BSP Autumn Symposium 2019 – Belfast

Post-genomic progress in helminth parasitology

Helminth parasites are pervasive pathogens that infect humans, animals and plants worldwide. They impact our agricultural productivity and are a significant cause of morbidity for humans in large parts of the developing world, where they are responsible for eight of the 17 neglected tropical diseases recognized by the WHO. Over the last 10-12 years, helminth parasitology research has been transformed by the appearance of genome, transcriptome, proteome and other "omics" datasets. These in turn have spurred the creation of a suite of functional genomic tools, allowing exploration of the vast amounts of data now available. These tools include RNA interference platforms in several species, while the first applications of CRISPR-based genome editing methods have recently been reported. Meanwhile, large-scale genome sequencing and re-sequencing efforts have proceeded apace, shedding new light on our understanding of evolution on a phylum-wide scale, and on development of anthelmintic resistance mechanisms. This one-day BSP Autumn Symposium aims to highlight some of these exciting research areas, where state-of-the-art technologies are being applied to helminth genome research.

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11:00 (30 mins) - Defining the genetic and molecular basis of triclabendazole resistance in *Fasciola hepatica* (Jane Hodgkinson)

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Session 2

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12:30 (30 mins) - Host-seeking behaviours of skin-penetrating nematodes (Elissa Hallem)
13:00 (30 mins) - Probing *in-vivo* liver fluke biology *in vitro* (Aaron Maule)

13:30 (60 mins) Lunch Break and Posters

Session 3

14:30 to 16:00
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Poster Session

16:00 to 18:00 – Poster viewing includes drinks and snacks

Oral Abstracts

Session 1

Multi-Omics Approaches to Unravelling the Complexity of Neuropeptide Signalling in Nematodes

Dr Angela Mousley, *Queen's University Belfast, UK*

A Mousley[1];
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The recent advances in genomic, transcriptomic, proteomic, and peptidomic technologies have driven significant expansion in parasitic nematode 'omics data, providing a welcome gateway to the identification of putative drug targets, vaccine candidates, and biomarkers for the control and diagnosis of nematodiasis. In the face of rising multi-drug resistance to nematode parasites, we must harness these recent advances to enhance our basic understanding of parasitic nematode biology that will inform drug discovery pipelines. Whilst the nematode nervous system is a proven drug target, it remains underexploited. Indeed, the neuropeptidergic component is an untapped resource and thus a key focus for novel drug target discovery. This presentation will provide an overview of the progress in application of 'omics technologies to inform nematode neuropeptide biology and enhance understanding of the complexity of the nematode signalling system. Specifically: (i) Pan-phylum genomic analyses have enabled the identification of neuropeptides, and neuropeptide-GPCRs, and facilitated the prediction of putative ligand-receptor interactions; (ii) Species-, life- and tissue-specific transcriptomic data have facilitated novel *in silico* deorphanisation approaches and target prioritization strategies based on spatial and temporal expression of neuropeptide ligands and their putative receptors; and (iii) The novel application of peptidomics tools to the extrasynaptic component of the nematode nervous system has revealed a novel route for neuropeptide signalling in parasitic nematodes. Integration of these multi-omics-derived datasets collectively unravel the complexity of neuropeptide signalling, provide a springboard to better understanding of basic worm biology, and support novel anthelmintic discovery.

From Genomics to Molecular Diagnostics of Anthelmintic Resistance

Dr John Gilleard, *University of Calgary*

J Gilleard[1];
[1] University of Calgary, Canada

Anthelmintic resistance is a major clinical and economic problem for numerous helminth species of domestic livestock and companion animals and an emerging concern for helminth control in humans. Recent improvements in helminth reference genomes, sequencing technologies and informatic approaches are now enabling genome-wide approaches to identify the genetic loci in helminth genomes that underly anthelmintic resistance. Once such loci are identified, sequencing technologies can also be used for diagnosis and surveillance of anthelmintic resistance and to study its molecular epidemiology in the field. In this presentation, I will focus on the recent progress for benzimidazole and ivermectin resistance in *Haemonchus contortus* and related nematode species of livestock. The chromosomal scale reference genome assembly of *H. contortus* has transformed our ability to study the molecular genetics of resistance in this species. I will review some recent data from genetic crosses and genome-wide scans of natural field populations that identify the major benzimidazole and ivermectin resistance loci. I will also discuss the use of amplicon sequencing to detect anthelmintic resistance mutation in field populations and its practical application to improve diagnostics and surveillance.

Defining the genetic and molecular basis of triclabendazole resistance in *Fasciola hepatica*

Prof Jane Hodgkinson, *Professor of Molecular Veterinary Parasitology, UOL*

J Hodgkinson[1]; R Hoyle[1]; D J Williams[1]; N J Beesley[1];
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The liver fluke, *Fasciola hepatica* is an economically important trematode pathogen of livestock worldwide and is regarded by the WHO as a re-emerging zoonosis. Predictions indicate an increased prevalence of *F. hepatica* infection, which is likely to exacerbate its impact on livestock production and human health in future. Control of *F. hepatica* relies heavily on drug treatment, in particular the drug triclabendazole (TCBZ), which targets the highly pathogenic juvenile fluke migrating through the liver. As with many helminth parasites drug resistance has emerged and poses a substantial threat to sustainable liver fluke control.
This paper will report a series of studies we have taken to define the genetic basis of TCBZ resistance in *F. hepatica*. We have characterised clonal parental lines of TCBZ-resistant (TCBZ-R) and TCBZ-susceptible (TCBZ-S) liver fluke. In order to identify areas of the genome with signatures of drug selection we have crossed TCBZ-S and TCBZ-R clones and carried out whole-genome mapping of TCBZ-R genes through subsequent F1 and F2 populations. By comparing the frequency of SNP alleles derived from the resistant parental clone and linked to the TCBZ resistance loci in pooled, phenotyped F2 recombinants we have localised a single genomic locus under selection, containing around 30 genes. Recently, we have complemented these mapping studies with metabolomic analysis of TCBZ-R and –S parasites with and without exposure to triclabendazole, *in vivo.*

Session 2

Genome editing with CRISPR/Cas9 provides new insight into pathogenesis and transmission of schistosomiasis

Prof Paul Brindley, *George Washington University, USA*

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CRISPR/Cas9-based genome editing had yet to be reported, until earlier this year, in species of the Platyhelminthes. We tested the approach by targeting the omega-1 (ω1) ribonuclease of the egg of *Schistosoma mansoni* as a proof of principle. This secreted enzyme is crucial for Th2 polarization and granuloma formation in the infected host. Schistosome eggs were exposed to Cas9 complexed with guide RNA complementary to ω1 by electroporation or by transduction with lentiviral particles. Some eggs were also transfected with a single stranded donor template. Sequences of amplicons from gene-edited parasites exhibited Cas9-catalyzed mutations including alleles arising from homology directed repair, and other analyses revealed depletion of ω1 transcripts and the ribonuclease. Gene-edited eggs failed to polarize Th2 cytokine responses in human macrophage/T-cell co-cultures, while the volume of pulmonary granulomas surrounding ω1-mutated eggs following tail-vein injection into mice was vastly reduced. Knock-out of ω1 and the diminished levels of these cytokines following exposure showcased the novel application of programmed gene editing for functional genomics in schistosomes.

Host-seeking behaviours of skin-penetrating nematodes

Dr Elissa Hallem, *Associate Professor, University of California, Los Angeles, USA*

E Hallem[1];
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Skin-penetrating gastrointestinal parasitic nematodes have an infective third-larval stage (iL3) that actively searches for hosts to infect. We are interested in understanding the host-seeking behaviors of iL3s, as well as the molecular and cellular mechanisms that underlie host seeking, using the human-parasitic threadworm *Strongyloides stercoralis* as a model system. We found that *S. stercoralis* iL3s display robust attraction to a diverse array of human-emitted odorants. The strongest attractants we identified for *S. stercoralis* iL3s are also mosquito attractants, suggesting that mosquitoes and worms target humans using many of the same olfactory cues. *S. stercoralis* iL3s are also robustly attracted to temperatures approximating mammalian body temperature. Both chemosensory behaviors and thermosensory behaviors are flexible such that *S. stercoralis* iL3s display experience-dependent and life-stage-specific sensory behaviors. To investigate the molecular mechanisms that drive sensory-driven host seeking, we first developed a method for introducing CRISPR/Cas9-mediated gene disruptions in *S. stercoralis*. We then used this system to identify genes required for host seeking. In C. elegans, many sensory behaviors require the cGMP-gated cation channel subunit gene *tax-4*. We found that targeted mutagenesis of the *S. stercoralis tax-4* homolog abolishes sensory-driven host seeking, demonstrating that parasitic host-seeking behaviors are generated through an adaptation of sensory cascades that drive environmental navigation in *C. elegans*. We also found that responses to carbon dioxide require the receptor guanylate cyclase gene *gcy-9*. Our results provide insight into the molecular mechanisms by which human-parasitic nematodes find human hosts to infect.

Probing *in-vivo* liver fluke biology *in vitro*

Prof Aaron Maule, *Academic, Queen's University Belfast*

A Maule[2]; E McCammick[2]; P McVeigh[2]; P McCusker[2]; E Gardiner[2]; D Wells[2]; M P Evans[2]; N Clarke[2]; J Coulter[2]; A Margariti[2]; J Hodgkinson[1]; N J Marks[2];
[1] Institute of Infection and Global Health, University of Liverpool, UK; [2] Queen's University Belfast, UK

We have developed an experimental toolkit for studies on juvenile liver fluke that is built upon their ability to survive and develop over long periods *in vitro*. The toolkit comprises robust reverse genetics and target localization tools complimented by a suite of bioassays that inform gene functions relating to survival, motility, growth/development and drug susceptibility (the availability of resistant and susceptible isolates facilitates studies on parasite-drug interplay). High RNAi penetrance extends across diverse neuronal, muscle, gut and stem cell targets. Here we pose the question, can our *in vitro* worms inform *in vivo* biology? Whilst they don't reach sexual maturity, *in vitro* worms develop *ex vivo* adult-like motility, morphology and excretory/secretory profiles. Also, during development *in vitro* worms display changes in drug sensitivity similar to those reported for *in vivo* studies. Worms grow more slowly *in vitro* than *in vivo*, a difference that appears to be driven by ~4-fold higher stem cell proliferation *in vivo*. Indeed, fluke drug susceptibility appears to diminish with increasing stem cell proliferation. Additionally, we find that the transcriptomes of 3-week old *in vivo* and *in vitro* juveniles show that ~11% of gene transcripts are differentially expressed, and many of those showing differential expression have proposed functions relating to growth/development. Further, only three of 42 miRNAs showed differential expression between *in vitro* and *in vivo* juveniles, with all three involved in developmental regulation. These data support our hypothesis that *in vitro* maintained juvenile fluke provide a valid model system for the study of parasite biology.

This work reported here has been supported by NC3Rs (NC/N001486/1) and the Department for the Economy Northern Ireland

Session 3

Parasitic nematode small RNAs: forms and functions

Dr Collette Britton, *University Reader, University of Glasgow*

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Knowledge of small RNA structure and function has greatly increased in the last decade, from higher organisms to parasitic helminths. Genome and transcriptome data have identified different classes of nematode small RNAs, including microRNAs (miRNAs), small interfering RNAs (siRNA) and PIWI-interacting RNAs (piRNAs). These are involved in negatively regulating expressed genes and/or transposable elements, however their precise functions are currently unknown. Here I will focus on miRNAs, which modulate gene expression by binding with partial sequence complementarity to sites predominantly in the 3'UTR of target mRNAs. Genetic studies in *C. elegans* first showed that miRNAs were required for correct development. We profiled the expression of miRNAs in all parasitic stages of the highly pathogenic gastrointestinal nematode *Haemonchus contortus* using microarrays. Micro RNAs enriched in L3 and L4 larval stages are conserved in nematodes or other organisms, and using miRNA inhibitors, we found that L3-enriched miRNAs act to suppress development and maintain an arrested state. In contrast, many miRNAs abundantly expressed in adult male and female worms are species-specific, suggesting potential roles in adaptation and survival within the host. miRNAs have also been identified in excretory-secretory (ES) products and in extracellular vesicles released from a range of parasitic nematodes, stimulating interest in small RNAs as possible modulators of host immunity. I will discuss recent work that aims to determine the functions of small RNAs within parasitic nematodes and in host-parasite interactions.

The tangled history of the association between *Wolbachia* symbionts and their filarial nematode hosts.

Prof M Blaxter, *Programme Lead, Wellcome Sanger Institute*

M Blaxter[3]; A J Reid[2]; G Oldrieve[2]; J Krücken[1];
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Several human-infective filarial nematodes carry an intracellular alphaproteobacterial symbiont, *Wolbachia*. In terrestrial arthropods, *Wolbachia* induce a variety of reproductive manipulations that promote *Wolbachia* transmission. In filarial nematodes, *Wolbachia* do not impact host reproduction, but show features that suggest a mutualist association. However, some filarial species lack *Wolbachia* entirely and others have a patchy infection. While A and B supergroup *Wolbachia* strains only infect arthropods and C and D strains only infect filaria, supergroup F *Wolbachia* have been found in arthropods and filaria. Thus the history of association between *Wolbachia* and filarial nematodes is complex (see LeFoulon et al PeerJ. 2016;4:e1840). When one sequences a *Wolbachia*-infected filarial nematode genome, one gets the endiosymbiont genome "for free". Fragments of *Wolbachia* genomic DNA are also found in the nematodes' nuclear genomes. These horizontally-transferred fragments likely arise because Wolbachia is present in the nematode germline. We use these *Wolbachia* insertions to track the history of *Wolbachia* in filarial nematodes. We find that currently *Wolbachia*-free species used to carry infections, that D *Wolbachia* have replaced C in some species, and that *Dirofilaria* species carried two distinct *Wolbachia* (C and F). We find evidence of current dual infection by C and F *Wolbachia* in some but not all *Dirofilaria repens*. These findings suggest that the relationship between *Wolbachia* and filaria is not simply mutualist, and that some filarial *Wolbachia* may be recently-acquired manipulators.

Peeking at the sexual development of female schistosomes through the 'omics' keyhole

Professor Christoph G. Grevelding, *Professor, Justus Liebig University Giessen*

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***** This abstract/presentation is in memoriam to our wonderful and inspiring colleague Paula Ribeiro, who passed away. One of the fascinating features of schistosomes is that the sexual maturation of the adult female depends on a permanent pairing contact with the male. Although males appear sexually mature before pairing, male-female interaction of schistosomes seems to be a bidirectional process. Although a number of studies have addressed this in the past, our knowledge about this part of schistosome reproductive biology and the underlying molecular principles is still fragmentary. By organ-isolation and subsequent comparative sub-transcriptomics with RNA of gonads from both paired and unpaired adult S.

mansoni, we identified transcripts of >7,000 genes in both sexes. Of these, many transcripts occurred pairing-dependently, 3,600 in ovaries and 243 in testes. Besides evolutionary aspects, bioinformatics provided new insights into the potential roles of kinases, GPCRs, and neuropeptides in schistosome development and differentiation. Evidence was obtained for a prominent role of kinases for female traits, whereas part of the GPCRs and neuropeptides may be involved in male-associated, gonad-independent processes. Furthermore, male-female interaction even influences neural activities in females, which downregulate the expression of some GPCR and neuropeptides following pairing. The obtained results suggest that the male-female interaction of schistosomes is more complex than envisaged before.

Posters

Poster 1 : Expression and localisation of Bone Morphogenic Proteins (BMPs) in *Fasciola hepatica*.

Miss Nada Albalawi, *phd student, University of Liverpool*

N Albalawi[1]; J E Hodgkinson[1]; R Flynn[1];
[1] *University of Liverpool, UK*

Background: *Fasciola hepatica*, the liver fluke, is a common trematode parasite throughout the globe (Musah-Eroje and Flynn, 2018). It infects multiple species of mammals, particularly ruminants, as well as humans. As such, it represents a significant economic burden in livestock and health care. Bone Morphogenic Proteins (BMPs) are the largest subfamily of the Transforming Growth Factor-β (TGF-β) group of cytokines (Herrera et al., 2018) and they can have roles in dorsal-ventral patterning, organogenesis and cell differentiation.

Aim: Preliminary work suggests that there are two FhBMP genes in the *F. hepatica* genome. Here we propose to determine expression levels and tissue location of FhBMP3 and FhBMP15.

Methods : Primers used for amplification of *F. hepatica* β -tubulin (BT) isotype-specific fragments βT2, βT3 were from Fuchs et al 2013. Primers were designed in house for amplification of FhBMP3 and FhBMP15 based upon our initial sequence characterisation. Reactions were performed using Bio-line SensiMix SYBR on the Bio Rad. The 2^{-DDC}T Method was used for analysis of gene expression.

Results: Two primer/probe sets targeting BMP3 and 3 primer/probe sets for BMP15 were tested to pick the optimal set for amplification. Assays on *F. hepatica* adult samples showed that relative expression of FhBMP15 is higher in adults compared with FhBMP3.

Conclusions: FhBMP15 shows higher expression in adults compared with FhBMP3 which suggests a lifestage specific role of FhBMP15. Mammalian BMP15 is known to be important in reproductive function and ovarian development. We will apply *in situ* hybridisation to localise the tissues expressing FhBMP15 and in the future attempt to block signalling.

Poster 2 : The pentameric ligand-gated cation channels

Prof Robin Beech, *Assoc Professor, McGill University*

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The pentameric ligand-gated cation-channel (pLGCC) family encodes neurotransmitter receptors that regulate fast-synaptic signalling. Analysis of inherited diseases, electrophysiology, mutagenesis, structural modelling and transgenic animals have all been used to create a detailed understanding of the structure and function of this class of receptors in vertebrates. The origin of the major functional classes traces back prior to the Cambrian Explosion, some 600 MYA. As a result, the subsequent sequence change has obscured the mechanistic details of how these classes arose. An ideal model is one where the recent appearance of new subunit types through gene duplication demonstrates functional adaptation with minimal sequence divergence allowing the molecular details behind the change to be identified. Such a model would need to demonstrate characteristics applicable to this class of receptors as a whole.

The Helminth Genome Initiative provides high quality genome data for more than 70 species of nematode. These organisms have a greatly expanded pLGCC family that continues to produce new subunit types. Mining this resource identifies multiple examples of subunits where new functionality has appeared within the last 100 MY with protein sequence divergence less than 10 %. Substitution rate analysis shows that adaptive sequence change follows each example of gene duplication. Experimental verification of reconstituted receptors in *Xenopus* oocytes confirms that subunit function has changed. Minimal changes in neurotransmitter affinity are apparent and sensitivity to many agonists and antagonists is very similar. The major functional difference is in the ability of derived subunits to occupy specific positions in the new receptor. This is consistent with a model in which the

products of gene duplication, with identical sequence, will compete with each other for assembly into a functional receptor. Evolutionary adaptation would be strongest initially to exclude duplicate subunits from occupying the same structural position in the receptor and this would then allow adaptation for specific changes in pharmacological response. These newly derived subunits therefore provide an ideal system in which to investigate the mechanisms that regulate subunit assembly and structural organization of the receptor. In order to model the pLGCC family across all organisms, the way in which receptors are assembled and the cellular machinery involved should be similar.

We have been able to show that assembly of nematode acetylcholine receptors follows the same general pattern as assembly of the vertebrate receptors. This includes the involvement of orthologous accessory proteins that mediate assembly and processing as well as the pathway of assembling a pentameric receptor from its monomer components. These receptors require the RIC-3, UNC-50 and UNC-74 accessory proteins orthologous to their vertebrate counterparts. Receptor assembly begins with the rapid formation of a trimer with sequential addition of two remaining subunits to produce the final receptor, exactly as described for the vertebrate receptor. We believe that the resource of nematode genomes available and the unique, continuing expansion of the pLGIC family in the nematodes make these an ideal model for pLGICs more generally.

Poster 3 : Interfering with ABC-Transporters as a means to Potentiate the Efficacy of Flukicides

Mr Nathan Clarke , *PhD Research Student, Queen's University Belfast*

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Genus *Fasciola* contains liver fluke parasites, the causative agents of fascioliasis/fasciolosis, a disease of socioeconomic importance through its impact upon global agriculture and as a neglected tropical disease of humans. Treatment relies upon a small arsenal of flukicides, where triclabendazole (TCBZ) exhibits unique efficacy in treating both acute and chronic infections. With increasing reports of TCBZ resistance both in animals and humans comes an increasing necessity for the exploration of novel drug targets, or novel ways to potentiate the efficacy of current flukicides, e.g. limiting their metabolism and or efflux within the parasite. Utilising our *in vitro* culture platform, TCBZ and its metabolites (triclabendazole sulphoxide (TCBZ.SO) and triclabendazole sulphone (TCBZ.SO2)) are demonstrated as being active against juvenile *Fasciola hepatica*. Interestingly, TCBZ-resistant isolates exhibit increased tolerance to TCBZ.SO, the hypothesised active form of the compound. Co-incubation of TCBZ and its metabolites with the ABC-transporter inhibitor verapamil potentiates drug efficacy against *F. hepatica*, as well as the effectiveness of TCBZ.SO against a TCBZ-resistant isolate. Probing the available genomic data for *F. hepatica* revealed 23 putative ABC transporter sequences in the Liverpool genome, whereby orthologues of well-known drug resistance-associated proteins including p-glycoprotein 1, multidrug resistance associated protein (Fhmrp1) and breast cancer resistance protein were found. These transcripts were found to be highly amenable to RNAi-based knockdown, with a reduction in *Fhmrp-1* potentiating the efficacy of TCBZ.SO against a TCBZ-R isolate. These data highlight the drug target candidature of ABC transporters as a way to preserve the efficacy of currently used flukicides.

We acknowledge funding provided by the Biotechnology and Biological Sciences Research Council and Merial Ltd. (BB/K009583/1), the National Centre for the 3Rs (NC/N001486/1) and the Department for the Economy of Northern Ireland.

Poster 4 : Characterising the Endocannabinoid Signalling Pathway in Parasitic Nematodes

Miss Bethany Crooks, *PhD student , Queen's University Belfast*

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Neglected Tropical Diseases (NTDs) caused by parasitic nematodes (PNs) impose a global disease burden surpassing both malaria and tuberculosis. Overreliance on a limited range of anthelmintics heightens the threat of drug resistance and drives the need for novel nematode-NTD control options. Therapeutic exploitation of endocannabinoid (EC) signalling is receiving significant attention in human medicine due to its role in neuromodulatory processes including fertility, motor control and feeding. Knowledge on nematode EC biology is primarily limited to *Caenorhabditis elegans* where data demonstrate that EC signalling influences key aspects of neurobiology including homeostasis, ageing and locomotion. Crucially, mammalian and nematode EC G-protein coupled receptors (EC-GPCRs) appear to differ, providing the potential for selective anthelmintic drug design. In this study we have exploited nematode genome and transcriptome data to demonstrate that non-mammalian-like EC-GPCRS and EC signalling pathway enzymes display pan-phylum conservation across 96 nematode species (113 genomes), including key PNs of medical and agricultural significance. ECS pathway protein complement appears to be broadly clade specific; clade 2 nematodes display a reduced complement of EC-GPCRs, clade 12 species predominantly lack the EC-GPCR NPR-19 and degradation enzymes FAAH-1, and -2, and clade 8 nematodes display fewer EC pathway components overall. In addition, life-stage specific transcriptome analyses for 28 nematode species including *Caenorhabditis elegans*, *Strongyloides stercoralis* and *Meloidogyne incognita,* indicate that EC-GPCRs are expressed in therapeutically relevant life stages of PNs. Interestingly, EC-biosynthesis enzymes and EC-GPCRs are significantly upregulated in the infective larval stages of multiple PN species including *S. stercoralis* (Ss). *S. stercoralis* targeted mutagenesis studies will reveal the functional importance of EC signalling in nematodes. Together these data suggest a putative role for EC signalling in PN host seeking and invasion and underscore the appeal of EC signalling as a new, unexploited, avenue for novel nematode-NTD drug target discovery pipelines.

Poster 5 : Transcriptomic approaches to reveal praziquantel's mode of action in immature and adult *Schistosoma mansoni* in vivo

Dr Paul McCusker, *Postdoctoral Researcher, Medical College of Wisconsin*

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Despite decades of use and research the mode of action of the frontline anti-schistosomal drug praziquantel (PZQ) remains a mystery. However, recent advances in schistosome genomics provide us with new opportunities to investigate the action and impact of this drug. Here, we examine gene expression differences in immature (PZQ-insensitive) and adult (PZQ-sensitive) worms following treatment with PZQ in vivo. In combination with this approach we investigated the damage caused by PZQ through TEM and confocal microscopy, correlating transcriptional changes with physical damage. 778 genes were affected by PZQ in vivo, many of which were flatworm or *Schistosoma* specific. Of these, 327 genes showed differential expression between immature and adult worms. These genes were principally associated with worm structure (muscle tissue and tegument), fitting with damage to the basement membrane and muscle tissue observed through TEM and confocal microscopy. In adult worms, genes upregulated following treatment with PZQ were localised to identify tissue types most affected by drug exposure. Additional assays revealed cell death does not occur within the worm until around six hours after PZQ exposure. It is possible that structural disruption leads to host immune exposure which ultimately results in worm death.

Many studies on PZQ focus on drug effects and known genes/signaling pathways identified in other organisms, whilst here we have highlighted how many uncharacterised genes with no annotation or known homolog are also enriched by PZQ exposure. Together these data both provide a path to better understand the action of PZQ and also aid identification of potential novel drug targets, especially those involved in maintaining worm structure, where disruption could cause damage similar to that of PZQ.

Poster 6 : Ion channels as anthelmintic drug targets in parasitic flatworms

Dr Emily Robb, *Postdoctoral Researcher, Queen's University Belfast*

E Robb[1]; P McVeigh[1]; E McCammick[1]; D Wells[1]; M P Evans[1]; A Mousley[1]; N J Marks[1]; A Maule[1];
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Greater than 300 million people are infected with parasitic flatworms each year. Flatworm diseases sit among 20 neglected tropical diseases (NTDs) recognised by the United Nations World Health Organization (WHO), with recent estimates attributing losses of >6.2 million disability-adjusted life years (DALYs) annually to such infections. Limited flatworm vaccines and increasing anthelmintic resistance threaten the sustainability of control and underscore the need for novel flatworm anthelmintic development. Ion channels are involved in a vast array of cellular functions, underpinning behaviours key to survival including neuromuscular function. Despite the rich pharmacology of ion channels and their current use in the treatment of nematode infections, praziquantel is the only drug linked to ion channel dysregulation in parasitic flatworms. A primary limitation to further exploiting ion channel networks in flatworm drug development is a lack of knowledge on their fundamental biology within these organisms. This work showcases the use of recent genomic datasets to identify and evaluate ligand-gated ion channels (LGICs) as potential anthelmintic drug targets for parasitic flatworms. Using a Hidden Markov Model (HMM) based approach, predicted protein datasets were mined for LGIC sequences. Initial work focussed on characterising ion channels in liver fluke, *Fasciola hepatica*, describing 53 high confidence LGICs. The dataset includes obvious potential drug targets such as members of the Cys-loop superfamily (nicotinic acetylcholine and glutamate-gated chloride channels) and ionotropic glutamate receptors. Less obvious drug targets such as amiloride-sensitive sodium channels, ATP-gated channels and inositol 1,4,5-trisphosphate binding receptors were also identified. *In silico* analysis was expanded to include datasets for 30 other flatworm species (free living and parasitic) allowing for a deeper characterisation of ion channel structure and function. Combining *in silico* analysis with our functional genomic platform offers a much-improved ability to characterise ion channel function and evaluate flukicide-target and spectrum potential.

Poster 7 : AL-PHA beads: bioplastic-based protease biosensors for global health

Dr Alexander Webb, *Postdoctoral Research Associate, Imperial College London*

A J Webb[1]; R J Kelwick[1]; Y Wang[1]; A Heliot[1]; M R Templeton[1]; P S Freemont[1];
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Proteases are multi-functional, proteolytic enzymes that have complex roles in human health and disease, and therefore, detecting the activities of these enzymes can yield important insights into communicable and non-communicable diseases. Thus, the development of novel detection strategies for proteases may be beneficial to a host of global health applications. Using a synthetic biology approach, systematically, we have developed **A**dvanced proteo**L**ytic detector **P**oly**H**ydroxy**A**lkanoates beads (AL-PHA) – a library of low-cost, biodegradable, bioplastic-based protease

biosensors. These biosensors utilise PhaC-superfolder GFP (sfGFP) reporter fusion proteins that are bound to microbially manufactured polyhydroxyalkanoate (PHA) bioplastic beads. The design of the PhaC-sfGFP reporter fusions also incorporate modular, specific protease cleavage sites, thereby enabling the detection of specific proteases of choice. In the presence of a specific protease, sfGFP reporter proteins are cleaved off of the AL-PHA beads - resulting in a loss of bead fluorescence. These AL-PHA biosensors were initially optimised using a commercially available Tobacco Etch Virus (TEV) protease. Our third generation TEV biosensor (PhaC-112L-T-G) detected 0.5-1 U of AcTEV activity and 10 units of AcTEV protease activity resulted in a visually noticeable loss in AL-PHA bead fluorescence. It is known that parasites employ proteases to support pathogenesis. In the case of schistosomiasis (bilharzia), a disease that affects over 200 million people, the invasive *Schistosoma* cercariae release a cocktail of proteases, including elastase, that help the parasite to invade into a host through the skin. We have therefore, designed AL-PHA beads that contain a protease cleavage site specific for *Schistosoma mansoni* cercarial elastase. These *S. mansoni* cercarial elastase specific AL-PHA beads were validated using *S. mansoni* cercarial transformation fluid (SmCTF) samples, and were able to detect elastase. We envision that AL-PHA beads could be adapted into a low-cost and high-throughput protease detection assay for global health applications.

Poster 8 : The anthelmintic praziquantel activates a schistosome transient receptor potential channel

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Schistosomiasis is a parasitic flatworm infection that afflicts over 200 million people worldwide. The drug praziquantel (PZQ) remains the key clinical therapy to treat schistosomiasis and has been used for several decades. PZQ is also used to treat other diseases caused by parasitic flatworms. The clinical formulation of PZQ is a racemate (±PZQ) composed of the enantiomers (*R*)-PZQ and (*S*)-PZQ. (*R*)-PZQ causes Ca^{2+} influx, spastic paralysis and tegumental damage of adult schistosomes – however the molecular targets that mediate these effects in the worm remain unknown. Here, we have identified a Ca^{2+}-permeable ion channel that is activated by (*R*)-PZQ in the nanomolar range. This channel, christened *Sm*.$TRPM_{PZQ}$, is a member of the transient receptor potential melastatin (TRPM) channel subfamily. In heterologous expression assays, PZQ caused a long-lasting activation of *Sm*.$TRPM_{PZQ}$ in the absence of clear desensitization. Concentration response analysis revealed that (*R*)-PZQ activated *Sm*.$TRPM_{PZQ}$ with an EC_{50} of 597±10nM in Ca^{2+} imaging analysis and this activation was stereoselective, with the (*R*)-PZQ evoking Ca^{2+} signals over a considerably lower concentration range than (*S*)-PZQ (EC_{50} of 27.9±3.1μM). *Sm*.$TRPM_{PZQ}$ was expressed across various schistosome life cycle stages and was also present in other free-living and parasitic flatworms that exhibit sensitivity to PZQ. These data provide the first report of a schistosome target activated by PZQ and are consistent with *Sm*.$TRPM_{PZQ}$ being a target of this clinically important therapeutic.

Poster 9 : Defining Reference Genes for Gene Expression Analysis in *Fasciola hepatica* – Expression of Kinases During Fluke Development

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In *Fasciola hepatica* research, comparative gene expression analysis of the different life-stages is of major interest. This includes expression analysis of potential drug target genes: a new active compound should ideally target all life stages within the mammalian host, from NEJ and immature to adult flukes. Other research approaches include the *in vitro*-culture of flukes for several days to weeks, such as for or RNA interference-mediated knockdown of gene transcripts, which again needs a reliable quantification of expression. For the relative quantification of gene expression by quantitative real-time PCR (qRT-PCR), the accuracy is largely dependent on the stable expression of the reference genes used for normalisation. However, classical housekeeping genes such as glyceraldehyde-3-phosphate dehydrogenase (*gapdh*) and ribosomal RNA (rRNA) are often used based on tradition rather than being experimentally validated as stably expressed genes for the species or parasite stage of interest. In this study, we defined a set of stably expressed reference genes for qRT-PCR in *Fasciola* studies. We compared the expression stabilities of eight candidate reference genes, pre-selected from transcriptome data, by the algorithms NormFinder, geNorm, BestKeeper, and comparative DCT method. The most stably expressed reference genes for the comparison of intra-mammalian life stages were glutamyl-prolyl-tRNA synthetase (Fh*eprs*) and tubulin-specific chaperone D (Fh*tbcd*). The two best reference genes for analysis of *in vitro*-cultured juveniles were Fh*tbcd* and proteasome subunit beta type-7 (Fh*psmb7*). These genes may be suited to replace the housekeeping gene *gapdh* which has been used as standard in most *Fasciola* studies to date, but in fact was differentially expressed in our analysis. As a first application of the newly defined reference genes, we determined the expression levels of five kinases (Abl1, Abl2, PKC, Akt1, Plk1) which are discussed as potential druggable targets in other parasitic flatworms. Distinct expression patterns throughout development were found pointing to interesting biological functions, such as a predominant expression of the polo-like 1 kinase in

adult worms. Our results may motivate using this set of validated reference genes for future studies with *Fasciola* dealing with drug targets or parasite development.

Poster 10 : Novel hybrids of *Cinchona* alkaloids and bile acids with high anti-*Trypanosoma* activity

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Chagas disease, caused by *Trypanosoma cruzi*, is a major public health problem in Latin America. According to the World Health Organization, around 20 million people are infected and another 40 million are at risk of acquiring the disease. Currently, chemotherapy of the disease is based in two old drugs, Benznidazole and Nifurtimox, which are not effective and highly toxic especially in the chronic stage of the disease. For these reasons, new drugs to replace the outdated and ineffective therapy are required. To this end, we have produced a series of hybrids of *Cinchona* alkaloids and bile acids, expecting to combine their anti-parasitic activity and the known properties as drug transporters, respectively. These chimeric compounds were synthesized by a Barton-Zard decarboxylation reaction and have shown promising activity against *T. brucei*, *L. mexicana mexicana* and *P. falciparum*. Moreover, we have recently demonstrated that these compounds have antiparasitic activity against different *Trypanosoma cruzi* DTUs. In this work, new hybrids, with modifications in the bile acid fraction, have been tested for cytotoxicity assayed on HeLa cells. Half maximal inhibitory concentration (IC50) against these cells were estimated ranging between [1.0 – 3.0 ug/ml]. Besides, we tested the compounds against two *T. cruzi* strains (DTUs II and VI) tripomastigotes forms and the IC50 were estimated between [0.15 – 0.6 ug/ml]. Compounds activity against amastigote forms was less effective than the observed against tripomastigote forms. Selectivity of the hybrids were calculated as IC50HeLa/IC50tripomastigote form and some compound showed values of 10 or higher. This study opens the door to new possibilities in the screening of alternative drugs used traditionally in the Chagas disease's treatment.

Poster 11: Prevalence of hemoprotozoan parasites in small ruminants along a human-livestock-wildlife interface in western Uganda.

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Small ruminants are important to community livelihood in developing countries; however information on the role of hemoprotozoan parasites is scant. The objective of the study was to determine hemoprotozoan parasitic prevalence in western Uganda and identify major areas associated with these infections. This was a cross sectional study conducted at the edge of Budongo Conservation Forest in Masindi district of western Uganda in which 712 small ruminants were sampled. Blood from the jugular vein was collected from caprines and ovines and placed in an EDTA tube, and transported to the laboratory for examination. Thin and thick smears were prepared and examined by microscopy for hemoprotozoan parasites, and DNA was extracted and examined by PCR for Trypanosoma spp. A total of 13 villages in Budongo sub-county were surveyed and the study showed that caprines were the major small ruminants of importance to the community. Prevalence of hemoprotozoan parasites was as follows; anaplasmosis (3.65%) > theileriosis (0.45%) > trypanosomiasis (0.15%) and babesiosis (0%) by microscopy. Infections were found in the young with the exception of Anaplasma spp. while coinfections of anaplasmosis and theileriosis were high. Molecular analysis showed an overall trypanosome prevalence of 9.27% (PCR), mainly due to Trypanosoma brucei and T. congolense forest. Villages with trypanosomiasis were found in lowlands and swamps. The current trypanosomiasis prevalence in small ruminants of Uganda was 10 times greater than that previously reported showing that the disease burden has increased overtime within Uganda. A prevalence of 0.14% (95% CI: 0.00, 0.78) for the SRA gene showed that small ruminants would be important reservoirs of infection to humans. Hemoprotozoan parasites are a threat to community livelihood in developing countries and the role of molecular diagnostic techniques in disease monitoring was re-emphasized by this study. Information on primary hosts involved in the propagation of hemoprotozoan parasites in Uganda would help streamline prospective disease surveillance and control efforts.

Poster 12 : Laser Capture Microdissection RNAseq in *Brugia malayi*

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Spatially resolving gene expression is a key step in determining gene function in multicellular animals. In nematodes, including the parasitic filarial worm *Brugia malayi*, there is great interest in identifying proteins present at druggable or hidden antigenic sites for the development of new anthelmintics and vaccines. However, determining tissue specific gene expression in parasite nematodes has been limited by a lack of tissue accessibility and streamlined genetic tools. Here we present use of Laser Capture Microdissection (LCM) to isolate tissues in *B. malayi* including the excretory-secretory apparatus in microfilariae as well as pharyngeal tissue in adult females. Collected tissue from larval and adult stages can be used for 'omics' discovery platforms, including RNAseq.

Poster 13 : ZooTRIP. Zoonotic transmission of intestinal parasites: Implications for control and elimination.

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Zoonotic intestinal helminthiasis affects more than 949 million people globally, collectively contributing to an estimated 9.68 million disability adjusted life years (DALYs) lost per annum. Endemicity is focused in rural and poor urban areas of low-and-middle-income countries, where access to sanitation, hygiene, health care and education on parasite transmission is lacking. Zoonotic intestinal helminths include the *Schistosoma spp*. (*Schistosoma japonicum*), soil transmitted helminths, foodborne trematodes, and *Taenia spp*. Each having varying degrees of lifecycle complexity, but all utilising animal reservoirs as well as human definitive hosts to maintain transmission, complicating control strategies. The mainstay of control for helminth infections is mass drug administration with a handful of anthelmintic chemotherapies (praziquantel, and benzimidazoles). Despite great efforts involving pharmaceutical companies and non-governmental organisations to distribute these treatments freely in endemic regions to relieve morbidity, it is believed a more multidisciplinary approach to control, a One Health approach, will be required to successfully eradicate helminthiasis completely. This project will focus on endemicity in southeast Asia, applying a multidisciplinary approach to investigate the prevalence of zoonotic intestinal helminthiasis in the Philippines. Sampling from animals, humans and the environment will be integrated with parasitological, molecular diagnostic, and genomics and mathematical modelling approaches to investigate helminth transmission dynamics. The end focus, to predict whether a One Health approach involving integrated human/animal control and surveillance programmes can provide more effective management options than solely human-focused control.

Poster 14 : Deorphanizing G protein-coupled receptors in *Schistosoma mansoni* utilizing the MALAR yeast two-hybrid system

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Schistosomiasis is a neglected tropical disease caused by platyhelminths of the genus *Schistosoma*. The disease has global impact on human and animal health. According to the WHO, approximately 600 million people live in endemic areas, of which > 200 million require treatment [1]. Schistosomes are the only platyhelminths that have evolved separate sexes, and they exhibit a unique reproductive biology because the female's sexual maturation depends on a constant pairing-contact with the male. Because medical treatment is based upon a single drug, praziquantel, there is urgent need for the development of alternative control strategies. Due to their proven druggability, G protein-coupled receptors (GPCRs) are promising targets for anthelmintics. However, to identify candidate receptors, a deeper understanding of GPCR signaling in schistosome biology is essential. Comparative transcriptomics of paired and unpaired worms and their gonads revealed 39 differentially regulated GPCR genes putatively involved in neuronal processes [2-3]. In general, the diversity among GPCRs and their integral membrane topology make it difficult to characterize and deorphanize these receptors. To overcome existing limitations we utilized the innovative MALAR yeast two-hybrid assay to associate neuropeptide ligands with their cognate receptors. This method allowed us to identify putative neuropeptides for two GPCRs, which are differentially expressed in a pairing-dependent manner. Besides their value for basic research, insights into the participation of GPCRs/neuropeptides in schistosome biology may also support applied research aspects.

[1] www.who.int/news-room/fact-sheets/detail/schistosomiasis

[2] Lu, Z. et al. (2016). Schistosome sex matters: a deep view into gonad-specific and pairing-dependent transcriptomes reveals a complex gender interplay. Scientific Reports 6, 31150.

[3] Hahnel, S. et al. (2018) Tissue-specific transcriptome analyses provide new insights into GPCR signaling in adult *Schistosoma mansoni*. PLoS Pathogens 14(1): e1006718

Poster 15 : Investigating the genetic diversity and molecular epidemiology of *Schistosoma haematobium* in Eswatini and other endemic African countries

Mr Felix Llanwarne, *Student, Felix Llanwarne*

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Human schistosomiasis is a neglected tropical disease affecting millions of people predominantly in South America, Asia and Sub-Saharan Africa (SSA). Of the several species causing this debilitating disease, *Schistosoma haematobium*, the causative agent of urogenital schistosomiasis, has been somewhat overlooked in terms of research efforts, yet infects more people than all other schistosomes combined and displays unexplained genetic homogeneity throughout SSA. Eswatini (formerly Swaziland) is a schistosomiasis endemic country that has not been genetically characterised, and through observational research on egg morphology, exhibits high levels of inter-species hybridisation. *S. haematobium* miracidia were collected in May 2019 from 19 infected children in five known transmission localities from all four administrative areas of Eswatini (Hhohho, Manzini, Shiselweni and Lubombo). A partial fragment of the mitochondrial cytochrome oxidase subunit I (*cox*1) and NADH-dehydrogenase subunit 5 (*nad*5) genes were amplified for each miracidia to confirm species identification and genetic diversity. Furthermore, the nuclear internal transcribed spacer 1 (ITS1) was sequenced to identify if inter-species hybridisation events had occurred From the 148 *S. haematobium* miracidia molecularly analysed, there was no evidence of species hybridisation in the schistosomes as is observed elsewhere in SSA. However, *cox*1 haplotype diversity in the Eswatini *S. haematobium* larvae was shown to exhibit 11 unique haplotypes in just a small subset of samples collected from the 19 infected individuals. This is in stark contrast to the one predominant *cox*1 haplotype seen throughout continental African strains. It was also observed that *nad*5 sequence data offered less genetic diversity than *cox*1 data, displaying six unique haplotypes. The high genetic diversity of *S. haematobium* in Eswatini is unlike the norm seen throughout mainland Africa that displays unusual uniformity. This suggests that schistosomes being transmitted in Eswatini have experienced a different evolutionary past to the rest of the continent. For example, not being subjected to the same selection pressures such as Mass Drug Administration (MDA) or not undergoing the same species mixing due to a restricted freedom of movement. In addition, *nad*5 was found to have lower genetic diversity and offered no further molecular information than *cox*1, and therefore is proposed here as being less useful for exploring the genetic diversity of schistosomes in molecular epidemiology studies.

Poster 16 : 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 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 EVs 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. In order to identify glycosylated exosomal proteins, we have performed mass spectrometry analysis on glycoproteins segregated using a lectin-bead approach with DSA, RCA-I and SNA-I. Moreover, in an effort to identify worm structure(s) responsible for the release of EVs, we have performed lectin histochemistry assays on whole adult worms.

We are currently working to profile glycans at the surface of S.m. exosomes and to identify the glycosylated exosomal proteins, 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 results diagnostic tools as well as new targets for the potential prevention and therapy of schistosomiasis.

Poster 17 : Progress Towards Sensitive Molecular Diagnosis of Human Helminth Infections at the Point-Of-Care via Non-Invasive Urine Sampling: A Review

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Rapid, straightforward and reliable diagnosis of human helminth infections is essential for ongoing disease surveillance and successful disease control, particularly as control programmes advance towards disease elimination within endemic areas. Current WHO-recommended 'gold standard' diagnostic assays are unreliable in these low-endemic settings and typically involve the cumbersome or invasive sampling of bodily fluids such as stool and blood, as well as tissue via biopsy. Not only are these procedures often onerous, painful and carry a risk of infection, but many also require specific equipment and specialist health workers seldom available in endemic areas. A reliable assessment of disease prevalence within a given community can therefore often prove challenging as a result of patient aversions to being assessed, as well as through a lack of resources. In contrast, the sampling of urine is generally painless, low risk and relatively inexpensive. It negates the need for specialist staff, can usually be obtained immediately and is better tolerated by communities. Further to these clear practical advantages, in some instances, urine-based diagnostic assays have also been shown to provide a much more sensitive diagnosis of helminth infection when compared to gold standard methods that require alternative and more invasive bodily samples, particularly in low-endemicity settings. One approach to detecting helminth infections using urine samples is to target and amplify trans-renal helminth-derived DNA. Although highly sensitive even in low-endemicity settings, assays traditionally used to achieve this, such as conventional- and quantitative-PCR, are expensive and rely on specially trained technicians and sophisticated laboratory infrastructure; often preventing their application at the point-of-care. The recent development of alternative and field-deployable methods to target and amplify DNA, such as LAMP and RPA assays, however, offer a promising means of rapid, straightforward and reliable diagnosis of human helminth infections using non-invasive urine samples at the point-of-care in low-endemicity settings. Given the relative benefits in ease of collection, better community acceptance and improved diagnostic performance, we review current research literature and evaluate whether non-invasive urine sampling is currently exploited to its full potential in the development of molecular diagnostic tools for human helminthiases.

Poster 18 : The *C. elegans* L-AChR as a model for studying Helminth ion channels

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Pentameric ligand-gated ion channels (pLGICs) represent an ancient, fundamental signaling mechanism. These receptors at the Helminth neuromuscular junction are of particular interest as anthelmintic drug targets. The response of specific receptors depends on the composition and arrangement of their subunits. The nematodes have an expanded family of pLGIC subunits that allows for receptor subunit diversity. The levamisole-sensitive acetylcholine receptor (L-AChR) from *C. elegans* is used as a model to study Helminth receptors due to high structure conservation across subunit orthologs. The *C. elegans* L-AChR requires subunits encoded by five different genes; two non-alpha subunits LEV-1 and UNC-29, and three alpha subunits ACR-13, UNC-38, and UNC-63. In *Haemonchus contortus* the L-AChR does not require LEV-1, suggesting it is replaced by one of the other subunits. The appearance of new subunits through gene duplication reveals a pair of alpha-type subunits (*acr-8* and *acr-13*) and a pair of non-alpha type subunits (*unc-29.1* and *unc-29.2*). Interaction between these regulates whether the non-alpha subunit can occupy multiple positions within the receptor. The non-alpha UNC-291 and the alpha ACR-8 do not require the presence of LEV-1 to form a functional channel. Chimera subunits were made by exchanging the intracellular parts between UNC-29.1 and UNC-29.2, and between ACR-8 and ACR-13. Expression of the ion channels in *Xenopus* oocytes and characterization by two-electrode voltage clamp electrophysiology shows different regions contributing to subunit positioning between alpha and non-alpha subunits. The intracellular loop meditates non-alpha UNC-29.1 to function without LEV-1, whereas the extracellular domain of ACR-8 allows it to function without LEV-1. These results suggest a complex regulatory mechanism for pLGICs. Future studies will use subunit concatemers to identify the subunit that replaces LEV-1.

Poster 19 : Neuropeptide Biology in Fasciola hepatica

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Increasing resistance to existing flukicides continues to compromise the sustainable control of liver fluke. New flukicides are needed to suppress the impact of fluke infections in animals and humans. G protein-coupled receptors (GPCRs) are established targets for drugs used in human medicine. Within the GPCR complement of *Fasciola hepatica*, at least 47 are putative peptide receptors with many of the associated ligands likely to be neuropeptides (NPPs). Neuropeptidergic-signalling systems are evolutionarily ancient and have been shown to play key roles in a variety of fundamental processes including motility, reproduction, growth and development. The neuropeptide complement of *F. hepatica* has more than doubled relative to the 17 putative NPP genes identified previously. Analysis of transcriptomic data across multiple studies from various flatworm species has revealed distinct expression patterns, allowing for basic hypotheses on function to be formed. We have adapted a planarian wholemount *in situ* hybridisation protocol for *F. hepatica* and used it to examine the spatial expression patterns of putative neuropeptide-F/Y-like gene transcripts. We show that these have distinct expression patterns suggesting differential functions. In order to elucidate NPP function, we have also performed an RNAi screen of the predicted NPP complement in *F. hepatica*. Resultant phenotypes have revealed intrinsic roles for NPPs in behaviour and physiology of the developing juvenile. Expression, localisation and RNAi phenotypes of NPPs and peptide GPCRs will prove to be useful tools in directing deorphanisation approaches. This in turn will reveal GPCRs that represent the most attractive drug targets.

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