is induced via the Dectin-1 receptor but independent of the presence of antibodies. DNA-dependent inhibition of microfilariae motility and microfilariae-induced DNA release by eosinophils appears to be a conserved mechanism since murine and human eosinophils respond to microfilariae derived from the rodent filarial nematode *L. sigmodontis* and the canine heartworm *Dirofilaria immitis*. *In vivo* results show an increase in local DNA concentration upon *L. sigmodontis* infection and raised systemic DNA concentrations upon intravenous microfilariae injection, which is partly mediated by eosinophils, indicating a potential role of EETosis *in vivo* as well.

8. Deducing the protective mechanism of the *Schistosoma mansoni* glycoprotein Omega-1 in the mouse model of allergic airway inflammation.

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Chronic infection with Schistosoma mansoni is known protect against allergic airway inflammation (AAI) in mice, and to be associated with reduced Th2 responses to inhaled allergens in humans, despite a robust schistosome-specific Th2 responses. We have previously shown the protective capacity of S. mansoni eggs in an ovalbumin (OVA)/alum mouse model of AAI, highlighting schistosomes as an potential source of novel AAI therapeutics. An abundant secreted egg glycoprotein, omega-1 (ω1) has been shown to target mannose receptor (MR)-expressing dendritic cells via Lex-glycans and suppress protein translation via its RNAse activity. Here, we investigate the immunoregulatory properties of ω1 in the OVA/alum AAI model. Mice treated with plant-derived recombinant ω1 exhibited lower pulmonary eosinophilia upon allergic challenge and less Th2 cytokines (IL-5, IL-13) in the lavage and the mediastinal lymph nodes. Additionally, mice pretreated with ω1 exhibited reduced influx of proinflammatory, monocyte-derived dendritic cells and lower levels of the monocyte-attractant chemokine CCL2 in the lungs, upon allergen challenge. The induction of allergic inflammation in the OVA/Alum model depends on the trafficking of OVA from the peritoneal cavity (the injection site) into the mediastinal lymph nodes by inflammatory monocytes. Pre-treatment with ω1 resulted in a significantly higher number of OVA-positive cells remaining in the peritoneal cavity 24 hours following OVA/alum injection and reduced trafficking of OVA-positive cells into the mediastinal lymph node and bone marrow compartment. Therefore, these data suggest that pretreatment with ω1 may prevent the induction of an allergic response to OVA by reducing allergen trafficking towards the mediastinal lymph nodes. Further experiments aim to investigate the MR-expressing cell types targeted by ω1 and how cellular function is influenced to inhibit migration of OVA carrying inflammatory monocytes towards the lung- draining lymph nodes.

9. Evaluation of derivative-specific pharmacokinetics and potencies of anthelmintic drug classes by assessing nematode motility and pharyngeal pumping

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Despite the tremendous impact of parasitic nematodes on global health and socio-economic development, only a limited repertoire of anthelmintic compounds is available to control nematode infections in humans, animals and plants. Major anthelmintic drug classes targeting nematodes, including the macrocyclic lactones (ML) and the chemically highly diverse nicotinic acetylcholine receptor (nAChR) modulators, were originally discovered more than three decades ago. However, new representatives of both classes are still entering the market. Regardless of uniform molecular targets, derivatives within a drug class differ in their physicochemical properties, which potentially influence potency (e.g. target affinity) as well as pharmacokinetics (e.g. solubility, permeability, metabolism, distribution) in the host, but also in the worm itself. To examine both of these aspects on the worm in vitro, we used a two-dimensional read-out approach, to track individual drug effec in a time-dependent manner. We applied two assay systems to elucidate intra- and inter-class specific differences of ML and nAChR modulators on the free-living nematode *Caenorhabditis elegans.* Using the motility-based WMicroTracker system (NemaMetrix, USA), we were able to detect significant differences for both drug classes with respect to derivative-specific potencies (determined by EC50 values) and kinetics (onset and persistence of the anthelmintic effect). Additionally, we used the ScreenChipTM system (NemaMetrix, USA) which allows the recording of electropharyngeograms (EPGs) from the nematode pharynx. By using the WMicroTracker system and the ScreenChipTM system we were able to evaluate drug effects on multiple tissues. In summary, both assay systems we used provided very helpful information suggesting that both kinetics as well as selection of read-out is highly relevant when characterizing the potency of ananthelmintic compound.

10. Structure-function studies of the *H. polygyrus* TGF-β mimic TGM

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TGF-β is a secreted signaling protein involved in regulation of the adaptive immune system. As an immune modulator, TGF-β stimulates the differentiation of CD4+ cells into regulatory T-cells (T-regs) to promote peripheral immune tolerance. The parasite *Heligmosomoides polygyrus* takes advantage of its host's immune system through the induction of Fox3p+ T-regs using a five domain (D1-D5) TGF-β mimic, TGM. Each domain of TGM is about 90 amino acids in length and has either two or three disulfides, with similarity to the complement control protein (CCP) family. Despite having no homology to TGF-β family proteins, TGM binds directly to both the type I and type II TGF-β receptors, TβRI and TβRII. To determine how TGM binds and assembles TβRI and TβRII into a signaling complex, we followed up on the recent finding that domains 4 and 5 are dispensible for binding TβRI and TβRII and signaling by producing TGM-D1, TGM-D2, and TGM-D3 as separate proteins in *E. coli* and refolding them into native monomers. Through SPR- and ITC-based binding studies, we showed that TbRII is bound exclusively by TGM-D3, whereas TbRI is directly bound by TGM-D2, but its binding is indirectly potentiated by TGM-D1. NMR titration experiments showed that TGM-D3 and TGM-D2 exhibit slow-exchange binding with TβRII and TβRI, respectively, consistent with the binding studies that TGM-D3 and TGM-D2 represent the primary binding sites for TβRII and TβRI. NMR titrations further revealed fast-exchange binding of TGM-D2 by TGM-D1, but no binding between TGM-D1 and TbRI. Taken together, TβRII directly binds TGM-D3 without involvement of other domains of TGM, while TGM-D2 binds TbRI directly, but in a manner indirectly influenced by TGM-D1. The information derived from these studies is important not only for guiding efforts to determine the structure of TGM with TbRI and TβRII, but also will also aid in the development of TGM and other parasitic TGF-β mimics as immunosuppressive therapeutics.

11. The circRNAs expression profiles of different developmental stages of the parasitic nematode, *Haemonchus contortus*

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CircRNAs commonly expressed in animals and plants are a new type of long non-coding RNAs generated by back-splicing and with a continuous, covalently closed stable loop structure. They are involved in miRNA or protein sequestration, transcription modulation and even polypeptide translation. Nevertheless, almost nothing is known about circRNAs of parasitic nematodes. In the present study, we comprehensively explored the circRNA repertoire of parasitic nematode *Haemonchus contortus* via deep-sequencing and stringent bioinformatic analyses. A total of 20,073 circRNAs were identified and annotated from three different developmental stages, including infective third stage larvae, parasitic female and male adults. These circRNAs were non-randomly distributed in all chromosomes and derived from 4937 parental genes, among them, 41.74% of the genes only produce one circRNA, and 94.75% of the genes produce no more than 10 circRNAs. In addition, among 20,073 identified circRNAs, 14,314, 1,283, 4,476 were arising from coding exons, introns and intergenic regions, respectively. Comparison between three developmental stages revealed that 376 circRNAs were commonly expressed in three stages, 561 circRNAs only expressed in female adults, 329 circRNAs only in male adults, and 15,982 circRNAs only in infective third stage larvae. Functional annotation revealed that parental genes of differentially expressed circRNAs were predicted to be mainly involved in the longevity regulating pathway, such as MAPK signaling pathway, mTOR signaling pathway, phosphatidylinositol signaling system and FoxO signaling pathway. The back-splicing junctions of randomly selected twenty-four circRNAs were verified using reverse transcription PCR. The expression levels of selected fifteen circRNAs were assessed by real-time quantitative PCR, demonstrating a consistent expression pattern between RNA-Seq and real-time PCR data. Then, ten circRNAs were demonstrated resistant to RNase R digestion using qRT-PCR. Taken together, our results provided a basis for studying the functions of circRNAs in *H. contortus,* and advanced our understanding of circRNAs in parasitic nematodes.

12. The major pseudocoelomic proteins of the giant kidney worm, *Dioctophyme renale,* and what they can tell us about other Clade 1 nematodes

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The giant kidney worm, *Dioctophyme renale*, is a Clade I nematode that includes pathogens such as *Trichuris, Trichinella,* and *Capillaria.* The latter parasites are small and studying their proteins without resorting to recombinant methods can be difficult. *D. renale* is a very large Clade I nematode that infects and destroys typically the host's right kidney, and is usually found in ectopic sites. It is circumglobally distributed, mainly in dogs, and is increasingly regarded as a threat to wildlife, domestic animals, and humans. We are investigating the soluble proteins of body wall, intestine, gonads and pseudocelomic fluid (PCF) of adult *D. renale*, which have already been found to be different from a similarly-sized Clade III worm, *Ascaris.* Two *D. renale* proteins stand out. P44 is closely related to the cysteine- and histidine-rich protein of *Trichinella,* and the 'immunologically silent' and potentially immunomodulatory p43 of *Trichuris*, the biological activity of neither of which is known. We present new information on the ligand-binding activity of P44. P17 accounts for the intense red colour of the parasites and we show that P17 is a haem-containing member of the nemoglobins that are widespread in other nematode Clades. Hence, while nemoglobins appear to have evolved before the split in nematode clades, P44-types may be a specialism for Clade I in substituting bulk lipid transport inside the parasites and potentially interacting with host tissues.

13. Study of the role of IL-9 in basophil differentiation and function

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Basophils are one of the main effector cellular subsets responsible for type 2 immune protection during helminth infections and key mediators in the pathogenesis of asthma by secreting soluble mediators contained in specialized granules. Among the prototypical type 2 cytokines, basophils express IL-4, IL-9 and IL-13; however the potential to respond to them as an autocrine signal has not being studied in detail. Our recent work on studying the role of IL-9 against helminth infection, demonstrated for the first time the potential role for IL-9 as a regulator of basophil differentiation and/or function. Basophils express high levels of IL-9 receptor (IL-9Ra) and IL-9 deficient mice showed decreased basophilia in infected tissues using a hookworm infection model. Hence, our aim is to identify the signals promoting IL-9 receptor expression in basophils and to characterize the role of IL-9 in the control of basophil function during helminth infection. We hypothesize that IL-9 regulates basophil differentiation and function including degranulation, proliferation and survival to assure an efficient helminth expulsion. We expect to identify the molecular pathways controlling IL-9R expression in basophils as well as the functional consequences of IL-9 signaling in these cells. Our data could provide new strategies to potentiate and/or inhibit basophil functional responses induced by IL-9 in vivo. Studying the role of IL-9 in basophil biology is imperative and could provide new venues on the search for alternative strategies to control this important effector subset in type 2 immune pathologies.

14. Immunomodulation of human cytokine responses by the porcine whip worm, *Trichuris suis*, and the porcine nodular worm, *Oesophagostomum dentatum*

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Trichuris suis and *Oesophagostomum dentatum* are parasitic gastrointestinal nematodes that occupy the cecum and colon of their porcine host. While *T. suis* promotes a Th2 response resulting in expulsion from the host about 9 weeks after infection, *O. dentatum* is able to establish a chronic infection. *O. dentatum* seems to induce a Th1 response and a delayed Th2 response. Co-infections with *T. suis* and *O. dentatum* do occur, and studies have shown that *T. suis* can suppress *O. dentatum* and *O. dentatum* can prolong infection by *T. suis*. We are interested in dissecting the responsible molecules of *T. suis* and *O. dentatum* focusing on their excretory-secretory products (ESP) and their worm extracts (soluble products=SP). We size-fractionated the molecules in the ESP of both parasites separately. In addition, as recent studies have shown that parasite extracellular vesicles (EVs) may have potent immune stimulatory properties, we enriched our samples for EVs and investigated their immunomodulatory effects *in vitro*. Interestingly, we find that the *O. dentatum* ESP and EVs stimulate TNF production in the human monocytic leukemia cell line THP-1, whereas *T. suis* does not. In the presence of the TLR4 agonist, LPS, *T. suis* ESP, but not EVs, is able to suppress TNF production in a dose-dependent manner, whereas *O. dentatum* ESP or EVs cannot. These findings indicate that both SP, ES, and EVs contribute to host modulation. If we can unlock the underlying mechanisms of how *T. suis* and *O. dentatum* interact with their host we may identify new targets for intervention and control.

15. Orthologues of the immunodominant antigens of *Onchocerca volvulus* in the rodent onchocerciasis model *Cercopithifilaria johnstoni*.

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The pathology associated with onchocerciasis is driven by host immune responses to the presence of dead or dying microfilariae in the skin and eyes. Rodent infection with *Cercopithifilaria johnstoni*, a rodent filarial parasite with skin and eye dwelling microfilariae, provokes similar skin and eye inflammatory pathology. The similarity in immunopathology between these two related parasites raises the question of whether similar antigens, or antigenic epitopes, are responsible for the similarities in immune responses directed against the two species. Immune responses against *Onchocerca volvulus*, the causative agent of onchocerciasis, are dominated by a small number of immunodominant motifs. We are taking the immunodominant motifs and epitopes identified in *O. volvulus* and exploring whether these are present in the *C. johnstoni* genome. We report the general characteristics of the *C. johnstoni* genome, which is similar in size and organisation to that of other filarial nematodes, and show the relationship between *C. johnstoni* and other filarial nematodes using whole mitochondrial genome and nuclear data. We have identified a minimum of 8 one-to-one *C. johnstoni* orthologous of immunodominant *O. volvulus* proteins with high confidence. These proteins tend to be conserved across all available filarial genomes, and we report also the phylogenetic relationships between *C. johnstoni* and other filarids for these orthogroups. We have also identified candidate predicted proteins in the *C. johnstoni* genome contain conserved immunodominant *O. volvulus* motifs, and present structural predictions for both the one-to-one ortholgues and the "motif-containing" sequences. The apparently extensive conservation of these antigenically important sequences between *O. volvulus* and *C. johnstoni* supports the hypothesis that the similarity in immunopathology each species provokes may be at least in part due to similarity in the antigenic profiles each presents to the host.

16. The gastrointestinal helminth *Heligmosomoides polygyrus* suppresses inflammation in a model of atopic dermatitis

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Helminths are well known to modulate the host's immune system to prolong their own survival and have shown therapeutic potential in allergic conditions. One of the most fascinating aspects of helminth infection is the impact they can have on organs distant to where they reside in the body. Atopic dermatitis is a chronic Th2-driven inflammatory skin condition. After a topical application of the chemical hapten dibutyl phthalate-fluorescein isothiocyanate (DBP-FITC), we found *Heligmosomoides polygyrus* (*Hp*) is capable of suppressing classical allergic response caused by this atopic dermatitis model. We further sought to identify the mechanism of how Hp is inducing a suppressive phenotype at a distal site. We observed a significant reduction of CD45+ cells and increased proportions of T regulatory cells (Treg) in the skin associated lymph nodes; suggesting suppressive capabilities of Hp. However, Treg depletion did not dampen the suppressive capabilities of Hp. Instead, Hp infection may be acting to limit infiltration and the release of chemokines and cytokines in the skin. We also postulate that Hp is inhibiting or skewing the abilities of inflammatory cells to enter the skin. This research further demonstrates the potential use of helminths as therapy for allergic diseases, including those of the skin.

17. Understanding the molecular basis of hatching in *Trichuris* species

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Trichuriasis is a disease caused by infection with *Trichuris trichiura,* the human whipworm. It affects over 700 million people in parts of Asia, Africa, and the Americas, often from low- and middle- income brackets. Infection occurs upon ingestion of whipworm eggs that hatch and establish in the cecum and proximal colon. Trichuriasis has been studied in animal models, in particular the mouse and pig whipworms, *T. muris* and *T. suis,* respectively. Experiments using these models indicate a role of the host intestinal microbiota in inducing hatching of whipworm eggs. *Escherichia coli* surface molecules have been shown to play a role in the hatching of *T. muris,* and we hypothesise that the same or similar molecules found in specific conformations on defined members of the host gut microbiota trigger hatching across other *Trichuris* species, through both physical interaction and enzymatic activity around the polar plugs. To understand the molecular basis of hatching in *Trichuris* species and identify human gut microbiota capable of hatching *T. trichiura* we are quantifying the interactions between *E. coli* and *T. muris*; using a variety of microscopy techniques including: live cell imaging, laser capture microdissection, light, fluorescence, and electron microscopy. We have identified classes of proteins and proteases important to the hatching process; observing reductions in the hatching of *T. muris* when using *E. coli* fimbria knockout strains, or inhibiting serine protease activity. We hope to build a picture of hatching across *Trichuris* species by looking for expression of these molecules in purified gut microbiota and faecal samples, and studying their effect on hatching in *T. muris*, *T. suis,* and *T. trichiura*. Additionally, we are using these techniques to build a high throughput hatching screening platform that will be used to test and validate our candidate human gut microbiota, and can be applied to other large-scale investigations.

18. Immunosuppressive effects of helminth antigens on TLR-mediated inflammatory responses in macrophages

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Toll-like receptors (TLRs) are an important compartment of the innate immunity which highly expressed by macrophages. TLRs are able to initiate inflammatory signals and polarize macrophages toward overactivation that leads to onset of an autoimmune condition. Helminths exploit manifold approaches to interfere host immunity and setting up a permissive infection. One of the striking mechanisms is manipulating TLRs signalling. In this study, we explored the effects of antigens from the porcine nematode *Trichuris suis* on mouse bone-marrow derived macrophages (BMDMs) in the presence of different TLRs agonists. *T. suis* antigens displayed a significant attenuation of NLRP3 activation and most TLR-induced pro-inflammatory cytokines (TNFα, IL-1β, IL-6, IL-12p70) in BMDM while upregulated IL-10 production. We aim to narrow down our findings to dissect intracellular downstream pathways and address the suppressive machinery mediated by worm antigens against TLR signalling. In line of modulatory activities of these antigens, we also conducted an *in vitro* experiment to explore whether treatment of BMDMs with the worm antigens can modify extracellular vesicle (EV)-associated immune cells communication. Characterization of EVs released from antigen-treated BMDCs has a size range of 70-150nm. In terms of functionality, we observed that EVs released from *T. suis* treated BMDMs have regulatory function as they were able to inhibit LPS induced proinflammatory cytokines in naïve BMDMs. We plan to characterize the content of the EVs and the way they specifically interact with naïve macrophages.

19. Helminth-allergy associations in rural and urban Uganda: insights from carbohydrate-specific IgE studies

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Antibodies, especially IgE, are key constituents of the analogous immune responses to helminths and allergens, and are among the chief mediators of the effector cell activation underlying allergy-related disease. We hypothesized that helminth-induced antibody profiles influence the profile of allergic sensitisation and disease, and consequently the epidemiological trends pertaining to allergy in tropical low-income countries (LICs). Among Ugandan residents of rural *Schistosoma mansoni* (*Sm*)-endemic islands, proximate urban communities with lower helminth exposure, and asthmatic and non-asthmatic schoolchildren, we measured IgE against crude house dust mite (HDM), cockroach and peanut allergen extracts by ImmunoCAP®. In subsets of participants, we measured IgE to established major allergenic components purified from 51 allergen sources, by ISAC® microarray. Helminths have a small range of antigens that are strikingly homologous to common allergens, such as cross-reactive carbohydrate determinants (CCDs) characterized by presence of core β-1,2-xylose and α-1,3-fucose moieties. We measured IgE to these moieties using synthetic glycan microarray technology. Analyses showed distinctive relationships between IgE reactivity to core β-1,2-xylose/α-1,3-fucose and *Sm* infection intensity and the rural (versus urban) environment, and implied that they are abundant on HDM, cockroach and peanut allergen extracts, which likely results in false diagnosis of allergic sensitisation in tropical helminth-endemic settings. ISAC® microarray analyses showed that rural participants had lower responses to non-glycosylated protein allergens, but higher reactivity to CCDs, than urban participants. Elevated anti-CCD IgE did not translate into clinical allergy. Instead, we found an inverse association between asthma and reactivity to core α-1,3-fucose substituted N-glycans. Our studies provide important insight into mechanisms underlying the complex epidemiological helminth-allergy associations in LICs. Notably, the finding that schistosomiasis-associated core α-1,3-fucose-specific IgE is inversely associated with asthma is suggestive of a potential role of specific carbohydrate epitopes in protection against clinical allergy.

20. *In silico* methods and drug reposition for the identification of new anti-schistosomal drugs

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The current treatment for schistosomiasis exclusively relies on praziquantel despite its unknown mechanism of action, inefficacy to kill juvenile schistosomes and inability to prevent reinfection. Therefore, the identification of new drugs or drug targets are crucial for the control of this parasitic disease. Through its digenetic life cycle, *Schistosoma mansoni* shows a phenotypic plasticity heavily influenced by both biotic and abiotic stresses. Hence, the analysis of schistosome adaptation in response to different environmental stimuli may highlight key targets for anti-schistosomal drug development. In this context, epigenetic processes present an interesting area of research due to their role in regulating both heritable and environmentally influenced phenotypes. While previous studies have implicated histone modifying enzymes in various aspects of schistosome biology, little is known about the specific contribution of histone methylation machinery components. Therefore, we have begun the characterisation and validation of histone methyltransferases/demethylases as novel anti-schistosomal drug discovery targets. Genomics and bioinformatics-based analyses identified 27 putative protein methyltransferases in *S. mansoni* including 20 Protein Lysine Methyl Transferases (PKMTs), 1 PR Domain-containing Methyltransferase (PRDM), 1 DOT1 Like Histone Lysine Methyltransferase (DOT1L) and 5 Protein Arginine Methyl Transferases (PRMTs). Similarly, 14 histone demethylases were found, including three Lysine Specific Demethylases (LSDs) and eleven JuMonJi Demethylases (JMJDs). In total, 6 new members were identified including 3 histone methyltransferases and 3 histone demethylases. Using a combination of homology modelling, *in silico* virtual screening, whole organism assays and drug repositioning, promising anti-schistosomal candidates targeting selected components of the *S. mansoni* histone methylation machinery were identified. We will present how these synergistic approaches have been used to progress SmLSD1 (Smp_150560) as a novel anti-schistosomal drug target.

Poster Session 2 Wednesday 4 September 2019

21. Role of small RNAs in Fasciola hepatica development and the interaction with the host

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MiRNAs are relevant post-transcriptional regulators of gene expression involved in many biological processes. Recent studies show that miRNA from parasites can be released to the host medium regulating the expression of host genes. In the present work, we studied the pattern of expression of small RNAs through the intramammalian stages of the life cycle of the parasitic platyhelminth *Fasciola hepatica*. Furthermore, with the miRNAs detected we made *in-silico* predictions of targeted genes in the 3'UTR region of *F.hepatica* as well as the mammalian host. We found that the profile of expression of miRNAs show important variations through the stages analyzed however there was a set of miRNAs that were always highly expressed. Additionally, we found no substantial variations in miRNA expression between dormant and activated metacercariae, is possible that miRNA form part of an slow response mechanism that do not take part in the quick changes that occur in the metacercariae after activation stimuli. Between the host targeted genes we found that several functions related to inmuno-response or modulation were enriched among most expressed parasite miRNAs, is possible to hypothesize that high levels of expression of these miRNAs is relevant to establish and maintain the infection. Additionally, we detected fragments derived from the processing of tRNAs, named tRNAhalves, in all stages analyzed. tRNAhalves have been reported to act as post-transcriptional regulators and could also be involved in the invasion process. The presence of some of these parasite generated small RNA regulators in host circulation is under scrutiny.

22. Serum parasite-derived microRNAs as novel biomarkers for the detection of *Schistosoma japonicum* infection: lessons learned from a setting of low infection intensity

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Schistosomiasis remains a major public health problem afflicting more than 200 million people worldwide. Novel tools for early diagnosis and monitoring of schistosomiasis are urgently needed. Previously, a number of parasite-derived microRNAs (miRNAs) in plasma/serum showed promise for the diagnosis of schistosomiasis, yet were validated with only a limited number of patient samples. Recent studies in characterizing miRNA profiles in extracellular vesicles secreted by *Schistosoma* species have raised the possibility of validating more parasite-derived miRNAs as potential novel biomarkers for the detection of schistosome infection. In this study, a total of 21 miRNAs were initially validated by RT-PCR using serum samples of *Schistosoma japonicum*-infected BALB/c mice, as a result of which 12 miRNAs were further tested with a small number of clinical samples. Of these, 6 miRNAs were further validated with a human cohort of individuals from a schistosomiasis-endemic area of the Philippines; these included fifty-three Kato Katz (KK) positives and twenty-five KK and SjSAP4 + Sj23-LHD-ELISA negatives. RT-PCR analysis showed that two parasite-derived miRNAs (sja-miR-2b-5p and sja-miR-2c-5p) could detect infected individuals with low infection intensity with moderate sensitivity/specificity values of 66%/68% and 55%/80%, respectively. Analysis of the combined data for the two parasite miRNAs revealed a specificity of 77.4% and a sensitivity of 60.0%; however, a duplex RT-PCR targeting both sja-miR-2b-5p and sja-miR-2c-5p did not result in an increased diagnostic performance compared with the singleplex assays. Furthermore, the serum level of sja-miR-2c-5p correlated significantly with faecal egg counts, whereas that of sja-miR-2b-5p did not. In conclusion, targeting serum parasite-derived miRNAs resulted in a moderate diagnostic performance when applied to a setting of low schistosome infection intensity. Consequently, assay optimization, such as increasing the starting serum volume for RNA extraction and using locked nucleic acid (LNA) primers, will be required to improve overall diagnosis accuracy using this approach.

23. Elucidating the function of an extracellular Argonaute secreted from a gastrointestinal nematode

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Parasites manipulate their hosts to promote infection by secreting various bioactive molecules, many of which have immunomodulatory abilities. We have found that the rodent-infective gastrointestinal nematode *Heligmosomoides bakeri* secretes a worm-specific Argonaute protein (Hb-exWAGO) and this protein facilitates the export of a specific set of small RNAs into extracellular vesicles (EVs). *H. bakeri* EVs are internalised by mouse host cells permitting the parasite-derived cargo to interfere with host processes. We want to understand the putative role of Hb-exWAGO in mediating cross-species gene silencing and how this affects the local gut environment. To do this we are developing a method to immunopurify the Hb-exWAGO protein from the gut of *H. bakeri* infected mice and directly identify the RNA targets of parasite small RNAs. Additionally, using *in vitro* and *in vivo* approaches, we will examine whether the Hb-exWAGO protein operates solely through EVs or whether another environment-exposed (non-vesicular) form of this protein is involved in RNA transmission. We hypothesise that the vesicular and non-vesicular forms of Hb-exWAGO could have different targets in the mammalian gut and we will test whether the non-vesicular form is internalised by mouse cells. Finally, to understand the mechanism of action of the Hb-exWAGO protein in mouse cells we have identified its host protein interactors *in vitro* (epithelial cell line) and *in vivo* (infected mouse guts) using liquid chromatography-tandem mass spectrometry. Our initial data suggest that Hb-exWAGO interacts with proteins known to be involved in gene silencing pathways in mammals. We will further examine how the putative function of Hb-exWAGO is affected when such interactions are abrogated.

24. Comparative analysis of microRNA levels in *Haemonchus contortus* strains with different drug-susceptibility

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Haemonchus contortus, also known as the barber's pole worm, is recognized as one of the most important parasite of small ruminants. The main clinical signs of haemonchosis relate to its blood-feeding activity, leading to anaemia, weakness and in case of large burdens even to death, unless treatment is provided. However, chemical control is becoming less effective due to the occurrence of resistance in the worm to one or multiple drugs. To maintain an ability to control this parasite it is important to gain a much better understanding of the mechanisms of drug resistance development. Up to date, the mechanisms of drug resistance at the molecular level have not been fully elucidated. Among others, microRNAs (miRNAs) might be involved in drug resistance development. The miRNAs are a class of small non-coding RNAs with important roles in regulating gene expression on post-transcriptional level. The dysregulation of miRNAs have been intensively studied in tumour cells where some miRNAs have been proposed as targets for novel chemotherapy as well as diagnostic biomarkers. We recently undertook the small RNA sequencing from RNA obtained from isolates with various level of resistance to anthelmintics, namely the susceptible strain, the benzimidazole resistant strain and the multi-drug resistant strain. Our results suggest involvement of miRNAs in the development of drug resistance in *H. contortus.*

25. Identification of developmental genes and pathways involved with cestode strobilation

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The Cestoda class of the Platyhelminthes phylum is exclusively formed by parasitic flatworms. Some interesting aspects are evident in the evolution of cestodes, as the increase of their reproductive capacity by larval asexual reproduction, and the serial repetition of reproductive organs in adult worms, often associated with external segmentation (strobilation). However, the developmental mechanism underlying adult body strobilation is still unknown. To assess this issue, an evolutionary comparative study among segmented and non-segmented species of platyhelminths was performed, in order to identify strobilation-related genes and proteins. Ten parasitic platyhelminth species with sequenced and annotated genomes were included in this study, 5 segmented and 5 non-segmented ones, along with 6 nematode species, one annelid, and one mollusk. A comparative genomic approach was used to identify genes with orthologs present in all cestode genomes, but absent from at least one trematode genome. The identified set, including both development-related genes and genes of unknown function (UF), had their transcriptional profiles analyzed to identify those differentially expressed between the larval (non-segmented) and adult (segmented) stages in at least one cestode species. This allowed to propose 34 genes as strobilation-related, 12 with annotated function, including genes of the Wnt, TGF-β/BMP and G-protein coupled receptor signaling pathways, and 22 UF-genes. To assign functions to at least some these strobilation-related UF-genes putatively related to, a global gene co-expression analysis was performed for the cestode *Echinococcus multilocularis*. This allowed to predict the involvement of 8 of the UF genes with developmental and reproductive processes. Overall, our data provided evidences of genes and developmental pathways involved with cestode strobilation. Some of these genes are expected to be useful as developmental markers and/or as targets for novel anthelmintic drugs.

26 Metabolic signatures of urinary schistosomiasis and praziquantel pharmacokinetics in pre-school and school aged children from rural Côte d'Ivoire

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Schistosomiasis has been linked to paediatric malnutrition and stunting in children, yet the causal pathways are not known. Moreover, praziquantel is the only treatment, yet its erratic absorption and excretion may play a role in variable treatment efficacy and can hamper efforts to inform proper dosing for pediatric populations. Metabolic profiling of biofluids has been successfully applied to elucidate the metabolic perturbations resulting from infections and their links to consequent morbidities, while pharmacometabolomics is an important tool for predicting and enhancing treatment outcomes. In this work, we characterized the metabolic effects of Schistosoma hameatobium infection (urinary schistosomiasis) before and after praziquantel treatment, in a clinical trial cohort of pre-school aged and school-aged children. In the health district of Azaguié, Côte d'Ivoire, 170 pre-school aged and 174 school aged children were screened, shown to be positive for S. haematobium and enrolled in the study. Children were stratified according to age category and infection intensity and randomized to receive 20, 40 or 60 mg/kg of praziquantel or a placebo. Urine samples were taken pre-treatment, 24 hours post-treatment, and at the 3-week follow up time-points of infected and 42 non-infected children. Samples were analysed using 1H NMR spectroscopy followed by unsupervised and supervised multivariate statistical analysis to investigate variation between groups. Infection was associated with TCA cycle and ketogenic metabolic pathways and microbial co-metabolism. Moreover, a cross-analysis with matching pharmacokinetic data showed microbial co-metabolites were associated with higher praziquantel exposure both before and after treatment, hinting at a gut microbial role in praziquantel absorption. The results are compared to our previous findings in intestinal schistosomiasis to outline potential mechanisms of schistosomiasis-associated paediatric stunting as well as a potential role for gut microbial modulations to standardize praziquantel treatment outcomes.

27. Differential gene expression of *Dirofilaria immitis* larvae exposed to dog macrophage cell culture

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The parasitic nematode *Dirofilaria immitis* causes cardiopulmonary dirofilariosis, or heartworm disease, in domestic dogs. This nematode can infect a variety of mammal species, with varying levels of disease severity, but can be fatal in dogs. Heartworm has two hosts during its life cycle. Its initial stages of development occur in various species of mosquito, which act as vectors in its transfer to the mammalian host, in which it completes its life cycle. Mosquitoes acquire *D. immitis* microfilaria when ingesting the blood of an infected mammal. The microfilaria develop until the third-larval (L3) stage in the mosquito, which then transfer the nematodes back to a mammalian host when feeding again. In dogs, the nematodes migrate through the host for several weeks until they establish themselves in the pulmonary artery or right ventricle, where they become reproductive adults. Current canine preventative heartworm medications target the still developing worms in the first weeks following infection. Nevertheless, little is known about the biological processes occurring in third- and fourth-stage larvae of the nematode as it establishes itself in the host. In this novel work, we have investigated transcriptional changes in the nematode resulting from exposure to dog cells. We exposed individual L3 *D. immitis* to the dog macrophage cell line DH82 *in vitro* and subsequently performed RNA-seq of the transcriptome of individual worms +/- exposure to cells. Worms undergo a significant shift in transcriptional profile upon exposure to cells compared to those cultured in media alone. For example, gene set enrichment analysis indicates that genes of intestinal muscle tissue are upregulated, while those with gene ontology classifications of protein heterodimerization and actin-filament based processes are downregulated in worms exposed to cells relative to those unexposed. This work provides a new avenue for understanding mechanisms by which parasitic nematodes establish themselves in their hosts.

28. Biochemical pathways supporting *Wolbachia* population growth and regulation in *Brugia malayi*

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Most human parasitic filarial nematodes maintain a mutualistic association with the obligate intracellular bacterium *Wolbachia. Wolbachia* populations in nematodes are known to expand significantly following infection of the mammalian host, to support subsequent larval growth and development. Utilising transcriptomic data from *Brugia malayi* at 3, 7, 11, and 14 days post-infection in an animal model, we present an analysis of the biochemical pathways in both endosymbiont and nematode that are involved in *Wolbachia* population growth and regulation. In *Wolbachia*, we observe coordinated regulation of carbon metabolism with an alternating pattern of regulation in the Glycolysis and TCA cycle pathways in a manner reminiscent of the 'Warburg effect' in cancer cells. *Wolbachia*'s purine and pyrimidine biosynthesis pathways are also upregulated and correlate with the upregulation of *Brugia malayi*'s DNA replication pathway, suggesting the provision of nucleotides from the symbiont is key to larval growth and development. In the nematode we observe regulation of the autophagy pathway, coordinated to regulate overgrowth of *Wolbachia* population loads. The findings of this study represent an important step in understanding the biological basis of the mutualistic association of *Wolbachia* and its nematode host.

29 Microfilariae and lung pathology

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Filariases are tropical diseases caused by parasitic nematodes of the Onchocercidae family. Tissular localization of adults in the host depends on the species, but after reproduction females lay microfilariae, which will either join the blood circulation or the dermis. The development of the parasite until the patent phase and microfilarial status depends on the immune status of the host. Rodent filariasis due to *Litomosoides sigmodontis* occurs as a bronchoalveolar and lung inflammation, leading to chronic infection of the pleural cavity. A fraction of hosts is amicrofilaremic. This ressembles a *Mansonella perstans* infection in which some adults live in the pleural cavity, some hosts are amicrofilaremic, and pulmonary manifestations have been reported. However only a few studies have compared the pulmonary responses of microfilaremic and amicrofilaremic host. Wild-type (WT) and Th2-deficient (IL4receptor(r)$^{-/-}$/IL5$^{-/-}$) BALB/c mice which are known to display different microfilaremic status were infected with *L. sigmodontis*. Survival and growth of filariae, prevalence and density of their offspring were evaluated. Lungs from 70-days-infected mice were analyzed for mucus production, collagen fibers, mesothelial cells and immune cells. Finally, pleural and bronchoalveolar cells recruited during the infection were characterized. 40% of WT mice were amicrofilaremic whereas all mutant mice shown blood microfilaremia. Moreover, filariae from mutant mice displayed a better fertility and survival. The number of cells in the pleural cavity increased in infected WT mice with mainly eosinophils and macrophages. Microfilaremic WT mice present an accumulation of macrophages in the perivascular space. We also showed that microfilariae induced pleural, bronchoalveolar and lung-tissue inflammation associated with production of mucus, visceral pleura alterations and fibrosis in mice. Finally, the results presented here describe lung inflammation and pathology in a chronic filariasis and emphasize the role of Th2 responses according to the presence of microfilariae.

30. Exploring helminth gene expression data using WormBase ParaSite

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WormBase ParaSite (http://parasite.wormbase.org) aims to integrate, organise and present data for nearly all nematode and platyhelminth genomes. Five years on from the start of the project, we now include data for over 150 nematode and platyhelminth genomes, adding value by way of systematic and consistent functional annotation (e.g. protein domains and Gene Ontology terms), and comparative analysis (e.g. orthologues and paralogues). We provide several ways to explore the data, including genome and gene summary pages, text search, sequence search, a query wizard, bulk downloads, a choice of genome browsers, and a programmatic interface. WBPS13 (released in spring 2019) includes a major update: a gene expression sub-portal. This has been underpinned by three key components of work: (a) curation of the majority of published helminth RNASeq expression studies, inferring the experimental design and standardising on sample labels and descriptions; (b) quantification of samples on a per-gene and per-transcript basis using established tools; and (c) for selected studies, differential expression analysis. The results are made available via a number of new views in WormBase ParaSite, including a per-genome study explorer, and per-gene summaries of expression across all studies. As ever, we encourage the community to describe your use-cases and make suggestions for improvements to help us prioritise future work.

31. Can parasite TGF-β superfamily mimics bind and signal via Activin or BMP receptors?

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The parasitic nematode *Heligmosomoides polygyrus* successfully establishes long-term infections in mice. Despite the parasite causing extensive damage to the intestinal epithelium during its lifecycle, mice infected with *H .polygyrus* do not show significant pathology, and remain healthy through infection. How the parasite evades a protective anti-parasite immune response, whilst also ensuring healing of the intestinal barrier, remains enigmatic. We recently described one mechanism to achieve this a novel secreted TGF-β mimic (TGM-1) that binds to the heterodimeric mammalian TGF-β receptor and directly induces regulatory T cells (Tregs). We subsequently established that TGM-1 is part of a family of proteins comprising of 10 distinct members, some of which are highly expressed and secreted by parasite larvae at the time they are invading the intestinal submucosa. The wider superfamily of TGF-β molecules includes activin and bone morphogenetic protein (BMP), which signal through related but distinct heterodimeric receptors, employing an overlapping set of Smad transcription factors. These superfamily members are pleotropic cytokines, with extensive roles outside of the immune system including the maintenance of epithelial homeostasis, proliferation, differentiation and wound repair, which are highly relevant to survival of *H. polygyrus* in the intestinal tract. Here we report the binding capabilities of the *H. polygyrus* TGM family members to a series of mammalian type I and type II receptors, firstly to determine which ligate to each specific receptor, and secondly, using in cellular bioassays specific to the receptor subtypes, to test whether functional binding and signaling occur.

32. *Spirometra erinaceieuropaei* in a free-roaming cat from the lower portion of Thailand's northern region

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Parasitic zoonoses remain a worldwide public health concern. The free-roaming cat has been identified as a potential reservoir host of parasites that cause sparganosis and opisthorchiasis; these parasites have caused an endemic in the lower portion of Thailand's northern region. This study obtained and characterized Spirometra sp. that was collected from the vomitus of a free-roaming cat from the Khao Thong district of Nakhon Sawan Province, Thailand. Prior to performing a surgical sterilization procedure which was supported by the Faculty of Veterinary Science and One Health Clinic mobile team, the three-year-old male cat was sedated. Spirometra sp., which was obtained from the cat's vomitus, was preserved in 70% ethanol for morphological and molecular studies. A simple flotation examination produced a positive result for Spirometra sp. eggs. Additionally, microscopic findings of the proglottid structure and the coiled uterus revealed that the cestode was a Spirometra sp. A polymerase chain reaction (PCR) technique was used to perform a molecular analysis of the cytochrome c oxidase subunit 1 (cox1) gene; then, the PCR product was sequenced. The cox1 sequence data, which consisted of 343 base pairs, was compared with the various helminth sequences provided by the BLAST program—the analysis indicated that the cox1 data was 100% identical to the Spirometra erinaceieuropaei sequences provided by the database. It is believed that the present study is the first to use morphological examinations and mitochondrial cox1 sequence analyses of the adult worm to identify the presence of S. erinaceieuropaei in a free-roaming cat from the lower portion of Thailand's northern region. However, community members should be educated regarding the risks involved in consuming undercooked food and drinking untreated water, as this practice may lead to a parasitic infection. Finally, a deworming program should be provided to animal owners.

33. A novel cell-free method to culture *Schistosoma mansoni* from cercariae to juvenile worm Stages For *In Vitro* Drug Testing

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The anthelminthic treatment against schistosomiasis is limited and relies almost exclusively on a single drug, praziquantel. Even though praziquantel is potent in killing adult worms it has been shown to have limited activity against earlier developmental stages. Current *in vitro* drug screening strategies depend on newly transformed schistosomula for initial hit identification, thereby limiting sensitivity to new compounds predominantly active on later developmental stages. This study aimed to establish a highly standardized, straightforward and reliable culture method to generate and maintain advanced larval stages *in vitro*. Cercariae were mechanically transformed into skin stage schistosomula and successfully cultured under cell- and serum-free conditions for up to four weeks. Under these conditions, larval development halted at the lung stage. Addition of human serum propelled further development into juvenile worms within eight weeks. Skin and lung stages, as well as juvenile worms (late liver stage), were tested with praziquantel, oxamniquine, mefloquine and artemether. Our findings showed stage-dependent differences in larval susceptibility to the tested drugs. The phenotype of juvenile worms, when exposed to reference drugs, was comparable to previously published works for *ex vivo* harvested adult worms. This *in vitro* assay can help reduce reliance on animal experiments in the search for new anti-schistosomal drugs and provide a platform for the investigation of the host protein- or cell-mediated effects on the parasite's development.

34 Immune networks in wild mice, *Mus musculus domesticus*

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The myriad components of the mammalian immune system – cells, receptors and signalling molecules – orchestrate the immune response that animals make given their antigenic challenge, but within the broader context of their lives. To better understand the immune state of wild mice – which differs from that of wild mice – we wanted to understand the whole immune system-level function of wild mice. We did this by network analysis of 120 measures of the immune state of mice, and we compared these immune networks between wild and laboratory mice, and between male and female wild mice. We find that there are mesoscale structural similarities of these immune networks, though substantial fine-scale differences across these two axes, reflecting how the same immune system can be used differently.

35. Omics analyses and functional proteins of *Eudiplozoon nipponicum* (Monogenea)

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Ectoparasitic flatworms from the group Monogenea represent serious fish pathogens and their presence in the stocks can lead to significant losses in the fish host populations. Despite this fact information related to the biochemical and molecular nature of the physiological processes of these parasites is rather sporadic. Here we introduce novel sequential data set for selected representative *E. nipponicum* (parasite of common carp) for the purpose to identify functional protein molecules involved in the interaction with the fish host. We started with analyses leading to the generation of *E. nipponicum* genome, mitochondrial genome, transcriptome and secretome by multiple sequencing techniques (454/Roche, Illumina, Oxford Nanopore) and mass spectrometry analysis, followed by appropriate bioinformatic processing of obtained data. In the *E. nipponicum* transcriptome (37,062 transcripts) we identified numerous translated proteins potentially involved in the host-parasite interaction (e.g. in the digestion of the host blood), such as e.g. cathepsins B, L1, L3, anticoagulation e.g. Kunitz-type inhibitors, serpins and stefins. Using the mass spectrometry analysis and MEROPS database (BLASTp, E-value 1e-5), 501 *E. nipponicum* secreted proteins were identified as peptidases and next 107 as inhibitors. Additionally, we made the first bioinformatic steps leading to the second draft of assembly (improved by long Oxford Nanopore reads) and detailed annotation of *E. nipponicum* genome. According to our data (k-mer counting and flow cytometry), the *E. nipponicum* genome size was estimated for 1.5 Gb and thus could be one of the biggest within platyhelminths.

36. *Cytokine signals and immune competition in the Th2 response to helminth infection*

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More than 1.5 billion people per year are infected with helminths, presenting a global health problem that requires urgent new treatment strategies. Helminths induce potent type 2 immunity as well as a strong regulatory response. An important component of the type 2 immune response is T helper 2 (Th2) cells. IL-10 is a regulatory cytokine that is highly expressed during human and murine helminth infection, however, the role of this cytokine during infection remains unclear. Our hypothesis is that IL-10 acts directly to promote Th2 differentiation while also suppressing counter-active Th1 development. We examined this hypothesis both by direct addition of IL-10 *in vitro* and through IL-10R blockade in mice infected with the murine helminth *Heligmosomoides polygyrus. In vitro*, IL-10 can induce both Th2 cytokines and the Th2 master transcription factor GATA3 in CD3 and CD28 stimulated $CD4^+$ T cells. *In vivo,* IL-10R blockade during *H.polygyrus* infection correlated with a significant decrease in the Th2 cytokines, IL-13 and IL-5 in $CD4^+$ T cells in the small intestine, as well as the master Th2 transcription factor GATA3. This decrease was not observed in the draining lymph node, suggesting that the function of IL-10 during a Th2 response may be tissue-dependant. We are now investigating the molecular mechanisms by which IL-10 acts in these different tissues and aim to establish the importance of this cytokine for a successful Th2 response. Understanding how regulatory cytokines optimise the Th2 response during *H. polygyrus* infection may allow us to identify therapeutic targets to enhance anti-helminth immunity. In addition, new insight into the mechanisms by which IL-10 controls $CD4^+$ T cells will be translatable to shedding light on immune regulation in many other disease contexts.

37. Host modulation by helminth parasite *Heligmosomoides polygyrus bakeri* extracellular vesicles.

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Helminths are known to have potent immunomodulatory effects on their host that are at least partially recapitulated by their secretory products alone. *Heligmosomoides polygyrus bakeri*, a helminth that naturally infects mice, is known to actively secrete extracellular vesicles (EV) which contain lipids, proteins and small RNAs. Excitingly, vaccination with *H. polygyrus bakeri* derived EVs results in protective immunity to subsequent *H. polygyrus bakeri* infection in mice. We therefore suspect EVs and their contents target the host epithelial response to infection in a manner that benefits the parasite. However, what host pathways *H. polygyrus bakeri* derived EVs specifically target is unknown. EV uptake has been demonstrated in cell line models, and results in differential gene expression. To uncover the natural cellular target of *H. polygyrus bakeri* EV within the host we utilised small intestine flat organoids to identify whether particular cell lineages within the barrier site closest to infection, the gut epithelium, preferentially uptake labelled-EV. Additionally, we have employed RNA sequencing to identify differential gene expression patterns between recipient cells and either non-recipient, or naïve cells. We will present data on gene expression changes that inform further investigation into the function of helminth EV during infection.

38. Mass spectrometric characterisation of novel glyco-epitopes in free-living and parasitic nematodes

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Nematodes constitute a large number of different species on earth and many of them parasitise animals and humans. Glycoproteins synthesised by nematodes possess glycan structures, which are very different from those made by mammals. It is known that some epitopes of nematode glycans, such as the core α1,3-linked fucose on N-glycans, are immunogenic as they cross-react with antisera of infected mammals. Therefore, we set focus on analysing the N-glycomes of nematode species. In the past few years, we have studied the N-glycomes of free-living *Caenorhabditis elegans* and *Pristionchus pacificus*, of the sheep parasite *Haemonchus contortus*, the porcine parasites *Oesophagostomum dentatum* and *Trichuris suis*, and more recently the canine heartworm *Dirofilaria immitis.* Using an off-line HPLC-MALDI-TOF-MS/MS approach coupled with glycosidase and chemical treatments, worm N-glycans were separated on HPLC columns and analysed by mass spectrometry in great detail. In addition, we employed *C. elegans* as a model and generated a set of mutants to investigate the function of genes which are involved in its N-glycan biosynthesis. A repertoire of novel glyco-epitopes were revealed, for instance, different types of Gal-Fuc modifications of the chitobiose core found in the clade V nematodes, phosphorylcholine (PC) substitution of the LacDiNAc residues on the antennae (*Trichuris*), and long fucosylated HexNAc-based repeats as well as glucuronylated glycans (*Dirofilaria*). By using a novel PNGase A from rice or hydrazine, we were able to release N-glycans with heavily modified core structures from the wild type *C. elegans*, which carry up to five fucose or galactose residues. The findings of novel glyco-epitopes in nematodes not only provide clues about the molecular basis of host-parasite interactions, but also shed light on new diagnostic markers as well as potential therapeutic targets.

39. *In vitro* development of *Nippostrongylus brasiliensis* larvae on nutrient agar plates

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We report here a method for the *in vitro* development of rat hookworm (*Nippostrongylus brasiliensis*) larvae on Nematode Growth Medium (NGM) agar plates. Eggs were isolated from rat faeces using a sugar – salt gradient, disinfected and sorted on a BD Influx Cell Sorter, before seeding on NGM agar plates supplemented with bacterial food. Third stage infective larvae (iL3) typically developed by day 7 post seeding on NGM agar plates, and displayed similar morphology and size compared to larvae reared using the standard charcoal coproculture method. Infectivity assessed in permissible rodent hosts revealed a similar magnitude in larval burden in the lung and intestines at different days post infection for both methods. Patent infection was detected in both mice and rats infected with agar cultured iL3. Cryopreserving the larvae was also investigated. The method described here is useful for characterizing the *in vitro* growth requirements of hookworm larvae and their metabolic activity in a defined setting.

40 Prevalence of malaria and soil-transmitted helminth infections and their association with undernutrition in schoolchildren residing in Mfou health district in Cameroon.

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Malaria and soil-transmitted helminths are common parasitic diseases found in school children in subSaharan Africa. We determined the prevalence and intensities of these infections in apparently healthy schoolchildren living in Mfou health district, where malaria and intestinal infections are among the first causes of morbidity. In a cross-sectional study involving 503 schoolchildren, anthropometric parameters were measured. Finger-prick blood and fresh stool samples were collected for malaria parasites determination, assessment of anaemia and detection of helminths' eggs. Logistic regression analysis was used to investigate the association between these infections and other factors. Overall, 40.6 and 29.6% of children harboured malaria parasites and worms respectively. Prevalences of mild, moderate and severe undernutrition were respectively 22.2, 2.3 and 0.5% for underweight, and 21.9, 7.6 and 2.0% for stunting. In logistic regression analysis, anaemia (OR=2.64, 95% CI: 1.71-4.07) and infection with *Ascaris lumbricoides* (OR=1.72, 95% CI: 1.01-2.91) were significantly associated with malaria infection. Infection with *Trichuris trichiura* was significantly associated with increased risks of underweight (OR=2.11, 95% CI: 1.11-4.01). Moreover, rural schoolchildren showed increased chances of carrying worms, compared to their urban counterparts (OR=2.60, 95% CI: 1.75-3.86). Malaria prevention and school-based deworming activities should be re-enforced in Mfou health district to reduce the burden of these infections in children.

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