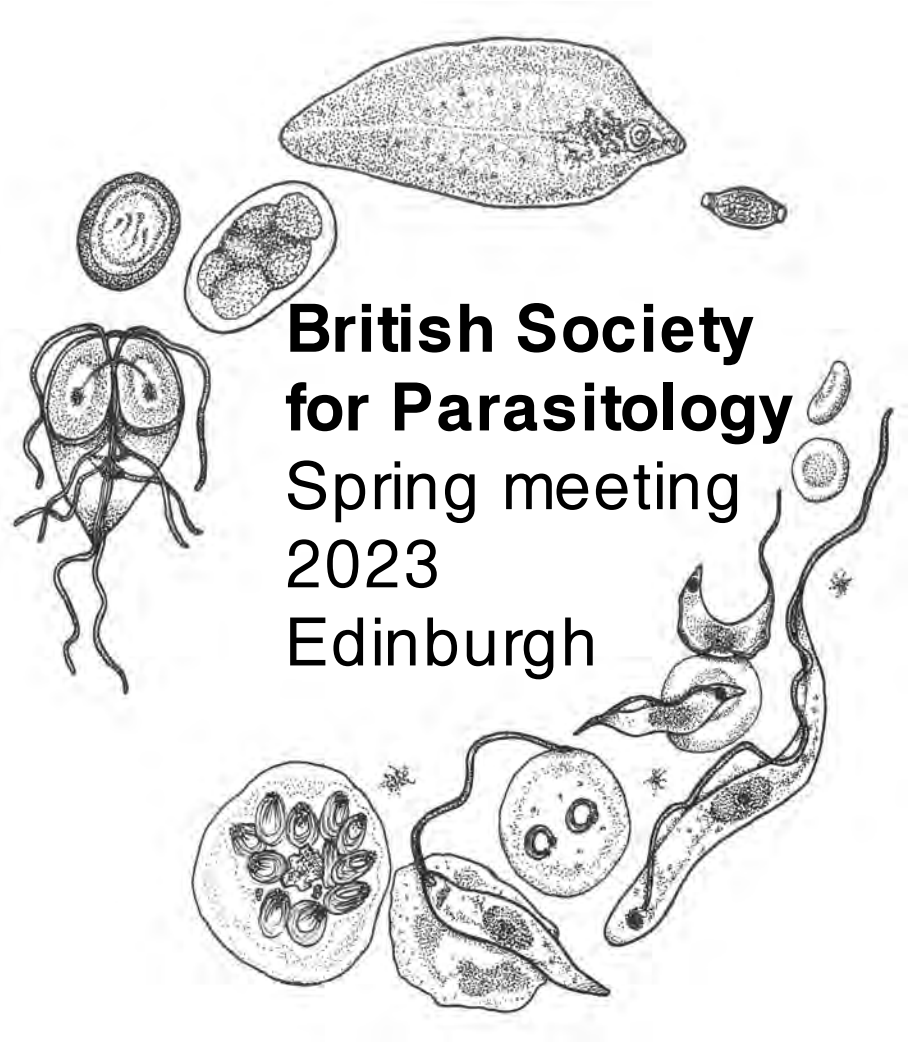

British Society for Parasitology

Spring meeting 2023

Edinburgh

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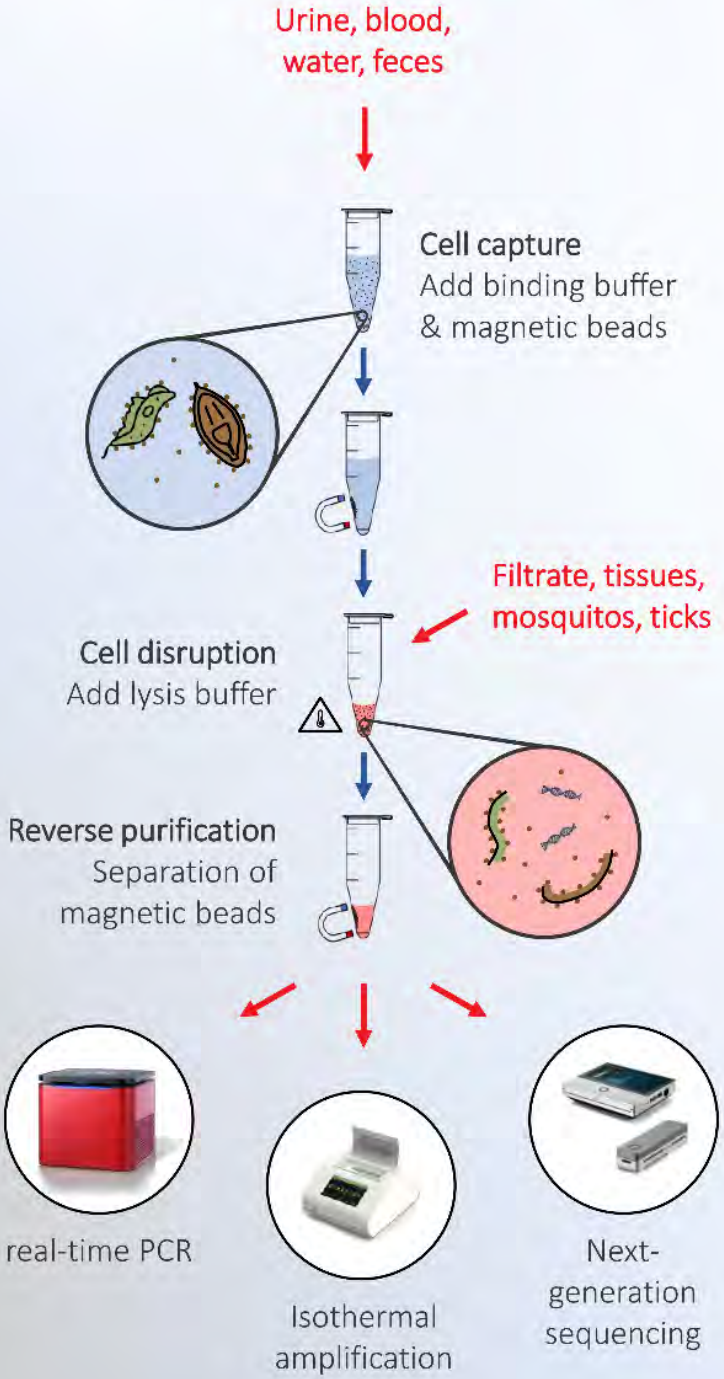

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Welcome to Edinburgh

Welcome to the 2023 British Society for Parasitology Spring Meeting, and welcome to the University of Edinburgh! Edinburgh has a long and proud history of research into parasites [1], and this strength continues today. Notable contributions came from those who helped elucidate the life cycles of African trypanosomes (Sir David Bruce), *Leishmania* (David Cunningham), *Onchocerca* (Donald Blacklock) and *Wuchereria* (George Low), with further important work on the biology and treatment of *Loa loa* (Douglas Argyll Robertson). These pioneers were graduates of our Medical School, and the budding parasitologists were taught in the Anatomy lecture theatre immediately adjacent to where our conference is held (and where we still teach parasitology). In the 20th century, the foundation of malaria genetics can be traced to the Edinburgh Protozoan Genetics unit, with its alumni (David Walliker, Richard Carter and Andy Tait) each going on to make substantial contributions as the genetic study of parasites came to the fore in the 1970s-1980s. This all took place in the crucible of Edinburgh's unique contribution to the birth of molecular biology (involving Edwin Southern, Kenneth and Noreen Murray, Paul Nurse and, as a young PhD student, the doyen of *Toxoplasma* research, John Boothroyd!).

Life cycles form an intrinsic component of any study of parasites and we're proud to reflect on the ongoing life cycle of parasitology in Edinburgh. We have continued to build on the legacies of these 19th and 20th century discoveries by increasing the diversity of the parasites we study and – importantly – the diversity of Edinburgh parasitologists. This diversity and excellence is also a key characteristic of meetings of the British Society for Parasitology, where our community from across the globe is connected through our fascination with, and study of, a lifestyle (parasitism) in all its forms, rather than any one individual 'ology'. We very much hope you will enjoy the diversity of science of the meeting and the chance to meet old friends and to make new ones. Equally, we hope you take the full opportunity to experience this amazing city. Welcome to Edinburgh!

[1].Cox, F. E. G. The Golden Age of parasitology-1875-1925: the Scottish contributions. Parasitology 144, 1567-1581, doi:10.1017/S0031182016001566 (2017).

Prof Achim Schnaufer
Prof Keith Matthews

Full Abstract online: https://bsp.uk.net/media/2023-Edinburgh-Abstracts.pdf or

Download the **BSP Eventflo App** for Interactive engagement with the event. –
Apple http://itunes.apple.com/gb/app/eventflo/id517585490?mt=8
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Cover art Dr Laura Jeacock

We are very grateful to Laura who provided us with the cover image. Laura Jeacock is a Visual Artist from Edinburgh, who has trained as a Scientist. She holds a PhD and MSc in Parasitology, and a BSc in Zoology, and has recently been reminded how much fun it is to draw parasites! You can find more of her work here: www.linktr.ee/laura.jeacock

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Presentations

Information for Oral Presentations

Oral presentations need to be provided to the AV technician on a USB stick at least 4 hours in advance, or the day before for presenters in morning sessions. AV technicians WILL NOT BE waiting in the session room to upload presentations at the start of the sessions.

Speakers are respectfully requested to keep to their time slot so that delegates who wish to move between sessions can do so.

All presentations should be as follows

30 mins – 25 mins oral with 5 mins for questions.

15 mins - 10-12 mins oral with 3-5 mins for questions

12 mins – 9-10 mins oral with 3-2 mins for questions.

Presentation Guidelines

1. All presenters should make sure their presentation is compatible with **PowerPoint for Windows (.pptx format).** All presentations should be named as per the schedule - Date/day – presentation time – Speaker name.
2. Widescreen projectors will be used in all meeting rooms. PowerPoints should be formatted in **16:9 aspect ratio.**
3. Comfort monitors in Appleton Tower venues will show the current slide, upcoming slide and notes. The comfort monitor in McEwan Hall will only show the current slide.
4. For presentations created on an Apple computer, please be aware of the following:
    a. **Videos**: QuickTime formats such as .mov are not native to Windows computers. Please convert videos to .mp4.
    b. **Animations**: Use simple entry animation effects such as: fly in/out, appear, and dissolve.
    c. **Fonts**: Use common fonts. Pre-installed and custom fonts will not translate properly on a Windows machine.
    d. **Keynote**: If creating your presentation in Keynote, please **export as .pptx and check it on a Windows machine.**

Information for Poster Presentations

Posters will be displayed in the concourse and foyers of McEwan Hall

Posters should be A0 size (841 mm wide x 1189 mm high) in portrait format, and should be no larger than 900 mm wide x 1200 mm high).

You will find Velcro coins on the board ready to attach your Poster. Please put up your poster on Wednesday before the first session and remove it on Friday morning.

There are two sessions during the conference: Wednesday from 17:00 to 19:00, Odd numbers presented and Thursday from 16:30 till 18:30, Even numbers presented. Presenters should stand by their Poster their sessions, we are flexible if they wish present both days.

Schedule In brief

Day 2 - 12-April - 2023

Plenary 1- Prof. Gloria Rudenko - (McEwan Hall) at 09:00 to 09:35

09:00 (30 mins) – Invited Speaker - A celebration of Prof. Gloria Rudenko within the saga of Antigenic Variation in Trypanosomes (Keith Gull FRS)

Plenary 2 - (McEwan Hall) at 09:45 to 10:30

09:45 (45 mins) – Invited Speaker - Hitting transmission - a quest for drugs that target malaria parasites in the mosquito (Jake Baum)

Protists 1: Development & Signalling - (McEwan Hall) at 11:00 to 12:30

11:00 (30 mins) Invited Speaker - Host-*Plasmodium* interaction: a tale of symbiosis and pathogenesis (Maria Mota)

11:30 (15 mins)- When is a slender not a slender? The irreversible arrest and scarcity of replicative parasites challenges the maintenance of trypanosome infections in the bloodstream (Stephen Larcombe)

11:45 (15 mins) - The role of cyclic nucleotide signalling in *Leishmania mexicana* flagellar motility. (Sophia Fochler)

12:00 (15 mins) - Ubiquitin controls form and function in malaria parasite transmission stages (Nila Johnson)

12:15 (3 mins) – Speed Talk -TUSK: A ubiquitin hydrolase complex modulating surface protein abundance in trypanosomes (Mark Field)

12:18 (3 mins) - Speed Talk -The *Leishmania mexicana* cell cycle\: Patterns of organelle duplication and segregation revealed by 3D electron microscopy and their implications for parasite biology (Molly Hair)

12:21 (3 mins) - Speed Talk -CEP43 – a protein with unexpected and divergent functions in the assembly and stability of the trypanosome flagellum (Aro Nugawela)

12:24 (3 mins) - Speed Talk -Environmental sensing and metabolism and growth control by trypanosome QIQ1 (Anna Trenaman)

Protists 2: Host-Parasite Interactions - Sponsored by the Royal Society - (McEwan Hall) at 13:30 to 15:00

13:30 (30 mins)- Invited Speaker - Mapping the antibody response to VSG using phage immunoprecipitation sequencing (PhIP-seq) (Monica Mugnier)

14:00 (30 mins) - Reassessing merozoite invasion processes using *Plasmodium knowlesi* (Robert Moon)

14:30 (15 mins) - Defining the spatial proteome of the apicomplexan parasite *Plasmodium falciparum* and its interaction with its host (Scott Chisholm)

14:45 (3 mins) - Speed Talk -The hematopoietic stem cell as a parasitological niche responsible for antileishmanial treatment failure (Laura Dirkx)

14:48 (3 mins) - Speed Talk -Malaria parasite development is rhythmic and is synchronised with host feeding-fasting rhythms: How? Why? Huh? (Aidan O'Donnell)

14:51 (3 mins) - Speed Talk -Structure of the PfRCR complex which bridges the malaria parasite and erythrocyte during invasion (Brendan Farrell)

14:54 (3 mins) - Speed Talk -Genetic validation of the function of PfEMP1 in *Plasmodium falciparum* rosette formation using CRISPR-Cas9 genome editing. (Stanley Otoboh)

Protists 3: Epigenetics and Gene Expression - Sponsored by Informatics Unlimited - (McEwan Hall) at 15:30 to 17:00

15:30 (30 mins)-Invited Speaker - SPARCing analyses of chromatin contexts in *Trypanosoma brucei* (Robin Allshire)

16:00 (15 mins) - Direct demonstration that histone modification impacts gene expression and DNA compaction in trypanosomes (Marketa Novotna)

16:15 (15 mins) - Multi-allelic exclusion by an allele-selective helicase in African trypanosomes (Joana Correia Faria)

16:30 (15 mins) - The mechanism of codon mediated mRNA turnover in trypanosomes (Mark Carrington)

16:45 (3 mins) -Speed Talk - mt-LAF3 is a pseudouridine synthase ortholog required for mitochondrial rRNA and mRNA gene expression in *Trypanosoma brucei* (Suzanne McDermott)

16:48 (3 mins) - Speed Talk - The ATAD2/Abo1/Yta7 homologue, Bromodomain Factor 7, is essential for macrophage infection by *Leishmania mexicana*. (Nathaniel Jones)

16:51 (3 mins) - Speed Talk -Competition among variants is predictable and controls the antigenic variation dynamics of African trypanosomes (Douglas O Escrivani)

16:54 (3 mins) - Speed Talk -*Leishmania (Viannia) braziliensis* long non-coding RNAs are enrolled in parasite fitness and interact with proteins in a structure-dependent manner. (Caroline Ricce Espada)

BES Ecology 1: Within-host Interactions - Sponsored by Quadratech Diagnostics - (Appleton Tower 1) at 11:00 to 12:30

11:00 (30 mins) – Invited Speaker - Drivers and fitness consequences of gut community dynamics in wild Soay sheep (Amy R Sweeny)

11:30 (15 mins) - *Galba truncatula* and Helminths, the Importance of Microbes (Peter McCann)

11:45 (15 mins) - Molecular typing of *Giardia duodenalis* detected in UK cats and dogs using an improved marker (Rossella Panarese)

12:00 (15 mins) - Hybridization between human and livestock schistosomes – Ancient or ongoing? (Roy Platt)

12:15 (15 mins) - A novel *Cryptosporidium hominis* outbreak during British military training in Kenya (Romeo Toriro)

BES Ecology 2: Quantitative Methods in Parasitology - (Appleton Tower 1) at 13:30 to 15:00

13:30 (30 mins) – Invited Speaker - Causal Analysis of the Relationship Between Helminth Infections and Vaccine Responsiveness in a Wild Rodent Model (Simon Babayan)

14:00 (15 mins) - Alternative roles of heterogeneities in driving transmission dynamics: analysis of contrasting compartmental models (Jacob Cohen)

14:15 (15 mins) - Using mathematical models to understand schistosomiasis transmission in a Ugandan hotspot (Gregory Milne)

14:30 (15 mins) - *Strongyloides stercoralis* in the United Kingdom: A systematic review and meta-analysis of published cases (Cansu Ozdemir)

14:45 (15 mins) - Is *Strongyloides stercoralis* in people a zoonosis from dogs? (Yuchen Liu)

BES Ecology 3: Wild Immunology - (Appleton Tower 1) at 15:30 to 17:00

15:30 (30 mins) – Invited Speaker - The adaptive immune response to *Trichuris* in wild versus laboratory mice: An established model system in context (Iris Mair)

16:00 (15 mins) - Longitudinal trends of T-helper cell immune phenotypes in the Soay sheep from St Kilda: can we predict parasite burden? (Yolanda Corripio-Miyar)

16:15 (15 mins) - The impact of host-pathogen coevolution on *Trypanosoma* infection rates within wild *Glossina morsitans morsitans*. (Calam Bruce)

16:30 (15 mins) - Hybridization in UroGenital Schistosomiasis (HUGS): A novel real-time PCR assay, with high resolution melt profiling, useful for the detection of hybrid schistosomes in Malawi (Lucas Cunningham)

16:45 (15 mins) - Exploiting the mosquitocidal properties of nitisinone as a novel strategy for malaria control (Alvaro Acosta Serrano)

Immunoparasitology 1: Immune Response to Unicellular Parasites - (Appleton Tower 2) at 11:00 to 12:30

11:00 (30 mins) – Invited Speaker -To the skin and beyond: the impact of the immune system on African trypanosome infections (Neil Mabbott)

11:30 (15 mins) - IL-27 in African trypanosomes: a double-edged sword in parasite control. (Mathieu Claes)

11:45 (15 mins) - The local immune response to *Trypanosoma brucei* in the tissues of the abdomen. (Chloe Barnes)

12:00 (15 mins) - Transcriptomics of the immune response in Chagas heart disease (Damian Perez Mazliah)

12:15 (15 mins) -What are the drivers of enteric neuropathy in experimental Chagas disease? (Harry Langston)

Immunoparasitology 2: Immune-Helminth Interactions - (Appleton Tower 2) at 13:30 to 15:00

13:30 (30 mins) – Invited Speaker - Macrophages & B cells - partners in nematode immunity (Judith Allen)

14:00 (15 mins) - Characterising the ovine small intestinal tuft cell response following parasitic nematode infection. (Katie Hildersley)

14:15 (15 mins) - A family of helminth-derived TGF-β mimics provide key insights to Treg and innate immune cell activation. (Kyle Cunningham)

14:30 (15 mins) - Rapid induction of clinical tolerance in a placebo-controlled clinical trial investigating repeated controlled exposure to *Schistosoma mansoni* (Jan Pieter Koopman)

14:45 (3 mins) - Speed Talk -The interaction of *Schistosoma mansoni* infection with diabetes mellitus and obesity in mice (Alaa Saed Anwer Amer)

14:48 (3 mins) - Speed Talk -Structural basis for IL-33 recognition and its antagonism by the hookworm effector HpARI (Abhishek Jamwal)

Drugs, Vaccines and Diagnostics - (Appleton Tower 2) at 15:30 to 17:00

15:30 (30 mins) – Invited Speaker - Can RH5-based vaccines succeed against blood-stage *P. falciparum*? (Simon Draper)

16:00 (15 mins) - Benznidazole uptake by *Trypanosoma cruzi* is a determinant of variable drug efficacy and treatment failure (John Kelly)

16:15 (15 mins) - Development of a SHERLOCK molecular diagnostic toolbox for the detection of trypanosomatid parasites (Elena Perez Anton)

16:30 (15 mins) - Drug tolerance and quiescence in *Leishmania* (Jean-Claude Dujardin)

16:45 (3 mins) - Speed Talk -Deep mutational resistance profiling of an anti-trypanosomal proteasome inhibitor (Simone Altmann)

16:48 (3 mins) - Speed Talk -Safety and preliminary protective efficacy of immunisation with genetically attenuated Pf mei2 (GA2) malaria parasites in healthy Dutch volunteers (Olivia Lamers)

16:51 (3 mins) - Speed Talk -Preclinical evaluation of a novel nucleoside analogue for the treatment of animal trypanosomiasis (Kayhan Ilbeigi)

16:54 (3 mins) - Speed Talk -Reticulocyte Binding-like Proteins as potential vaccine targets for *Plasmodium knowlesi* and *Plasmodium vivax* (Sophia DonVito)

Day 3 - 13-April - 2023

Early Career Researchers Workshop - Sponsored by Cambridge University Press - (McEwan Hall) at 09:00 to 10:30

Plenary 3 - (McEwan Hall) at 11:00 to 11:45

11:00 (45 mins) – Invited Speaker - Hookworm dynamically respond to Type 2 immune pressure (De'Broski Herbert)

Plenary 4 - (McEwan Hall) at 11:45 to 12:30

11:45 (45 mins) – Invited Speaker - Anthelmintic treatment dampens immunosenescence in a wild mammal with consequences for survival (Vanessa Ezenwa)

Equality & Diversity - (McEwan Hall) at 13:30 to 14:30

13:30 (12 mins) - Invited Speaker - Race and Gender in Science (Annamaria Carusi)

13:42 (12 mins) - Invited Speaker – Equality, Diversity and Inclusion at UKRI: research and innovation by everyone, for everyone (Jo O'Leary)

13:54 (12 mins) - Invited Speaker – Panel Discussion with Karen Halliday, Jo O'leary and Annamaria Carusi. (Karen Halliday)

BSP Presidents Medal - (McEwan Hall) at 15:00 to 15:30

15:00 (30 mins) – Medalist - Biomonitoring and long-term studies on sylvatic rodents in Poland – the good, the bad and the effort (Maciej Grzybek)

BSP Wright Medal - (McEwan Hall) at 15:30 to 16:30

15:30 (60 mins) – Medalist - Supporting NTD drug discovery through comprehensive mechanism of action studies and Tales from the world's longest postdoc! (Susan Wyllie)

Day 4 - 14-April - 2023

Protists 4: Metabolism and Physiology - (McEwan Hall) at 09:30 to 11:00

09:30 (30 mins) – Invited Speaker - Mitochondrial reactive oxygen species control cellular differentiation of *Trypanosoma* parasites (Alena Zikova)

10:00 (12 mins) - The role of MICOS in mitochondrial maturation during *Trypanosoma brucei* differentiation (Corinna Benz)

10:12 (12 mins) - Mitochondrial DNA dynamics in trypanosomatid parasites: a story of loss and gain (Zihao Chen)

10:24 (12 mins) - The role of unique *Leishmania* respiratory enzymes in mice infections (Margarida Duarte)

10:36 (12 mins) - Dissecting fatty acid metabolism in the livestock parasite *Trypanosoma congolense* (Pieter Steketee)

10:48 (12 mins) - Kinetoplastid pantothenate kinase is a unique and essential multi-functional enzyme (Martin Taylor)

Protists 5: Genomics and Evolution - (McEwan Hall) at 11:30 to 13:00

11:30 (30 mins) – Invited Speaker - Darwin in a dish: Experimental Evolution reveals novel mechanisms of *Leishmania* fitness gain (Gérald Spaeth)

12:00 (12 mins) - Designing genome-scale strategies for knockout life cycle fitness phenotyping in LeishGEM (Ulrich Dobramysl)

12:12 (12 mins) - A central role for the amino acid transporter (AAT1) in Chloroquine resistance evolution in *Plasmodium falciparum* (Timothy Anderson)

12:24 (12 mins) - Emerging parasite resistance in Africa - are we about to see a resurgence in *falciparum* malaria across the continent? (Colin Sutherland)

12:36 (12 mins) - Comprehensive investigation of the *Trypanosoma brucei* kinetoplast and the discovery of a slew of new protein constituents. (Michael Hammond)

12:48 (12 mins) - To Per-Cyst or Not: unravelling the secrets behind an attenuated *Toxoplasma* strain (Saniya Crouch)

BES Ecology 4: Wild Parasitology: Impacts of Infection on Health and Fitness -Sponsor -Xpedite Diagnostics GmbH (Appleton Tower 1) at 09:30 to 11:00

09:30 (30 mins) – Invited Speaker - The impact of parasitism within the family (Emma Cunningham)

10:00 (15 mins) - Resource quality and distribution impacts vectors and vector-borne infections in wild wood mice (Agata Delnicka)

10:15 (15 mins) - The potential mechanistic pathways leading from parasite infection to childhood stunting. (Isobel Gabain)

10:30 (15 mins) - Giardiasis and intestinal pathology: Molecular detection and taxon assemblage typing of *Giardia duodenalis* in school-aged children along the shoreline of Lake Malawi, Malawi (John Archer)

10:45 (15 mins) - A droplet digital PCR (ddPCR) workflow for the detection of helminth and snail host eDNA in water and soil (Christopher McFarland)

BES Ecology 5: Host-Parasite Interactions - Sponsored by Bio Molecular Systems Ltd - (Appleton Tower 1) at 11:30 to 13:00

11:30 (30 mins) – Invited Speaker - The transmission modifying effects of parasite coinfections: insights from wild mice (Andy Fenton)

12:00 (15 mins) - The epidemiology of periportal fibrosis (Seun Anjorin)

12:15 (15 mins) - Prevalence of *Trichomonas vaginalis* and associated risk factors among pregnant women attending antenatal care in government health facilities, Ambo town, Western Oromia in Ethiopia. (Chala Kumsa)

12:30 (15 mins) - Associations of water contact with schistosome infection: A systematic review and meta-analysis (Fabian Reitzug)

12:45 (15 mins) - Effect of co-habitation on gastrointestinal parasite prevalence and burden in wild and domestic herbivores in Maasai Mara National Reserve, Kenya (Kim van de Wiel)

Veterinary Parasitology - (Appleton Tower 2) at 09:30 to 11:00

09:30 (30 mins) – Invited Speaker - Making roundworm data ewe-niversal for all (Fiona Kenyon)

10:00 (15 mins) - Integrating multi-species swards into parasite management in sheep under climate change (Nicole Henry)

10:15 (15 mins) - RNA interference: a functional tool for screening potential vaccine targets in the poultry red mite *Dermanyssus gallinae* (Wan Chen)

10:30 (15 mins) - Co-culture with HepG2 spheroids spurs *in vitro* growth and development of the infective stages of the helminth pathogen *Fasciola hepatica* (Nichola Calvani)

10:45 (15 mins) - Exploration of the sensitivity to macrocyclic lactones in the canine heartworm (*Dirofilaria immitis*) in Australia using phenotypic and genotypic approaches (Rosemonde Power)

Vectors and Transmission - Sponsored by Current Research in Parasitology and Vector-Borne Diseases - (Appleton Tower 2) at 11:30 to 13:00

11:30 (30 mins) – Invited Speaker - Evolution of Insecticide resistance and efficacy of malaria control in Africa (Charles Wondji)

12:00 (15 mins) - From spillover to persistence: hybridization and schistosomiasis transmission dynamics at the human-animal interface (Anna Borlase)

12:15 (15 mins) - Functional dissection of the *Leishmania* - sand fly attachment interface (Ryuji Yanase)

12:30 (15 mins) - A decade of Trypanosomiasis research in Malawi: is the battle lost or won? (Janelisa Musaya**)**

12:45 (15 mins) - Tsetse transmitted trypanosomes: from the skin to a systemic infection (Dorien Mabille)

Invited & Speaker Abstracts

Day 2 - 12-April - 2023

Plenary - Prof. Gloria Rudenko - (McEwan Hall)

09:00 (30 mins)

A celebration of Prof. Gloria Rudenko within the saga of Antigenic Variation in Trypanosomes

Presenter: **Prof Keith Gull FRS**, *Sir William Dunn School of Pathology*

A celebration of the life and works of Gloria Rudenko.

Plenary 2 - (McEwan Hall)

09:45 (45 mins)

Hitting transmission - a quest for drugs that target malaria parasites in the mosquito

Presenter: **Prof Jake Baum**, *Professor Infectious Diseases, UNSW*

J Baum[1];

[1] UNSW, Australia

Malaria was estimated to cause more than 240 million cases worldwide in 2020. This translated into 640,000 deaths, primarily among children under the age of 5, with 80% of these deaths in sub-Saharan Africa. The WHO has set itself the goal of a 90% reduction of malaria incidence and mortality by the end of this decade. Whilst RTS,S/AS01, the malaria vaccine developed by GSK and recommended by WHO, does provide partial protection, as a singular tool it won't be enough alone. Bending the curve to get malaria rates back on a path towards control and eventual eradication will require new innovations, in particular new strategies in diagnostics, treatments and a long-lived efficacious vaccine. In this talk Professor Jake Baum will discuss one central project from his lab that has sought to find drugs that target the parasite in the mosquito - transmission blocking drugs. From developing a screen using high throughput imaging to medicinal chemistry of compounds found and identification of their targets, this talk will trace the drug discovery journey to its most recent end-point, presenting some potentially very new ways of thinking about antimalarial drug design, development and delivery.

Protists 1: Development & Signalling - (McEwan Hall)

11:00 (30 mins)

Host-*Plasmodium* interaction: a tale of symbiosis and pathogenesis

Presenter: **Prof Maria Mota**, *IMM Lisborn*

11:30 (15 mins)

When is a slender not a slender? The irreversible arrest and scarcity of replicative parasites challenges the maintenance of trypanosome infections in the bloodstream

Presenter: **Dr Stephen Larcombe**, *Postdoctoral Researcher , University of Edinburgh*

***S Larcombe**[1]; E Briggs[1]; B Szoor[1]; K Matthews[1];*

[1] University of Edinburgh, UK

The development of *Trypanosoma brucei* in its mammalian host is marked by a distinct morphological change as replicative "slender" for differentiate into cell-cycle arrested "stumpy" for in a quorum-sensing dependent manner. Although stumpy for dominate chronic infections at the population level, the proportion of replicative parasites at the individual cell level and the irreversibility of arrest in the bloodstream is unclear. Here, we use an *ex vivo* assay and a developmentally-deficient mutant to demonstrate that developmental cell cycle arrest is definitively irreversible in both acute and chronic infection stages in mice. Further, an analysis of replicative capacity and the transcriptome profile at the single cell level demonstrates a temporal hierarchy exists whereby cell cycle arrest and transcriptomic adaption to stumpy development precedes irreversible commitment and morphological change. Unexpectedly, we show that once trypanosome infections are established, proliferative parasites are exceptionally scarce. This challenges the ability of trypanosomes in the circulatory bloodstream to sustain the infection by proliferation or antigenic variation, these parasites instead being overwhelmingly adapted for transmission.

11:45 (15 mins)

The role of cyclic nucleotide signalling in *Leishmania mexicana* flagellar motility.

Presenter: **Sophia Fochler**, *University of Bern*

S Fochler[1]*; R Wheeler*[2]*; E Gluenz*[3]*;*

[1] University of Bern, Switzerland; [2] Peter Medawar Building for Pathogen Research, Nuffield Department of Medicine, University of Oxford, UK; [3] Institute of Cell Biology, University of Bern, Switzerland

The second messenger cyclic AMP (cAMP) has been linked to modulation of flagellar movement in diverse eukaryotic species including *Trypanosoma* and *Leishmania* but the specific transduction pathways from second messenger to dynein driven force remains in most cases unclear. Trypanosomatids are recognised to have an unconventional cAMP signalling pathway, lacking canonical downstream effectors of cAMP and harbouring protein kinase A (PKA) complexes that are insensitive to cAMP. We aimed here to elucidate how the cAMP signalling pathway impacts on flagellar motility in *L. mexicana*. We compiled all proteins predicted to act in cAMP signalling pathways from analysis of the *L. mexicana* genome sequence, including known cAMP producers (adenylyl cyclases, ACs) and degraders (phosphodiesterases, PDEs) and proteins with predicted cyclic nucleotide binding domains. We then tagged these with mNeonGreen (mNG) to determine their subcellular localisation and generated knockout mutants to study the effect on motility. These screens identified over two thirds (24) of our proteins localised to flagellar domains. KO of tip-localised AC (RAC-a) swam slower, KO of PDEs in the distal flagellum swam faster, suggesting cAMP in the distal flagellum modulates swim speed. Our population motility screen found that the deletion mutant of the cyclic AMP response protein 1 (*dCARP1*) had the slowest swim speed. High speed video microscopy and Fourier analysis of the tip-to-base flagellar beat revealed that *dCARP1* flagella were unable to reach frequencies over 30 Hz and the *dCARP1* population showed an increased prevalence of cell turning via the asymmetric base-to-tip ciliary beat. A higher propensity for ciliary beats was also observed upon deletion of PDE B1 or treatment with high concentrations of the PDE inhibitor CpdB. mNG::LmxCARP1 localised to the detergent insoluble fraction of the flagellar axoneme and conservation of key residues in its CNBD suggests it binds cAMP. Our data identifies CARP1 as a cAMP-binding protein that may directly or indirectly modulate the activity of axonemal dyneins, which ultimately dictate swimming behaviour. Together, these data indicate that cAMP signalling modulates beat frequency and cell swim speed and contributes to the balance between flagellar beating, and ciliary beating.

12:00 (15 mins)

Ubiquitin controls form and function in malaria parasite transmission stages

Presenter: **Nila Johnson**, *PhD Student, University of Edinburgh*

N Johnson[1]*; EJ Marr*[1]*; B Orosa-Puente*[2]*; D Kwecka*[1]*; A von Kriegsheim*[3]*; S Spoel*[2]*; S Mitchell*[4]*; N Philip*[1]*;*

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During transmission from the vertebrate host to mosquito vector malaria parasites must adapt to a rapid change in environment when encountering the hostile mosquito gut. To establish a successful mosquito infection, the parasite develops into a specialised motile ookinete form capable of invading the mosquito midgut. Ookinete formation is a strictly regulated developmental process orchestrated by dynamic signalling networks. In eukaryotes, signalling networks are often regulated by post-translational modifications (PTMs) that can significantly diversify protein function. Ubiquitylation is a reversible PTM utilised by eukaryotes to regulate protein activity, localisation and stability. While ubiquitylation has been explored in *Plasmodium* erythrocytic stages, the role of this crucial PTM in malaria transmission is poorly understood. Leveraging *Plasmodium berghei* to investigate early host-vector transition stages of the parasite, we identified ~1400 ubiquitin associated proteins, and >600 ubiquitylation sites in 240 unique proteins. We discovered several E3 ligases (writers of ubiquitin) in the ubiquitylome interaction networks indicating a vital role during parasite transmission. A systematic genetic and molecular analysis revealed five E3 ligases are essential for *Plasmodium* transmission. In particular two E3 ligases (RING-E3, U-Box-E3) regulated ookinete development where removal of the E3 ligases revealed crucial roles in maintaining cell morphology and motility. The E3-deficient parasites exhibit a severely reduced or complete inability to establish a mosquito infection. Currently we are investigating the spatial and temporal expression of the E3 enzymes and how they regulate the ookinete's cytoskeleton. Our results demonstrate prevalent ubiquitylation in the parasite proteome during transmission, uncovering essential roles for E3 ligases that could inform new transmission blocking strategies.

12:15 *(3 mins) Speed Talk*

TUSK: A ubiquitin hydrolase complex modulating surface protein abundance in trypanosomes

Presenter: **Prof Mark Field**, *Professor, University of Dundee*

12:18 *(3 mins) Speed Talk*

The *Leishmania mexicana* cell cycle: Patterns of organelle duplication and segregation revealed by 3D electron microscopy and their implications for parasite biology

Presenter: **Molly Hair**, *Oxford Brookes University*

12:21 *(3 mins) Speed Talk*

CEP43 – a protein with unexpected and divergent functions in the assembly and stability of the trypanosome flagellum

Presenter: **Aro Nugawela**, *Lancaster University*

12:24 *(3 mins) Speed Talk*

Environmental sensing and metabolism and growth control by trypanosome QIQ1

Presenter: **Dr Anna Trenaman**, *University of Dundee*

Protists 2: Host-Parasite Interactions - Sponsored by the Royal Society - (McEwan Hall)

13:30 (30 mins)

Mapping the antibody response to VSG using phage immunoprecipitation sequencing (PhIP-seq)

Presenter: **Monica Mugnier**, *John Hopkins*

M Mugnier[1];

[1] John Hopkins, United States

Trypanosoma brucei, the causative agent of human and animal African trypanosomiasis, lives an entirely extracellular life cycle in its mammalian host. Despite constant exposure to host antibody, this parasite manages to maintain chronic infections that can last for years. *T. brucei* manages to sustain such long infection through antigenic variation of its dense variant surface glycoprotein (VSG) coat. Using an enormous repertoire of VSG-encoding genes, the parasite continually "switches" expression to new VSGs, escaping recognition by host antibody. Work from our lab using high-throughput sequencing to characterize the repertoire of VSGs expressed during chronic infections in humans has revealed that the VSG repertoire is quickly evolving, and parts of the VSG protein that are suspected to be targeted by host antibody appear most likely to mutate. Thus, the host antibody response is critical for shaping this parasite's antigenic repertoire. Despite its importance, the principles governing antibody recognition of VSG remain poorly understood. Here, we present the development of VSG Phage Immunoprecipitation Sequencing (PhIP-seq), an approach for high-throughput epitope mapping of VSGs. Using a phage display library containing peptides from 13,171 VSGs, we are able to track the specificity and dynamics of the antibody response to VSG in high resolution, in both experimental and natural infections. In addition to informing the design of serodiagnostics for trypanosomiasis, this experimental system is likely to reveal fundamental insight into the co-evolution of the host-pathogen interface over time.

14:00 (30 mins)

Reassessing merozoite invasion processes using *Plasmodium knowlesi*

Presenter: **Dr Robert Moon**, *London School of Hygiene and Tropical Medicine*

***R Moon**[1];*

[1] London School of Hygiene and Tropical Medicine, UK

The adaptation of *P. knowlesi (Pk)* to culture in human erythrocytes has provided exciting opportunities to investigate erythrocyte invasion biology. Two major protein families, the erythrocyte binding-like proteins (EBPs/EBAs) and the reticulocyte binding-like proteins (RBLs/RHs) have been studied extensively in *P. falciparum (Pf)* and are hypothesized to have overlapping, but critical roles during the invasion process. *Pk* has a smaller repertoire, including one EBL(DBPa) and one RBL(NBPXa) that are both essential for invasion of human erythrocytes. By taking advantage of the unique biological features of *Pk*, which have merozoites double the size of *Pf*, we have used live microscopic analysis to develop a deeper understanding of the distinct stages of invasion, as well as the roles of the *Pk* EBP/RBL families within this process. Using a conditional DiCre approach we have demonstrated distinct roles for the two families at different stages in invasion. We also uncovered new features that caused us to reassess invasion including that *Pk* merozoites are able to undergo productive gliding motility prior to invasion and merozoite topology was the opposite to how we expected! These findings have revealed new features of this complex process, as well as new tools and techniques to deepen our understanding of this process in all malaria parasite species.

14:30 (15 mins)

Defining the spatial proteome of the apicomplexan parasite *Plasmodium falciparum* and its interaction with its host

Presenter: **Dr Scott Chisholm**, *Research Associate, University of Cambridge*

S Chisholm[1]*; K Barylyuk*[1]*; A Kemp*[1]*; M Paoletta*[2]*; E Bushell*[2]*; K Lilley*[1]*; JC Rayner*[1]*; RF Waller*[1]*;*

[1] *University of Cambridge, UK;* [2] *Umeå University, Sweden*

Malaria is caused by apicomplexan parasites of the genus *Plasmodium*. Our understanding of the biology of the malaria parasite is hampered by the large proportion of the cell's proteome that is of unknown cellular location or function. Apicomplexans, as for all of Myzozoa, have developed new cell compartments and structures along with great proteomic novelty, and this hinders interpretation of these cells from classical organism models. To address this problem, we are generating comprehensive high-resolution maps of protein subcellular localisation for the blood stages of the life cycle of *Plasmodium falciparum*. To achieve this, we have used the spatial proteomics technique hyperplexed Localisation of Organelle Proteins by Isotopic Tagging (hyperLOPIT) and generated data for 3916 *P. falciparum* proteins in the schizont stage and 3508 *P. falciparum* proteins in the trophozoite stage. We have also recently generated data on extracellular merozoites. With these data, we have performed high-resolution mapping of the subcellular localisations of these proteins. A curated list of marker proteins with experimentally validated localisation reveals that our hyperLOPIT maps clearly resolve numerous compartmental proteomes across the asexual developmental stages, including some sub-compartmental resolution. In addition to resolving the structures within the parasite, these maps also resolve parasite-derived structures exported into the host erythrocyte and parasite proteins that target to the host's plasma membrane. Furthermore, we find evidence of proteins from the host cell that map to compartments within the parasite, providing further insight into the interactions between parasite and host. Using supervised machine-learning classification methods we have attributed proteins of unknown localisation to the known cellular niches, providing localisation assignments to approximately 60% of the proteins analysed in our datasets. We are currently experimentally validating the results of these analyses through epitope tagging of a number of assignments of uncharacterised proteins. At least a half of the proteins in our data are uncharacterised or hypothetical proteins, and information about the localisation of these proteins across different developmental stages offers a major advance in our understanding of the organisation and biology of the malaria parasite.

14:45 *(3 mins) Speed Talk*

The hematopoietic stem cell as a parasitological niche responsible for antileishmanial treatment failure

Presenter: **Laura Dirkx**, *PhD student, University of Antwerp*

14:48 *(3 mins) Speed Talk*

Malaria parasite development is rhythmic and is synchronised with host feeding-fasting rhythms: How? Why? Huh?

Presenter: **Aidan O'Donnell**, *University of Edinburgh*

14:51 *(3 mins) Speed Talk*

Structure of the PfRCR complex which bridges the malaria parasite and erythrocyte during invasion

Presenter: **Dr Brendan Farrell**, *Postdoctoral Research Associate, University of Oxford*

14:54 *(3 mins) Speed Talk*

Genetic validation of the function of PfEMP1 in *Plasmodium falciparum* rosette formation using CRISPR-Cas9 genome editing

Presenter: **Stanley Otoboh**, *PhD researcher, University of Edinburgh*

Protists 3: Epigenetics and Gene Expression - Sponsored by Informatics Unlimited - (McEwan Hall)

15:30 (30 mins)

SPARCing analyses of chromatin contexts in *Trypanosoma brucei*

Presenter: **Prof Robin Allshire**, *, Wellcome Centre for Cell Biology, University of Edinburgh*

R Allshire[2]*; R Carloni*[2]*; A Valera*[2]*; T Devlin*[2]*; DP Staneva*[2]*; KR Matthews*[1]*;*

[1] *Institute of Immunology & Infection Research, University of Edinburgh, UK;* [2] *Wellcome Centre for Cell Biology, University of Edinburgh, UK*

Kinetoplastids such as *Trypanosoma brucei* possess unusual mechanisms for regulating gene expression relative to other eukaryotes. Fungi (e.g. yeasts), flies and mammals all exhibit specialized chromatin on repetitive elements at centromeres (i.e. heterochromatin), adjacent to telomeres and at promoters. In general, analysis of writers, readers, and erasers of histone post-translational modifications in kinetoplastids is at a relatively early stage. Moreover, little is known about the nature of chromatin that is assembled on repetitive elements in *T. brucei* or other kinetoplastids. We previously surveyed 65 putative chromatin-associated factors in *Trypanosoma brucei* [1]. Our analyses revealed that the

predicted histone methyltransferase SET27 and the Chromodomain protein CRD1 are tightly concentrated at RNAPII transcription start regions (TSRs). We have found that SET27 and CRD1, together with four previously uncharacterized constituents (CSD1, PHD6, PWWP1, PBP1) form what we refer to as the SET27 promoter-associated regulatory complex (SPARC), which is specifically enriched at TSRs [2]. SET27 loss leads to aberrant RNAPII recruitment to promoter sites, accumulation of polyadenylated transcripts upstream of normal transcription start sites, and conversion of some normally unidirectional promoters to bidirectional promoters. Our analyses uncover a novel chromatin-associated complex required to establish accurate promoter position and directionality. We are currently undertaking detailed analyses of SPARC components to determine their activities and, ultimately, the overall structure of this complex. To probe the composition of chromatin that is associated with repetitive elements at various locations in the *T. brucei* genome we have expressed synthetic nuclear TALE-YFP proteins designed to bind terminal telomere (TTAGGG)n repeats (Tel-TALE) or other distinct types of repetitive sequences. Proteomic analyses of Tel-TALE protein affinity selected from bloodstream form cell extracts provides proof-of-principle in that many known telomere-associated proteins are identified as being significantly enriched. Thus, locus-specific proteomics provides a potentially useful tool for investigating specific chromatin contexts in trypanosomes. Ongoing analyses of synthetic TALE-YFP proteins designed to bind centromeric CIR147 repeats, VSG gene expression site 70 bp repeats, and small chromosome associated 177bp repeats will be discussed.

1. Staneva DP, Carloni R, Auchynnikava T, Tong P, Rappsilber J, Jeyaprakash AA, Matthews KR, Allshire RC. (2021) A systematic analysis of *Trypanosoma brucei* chromatin factors identifies novel protein interaction networks associated with sites of transcription initiation and termination. Genome Research 31:2138-2154. 2. Staneva DP, Bresson S, Auchynnikava T, Spanos C, Rappsilber J, Jeyaprakash AA, Tollervey D, Matthews KR, Allshire RC. (2022) The SPARC complex defines RNAPII promoters i

16:00 (15 mins)

Direct demonstration that histone modification impacts gene expression and DNA compaction in trypanosomes

Presenter: **Marketa Novotna**, *University of Dundee*

M Novotna[1]*; M Tinti*[1]*; D Horn*[1]*;*

[1] *University of Dundee, UK*

It remains unclear to what extent, and by which mechanisms, transcription, DNA replication and DNA repair rely upon chromatin-based controls in trypanosomatids. N-terminal histone tails, and tail modifications, such as lysine acetylation, play key roles in these processes in other eukaryotes.

However, trypanosomatid histone N-terminal tails are highly divergent relative to the usual model eukaryotes, suggesting potential novel mechanisms. Notably, interpretation of 'writer', 'reader' and 'eraser'-defective phenotypes is complicated by potential perturbation of diverse or non-histone substrates. Genetic manipulation and subsequent study of histone functions have also proven particularly challenging because core histone genes are typically present in polycistronic transcription units of many identical copies of each gene (there are approx. 40 copies of H4 histone gene, for example).

We used an inducible CRISPR/Cas9 system in *Trypanosoma brucei* to delete all native copies of the histone H4 genes, as confirmed by genome sequencing, complementing the defect with a single, recoded and highly expressed ectopic copy. Further templated editing was then used for site saturation mutagenesis of lysine residues (K4, K10 and K14) in the N-terminal tail of the ectopic H4 gene in these 'histH4one' strains. Multiplex amplicon-seq profiling was used to monitor relative fitness, revealing those tolerated H4-K4 or H4-K14 mutations; H4-K10 mutations were not tolerated. Remarkably, viability was maintained even when H4-K4 or H4-K14 residues were removed. Using these outputs, a panel of strains exclusively expressing novel histone H4 mutants, including arginine (R; non-acetylated mimic) or glutamine (Q; constitutively acetylated mimic) substitutions, was phenotypically profiled; using proteomic, microscopy, growth, protein blotting, flow cytometry and DNA-damage sensitivity assays. The results suggest H4K4 facilitates DNA compaction. We also observed a specific defect in Variant Surface Glycoprotein gene silencing and the DNA damage response in H4-K4Q mutants – the first direct evidence that histone tails and their modification impact these processes in trypanosomes.

16:15 (15 mins)

Multi-allelic exclusion by an allele-selective helicase in African trypanosomes

Presenter: **Dr Joana Correia Faria**, *University of York*

J Correia Faria[1]*; M Tinti*[2]*; CA Marques*[2]*; D Horn*[2]*;* [1] *University of York, UK;* [2] *Wellcome Trust Centre for Anti-Infectives Research, School of Life Sciences, University of Dundee, UK*

Large gene-families often exhibit monogenic expression, with contingent processes including antigenic variation for immune evasion in parasites, and olfaction and B-cell development in mammals. Despite intense study, the mechanisms that underpin these paradigmatic examples of stochastic gene choice and exclusion remain somewhat mysterious.

Substantial recent progress yielded Variant-Surface-Glycoprotein (VSG) exclusion factors in African trypanosomes and a new appreciation of the context in which they operate. Specifically, VSG-exclusion-2 (VEX2) accumulates at the active-*VSG* expression-site and binds VEX1 at a *trans*-splicing locus on another chromosome; other *VSGs* are excluded from this sub-nuclear expression-factory (PMIDs: 31289266; 33432154).

We performed single cell RNA-Seq following VEX1 or VEX2 depletion, revealing a surprisingly complex mixture of simultaneously active *VSGs* in single cells, and a striking difference between both factors. Further, this analysis showed: 1) the number of simultaneously active VSGs that can be tolerated; 2) a hierarchy of *VSG* transcriptional derepression.

ChIP-Seq indicated strong enrichment of VEX2, which for a native complex of ~1 MDa, at the active-*VSG* expression-site, particularly accumulating at the expression-site associated genes (ESAGs) coding regions. Using super-resolution microscopy, VEX2 N- and C-termini were distinctively visualised extending towards the active-*VSG* and the splicing locus, respectively, revealing an allele-selective inter-chromosomal bridge, via VEX1, to a *trans*-splicing locus on another chromosome.

Through a combination of super resolution microscopy and native gels, we found that most of VEX1 and VEX2 sub-nuclear pools are not in complex with one another, and their interaction is dynamic and cell-cycle regulated.

To further dissect VEX interactions, we generated several VEX1 truncated forms, and found that the VEX1 N-terminal fragment (1-289 aa) interacts with VEX2, stabilising it and sustaining exclusion. Additionally, we found that the VEX1 C-terminal fragment, which includes nucleic acid binding domains, also contains regions involved in protein stability and turnover.

Finally, VEX2 is a putative DNA:RNA helicase, thus to assess whether this activity was required for *VSG*-exclusion, we established a CRISPR/Cas9-mediated saturation-mutagenesis assay using FACS followed by amplicon-Seq profiling. Replacement of a critical amino acid with any other amino acid disrupted allelic exclusion.

This work begins to reveal the mechanisms by which the VEX complex promotes stochastic *VSG* gene choice and allelic exclusion in African trypanosomes.

16:30 (15 mins)

The mechanism of codon mediated mRNA turnover in trypanosomes

Presenter: **Prof Mark Carrington**, *PI, University of Cambridge*

*A Klein[1]; M Zoltner[2]; S Hester[3]; MC Field[4]; **M Carrington**[5];*

[1] University of Cambridge, Department of Biochemistry, UK; [2] BIOCEV, Department of Parasitology, Charles University, Czech Republic; [3] Nuffield Department of Medicine, University of Oxford, UK; [4] School of Life Sciences University of Dundee, UK; [5] University of Cambridge, UK

Gene expression in trypanosomes is regulated by post-transcriptional modulation of mRNA levels. The dominant mechanism that sets individual mRNA levels is codon use and a codon metric predicts the level of most mRNAs with considerable accuracy. Codon use determines the default half-life of an mRNA and it is likely that the extensive cohort of RNA binding proteins function by altering the levels of mRNAs and/or their rates of translation in response to stimuli including stresses, differentiation triggers, cell cycle transitions. The link between codon use and mRNA half-life is translation dependent and blocking the translation of an mRNA can increase its level more than 10-fold. How do variations in codon use lead to differences in mRNA stability? The major determinant is the speed of translation (codons translated/s) rather than the frequency of translation (initiations/s) and this is probably determined by the cognate tRNA availability.

Once a decision is made to degrade an mRNA then a series of events occurs starting with 3' to 5' exonucleolytic removal of the polyA tail. The next step is removal of the 5' cap followed by 5' to 3' exonucleolytic digestion of the mRNA. The key enzymes have been identified in trypanosomes: the 3' polyA tail is removed by CAF1, a component of the NOT complex; the cap is removed by ALPH1; and the mRNA is degraded 5' to 3' by XRNA. Throughout this process RNA binding proteins such as polyA binding protein, cap binding protein, ribosomes, and other hangers on, have to be displaced to allow the nucleases access.

We would like to understand the molecular mechanism of the decision to degrade an individual mRNA based on codon use. The approach described here is a screen to identify proteins associated with the enzymes of mRNA degradation followed by phenotype analysis after depletion. First, protein complexes and interactions were identified by pulldowns using five different proteins. Second, additional loosely associated proteins were identified using proximity biotinylation by TurboID tagging of eight proteins. This screen was successful and amongst

other findings it identified: (i) novel components of the NOT complex, (ii) a linear set of interactions: NOT complex->DHH1->SCD6->ALPH1->XRNA that is similar to the order of the steps in mRNA turnover, (iii) four further RNA helicases closely associated with mRNA turnover activities, and (iv) an association of the cap binding protein eIF4E1 and 4E-IP with most of the components in the mRNA turnover pathway. We have begun the analysis of selected components by determining phenotype after depletion. To do this, we have developed an effective degron system with degradation of tagged proteins triggered by the addition of 5-PhIAA resulting in rapid (minutes) depletion of the target protein. This overcomes the problem of distinguishing primary and secondary mRNA phenotypes that is unavoidable when RNAi is used. The effect of depletion on mRNA was determined using RNAseq quantitation over a time course after the addition of 5-PhIAA. The assay was tested by preventing translation initiation by eIF2alpha depletion to test a prediction that mRNAs with a low codon score (geCAI) would be stabilised relative to those with a high score, this was indeed the case. The effect of depleting DHH1 was tested and this resulted in the selective degradation mRNAs with long ORFs. This is evidence that DHH1 provides a general protection against a degradation machinery that non-specifically targets ORFs, so that longer ORFs are more susceptible in the absence of DHH1. This is a novel finding and one that validates using the same approach with further candidates.

16:45 *(3 mins) Speed Talk*

mt-LAF3 is a pseudouridine synthase ortholog required for mitochondrial rRNA and mRNA gene expression in *Trypanosoma brucei*

Presenter: **Dr Suzanne McDermott**, *Acting Assistant Professor, Seattle Childrens Research Institute*

16:48 *(3 mins) Speed Talk*

The ATAD2/Abo1/Yta7 homologue, Bromodomain Factor 7, is essential for macrophage infection by *Leishmania mexicana*

Presenter: **Dr Nathaniel Jones**, *Research Fellow in Drug Discovery, University of York*

16:51 *(3 mins) Speed Talk*

Competition among variants is predictable and controls the antigenic variation dynamics of African trypanosomes

Presenter: **Dr Douglas O Escrivani** , *University of Dundee*

16:54 *(3 mins) Speed Talk*

Leishmania (Viannia) braziliensis long non-coding RNAs are enrolled in parasite fitness and interact with proteins in a structure-dependent manner

Presenter: **Caroline Ricce Espada**, *Postdoctoral researcher, University of York*

BES Ecology 1: Within-host Interactions - Sponsored by Quadratech Diagnostics - (Appleton Tower 1) Chair: Dr Iris Mair

11:00 (30 mins)

Drivers and fitness consequences of gut community dynamics in wild Soay sheep

Presenter: **Dr Amy R Sweeny**, *Postdoctoral Research Associate, University of Shefield*

A Sweeny[1];

[1] University of Shefield, UK

Host-associated microbial communities (the microbiome) are intimately tied to host fitness, with important implications for wildlife disease dynamics. Despite growing interest, the relationship between microbiome community composition, host phenotypes, and subsequent consequences for population dynamics is difficult to determine due to the high resolution of longitudinal sampling and host metadata required. Furthermore, relationships between commensal and parasitic within-host communities are very poorly resolved due to the high complexity of the data. The Soay sheep of St Kilda are an unmanaged ungulate population monitored as part of an iconic long-term study system with an unparalleled wealth of host phenotype information. In this population, we monitored the gut microbiome & parasite communities of ~1600 samples from 400 unique individuals for 2 years using metabarcoding approaches on non-invasive faecal samples. Using modern mixed modelling approaches, we find that both gut microbiome and parasite communities vary substantially across seasons and especially between age classes. I will present causes and consequences of this and other variation in this longitudinal study and describe how dynamics of the microbiome align with those of the parasite community. Finally, I will discuss the implications of findings from this and other system for understanding how animal microbiomes may respond to changing environments, and how these effects might influence population and disease dynamics in the wild.

11:30 (15 mins)

Galba truncatula and Helminths, the Importance of Microbes

Presenter: **Peter McCann**, *PhD Student, Queen's Univeristy Belfast*

P *McCann*[1]*; C McFarland*[1]*; J Megaw*[1]*; C Cantacessi*[2]*; G Rinaldi*[3]*; G Gobert*[1]*;

[1] Queen's Univeristy Belfast, UK [2] University of Cambridge, UK; [3] Aberystwyth Uinversity, UK

Liver fluke (*Fasciola hepatica*) and rumen fluke (*Calicophoron daubneyi*) are endemic in the UK. Liver fluke is estimated to cost the UK agriculture industry approximately £300 million per year, particularly due to lamb deaths and liver condemnations. Rumen fluke is fatal in severe infections and only one flukicide, oxyclozanide, has been shown to effectively reduce rumen fluke burdens. The desirable potency of triclabendazole has stimulated its overuse for liver fluke control resulting in widespread anthelmintic resistance. Therefore, there is an urgent need to develop new control strategies for fasciolosis.

The microbiome is defined as the combined genetic material of the microorganis inhabiting a particular environment. A host's microbiome is known to play a key role in many aspects of health and disease, including susceptibility to parasitic infection. While most microbiome studies have focused on the mammalian hosts of helminths, their intermediate hosts should also be considered. The interaction between the snail microbiome and life stages of parasitic trematodes residing in their intermediate hosts has not been investigated to any large extent.

In this project we have shown that the snail host regulates its microbiota differently than the environmental microbiome and that the microbiome of infected snails differs from uninfected snails. These data indicate a decrease/increase in abundance of certain bacteria under a series of conditions. This novel study is a starting point for further study of the microbiota of the intermediate hosts of helminths.

11:45 (15 mins)

Molecular typing of *Giardia duodenalis* detected in UK cats and dogs using an improved marker

Presenter: **Dr Rossella Panarese**, *University of Glasgow*

*S Krumrie[1]; **R Panarese**[1]; P Capewell[1]; M McDonald[1]; D Dunbar[1]; F Katzer[2]; N El Sakka[3]; D MellorC Alexander[4]; W Weir*

[1] University of Glasgow, UK; [2] Moredun Research Institute, UK; [3] National Health Services Grampian, UK; [4] Scottish Microbiology Reference Laboratories (Glasgow), UK

Giardia duodenalis is a flagellated protozoa which causes enteric disease in both humans and animals. In the United Kingdom (UK), knowledge of the zoonotic impact of this parasite is limited due to the inconsistent diagnostic testing and the availability of only low-resolution molecular markers for genotyping with variable efficacy. Human giardiasis has long been considered a travel-associated condition in the UK. However, recent studies have suggested the presence of an endemic *Giardia* cycle in the country, although the source of human disease is still unclear in most cases. Therefore, this study aimed to (i) improve a commonly used *Giardia* genotyping assay that distinguishes assemblages (broad genetic sub-groups), the nested *topoisomerase phosphate* (*tpi*) PCR, to increase the amplification success rate in both human and animal *Giardia* positive samples and (ii) molecularly characterise *Giardia* strains circulating in the UK human and companion animal populations (feline and canine). After adjusting the *tpi* primers to account for additional sequence diversity present in published *Giardia* genomes and optimising the PCR conditions in a step-wise manner, a revised assay was used to test human (n=79) and companion animal (n=174) faecal samples, to evaluate the molecular epidemiology of *Giardia* in the UK. The overall genotyping success rate of the new assay was 37.4 % (65/174) and 46.8 % (37/79) for animal and humans, respectively. Humans were found to be infected with known human-infective assemblage genotypes AI (n=1), AII (n=11) and B (n=25). Assemblage AI genotypes were also found in three feline and one canine sample, while one feline sample contained assemblage AII. In addition, four feline samples were infected with assemblage B genotypes. Alongside these potentially zoonotic assemblages, canine samples were found to be infected with assemblage C (10/52) and F (10/52), while feline samples with F (38/122) assemblage genotypes. This study demonstrates the presence of zoonotic *Giardia* genotypes circulating in the UK companion animal population by an improved *tpi* PCR, which also resulted in an increased success rate compared to previous studies. Notably, 17.4 % (8/46) of feline-derived *Giardia* strains were identified as being zoonotic genotypes. Therefore, this work highlights the potential role of domestic pets in the endemic transmission of *Giardia* in the UK and underlines the need for appropriate hygiene measures to be observed when interacting with both symptomatic and asymptomatic animals. Further studies are needed to assess the zoonotic risk of *Giardia* associated with companion animals in high-income countries such as the UK.

12:00 (15 mins)

Hybridization between human and livestock schistosomes – Ancient or ongoing?

Presenter: **Dr Roy Platt**, *Texas Biomedical Research Institue*

***RN Platt II et al.**[1];*

[1] Texas Biomedical Research Institute, United States

Hybridization between human and animal parasites can transfer biomedically important traits between species and negatively impact human health. The human parasitic blood fluke, *Schistosoma haematobium* can hybridize with the livestock parasite *S. bovis* as evidenced by laboratory crossing experiments in rodent hosts and introgressed mitochondrial and nuclear markers in parasite samples from the field. These results have been widely interpreted to suggest that hybridization between these species occurs frequently. However, work from several, independent groups using exome sequence, single nucleotide polymorphism, or microsatellite markers suggest that hybridization events were ancient, rather than `ongoing, and have led to the adaptive introgression of *S. bovis* alleles into the *S. haematobium* population. Here, we expand on this work by analyzing 34.6 million genome-wide, single nucleotide variants in 167 *S. bovis* and *S. haematobium* samples collected from 18 countries across Africa and aided with a chromosomal-scale genome assembly. We found strong differentiation between *S. haematobium* and *S. bovis* populations and no evidence for recent or ongoing hybridization in these samples. Our results confirm the presence of an ancient introgression event(s) that occurred 421 – 22,108 (median=2,738) generations ago and was restricted to west African populations. Three introgressed *S. bovis* genome regions containing 52 genes on Chr. 4 and 6 are at, or near (>95% allele frequency), fixation in west African *S. haematobium* populations. Further, we identified some regions of the *S. haematobium* genome that are depleted of *S. bovis* alleles indicating selection against introgression. These results demonstrate (i) that strong reproductive barriers maintain species integrity between *S. haematobium* and *S. bovis* in wild populations (ii) that ancient hybridization has led to adaptive introgression between these two species and (iii) suggests caution when interpreting patterns of parasite epidemiology using limited numbers of genetic markers.

12:15 (15 mins)

A novel *Cryptosporidium hominis* outbreak during British military training in Kenya

Presenter: **Romeo Toriro**, , *Liverpool School of Tropical Medicine*

R Toriro[1];

[1] Liverpool School of Tropical Medicine, UK

Background: We report findings from a cryptosporidiosis outbreak during a British military training exercise between February and April 2022. Methods: Epidemiological and clinical data were collated from diarrhoea cases and symptomatic contacts amongst a 1200 homogenous population at risk of usually fit and well young adults. Stool samples were analysed using multiplex PCR BioFire® FilmArray®. Domestic and recreational water sources were tested using Colilert testing kits (IDEXX Technologies Ltd., UK). DNA was extracted from repatriated stool samples for typing by duplex real-time PCR targeting A135, Lib13 genes and ssu rRNA genes. *C. hominis* cases were further subtyped by DNA sequencing of the gp60 gene. Results: Of 106 primary stool samples, 63 (59.4%) were positive for *Cryptosporidium spp.* by PCR FilmArray® analysis. 38/63 (60.3%) of these had *Cryptosporidium spp.* alone and 25/63 (39.7%) were also positive for 1 other enteric pathogen. 27/106 had 1 other enteric pathogen only and 17/106 were negative on PCR. 54/74 (73.1%; 63 primary samples plus 11 repeat samples) were positive for the highly divergent *C. hominis* and none for *C. parvum*. Sympto settled within 10 days in 6/8 *Cryptosporidium* positive individuals with persistent diarrhoea who were offered nitazoxanide. A single sequence of the *Cryptosporidium* gp60 gene representative of identical ImA13G1 subtypes (previously assumed to be a non-human primate subtype) from 26 case specimens was placed on GenBank. Locally tested suspected point source recreational water contained faecal colifor >2419.6 cfu/100ml and E. coli 98 cfu/100ml, but repatriated water was unsuitable for *Cryptosporidium spp.* detection. Concurrent analysis of data on post infectious sequelae up to 12 months is underway. Common complaints include diarrhoea, abdominal aches and non-injury related or exercise induced joint aches. Conclusions: This report emphasises the potential for large point source outbreaks of cryptosporidiosis in an African setting, initially related to freshwater recreational activity. Such outbreaks are rarely reported in military populations. We have confirmed the specificity of on-site PCR stool testing in an otherwise resource limited diagnostic setting, use of which improved both clinical patient management and outbreak control measures in real time. The aetiology was a rare subtype of *C. hominis* only previously described in macaques in China, emphasising the need for sequencing studies to identify the widening host and geographic ranges of novel *Cryptosporidium spp.*

BES Ecology 2: Quantitative Methods in Parasitology - (Appleton Tower 1) Chair: Dr Emma Cunningham

13:30 (30 mins)

Causal Analysis of the Relationship Between Helminth Infections and Vaccine Responsiveness in a Wild Rodent Model

Presenter: **Dr Simon Babayan**, *University of Glasgow*

S Babayan[2]; S Venkatesan[1]; E Smith[2]; A Sweeny[1]; J Hall[2]; AB Pedersen[1];

[1] Institute of Evolutionary Biology, School of Biological Sciences, University of Edinburgh, Edinburgh, UK; [2] School of Biodiversity, One Health, and Veterinary Medicine, University of Glasgow, Glasgow, UK

Individuals vary substantially in their responses to vaccination due to a range of factors that include intrinsic host factors such as age, sex, and genetics, and acquired extrinsic factors such as pre-existing adaptive immunity, microbiota, and infections. The evidence for how parasitic helminths in particular might affect vaccine responsiveness is mixed, with some studies showing a range of effects from enhancement to suppression. Various factors, such as vaccine type and route of administration, the presence of distinct parasite species and the ability of helminths to alter the immune system, microbiota composition, and gut epithelium, have been cited as possible explanations for such apparent inconsistency. Yet issues with quantitative methods can also confound the interpretation of vaccine responses, leading to apparently contradictory effects of helminths on vaccine responses. Here, we present a causal analysis of the drivers and downstream effects of variation in helminth infection with respect to vaccine responsiveness using a paired laboratory-wild rodent system in which we manipulated diet and habitat. We found that under standard (and thus potentially confounded) analysis, helminth infection appeared to be associated with reduced vaccine responsiveness. However, in a causally explicit model where confounding was accounted for, we found no evidence that helminth infection was associated with reduced vaccine responsiveness. We also found that diet, sex, and reproductive status had negligible effects on parasite burdens in this set of experiments. These results suggest that while immunity to helminths and vaccine responsiveness may be context dependant, parasite burdens themselves may not be a direct driver of variation in vaccine responses.

14:00 (15 mins)

Alternative roles of heterogeneities in driving transmission dynamics: analysis of contrasting compartmental models

Presenter: **Jacob Cohen**, *PhD Student, University of Liverpool*

JA Cohen[1]*; ME Viney*[1]*; A Fenton*[1]*;*

[1] *University of Liverpool, UK*

Heterogeneities in host susceptibility and infectiousness are known to affect the dynamics of parasite spread. However, their combined effect on transmission is not well understood. We break down the classic Susceptible-Infected compartmental model to integrate heterogeneities in host susceptibility and infectiousness, under two alternative scenarios: Recipient Dependent (RD), where both susceptibility and infectiousness are fixed characteristics of the individual, and Donor Dependent (DD), where an individual's infectiousness is determined by the identity of the infecting individual. We show that these alternative mechanisms lead to different outcomes for transmission, and are influenced by heterogeneities in susceptibility and infectiousness in different ways. DD systems show highest variability in transmission outcomes (R0 and equilibrium host population size) at intermediate heterogeneity levels, determined purely by infectiousness. For RD systems, however, increasing host heterogeneity increases the range of possible transmission outcomes, influenced by both susceptibility and infectiousness, and the correlation between them. DD and RD models represent two ends of a spectrum in how host infectiousness is determined, with specific host-parasite systems likely lying at different points between them. Therefore, heterogeneities in susceptibility and infectiousness are both important drivers of transmission, but which of these traits is more consequential will be system dependent.

14:15 (15 mins)

Using mathematical models to understand schistosomiasis transmission in a Ugandan hotspot

Presenter: **Dr Gregory Milne**, *Research Associate, Royal Veterinary College*

GC Milne[1]*; R Oettle*[2]*; JP Webster*[1]*; M Walker*[1]*; S Wilson*[2]*;*

[1] *Royal Veterinary College, UK;* [2] *University of Cambridge, UK*

Schistosomiasis is a neglected tropical disease of profound medical importance, infecting approximately 240 million people, 90% living in Sub-Saharan Africa. Severe schistosomiasis is associated with periportal fibrosis and portal hypertension which can cause death without appropriate disease management. A cornerstone of international efforts to eliminate schistosomiasis as a public health problem is mass drug administration (MDA) with praziquantel. However, despite nearly two decades of MDA, the prevalence of infection and the incidence of periportal fibrosis remains high in communities along the shore of Lake Albert in Uganda, representing a conspicuous failure of the current intervention strategy. The FibroScHot Consortium is addressing this urgent public health need by conducting a randomised controlled trial to evaluate the effectiveness of delivering MDA at frequencies of up to four times per year. Reporting on research conducted as part of the Consortium, this talk presents progress from mathematical model-based analyses of the historical impact of MDA on the infection and morbidity dynamics of *Schistosoma mansoni* along the shore of Late Albert and discusses how severe schistosomiasis may be tackled in transmission hotspots.

14:30 (15 mins)

Strongyloides stercoralis in the United Kingdom: A systematic review and meta-analysis of published cases

Presenter: **Cansu Ozdemir**, *Medical Student , King's College London*

A Alam[1]*;* ***C Ozdemir***[1]*; N Reza*[2]*;*

[1] *Institute of Infection, Veterinary, and Ecological Science, University of Liverpool, Liverpool, & Barts Health NHS Trust, London, GKT School of Medical Education, King's College London, London, UK;* [2] *Tropical and Infectious Diseases Unit, Royal Liverpool University Hospital, Liverpool, UK*

Background: *Strongyloides stercoralis* is a soil-transmitted intestinal helminth which can cause lifelong infections in humans. Sympto of infection can vary, whilst many may be asymptomatic. When an infected host is immunocompromised, *S. stercoralis* has the potential to cause a 'hyper-infection' – a life-threatening disseminated disease with mortality up to 71%. Given treatment with anti-parasitic agents has a high eradication rate, successfully screening at-risk groups can reduce the threat of hyper-infection, particularly in those who may be immunocompromised. We conducted a systematic review and meta-analysis of *S. stercoralis* infections reported in the United Kingdom to describe the demographics and clinical features in those with this parasitic infection.

Methods: A systematic search of PubMed and Scopus was performed and studies describing patients in the United Kingdom with proven *S. stercoralis* infection were included. The outcomes studied were weighted pooled prevalence (WPP) of clinical features during illness,

demographics, and relevant investigation findings. We used the DerSimonian-Laird random-effects model to report prevalence of clinical variables and a Freeman-Tukey double arcsine transformation was applied to our data.

Results: Seventeen studies with 1361 patients were analysed. A third of cases reported were in returning travellers (454/1361, 33.3%), whilst 24.5% (334/1361) were cases in migrants. A total of 342/1361 (25.1%) cases were described in Armed Personnel who had returned to the United Kingdom. A minority of cases were in those living with HIV (8/1361, 0.6%) and 223/1361 (16.4%) were cases from a mixed cohort.

The weighted pooled prevalence (WPP) of asymptomatic cases was 31.0% [95%CI 27.5% - 34.6%, I^2=92.3%]. The most reported sympto were abdominal pain (WPP 30.8% [95%CI 27.4% - 34.3%], I^2=91.6%), rash (WPP 28.4% [95%CI 25.3% - 31.7%), I^2=98.8%) and diarrhoea [WPP 9.4% [95%CI 6.0% - 13.1%), I^2=80.7%].

Returning travellers were more likely to be asymptomatic with a WPP of 44.63% (95%CI 38.57 - 50.75%), whilst migrant groups commonly presented with abdominal pain (WPP 42.4% (95%CI 35.1% - 47.9%) and diarrhoea (WPP 65.3% [95%CI 25.2 - 96.8%]). Rashes were a frequent complaint in those diagnosed with *S. stercolaris* in the armed forces (WPP 75.3% [95%CI 70.2% - 80.1%]).

The most common diagnostic modality in reported cases was *Strongyloides* serology (51.8%), followed by stool culture (30.8%). A small number were diagnosed with the use of ELISA (9.1%). When analysing laboratory findings, the average eosinophil count was $1.75x10^9$/L (standard deviation $\pm 1.24x10^9$/L).

Of the 478 patients followed up, 255 were treated successfully (30.8%). There were only 4 reports of hyper-infection.

Conclusion: Our meta-analyses illustrates that a third of patients with *S. stercoralis* infectionin the UK were asymptomatic, whilst commonly reported sympto may include non-specific abdominal pain, diarrhoea, and a rash. Given these non-specific presentations, clinicians should have a low threshold in screening migrant groups and returning travellers for *S. stercoralis* – particularly if there are plans for immunosuppressive therapy.

14:45 (15 mins)

Is *Strongyloides stercoralis* in people a zoonosis from dogs?

Presenter: **Yuchen Liu**, *PhD Student, University of Liverpool*

Y Liu[1]*; B Sripa*[2]*; R Sarker*[3]*; ME Viney*[1]*;*

[1] *University of Liverpool, UK;* [2] *Khon Kaen University, Thailand;* [3] *University of Chittagong, Bangladesh*

It has been assumed that the parasitic nematode *Strongyloides stercoralis* transmits only among people. However, accumulating evidence suggests that *Strongyloides* from people and dogs are the same species, so that dogs can act as a source of human infection. To investigate the host range of *S. stercoralis* and the zoonotic potential of dog-derived *Strongyloides*, we sampled sympatric populations of wor from people and dogs in Bangladesh and in Thailand, which we then whole-genome sequenced. Population genomic analyses showed different genetic clusters of parasites, people in Bangladesh and Thailand were infected with closely related *S. stercoralis* genotypes, and the similar pattern was also found in infections of dogs. However, there was no evidence of *S. stercoralis* infection shared between people and dogs in Bangladesh and Thailand. Surprisingly, some parasites derived from people and dogs in Bangladesh were genomically identified as *S. venezuelensis*, a species normally thought to be a rat parasite, which we are continuing to investigate.

12-April-2023, at 15:30 (30 mins)

The adaptive immune response to *Trichuris* in wild versus laboratory mice: An established model system in context

Presenter: **Dr Iris Mair**, *Postdoctoral Research Associate, University of Manchester*

I Mair[1]*; J Fenn*[1]*; A Wolfenden*[2]*; A Lowe*[2]*; A Bennett*[1]*; A Muir*[1]*; J Bradley*[2]*; KJ Else*[1]*;*

[1] *University of Manchester, UK;* [2] *University of Nottingham, FMHS, Life Sciences, UK*

Infections caused by species of the gastrointestinal dwelling nematode parasite *Trichuris* tend to be chronic, and are associated with a significant health burden in humans, livestock and wildlife. A wealth of laboratory work using the model organism *Mus musculus* (commonly known as the house mouse) experimentally infected with *Trichuris muris* has established the balance of Type 1 and Type 2 immune responses as the major determinant of disease susceptibility versus worm expulsion. Single parameters such as sex, age, mouse strain, diet, or microbiome composition are known to affect the immune response and susceptibility to disease. However, what shapes the parasite-specific immune response in a multivariate environment? What are the consequences of infection and the strength and/or quality of the concomitant immune response for host health? Taking advantage of the knowledge and tools available for the house mouse, we present a novel study investigating the adaptive immune response to *Trichuris* in a wild, free-living island population of house mice naturally infected with this endoparasite. Immunological and ecological data were collected from over 200 mice on the Isle of May, UK, sampled across 2018-2019. *Trichuris* burden across the population showed a typical over-dispersed distribution, and infections were mostly of a chronic nature. The local parasite-specific cytokine response partially overlapped but was significantly different from laboratory mice experimentally infected with either a low or a high dose of *Trichuris* eggs, especially in response strength. CD4+ T effector/memory phenotypes were linked to cytokine expression but could not explain differences observed between wild and the laboratory setting. Importantly, age and worm burden affected individual Th1/Th2 balance in wild mice in an interrelated fashion. This interdisciplinary study gives first insights into the parasite-specific immune response in a natural system, and through wild-to-lab comparison is able to put the most used laboratory disease model in context. By looking at the immune response to parasites through an ecological lens, taking individual and environmental parameters, and their interactions, into account, we can start to bridge between the laboratory and the wild for more robust translatability of immnunoparasitological research.

16:00 (15 mins)

Longitudinal trends of T-helper cell immune phenotypes in the Soay sheep from St Kilda: can we predict parasite burden?

Presenter: **Dr Yolanda Corripio-Miyar**, *Moredun Research Insitute*

Y Corripio-Miyar[1]*; A Hayward*[1]*; X Bal*[2]*; J Pilkington*[3]*; F Kenyon*[1]*; JM Pemberton*[2]*; D Nussey*[3]*; TN McNeilly*[1]*;*

[1] *Moredun Research Insitute, UK;* [2] *University of Edinburgh, UK;* [3] *University of Edinburgh, Institute of Evolutionary Biology, UK*

The adaptive immune system is critical for appropriate responses to infections, with T helper (Th) cells playing a key role in orchestrating effective pathogen-specific responses. In contrast to controlled infections of laboratory animals, wild communities are challenged by multiple pathogens and consequently, discerning the responses to individual pathogens is more challenging. The long-term study of the Soay sheep on St Kilda offers a unique opportunity to investigate the variation in adaptive immune response to parasites under natural conditions and link this variation to infection pressures, health and fitness. Over the past four years we have investigated the main Th responses (Th1, Th2, Th17 and regulatory responses) in the population using ex-vivo T-cell stimulations to measure cytokine secretion and expression of Th transcription factors in CD4 T cells. Correlation of these immune responses with nematode and coccidian parasite burdens, were carried out to determine the animal's response to micro- and macro-parasites. We collected a total of 750 samples from 530 individuals, of which 149 were sampled more than once. Confirming previous results from analysis of a single year of data, we found that associations among the different immune markers were generally positive and that while CD4+ T cell counts were relatively stable with age, cytokine levels increased in older animals. We found that the canonical Th2 cytokine IL-4 was negatively associated with strongyle nematode faecal egg count, and that the canonical Th1 cytokine IFNγ was weakly negatively associated with coccidian faecal oocyst count. Overall CD4+ cell count and cytokine responses were moderately repeatable, with between-individual variation accounting for around 20% of variation in these traits, but CD4+ cell counts associated with different Th phenotypes were not repeatable.

16:15 (15 mins)

The impact of host-pathogen coevolution on *Trypanosoma* infection rates within wild *Glossina morsitans morsitans*.

Presenter: **Dr Calam Bruce**, *Research Assistant, University of Westminster*

RC Bruce[1]*; PM Hayes*[1]*; J Murphy*[1]*; W Gibson*[2]*;*

[1] *University of Westminster, UK;* [2] *University of Bristol, UK*

Tsetse flies (genera *Glossina*) are the sole biological vectors of African *Trypanosoma* species, the infectious agents of African Trypanosomiasis. Vector control is a key inhibitor of disease transmission; however, long-term control measures are economically and ecologically unsustainable and therefore, alternatives must be explored. Genetic interventions influenced by host-pathogen coevolution could present one such alternative. In this presentation, we explore the genetic variation and evolution within three immune genes Attacin-A (*AttA*), Defensin (*Def*) and Toll-like receptor 2 (*TLR2*) and the consequences for symbiont and parasitic interactions within a wild *Glossina morsitans morsitans* population. Nucleotide variation within *Def* and *AttA* was found to be similar, exhibiting eight and eleven polymorphic sites respectively, while nucleotide variation within *TLR2* was found to be considerably higher. A recent population expansion event and deviations from neutrality was also detected in all genes. Interestingly, genetic variation within *AttA* and *TLR2* was found to be maintained via purifying selection, while *Def* exhibited signs of the Red Queen ar race and balancing section. Trypanosome infection rates were unexpectedly high (69.35%), consisting of mixed species infections, although samples exhibiting Def variants under positive selection were observed to reduce infection rates within samples. Furthermore, these initial results indicate a potential correlation between *TLR2* variation and endosymbiont population variation. The results within show that further research is required to fully understand the interactions and impacts of genetic variation on *Trypanosoma* infection rates within wild tsetse, however that an understanding of host-pathogen evolution and interactions of the could be used to inform novel genetic control methods.

16:30 (15 mins)

Hybridization in UroGenital Schistosomiasis (HUGS): A novel real-time PCR assay, with high resolution melt profiling, useful for the detection of hybrid schistosomes in Malawi

Presenter: **Dr Lucas Cunningham**, *Liverpool School of Tropical Medicine*

LJ Cunningham[1]*; SA Kayuni*[2]*; P Makaula*[2]*; B mainga*[2]*; G Namacha*[2]*; D Lally*[2]*; D Kapira*[2]*; P Chammudzi*[32]*; S Jones*[1]*; S Rollason*[1]*; AL Reed*[3]*; J Archer*[1]*; A Juhasz*[1]*; EJ Lacourse*[1]*; J Musaya*[2]*; JR Stothard*[1]*;*

[1] *Liverpool School of Tropical Medicine, UK;* [2] *Malawi Liverpool Wellcome Trust Programme of Clinical Tropical Research, UK;* [3] *Lancaster University, UK*

Introduction: The ability of schistosomes to form inter-species mating pairs with resultant hybrid or introgressed offspring has been previously described. Such studies have relied upon sequence analysis of nuclear genome (nDNA) and/or the mitochondrial genome (mDNA) loci. Although highly accurate, sequence analysis of large numbers of targeted specimens is prohibitive, being both time-consuming and overtly costly. We have developed a rapid, low-cost two-tube real-time (rt)PCR multiplex assays, with high resolution melt profiling, screening both mDNA and nDNA loci. We have conducted a large-scale examination of recently collected material from two communities in Nsanje and Mangochi Districts.

Methods: Species-specific primers producing unique high-resolution melt-peaks were designed for mDNA gene targets (tRNA Lys, ND4 and ND6) for six schistosome species (*S. mattheei, S. currassoni, S. bovis, S. haematobium, S. mansoni* and *S. margrebowei*). To compliment the mDNA qPCR, a single-plex nDNA rtPCR targeting a 168bp variable region of the ITS2 gene was also developed. Comparison of the specific melt-peaks produced by each assay can be used to distinguish individuals of different species and determine if they have any mixed species parental signatures, especially maternal mitochondrial carriage.

Preliminary results: A combined total of 1,012 urine filter samples obtained from ~2,400 people were screened using the mDNA and nDNA qPCR assays. This resulted in the identification of 77 putative mixed infections and/or hybrid cases, equating to an overall prevalence of 7.3%. Furthermore, our assays identified gross under-reporting of mixed species infections with ectopic *S. mansoni* (n=95) versus microscopy (n=6), including 18 individuals with markers for triple-species infections.

Conclusion: With new assays, our ability to screen natural populations of schistosomes for introgressed for expands. Our study indicates the presence of various hybrid schistosomes capable of infecting local communities of endemic areas of Malawi in sub-

Saharan Africa. Further analysis of individual eggs on FTA cards is ongoing to clarify the distinctions between mixed infections versus introgressed genotypes. We also aim to complement these qPCR assays with development of discriminatory sex-specific loci to better interrogate the directional basis of hybridisation between male (ZW) and female (ZZ) genders.15:45 (15 mins)

Exploiting the mosquitocidal properties of nitisinone as a novel strategy for malaria control

Presenter: **Prof Alvaro Acosta Serrano**, *Faculty, University of Notre Dame*

*LR Haines[1]; A Trett[1]; C Rose[1]; M Sterkel[2]; D McGuinnessC RegnaultMP BarrettD Leroy[4]; J Burrows[4]; G Biagini[3]; R Lakshminarayan[5]; G Aljayyoussi[3]; **A Acosta-Serrano**[6];*

[1] Liverpool School of Tropical Medicine, UK; [2] Universidad de La Plata, Argentina, UK; [3] University of Glasgow, UK; [4] Medicines for Malaria Venture, Geneva, UK; [5] Royal Liverpool University Hospital, UK; [6] Department of Biological Sciences, University of Notre Dame, United States

One approach to interrupting the transmission of insect-borne diseases that is successfully used in veterinary medicine, is exploiting the ability of antiparasitic drugs to make vertebrate blood toxic to blood-feeding insects. Recent studies have identified an enzyme (4-hydroxyphenylpyruvate dioxygenase (HPPD)) that is part of the tyrosine detoxification pathway and essential for blood-feeding insect and tick survival. An FDA-approved HPPD enzyme inhibitor called nitisinone is a drug used to treat rare human inherited disorders of the tyrosine pathway. Building on the success of drug repurposing, here we demonstrate that feeding human blood containing nitisinone to the malaria-transmitting mosquito species, *Anopheles gambiae*, is mosquitocidal to young and old mosquitoes and insecticide-resistant strains. Proof-of-concept pharmacokinetic-pharmacodynamic (PK/PD) modelling of nitisinone's dose–exposure-response relationship, administered at the highest recommended doses for adults and children, shows improved efficacy against mosquitoes compared to the gold-standard endectocidal drug, ivermectin. In addition, blood from patients with alkaptonuria – a rare genetic metabolic disorder in the tyrosine degradation pathway – taking a daily low dose of nitisinone (2 mg), is lethal to mosquitoes. Together, these data show that inhibiting the *Anopheles* HPPD enzyme with nitisinone warrants further investigation as a new ectoparasitic intervention for malaria control.

Immunoparasitology 1: Immune Response to Unicellular Parasites - (Appleton Tower 2) Chairs: Prof Neil Mabbott, Dr Lucy Jackson-Jones

11:00 (30 mins)

To the skin and beyond: the impact of the immune system on African trypanosome infections

Presenter: **Prof Neil Mabbott**, *The Roslin Institute*

N Mabbott[1];

[1] The Roslin Institute, UK

African trypanosomes are single-celled extracellular protozoan parasites transmitted by tsetse fly vectors across sub-Saharan Africa, causing serious disease in both humans and animals. Natural mammalian infections begin when an infected tsetse fly penetrates the skin in order to take a blood meal, depositing trypanosomes into the dermis. Similarly, onward transmission occurs when differentiated and insect pre-adapted parasite forms are ingested by the fly during a blood meal. Between these transmission steps, trypanosomes access the systemic circulation of the vertebrate host via the skin-draining lymph nodes, disseminating into multiple tissues and organs, and establishing chronic, and long-lasting infections. However, most studies of the immunobiology of African trypanosomes have been conducted under experimental conditions that bypass the skin as a route for systemic dissemination (typically via intraperitoneal or intravenous routes). Therefore, the importance of these initial interactions between trypanosomes and the skin at the site of initial infection, and the implications for these processes in infection establishment, are often overlooked. I will discuss mechanisms involved in establishing African trypanosome infections via the skin, especially the role of innate immune cells in the skin and the local draining lymph nodes. I will also discuss data from studies of the subsequent immune interactions as the parasites migrate from the skin and the pathological consequences as they eventually establish infection in the central nervous system. The many studies of experimental systemic African trypanosome infections have of course helped to uncover important interactions between the parasite and the mammalian host's immune system. However, a thorough identification of the mechanisms involved in establishing African trypanosome infections in the skin and their subsequent progression through the host is essential for the development of novel approaches to block disease transmission.

11:30 (15 mins)

IL-27 in African trypanosomes: a double-edged sword in parasite control.

Presenter: **Mathieu Claes**, *PhD student, UAntwerp, LMPH*

M Claes[1]*; D Mabille*[1]*; YG Sterckx*[1]*; S Magez*[2]*; C De Trez*[2]*; G Caljon*[1]*;*

[1] *University of Antwerp, Belgium;* [2] *Vrije Universiteit Brussel, Belgium*

African trypanosomiasis (AT), caused by extracellular protozoan parasites of the genus *Trypanosoma*, is a neglected tropical disease affecting both humans and livestock. Left untreated, the disease is characterized by a chronic inflammatory response, often lethal for the host. Like in most infectious diseases, the host's immune system balances mounting an efficient immune response and limiting collateral damage. While the anti-inflammatory IL-10 cytokine is paramount in limiting the AT-associated immunopathologies, interest has increased in IL-27 as another key immunomodulating cytokine.

An initial study showed that abrogation of the IL-27 receptor (IL-27R) results in an increased mortality due to uncontrolled IFN-γ production by $CD4^+$ Th1 cells and accumulation of TNF/iNOS producing dendritic cells (TIP-DCs) in the liver. To investigate the role of IL-27 in tsetse-transmitted AT, our research relied on pharmacological and genetic models, including anti-IL-27 antibody-induced neutralization and genetic IL-$27^{-/-}$ mice. Ten days after inoculation by the bites of *Glossina morsitans* flies, trypanosome counts in peripheral blood and bio-luminescent imaging revealed significantly better parasitaemia control in the absence of IL-27, which contrasts previous observations in IL-$27R^{-/-}$ mice infected through an intraperitoneal route. Using IL-27 reporter mice, the immunological response was studied in skin exposed to the infectious bites, showing a strong influx of myeloid cells and $CD4^+$ T lymphocytes, with myeloid cells such as inflammatory monocytes and neutrophils representing the main sources of IL-27. Myeloid cells were also identified as the principle early producers of IL-27 in the blood, liver and spleen. Mortality occurred earlier in IL-27 depleted mice, associated with elevated IFN-γ, MCP-1 and TNF-a plasma levels from day 7 post-infection onwards, without affecting IL-10 levels.

Altogether, our data show that myeloid cell-derived IL-27 plays an essential role in the control of inflammation during tsetse transmitted AT, limiting host immunopathology at the expense of increased systemic parasite establishment.

11:45 (15 mins)

The local immune response to *Trypanosoma brucei* in the tissues of the abdomen.

Presenter: **Chloe Barnes**, *PhD Student, Lancaster University*

C Barnes[1]*; JJ Worthington*[1]*; L Jackson-Jones*[1]*; N Dawson*[1]*; MD Urbaniak*[1]*;*

[1] *Lancaster University, UK*

Trypanosoma brucei is the causative agent of human and animal African trypanosomiasis, creating a significant burden on health care services and the local economy in sub-Saharan Africa. The parasite is able to survive and develop a chronic infection in the host, despite being highly immunogenic and eliciting a strong anti-trypanosome immune response. In early infection, parasites reside in the blood, lymph, and a variety of other tissues whilst in later infection, trypanosomes enter the brain causing severe neurological disturbances. This work aims to develop the understanding of immune cell activation in the presence of *Trypanosoma brucei* by exploring the links between immune response, parasite localisation and the associated clinical symptoms.

A bioluminescent *in vivo* model of African trypanosomiasis was used to observe tissue tropism and colonisation at early and late stages of infection, with parasites identified in all studied tissues including the colon, small intestine and omentum. Further analysis of the colon showed alterations to gut structure through histological staining of naïve and infected sections. We have observed significant enlargement of the omentum, a specialised immunological adipose tissue located in the peritoneal cavity shown to capture contaminants such as translocating bacteria, which can enter the peritoneal space from the gut. Changes in immune cell populations of the colon lamina propria and mesenteric lymph nodes were also recorded. With an increasing understanding of the importance of the direct link between the brain and the gut, the presence of *Trypanosoma brucei* in the gut during infection provides a potential uncategorised link between perturbed brain function and immune response.

RNA sequencing analysis of tissues from naïve and infected mice revealed tissue specific differences in response to parasite presence, with many differentially regulated genes at both early and late stages of infection. There was a notable increase in macrophage related signatures in multiple tissues signifying a localised immune response to parasite presence. This was further studied *in vitro* comparing macrophage activation in the presence of trypanosomes and trypanosome secreted factors.

12:00 (15 mins)

Transcriptomics of the immune response in Chagas heart disease

Presenter: **Dr Damian Perez Mazliah**, *University of York*

A Khan[1]*; AF Francisco*[1]*; H Langston*[1]*; S Dey*[2]*; N Dey*[2]*; N Brown*[2]*; H Ashwin*[2]*; K Van Bocxlaer*[2]*; A Lima*[3]*; J Mottram*[2]*; JM Kelly*[1]*; PM Kaye*[2]*; MD Lewis*[1]*; **D Perez Mazliah***[2]*;*

[1] London School of Hygiene and Tropical Medicine, UK; [2] University of York, UK; [3] Instituto de Biofisica Carlos Chagas Filho, Universidade Federal do Rio de Janeiro, Brazil

Chagas disease, caused by infection with *Trypanosoma cruzi* parasites, is the most frequent cause of infectious cardiomyopathy in the world. Chagas heart disease (CHD), an inflammatory cardiomyopathy, develops in approximately one-third of chronically-infected people. Why some infected individuals develop cardiomyopathy while others remain asymptomatic for life remain poorly understood. Here, we used experimental mouse *T. cruzi* infections with differing degrees of cardiac pathology to model different pathological outcomes observed in the clinic. We then used single-cell RNA sequencing (scRNA-seq), spatial transcriptomics and flow cytometry to identify immune signatures associated with differing degrees of CHD. scRNA-seq showed that TcVI-CLBR-infected-BALB/c (mild CHD) and TcI-JR-infected C3H/HeN (severe CHD) mice develop strikingly different immune responses to the parasite in the spleen, with B cells dominating these differences. Severe CHD lead to accumulation of an unswitched, recently-activated B-cell subset with very high expression of *Nr4a1* (Nur77), previously identified as a marker of autoreactive B cells. To specifically track B cells reacting to the parasite vs those reacting to host cardiac tissues, we developed B-cell tetramers to detect, by flow cytometry and side-by-side, *T. cruzi*-specific and cardiac-specific B cells. Both parasite-specific and cardiac-specific B cell responses were robustly activated in the spleen of TcI-JR-infected C3H/HeN (severe CHD) mice. However, showing important differences in their functional profile, spleen-resident cardiac-specific B cells failed to develop germinal centre, class-switching and memory responses to the infection. Further, spatial transcriptomics of heart tissue sections showed a striking accumulation of plasma (B) cells dominating the immune infiltrate in the heart of TcI-JR-infected C3H/HeN (severe CHD) mice. Thus, our data shows a dominant B-cell immune signature associated with severe CHD, characterised by accumulation of short-lived autoreactive cardiac-specific B cells in the spleen and striking accumulation of plasma (B) cells in the heart of infected mice.

12:15 *(15 mins)*

What are the drivers of enteric neuropathy in experimental Chagas disease?

Presenter: **Harry Langston**, *London School of Hygiene and Tropical Medicine*

***H Langston**[1]; A Khan[1]; JM Kelly[1]; M Lewis[1];*

[1] London School of Hygiene and Tropical Medicine, UK

Chagas disease, caused by the protozoan parasite *T. cruzi*, affects ~7 million people worldwide. Around 30-40% of those infected develop chronic cardiac or gastrointestinal (GI) sequelae. GI disease is associated with a high morbidity, but the mechanisms responsible for the underlying enteric agangliosis and megasyndromes are largely unknown. The leading hypothesis for enteric nervous system pathology has been collateral damage induced by reactive nitrogen species, synthesised by iNOS-expressing myeloid cells during the acute phase immune response. This hypothesis has been questioned since bioluminescence imaging led to the discovery of chronic parasite reservoirs in the colon, which suggests an ongoing role for the infection in sustaining pathogenesis. Recent work has shown that C3H/HeN mice infected with TcI-JR parasites develop GI dysperistalsis, which is a common symptom of human digestive Chagas disease. This project aims to provide insight into the mechanisms responsible for enteric neuropathy in this mouse model. We conducted a histopathological analysis of colon tissue and found a significant increase in cellular infiltration in the smooth muscle of chronic Digestive Chagas Disease (DCD) mice, but not when a less pathogenic *T. cruzi* strain (TcVI-CLBR) was used. This inflammation was hyperfocal to the GI smooth muscle, adjacent to the adipose rich mesentery in the proximal colon. There was also evidence of focal fibrosis, high iNOS expression and neuronal damage. In ongoing *in vivo* experiments, we are investigating the effects of anti-parasitic and immunomodulatory treatments on the initiation of gut dysfunction in the DCD model, which occurs between 2 and 3 weeks post-infection. Benznidazole treatment suppressed the parasite burden below the limit of detection and prevented the initiation of gut dysfunction. Elimination of CD8+ T cells (which are critical for parasite control) by anti-CD8 immunotherapy increased the parasite burden by 19 fold and had no impact on the gut dysfunction phenotype. Interestingly, treatment with the broad immune suppressant cyclophosphamide completely reversed the GI peristalsis defect, even though the parasite load increased by 15 fold.

The results suggest an antagonistic interaction between parasites and immune action is key to the initiation of gut dysperistalsis, but one where CD8+ T cells are not critical pathological effectors. These results are generating insights into the early host-parasite interactions that drive digestive Chagas disease pathogenesis.

Immunoparasitology 2: Immune-Helminth Interactions - (Appleton Tower 2) Chairs: Prof Judith Allen, Dr Henry McSorley

12-April-2023, at 13:30 (30 mins)

Macrophages & B cells - partners in nematode immunity

Presenter: **Prof Judith Allen**, *Professor, University of Manchester*

J Allen[1]*;*

[1] *University of Manchester, UK*

The rodent nematode *Litomosoides sigmondontis*, which dwells in the pleural cavity, provides a unique model to study the host immune response to filarial infection. Resistance to infection is known to require adaptive immunity and IL-4 receptor alpha (IL-4Ra) signalling. However, the specific mechanisms that are involved in killing tissue-dwelling nematodes remain poorly understood. We recently found that during infection T cells and IL-4ra signalling drive the conversion of monocytes into tissue resident macrophages, which are essential for infection control. Infected mice that are genetically altered such that they cannot establish GATA6+ resident macrophages phenotypically mirror susceptible strains of mice. Unexpectedly, we found that the absence of resident macrophages results in a dramatic decline in B cell numbers at the infection site. Further experiments have revealed a surprising interdependence between B cells and macrophages that determines infection outcome.

14:00 (15 mins)

Characterising the ovine small intestinal tuft cell response following parasitic nematode infection.

Presenter: **Dr Katie Hildersley**, *Moredun Research Institute*

KA Hildersley[1]*; V Gillan*[2]*; TD Otto*[3]*; D Smith*[1]*; RM Maizels*[3]*; E Devaney*[2]*; C Britton*[2]*; TN McNeilly*[1]*;*

[1] *Moredun Research institute, UK;* [2] *Institute of Biodiversity, Animal Health and Comparative Medicine, University of Glasgow, UK;* [3] *School of Infection and Immunity, University of Glasgow, UK*

Tuft cells are of major interest in mucosal immunology due to their proposed function in sensing changes in the gut lumen environment and their role in initiating the Type-2 T-helper immune response to gastro-intestinal (GI) nematodes. Characterisation of murine tuft cells has built a profile of intestinal tuft cell specific markers, and differentiation of tuft cells following type-2 cytokine stimulation has been demonstrated in murine small intestinal (SI) organoids. As anthelmintic resistance is becoming an increasing challenge for controlling livestock parasites, it is important to improve our understanding of host immune mechanisms to aid development of new immune based control methods. We have previously demonstrated that tuft cell numbers in the ovine abomasum (equivalent of the gastric stomach) increase following infection with the economically important gastric nematode, *Teladorsagia circumcincta*. Characterisation of the gene expression profile of ovine gastric tuft cells by single cell RNA-sequencing (scRNA-seq) demonstrated that genes involved in tuft cell functions such as taste receptor signalling and eicosanoid biosynthesis are conserved between mice, humans and sheep; however, the cell surface receptor repertoire was different between gastric sheep and intestinal mouse and human tuft cells, suggesting either organ- or species-specific differences in receptor expression. The aim of this study was to characterise ovine intestinal tuft cell responses following intestinal GIN infection and compare ovine gastric and intestinal tuft cells at the transcriptomic level. Using antibodies to conserved tuft cell markers, we demonstrate that tuft cell numbers increase following infection with the ovine intestinal nematode, *Trichostrongylus colubriformis*. Transcriptomic analysis by scRNA-seq analysis identified an ovine SI tuft cell cluster which shares marker genes in common with the murine SI tuft cells, but more genes were conserved between the ovine SI and gastric stomach tuft cells. Furthermore, using recently established ovine duodenum organoid cultures, we demonstrate for the first time that stimulation with type-2 cytokines IL-4 and IL-13 is sufficient to induce ovine tuft cell differentiation. These results indicate that despite organ-specific differences in surface receptor expression, ovine tuft cells expand following both gastric and intestinal GIN infection. Furthermore, differentiation of tuft cells by type-2 cytokines appears to be conserved between different mammalian species.

14:15 (15 mins)

A family of helminth-derived TGF-β mimics provide key insights to Treg and innate immune cell activation.

Presenter: **Dr Kyle Cunningham**, *University of Glasgow*

KT Cunningham[1]*; M van Dinther*[2]*; SP Singh*[1]*; DJ Smyth*[3]*; MP WhiteT CampionV Shek*[3]*; A Mukundan*[4]*; S White*[4]*; AP Hinck*[4]*; P ten Dijke*[2]*; RM Maizels*[5]*;*

[1] *University of Glasgow, UK;* [2] *Leiden University Medical Centre, Netherlands;* [3] *The Division of Cell Signalling and Immunology, University of Dundee, UK;* [4] *University of Pittsburgh, United States;* [5] *School of Infection and Immunity, University of Glasgow, UK*

Helminth parasites have evolved sophisticated methods for manipulating the host immune response to benefit their long-term survival and circumvent therapeutic interventions. A pivotal mechanism for dampening protective immunity is through the secretion of immunomodulatory proteins. Studies on the secreted products of *Heligmosomoides polygyrus* have identified a novel mimic of TGF-β (TGM-1), organised as a 5-domain modular protein. *In vitro,* TGM-1 induces the differentiation of murine and human Foxp3+ T regulatory (Treg) cells via signalling through the canonical TGF-β receptor/SMAD pathway in both murine and human T cells, despite sharing no structural homology to any member of the

TGF-β family. Treg induction requires domains 1-3, while domains 4 and 5 increase the potency of the mimic through binding to co-receptors. Nine additional proteins with significant sequence similarity to TGM-1 are also found in the secretomes of adult (TG 2-6) and larval (TG 7-10) life stages. These TGM family members display contrasting abilities to induce or inhibit Treg cell induction *in vitro,* vary in TGF-β signalling in different cell types, and induce markedly disparate surface expression of key activation markers, including CD39, CD103 and PD-L1. Recently, through co-precipitation, followed by mass spectrometry, a novel co-receptor for TGM-1 has been identified as CD44, a cell surface marker found on many cell types, including effector T cells and macrophages, which is involved in the sensing of hyaluronan upon cellular damage. Therefore, *H. polygyrus* has evolved to secrete TGM-1 to act preferentially on cells which specifically co-express TGF-β receptors and CD44. Indeed, T cells from CD44 knockout mice have a significantly impaired ability to induce Treg cells in response to TGM-1, but have no such impairment in response to TGF-β. We have now identified several additional co-receptors and signalling pathways for each TGM which account for the cell-specific effects of each family member. In addition to Treg cell induction, *in vitro* stimulation of macrophages with certain TG induces an anti-inflammatory state, suppressing secretion of pro-inflammatory cytokines in response to LPS co-stimulation. Understanding these variances will provide key insights to helminth immunomodulation, including the identification of latent co-receptors as well as novel co-stimulatory and signalling pathways that may provide unique targets for drug discovery.

14:30 (15 mins)

Rapid induction of clinical tolerance in a placebo-controlled clinical trial investigating repeated controlled exposure to *Schistosoma mansoni*

Presenter: **Jan Pieter Koopman**, *PhD Student, Leiden University Medical Centre*

JP Koopman[1]*; JJ Janse*[1]*; EL Houlder*[1]*; OA Lamers*[1]*; GV Roozen*[1]*; A Van Diepen*[1]*; JC Sijtsma*[1]*; ST Hilt*[1]*; MY van der Stoep*[1]*; IM van Amerongen-Westra*[1]*; EA Brienen*[1]*; LJ Wammes*[1]*; L van Lieshout*[1]*; GJ van Dam*[1]*; PL Corstjens*[1]*; M Yazdanbakhsh*[1]*; CH Hokke*[1]*; M Roestenberg*[1]*;*

[1] *Leiden University Medical Centre, Netherlands*

Epidemiological data from endemic settings suggests that (partial) immunity to schistosomiasis develops over time, and is likely enhanced by repeated infections and treatments leading to enhanced or prolonged antigen exposure. Moreover, animal studies have demonstrated that protection can be achieved after repeated immunisation with irradiated cercariae. In this study, we aimed to investigate the protective efficacy and safety of consecutive exposure-treatment cycles with *Schistosoma mansoni* (*Sm*) in healthy, schistosome-naïve participants using the single-sex controlled human *Sm* infection model. We enrolled 24 participants who were randomised (1:1) to either three (reinfection) or one (infection control) exposures to 20 male cercariae. The infection control group received two mock exposures first. Treatment with praziquantel (or placebo for infection controls) was given 8 weeks after the first and second (mock) exposure. All participants were treated with praziquantel 12 weeks after the third exposure. Throughout the study, adverse events were collected as well as serum to measure circulating anodic antigen (CAA) secreted by juvenile and adult wor to determine infection status. All but one participant completed follow-up. The percentage of participants with detectable infection after the final exposure (CAA 1.0 pg/mL) in the reinfection group was 82% (9/11) and 92% (11/12) in the infection control group. In the reinfection group, more related adverse events were reported after the first infection (45%) as compared to the second (27%) and third infection (28%). Severe acute schistosomiasis (AS) was observed in both groups after the first infection (2 out of 12 in reinfection group and 2 out of 12 in infection control group), but no AS was reported after the subsequent infections. In conclusion, repeated *Sm* infection led to clinical tolerance, but did not result in (sterile) protection. Further investigation into the underlying immune response will result in better understanding of immunity to schistosomes.

14:45 *(3 mins) Speed Talk*

The interaction of *Schistosoma mansoni* infection with diabetes mellitus and obesity in mice

Presenter: **Dr Alaa Saed Anwer Amer**, *Faculty of medicine Tanta university*

14:48 *(3 mins) Speed Talk*

Structural basis for IL-33 recognition and its antagonism by the hookworm effector HpARI

Presenter: **Dr Abhishek Jamwal**, *Post Doctoral Fellow, University of Oxford*

15:30 (30 mins)

Can RH5-based vaccines succeed against blood-stage *P. falciparum*?

Presenter: **Prof Simon Draper**, *Professor of Vaccinology and Translational Medicin, University of Oxford*

S Draper*[1]*;

[1] University of Oxford, UK

Plasmodium falciparum malaria currently affects 200-300 million people annually, resulting in the death of about 0.6 million individuals. Thus, despite increasing implementation of control measures, the burden of malarial death and disease remains far too high. The most advanced subunit vaccines against *P. falciparum*, called RTS,S/AS01 and R21/Matrix-M, induce high level antibody responses that aim to block infection by the liver-invasive sporozoite. These vaccines have shown moderate efficacy against clinical disease in young children, but this efficacy then wanes over time. Moreover, with every single sporozoite that slips through the net at the liver, a new blood-stage infection is established that brings renewed risk of clinical disease. An effective subunit vaccine against the parasite's asexual blood-stage would thus be highly complementary to the existing anti-sporozoite vaccines. Such a vaccine would reduce mortality, morbidity and transmission of malaria, and offer the prospect for a multi-stage vaccine approach to tackle this parasite's complex lifecycle. However, an effective blood-stage vaccine has proved elusive. Recently, we have developed next-generation vaccines targeting the P. *falciparum* reticulocyte-binding protein homologue 5 (RH5) and its wider invasion complex, which mediate a highly conserved and essential invasion pathway into the human red blood cell. The rational design and delivery of these new vaccines has built on our understanding of how vaccine-induced anti-RH5 human antibody responses are able to inhibit parasite growth, coupled with learnings from human experimental medicine studies. This talk will describe our on-going work to understand anti-malarial antibody responses and present data from our most recent Phase I/II clinical trials of RH5-based blood-stage vaccines in the UK and Africa.

16:00 (15 mins)

Benznidazole uptake by *Trypanosoma cruzi* is a determinant of variable drug efficacy and treatment failure

Presenter: **Prof John Kelly**, *Professor of Molecular Biology, London School of Hygiene and Tropical Medicine*

*F Olmo[1]; FC Costa[1]; RL Atherton[1]; S Jayawardhana[1]; AF Francisco[1]; MD Lewis[1]; MC Taylor[1]; S Alsford[1]; **JM Kelly**[1];*

[1] London School of Hygiene and Tropical Medicine, UK;

Benznidazole (BZ) is the front-line treatment for Chagas disease. However, there is extensive variation in susceptibility within natural populations of the causative agent *T. cruzi*, and treatment failures are widely reported. The underlying reasons for this diverse efficacy are unknown. We used a range of genetic, cell biology and biochemical approaches to dissect the mechanisms of BZ resistance in *T. cruzi*. In combination with high resolution imaging and *in vivo* studies, this allowed us to identify BZ uptake as a major determinant of parasite susceptibility.

We show that BZ uptake by *T. cruzi* is mediated by endocytosis and that stage-specific and strain-specific differences in this process have important roles in drug efficacy. There is also considerable heterogeneity in drug accumulation by amastigotes, the replicative intracellular form of the parasite, even within the same infected host cell. Following uptake, BZ rapidly transits to the mitochondrial network, the site where it undergoes reductive activation. In the infectious, non-replicative trypomastigote life-cycle stage, low-level drug uptake is associated with reduced susceptibility. In addition, naturally resistant parasites have a reduced drug uptake capacity, a phenotype associated with treatment failure in experimental infections. To add further complexity, BZ uptake by mammalian cells, which is also endocytosis-mediated, varies between different host cell types. Our results therefore demonstrate that differences in BZ uptake, acting at several levels, provide a mechanism to explain the wide divergence in sensitivity within the *T. cruzi* population and highlight why sterile cure with this drug can be difficult to achieve.

16:15 (15 mins)

Development of a SHERLOCK molecular diagnostic toolbox for the detection of trypanosomatid parasites

Presenter: **Dr Elena Perez Anton**, *Institut Pasteur*

E Perez Anton[1]*; RJ Eloiflin*[2]*; A Dujeancourt-Henry*[1]*; A Camara*[3]*; JM Bart*[2]*; B Rotureau*[1]*; L Glover*[1]*;*

[1] Institut Pasteur, Paris, France; [2] Institut de Recherche pour le Développement (IRD), France; [3] Institut Pasteur Guinée, Guinea

New diagnostic tools with highly specific and sensitive detection are needed as we move towards control or elimination of a number of neglected tropical diseases. We have adapted SHERLOCK (Specific High-sensitivity Enzymatic Reporter unLOCKing) for the detection of different species of trypanosomatid parasites, including human and animal African trypanosomiasis (HAT and AAT), as well as American trypanosomiasis

(Chagas disease). SHERLOCK uses CRISPR-Cas13a to detect the presence or absence of RNA in a biological sample, allowing for the detection of an active infection. We have now developed (1) an 18S Pan-trypanosomatid SHERLOCK assay, capable of detecting the presence of any trypanosomatid parasites, (2) an 18S Pan-*Trypanozoon*SHERLOCK, (3) an 18S *T. congolense* SHERLOCK, (4) an IFX *T. vivax* SHERLOCK, and (5) an 18S *T. cruzi* SHERLOCK assay, which can discriminate between species of the same family with high specificity in a single sample. This methodology allows us to detect the presence of parasites in skin biopsies as well as in blood samples, at concentrations ranging from 0.5 to less than 0.05 parasites/mL according to the target. Furthermore, in order to adapt this technique to points of care, we are optimising an alternative nucleic acid extraction, and modifying the reaction conditions to reduce the cost, equipment needed, pipetting steps, and time to read-out.

16:30 (15 mins)

Drug tolerance and quiescence in *Leishmania*

Presenter: **Prof Jean-Claude Dujardin**, *Dept of Biomedical Sciences, Institute of Tropical Medicine, Antwerp, Belgium*

M Jara[1]; J Arevalo[2]; A Llanos-Cuentas[2]; F Van den Broeck[1]; MA Domagalska[1]; ***JC Dujardin[1];***

[1] Institute of Tropical Medicine, Antwerp, Belgium; [2] Universidad Peruana Cayetano Heredia, Peru

Drug resistance (DR) and drug tolerance (DT) are intrinsically different mechanisms leading to decreased drug susceptibility (measured by increase of half-maximal inhibitory concentration, IC_{50}) of pathogens. DR is a long-term adaptation acquired through genetic modification, it is heritable and high IC_{50} persist even in the absence of drug exposure. DT is a short-term adaptation resulting from a metabolic modulation and IC_{50} drops to low value, without drug exposure. DT is often associated with quiescence, a physiological state of cells triggered by environmental insults and involving a reversible cell division arrest driven by a dynamic and regulated cell and metabolic remodeling program. Studies on quiescence and DT in *Leishmania* are still in their infancy. In a previous study [Jara et al., 2022] on *L. lainsoni*, we developed two *in vitro* models of quiescence induction, under stationary starvation and Trivalent Antimonials (PAT) pressure, respectively. We found common traits (molecular and metabolic) of quiescence in both models. In a next study, we aimed to demonstrate *in vitro* the link between quiescence and DT in *L. braziliensis* . We exposed 9 strains to high doses of PAT and demonstrated that parasite survivors showed all the criteria of DT and not of DR: (i) under drug pressure, signatures of quiescence (reduced proliferation and significant decrease of rDNA transcription), (ii) the phenotype was reversible and IC_{50} dropped back after PAT removal, (iii) and albeit there were genetic differences between pre- and post-exposure lineages of each strain, they were not consistent and no reported markers of DR were encountered. Finally, when comparing different strains, we found different levels of quiescence and DT in the *L. braziliensis* sample under study. We discuss how DT may hamper the clinical efficacy of drugs and why/how it should be taken into account in R&D for new anti-*Leishmania* drugs.

16:45 *(3 mins) Speed Talk*

Deep mutational resistance profiling of an anti-trypanosomal proteasome inhibitor

Presenter: **Simone Altmann**, *University of Dundee*

16:48 *(3 mins) Speed Talk*

Safety and preliminary protective efficacy of immunisation with genetically attenuated Pf mei2 (GA2) malaria parasites in healthy Dutch volunteers

Presenter: **Olivia Lamers**, *Promovendus, Leiden University Medical Center*

16:51 *(3 mins) Speed Talk*

Preclinical evaluation of a novel nucleoside analogue for the treatment of animal trypanosomiasis

Presenter: **Kayhan Ilbeigi**, *PhD Student, University of Antwerp*

16:54 *(3 mins) Speed Talk*

Reticulocyte Binding-like Proteins as potential vaccine targets for *Plasmodium knowlesi* and *Plasmodium vivax*

Presenter: **Sophia DonVito**, *PhD Candidate, London School of Hygiene and Tropical Medicine*

Day 3 - 13-April - 2023

Plenary 3 - (McEwan Hall)

11:00 (45 mins)

Hookworm dynamically respond to Type 2 immune pressure

Presenter: **Prof De'Broski Herbert**, *University of Pennsylvania*

D Herbert*[1]*;

[1] University of Pennsylvania, United States

This lecture will review current controversies over the ability of gastrointestinal nematodes to develop drug resistance and adapt to an aging host. The core of the data presented will center on unpublished studies that take an experimental evolution approach to understand how the rodent hookworm *Nippostrongylus brasiliensis* adapts to different murine hosts that have various levels of Type 2 inflammatory capability. Using a variety of biochemical and transcriptional approaches, this work will highlight the dynamic interplay between a model metazoan and its mammalian host.

Plenary 4 - (McEwan Hall)

11:45 (45 mins)

Anthelmintic treatment dampens immunosenescence in a wild mammal with consequences for survival

Presenter: **Prof Vanessa Ezenwa**, *Yale University*

V Ezenwa*[1]*;

[1] Yale University, United States

Many organis show age-related declines in immune function (i.e., immunosenescence). In humans and some animals, immune aging has been linked to inadequate responses to infection and higher mortality risk. Interestingly, infection can also shape immunosenescence. For example, chronic viral infections (e.g., HIV, cytomegalovirus) in humans can accelerate immune system senescence. Thus, patterns of natural infection may play a non-negligible role in determining immunosenescence trajectories, and variation in individual infection history may help explain both the rate and survival consequences of immune aging. To understand whether common parasite infections contribute to immunosenescence and its potential survival costs, we examined the impact of long-term anthelmintic treatment on age-related changes in immunity in a wild mammal (African buffalo). We found that treatment slowed the rate of senescence for 6 out of 11 focal immune traits and buffered the mortality costs of age-related immune dysregulation. Our findings reveal a potential role for chronic helminth infection in driving patterns of immune aging and its fitness consequences in wild populations.

Equality & Diversity - (McEwan Hall)

13:30 (12 mins)

Race and Gender in Science

Presenter: **Dr Annamaria Carusi**, *Director, Inter-Change Research*

A Carusi*[1]*;

[1] Interchange Research, UK

The Emerging Research Cultures project supports a community of practice among Wellcome funded PhD programmes, reflecting on and sharing practices regarding research culture in PhD training and the biosciences broadly. As a core aspect of positive research culture, EDI has been a central topic of the community. In this presentation, Annamaria Carusi, PI of the project, will talk about ways of addressing EDI in the biosciences: considering both who is included among those selected to positions in science, and also the content of science, considering the different routes through which bias affects which topics and questions scientists focus on, and how they go about doing science.

13:42 (12 mins)

Equality, Diversity and Inclusion at UKRI: research and innovation by everyone, for everyone

Presenter: **Dr Jo O'Leary**, *Head of Equality, Diversity and Inclusion Strategy, UK Research and Innovation*

J O'Leary*[1]*;

[1] UK Research and Innovation, UK

UK Research and Innovation (UKRI) published its first Equality, diversity and inclusion (EDI) strategy in March 2023. It sets out UKRI's ambition for a more diverse and inclusive research and innovation system. Equality, diversity and inclusion are integral to UKRI's vision and mission. Including and valuing a broader range of people and talent will help to achieve the extraordinary potential of research and innovation to improve lives, promote economic growth, and support a knowledge economy that benefits everyone. This presentation will give an overview of the approaches UKRI is taking to create a more inclusive research and innovation system, where people, creativity, and ideas can flourish.

13:54 (12 mins)

Panel Discussion with Karen Halliday, Jo O'leary and Annamaria Carusi.

Presenter: **Prof. Karen Halliday**, *Dean of Systematic Inclusion, University of Edinburgh*

BSP Presidents Medal - (McEwan Hall)

15:00 (30 mins)

Biomonitoring and long-term studies on sylvatic rodents in Poland – the good, the bad and the effort

Presenter: **Prof Maciej Grzybek**, *Associate Professor, Medical University of Gdansk*

M Grzybek[1];

[1] Medical University of Gdansk, Poland

Introduction: Rodents, members of the most abundant and diversified mammalian order Rodentia, can pose a significant threat to the health of humans, livestock, and wildlife because they are hosts for a wide range of pathogens and, in some cases, constitute essential reservoir hosts for life-threatening zoonoses. Although short-term cross-sectional studies are useful as a starting point to obtain a comprehensive ecologic picture, long-term monitoring (several years and preferably a decade or longer) and a multisite approach are crucial to identify rodent species that can serve as reservoirs for zoonotic pathogens. Predicting regions where new outbreaks are most likely to happen is an essential step for preventing and minimizing the extent of zoonotic disease among humans. Here, I present the results of 22 years of biomonitoring of sylvatic rodents in NE Poland conducted within the PolVole project. I focus not only on the results per se but also on different issues linked to long-term studies. Methods:Our study sites are located in the Mazury Lake District region in the northeast corner of Poland. Over 22 years of study, our objectives were to monitor the prevalence/seroprevalence and abundance of a wide group of parasites in the four abundant vole species found in the region (Myodes glareolus, Microtus arvalis, Microtus agrestis, and Alexandromys oeconomus) and to assess variation in their ecology dynamics attributable to both intrinsic and extrinsic factors that were quantified. Results: We report an analysis of intrinsic and extrinsic factors on the seroprevalence, prevalence and abundance of a broad group of pathogens (both zoonotic and nonzoonotic). While some pathogen species have fluctuated markedly (e.g., some helminths and haemoparasites) or have even become locally extinct in our study sites, others have shown relative stability from year to year. Discussion: Results of our long-term biomonitoring provide a significant and novel contribution to our understanding of the ecology of parasites within vole populations. We underline the role of long-term studies that are necessary to comprehensively reveal the status of parasites in wildlife and assess the risk of possible infection, outbreaks, or spillovers. The World Organization for Animal Health recommends assessing infections in wild rodents to enable effective control and thereby reduce exposure of domestic animals and humans to zoonotic parasites. However, all appropriate action should be carried out with due regard for animal welfare and biodiversity.

BSP Wright Medal - (McEwan Hall)

15:30 (60 mins)

Supporting NTD drug discovery through comprehensive mechanism of action studies and Tales from the world's longest postdoc!

Presenter: **Dr Susan Wyllie**, *Principal Investigator, University of Dundee*

S Wyllie[1]

[1] University of Dundee, UK

The development of new drugs to treat kinetoplastid and many other infectious diseases has been hampered by a severe lack of robustly validated drug targets. This has left drug discovery progra heavily reliant upon phenotypic screening to identify suitable chemical start points. Development and optimisation of phenotypically-active compounds is also hindered by lack of information regarding mechanism(s) of action (MoA) and molecular target(s). Specifically, knowledge of molecular targets is often crucial in developing strategies to overcome issues such as poor pharmacokinetics and toxicity. When targets of phenotypically-active compounds are identified, target- and structure-based drug discovery programmes can be initiated allowing optimisation based on selectivity over human orthologues. Thus, comprehensive MoA studies can effectively integrate these two, often disconnected, approaches to drug discovery. Historically, MoA studies have been of secondary consideration for drugs being developed for neglected tropical diseases. If these studies were carried out at all, they were initiated after the development of pre-clinical or clinical candidates. Over the last 8 years, my group have developed an integrated drug target deconvolution platform, employing a range of methodologies encompassing high-throughput genetics, cell biology and chemical proteomics. Using this platform our aim is to provide MoA input and information in real-time for on-going drug discovery programmes. This enables the data we provide to guide and indeed drive the evolution of the best possible drug candidates. Details of my group's integrated approach to drug target identification in kinetoplastids will be provided and I will describe how we are transitioning this approach to study MoA in *Cryptosporidium*, *Plasmodium* and Schistosoma.

14-April-2023, at 09:30 (30 mins)

Mitochondrial reactive oxygen species control cellular differentiation of *Trypanosoma* parasites

Presenter: **Dr Alena Zíková** , *Biology Centre, Institute of Parasitology, Czech Academy of Sciences*

A Zikova[1]*; M Kunzová*[1]*; E Doleželová*[1]*; B Panicucci*[1]*;*

[1] *Institute of Parasitology, Czech Academy of Sciences, České Budějovice, Czech Republic*

Cellular differentiation of *Trypanosoma brucei* within its two distinct hosts includes at least five different life cycle forms. Their specific gene expression profiles point to differences in, for example, the cell surface proteome, carbohydrate metabolism, mitochondrial bioenergetics, and mitochondrial ultrastructure. The drivers and molecular mechanisms that control mitochondrial metabolic remodeling are still largely unknown. When characterizing the changes in mitochondrial metabolism of insect life cycle for generated in vitro, we found that parasite differentiation from procyclics to epimastigotes and metacyclics is accompanied by an increase in mitochondrial reactive oxygen species (mROS), known signaling molecules. When mROS were reduced by genetically introduced scavengers (catalase, mitochondrial catalase, superoxide dismutase), parasite differentiation was severely impaired. In contrast, when mROS production was artificially increased, the parasite differentiated more efficiently from epimastigote to metacyclics. We linked mROS production to higher proline consumption in epimastigotes, which generates high levels of NADH, suggesting involvement of NADH dehydrogenases in mtROS production. We have also shown that mROS, rather than an increase in the AMP /ATP ratio, is critical for activation of AMP activated protein kinase (AMPK), a cellular energy sensor that promotes cell survival under environmental stress. Our data suggest that the parasite has adapted generic stress pathways to drive its differentiation into metabolically quiscent metacyclic cells.

10:00 (12 mins)

The role of MICOS in mitochondrial maturation during *Trypanosoma brucei* differentiation

Presenter: **Dr Corinna Benz**, *Postdoctoral Researcher, Institute of Parasitology, Biology Centre, Czech Academy of Sciences*

C Benz[1]*; S Sheikh*[1]*; T Wagner*[2]*; U Koblar*[2]*; T Bílý*[1]*; M Tesařová*[1]*; J Lukeš*[1]*; H Hashimi*[1]*;*

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Invaginations of the inner mitochondrial membrane, so-called cristae, come in a variety of shapes and forms. They compartmentalise electron transport chain complexes, confined by diffusion barriers called cristae junctions (CJ), thus making them vitally important for efficient energy generation in mitochondria. The mitochondrial contact site and cristae organising complex (MICOS), found at these CJs, plays a role in crista formation and providing contact sites between inner and outer membrane through interaction with outer membrane protein Sam50.

While the mammalian bloodstream form (BSF) of the *Trypanosoma brucei* parasite has a tubular mitochondrion with tiny, stub-like cristae the insect procyclic form (PCF) possesses an elaborately branched, reticulated organelle with fully developed discoidal cristae harbouring electron transport chain complexes. Differentiation between these two life cycle stages can be induced *in vitro*, thus making the parasite an ideal model system to study cristae development. *T. brucei* MICOS consists of nine proteins and for two subcomplexes which differ in their localisation and function in the PCF. The membrane-embedded integral subcomplex of MICOS is important for CJ formation and maintaining cristae shape while the peripheral complex see to be essential for import of intermembrane space proteins by an oxidative folding pathway.

We show here that the core subunits of MICOS as well as interactions with SAM50 and ATP synthase are conserved in the BSF. Interestingly, four novel interactors were also discovered perhaps indicating additional or divergent functions of MICOS in this life cycle stage. Consistent with this notion, and in contrast to the situation in the PCF, gene deletion mutants of peripheral subcomplex subunits are viable in the BSF. Furthermore, the conserved trypanosome Mic10 homolog, which is part of the integral subcomplex, is also dispensable for differentiation to the PCF.

In contrast, the absence of peripheral trypanosome-specific MICOS components Mic34 or Mic40 compromises differentiation and mitochondrial morphology, correlated with defective electron transport chain complex assembly. This is most likely due to the two proteins' central yet still undefined role in protein import in the PCF life stage. This interdependency between mitochondrial protein import and morphology is also exemplified by the effects of RNAi-mediated depletion of Mic34 and Mic40 in the PCF. The highly branched mitochondrion normally found in this life cycle stage is rendered more simplistic and tubular like its BSF equivalent when Mic34 or Mic40 levels are reduced. This clear division of labour between the two trypanosome MICOS subcomplexes, with the peripheral one being vital for the stability of proteins imported into the

intermembrane space, and the dispensability of the Mic10 homolog sets *T. brucei* MICOS apart from its opisthokont (*e.g.* yeast and animal) counterpart.

10:12 (12 mins)

Mitochondrial DNA dynamics in trypanosomatid parasites: a story of loss and gain

Presenter: **Zihao Chen**, *University of Edinburgh*

Z Chen[1]*; N Savill*[1]*; E Wadsworth* *[1]*; F Van den Broeck*[2]*; P Buscher*[2]*; M Geerts*[2]*; A Schnaufer*[1]*;*

[1] *University of Edinburgh, UK;* [2] *Institute of Tropical Medicine, Antwerp, Belgium*

The flagellate protozoa of class Kinetoplastida are characterised by extraordinarily massive and complex mitochondrial DNA, the kinetoplast (kDNA). Members of genus *Trypanosoma*, important human and animal parasites, have kDNA comprised of two types of interlinked DNA molecules: a few dozen identical maxicircles, which are the equivalent of mitochondrial DNA in other eukaryotes, and thousands of highly heterogeneous minicircles, which encode short "guide RNAs" (gRNAs) that complement genetic information missing from maxicircle-encoded mRNAs. Hence, producing complete open reading frames (ORFs) from maxicircle encoded genes requires post-transcriptional RNA editing directed by gRNAs. Some genes, such as cytochrome oxidase subunit 3 and F1FO-ATP synthase subunit a, are edited extensively and involves dozens of different gRNAs and thus minicircle classes in restoring the ORFs.

Using next-generation sequencing and a bespoken bioinformatics pipeline for kDNA analysis, we demonstrate that different life-history, reproduction and transmission strategies, in closely related trypanosomatids have had profound impacts on their kDNA genome. Subspecies of *Trypanosoma brucei* that regularly cycle between their mammalian hosts and tsetse fly vectors have highly complex and redundant kDNA genomes, with hundreds of minicircle classes. Imperfect kDNA replication and segregation result in loss of minicircle classes, which is countered by sexual recombination in the tsetse fly vector given the bi-parental inheritance of kDNA. Consequently, kDNA complexity and redundancy (but not size) are streamlined in lab-adapted strains and chronic infections in the field that reproduce strictly clonally, as such strains contain only the minimal set of gRNA genes required to maintain viability. Extensive proliferation in the mammalian stage further reduces the gRNA repertoire, so that the strains have lost the ability to produce ORFs from genes required only for survival in tsetse vectors.

10:24 (12 mins)

The role of unique *Leishmania* respiratory enzymes in mice infections

Presenter: **Margarida Duarte**, *Research Assistant, i3s-Instituto de Investigação e Inovação em Saúde*

M Duarte[1]*; AM Tomás*[1]*;*

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Mitochondria are multifaceted organelles with a crucial role in energy production through the oxidative phosphorylation pathway. NADH and FADH2 electrons enter the respiratory chain (RC) and are transferred to oxygen in a process coupled to the translocation of protons into the mitochondrial intermembrane space that fuels ATP production. As in most eukaryotic cells, *Leishmania* RC is made up of complexes I through IV. Aside from these enzymes, *Leishmania* contains at least three extra and unique enzymes that cause RC bifurcations: i) type II NADH dehydrogenase (NDH2), that bypasses complex I and oxidizes NADH without coupled proton pump; ii) fumarate reductase (FRD) that, together with complex II, allows electrons from NADH to enter the RC; and iii) cytochrome c peroxidase (CCP) that bypasses complex IV and transfers electrons to H2O2. The relative contribution of these enzymes for *in vivo* parasite survival is yet unclear.

Expression of the different enzymes was evaluated in both *Leishmania infantum* (a visceralizing species, Li) and *L. major* (a cutaneous species, Lm) through western blot analysis and oxygen consumption assays with intact parasites. Subcellular localization was addressed by immunofluorescence studies and western blot analysis. Gene deletion was generated by CRISPR Cas9 techniques and mutants' ability to prosper in animal models of infection was evaluated in mice.

NDH2 protein is expressed in both the promastigote and amastigote stages of *L. infantum*, while complex I activity was not detected. Overexpression of *Li*NDH2 was found to increase basal oxygen consumption of intact parasites, confirming the enzyme as a respiratory chain component. Moreover, we found that *Li*NDH2 is essential in *L. infantum*, including in the disease-causing stage. In fact, i) deletion of both *ndh2* alleles is only possible upon complementation with an episomal copy of the gene, ii) knockout promastigotes and amastigotes do not lose the *Lindh2* episome after multiple passages in culture in absence of drug pressure, in contrast to a control episome that is lost after few cycles of parasite replication, and iii) single knockout ndh2$^{+/-}$ parasites are less virulent than the wild type in mice. Furthermore, NDH2 is also essential in *L. major* a species expressing active complex I. FRD is expressed in promastigotes *L. major* displaying higher levels than *L. infantum*. Attempts to delete the *Lifrd* gene reveal its non-essential character even for *in vivo* infections. Mutant Lifrd$^{-/-}$ parasites are, however, less virulent than wild type in mice. CCP is highly upregulated in amastigotes of both *L. infantum* and *L. major* although it is not essential for

either parasite survival. In fact, both Li and Lm ccp knockouts are not only able to infect mice but give rise to higher parasite burden in the liver (for Li) and increased footpad swelling (for Lm, Pal et al., 2010), when compared to wild type infections, suggesting that CCP controls parasite proliferation. Based on these results, our hypothesis is that the enzymatic activity of CCP is central for *Leishmania* to become persistent. Our working model predicts that, by acting as a complex IV competitor, CCP can lower the efficiency of the oxidative phosphorylation, and, consequently, reduce parasite numbers. This respiratory chain modulation promotes long-term parasite persistence and, hence, adaptation to host defensive strategies both immune and drug-mediated.

In short, NDH2 is essential for parasite survival *in vivo*, regardless of the presence of a functional complex I. FRD and CCP are non-essential genes that decrease or increase parasite virulence, respectively, when deleted. The current focus is on understanding the involvement of these proteins in persistence of both species.

Funding: This work was supported by National Funds through FCT - Fundação para a Ciência e a Tecnologia, I.P.,under the project UIDB/04293/2020.

10:36 (12 mins)

Dissecting fatty acid metabolism in the livestock parasite *Trypanosoma congolense*

Presenter: **Dr Pieter Steketee**, *BBSRC Discovery Fellow, The Roslin Institute, University of Edinburgh*

PC Steketee[1]*; E Dickie*[2]*; E Paxton*[1]*; S Young*[3]*; T Smith*[3]*; MP Barrett*[4]*; LJ Morrison*[1]*;*

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Animal African Trypanosomiasis (AAT) is a livestock disease prevalent across sub-Saharan Africa, primarily caused by *Trypanosoma congolense*. Whilst the closely related *T. brucei* has been studied for decades, there is a paucity of knowledge regarding the biology of *T. congolense*. We are using a combination of omics techniques to study core metabolism of bloodstream stage *T. congolense*. Like the bloodstream stage of *T. brucei*, glycolysis plays a major part in *T. congolense* energy metabolism. However, the rate of glucose uptake is significantly lower in *T. congolense*, and the glycolytic endpoints differ from those in *T. brucei*. Through the use of metabolic inhibitors we have shown that *T. congolense* is highly resistant to inhibitors of fatty acid synthesis, and instead, appears to rely on uptake to meet its lipid demands. These data have been used to explore fatty acid synthesis and metabolism in African trypanosomes, and to design media formulations that enable *in vitro* culture of the parasite in FBS-supplemented medium, which was previously not possible.

10:48 (12 mins)

Kinetoplastid pantothenate kinase is a unique and essential multi-functional enzyme

Presenter: **Dr Martin Taylor**, *Associate Professor, London School of Hygiene & Tropical Medicine*

RB Roscoe[1]*; F Olmo*[1]*; JM Kelly*[1]*; FC Costa*[1]*;* ***MC Taylor***[1]*;*

[1] London School of Hygiene and tropical Medicine (LSHTM), UK

Pantothenate kinase (PANK), a ubiquitous enzyme required for coenzyme A biosynthesis, is an antimicrobial drug target with proven essentiality to growth of bacteria, fungi and Apicomplexa. Kinetoplastids, including pathogens *Trypanosoma cruzi*, *T. brucei* and *Leishmania*, possess a unique PANK wherein the PANK gene has undergone fusion to two other enzyme domains. Homology suggests the presence of phosphodiesterase and adenylating activity within these multi-functional proteins and *in vitro* analyses demonstrate their essentiality to parasite growth and survival. Essentiality to *T. cruzi* epimastigotes was suggested by failure to generate homozygous null mutants using CRISPR-cas9 mediated editing. RNAi experiments revealed that PANK is essential to bloodstream-form *T. brucei* and is functionally homologous in the two organisms, allowing *T. brucei* to be used as a model for analysis of the *T. cruzi* PANK (TcPANK). Using this model, we showed that the amino acid residue R1270 in TcPANK is critical for enzyme activity and that the unique fused domains are required for growth. In addition, TcPANK undergoes potent negative feedback inhibition by coenzyme A and acetyl coenzyme A. These are the first characterisations of the unique kinetoplastid PANKs and evidence for therapeutic exploitability of the coenzyme A synthesis pathway in these organisms.

Protists 5: Genomics and Evolution - (McEwan Hall)

11:30 (30 mins)

Darwin in a dish: Experimental Evolution reveals novel mechanisms of *Leishmania* fitness gain

Presenter: **Prof Gérald Spaeth**, *Institut Pasteur*

G Spaeth[1];

[1] Institut Pasteur, France

Genome instability plays a central yet poorly understood role in human disease. Iterations between genetic amplification and environmental selection drive cancer development, microbial infection and therapeutic failure, thus increasing human mortality. The molecular mechanisms that harness the deleterious effects of genome instability to generate beneficial phenotypes in these pathogenic systems are largely unknown. An ideal disease model to address this important open question is provided by the protozoan pathogen *Leishmania* that causes devastating human and veterinary infections and exploits a wide variety of genetically heterogenous animal and insect hosts, thus undergoing constant adaptation. In the absence of transcriptional regulation, these early-branching eukaryotes exploit frequent variations in chromosome and gene copy number to regulate expression levels. We apply ecological genomics and experimental evolution approaches to assess how *Leishmania* genome instability generates genetic heterogeneity, how this is translated into selectable phenotypes, and how comparative systems analyses can inform on molecular markers underlying parasite fitness gain. Our results draw a complex picture of *Leishmania* evolutionary adaptation in the field and in culture that relies (i) on co-amplification of functionally related genes that establish complex fitness phenotypes, (ii) on frequent gene deletion and post-transcriptional, compensatory responses that significantly increase the parasite fitness landscape, and (iii) on dynamic changes of small nucleolar (sno) RNAs that can program epitranscriptomic and translational regulation, thereby providing proteomic robustness to genetically heterogenous parasite populations [1]- [5]. This complex adaptation process allows to maintain genetic heterogeneity and thus evolvability of the parasite population despite continuous selection inside equally heterogenous vertebrate and invertebrate hosts. Data will be presented that investigate the link between *Leishmania* genome instability and fitness gain in relevant animal models conducting Experimental Evolution in hamsters and sand flies. Novel insight into *Leishmania* adaptation will be likely applicable to other fast evolving eukaryotic systems with unstable genomes, such as fungi or cancer cells.

References [1] Prieto Barja et al., Nat Ecol Evol. 2017 [2] Bussotti et al., MBio, 2018 [3] Bussotti et al., PNAS USA. 2021 [4] Spath and Bussotti, NAR 2021 [5] Piel et al., PLoS Pathog. 2022

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12:00 (12 mins)

Designing genome-scale strategies for knockout life cycle fitness phenotyping in LeishGEM

Presenter: **Dr Ulrich Dobramysl**, *University of Oxford*

U Dobramysl[1]; RN Neish[2]; E Ferreira[2]; R Pereira[3]; R Etzensperger[3]; M Young[4]; J Smith[5]; J Damasceno[6]; JD Sunter[7]; J Mottram[2]; E Gluenz[3]; R Wheeler[8];

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Despite extensive efforts for the functional characterisation of protein-coding genes in *Leishmania*, the role of most is still unclear. Indeed, according to TriTrypDB only 14% of the 8267 *L. mexicana* protein-coding genes have been unambiguously named, with the large majority remaining of putative function or hypothetical. The major aims of the *Leishmania* Genetic Modification (LeishGEM) project are to address this by 1) determining the fitness of protein-coding gene deletion mutants (genome-wide, 8267 genes) and 2) visualising the sub-cellular localisation of the corresponding protein by tagging (if lacking an ortholog in or divergent from *T. brucei*, 2700 target genes). Using the LeishGEdit toolbox and CRISPR/Cas9, we generate uniquely genetically barcoded deletion cell lines for each gene. These can be mixed into pools and their fitness measured by barcode sequencing (Bar-Seq) as we previously showed. Here, we explain the fitness phenotyping strategies for pools in *in vitro* and *in vivo* models of life stages (promastigote culture, axenic amastigote culture, *in vitro* macrophage infection and mouse footpad infection). Using the Bar-Seq analysis of the first three pools of between 200 and 300 deletion mutants each, we test for A) linearity of the cell abundance using a dilution series of barcode parental cell lines; B) consistency of the fitness phenotypes of six control proteins with known behaviour in specific life stages; and C) potential bottlenecks for cell line survival in various life cycle stages. From this early, not fully randomised sample we detect a significant growth phenotype in 11% of mutants in amastigotes, 10% in macrophages and 21% in mouse footpads. This workflow is

quantitative and scalable and will be applied genome-wide over the coming three years for unbiased identification of the most important *Leishmania* cellular systems for pathogenicity.

12:12 (12 mins)

A central role for the amino acid transporter (AAT1) in Chloroquine resistance evolution in *Plasmodium falciparum*

Presenter: **Prof Timothy Anderson**, *Texas Biomedical Research Institute*

T Anderson[1]*;*

[1] *Texas Biomedical Research Institute, United States*

Malaria parasites break down host hemoglobin into peptides and amino acids in the digestive vacuole for export to the parasite cytoplasm for growth: interrupting this process is central to the mode of action of several antimalarial drugs. Mutations in the chloroquine (CQ) resistance transporter, *pfcrt*, located in the digestive vacuole membrane, confer CQ resistance in *Plasmodium falciparum* and typically also affect parasite fitness. However, the role of other parasite loci in the evolution of CQ resistance is unclear. Here we use a combination of population genomics, genetic crosses and gene editing to demonstrate that a second vacuolar transporter plays a key role in both resistance and compensatory evolution. Longitudinal genomic analyses of the Gambian parasites revealed temporal signatures of selection on a putative amino acid transporter (*pfaat1)* variant S258L, which increased from 0-87% in frequency between 1984 and 2014 in parallel with the *pfcrt1* K76T variant. Parasite genetic crosses then identified a chromosome 6 quantitative trait locus containing *pfaat1* that is selected by CQ treatment. Gene editing demonstrated that *pfaat1* S258L potentiates CQ-resistance but at a cost of reduced fitness, while *pfaat1* F313S, a common Southeast Asian polymorphism, reduces CQ-resistance while restoring fitness. Our analyses reveal hidden complexity in CQ-resistance evolution, suggesting that *pfaat1* may underlie regional differences in the dynamics of resistance evolution, and modulate parasite resistance or fitness by manipulating the balance between both amino acid and drug transport.

12:24 (12 mins)

Emerging parasite resistance in Africa - are we about to see a resurgence in *falciparum* malaria across the continent?

Presenter: **Dr Colin Sutherland**, *BSP President, London School of Hygiene & Tropical Medicine*

CJ Sutherland[1]*; DA van Schalkwyk*[1]*; S Pratt*[1]*; L Stewart*[1]*; D Nolder*[1]*;*

[1] *London School of Hygiene & Tropical Medicine, UK*

Clinical management of uncomplicated malaria caused by *Plasmodium falciprum* is reliant on the effectiveness of artemisinin-based combination therapy (ACT). New parasite genotypes encoding variants of the pfk13 gene are now emerging in Africa, and these are less susceptible to the artemisinin component drugs. This poses a risk of resistance selection against the partner drugs in ACT. Case histories from UK travellers with documented ACT treatment failure and field surveys of resistance gene variants will be presented, together with newly collected *in vitro* susceptibility data for parasites of African origin adapted to long-term culture in 2022. The implications of these findings for future drug strategies for African malaria chemotherapy, and management of imported UK cases, will be considered. Finally, we will consider the wider public health implications of a potential resurgence of artemisinin tolerant *P. falciparum* in Africa.

12:36 (12 mins)

Comprehensive investigation of the *Trypanosoma brucei* kinetoplast and the discovery of a slew of new protein constituents.

Presenter: **Dr Michael Hammond**, *Post-Doctoral Researcher, Biology Centre, Institute of Parasitology*

M Hammond[5]*; J Pyrih*[6]*; L Cadena*[4]*; M Svobodova*[4]*; A Alves*[7]*; S Dean*[8]*; JD Sunter*[1]*; R Wheeler*[9]*; K Gull*[3]*; C Benz*[4]*; V Raskova*[4]*; I Durante*[4]*; J Lukeš*[2]*;*

[1] University of Oxford, UK; [2] Institute of Parasitology, Biology Centre, ASCR, Czech Republic; [3] University of Oxford, Sir William Dunn School of Pathology, UK; [4] Institute of Parasitology, BioCenter, Ceské Budějovice, Czech Republic; [6] Department of Biochemistry, University of Cambridge, UK; [7] Oxford Brookes University, UK; [8] Division of Biomedical Sciences, Warwick Medical School, UK; [9] Peter Medawar Building for Pathogen Research, Nuffield Department of Medicine, University of Oxford, UK

The TrypTag project represents a methodological approach to tag every protein encoded in the nuclear genome of *Trypanosoma brucei*. A specialised mitochondrial investigation of this study (MitoTag), revealed for the first time a fluorescent tag association with the concentrated mitochondrial DNA structure known as the kinetoplast, documented in several hundred mitochondrial proteins. Combined with transmembrane

domain prediction, MitoTag enabled the sub-localisation of 1,053 mitochondrial proteins to this organelle's four compartments, circumventing the need for electron microscopy or other intensive sub-localisation methods. Furthermore, we demonstrate a method to distinguish genuine kinetoplast proteins from artificial tagging-induced associations among mitochondrial proteins, and accordingly demonstrate over a dozen novel kinetoplast components. From this, we expand the functions of the kinetoplast to metabolic pathways of dUMP synthesis and One Carbon metabolism. This constitutes the largest single expansion of the kinetoplast repertoire to date and represents an exciting development for a complex long considered an attractive drug target due to its clade-specific presence.

12:48 (12 mins)

To Per-Cyst or Not: unravelling the secrets behind an attenuated *Toxoplasma* strain

Presenter: **Saniya Crouch**, *Moredun Institute*

S Crouch[1]*; L Berna*[2]*; L Lemgruber Soares*[3]*; J Ovciarikova*[4]*; D Price*[1]*; S Shikha*[5]*; D Walsh*[5]*; D Beraldi*[6]*; L Sheiner*[4]*; D Smith*[1]*;*

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Toxoplasmosis is a zoonotic parasitic disease caused by the obligate intracellular protozoa *Toxoplasma gondii*, which threatens a range of warm-blooded mammals including humans, livestock and zoo animals. To date, the only commercial vaccine to treat toxoplasmosis is Toxovax, comprising an 'incomplete' tachyzoite S48 strain. The molecular and cellular basis of attenuation of the strain remains a mystery.

The aim of this project is to characterise the S48 strain, which will give a deeper understanding of apicomplexan biology and *Toxoplasma* persistence. It could uncover previously unidentified genes essential for parasite persistence and has the potential to identify new therapeutic targets. Furthermore, from a biotechnology and synthetic biology standpoint, understanding the genetic basis of *T. gondii* attenuation can inform the development of *Toxoplasma* as a gene delivery vehicle, in both humans and animals.

We show S48 has an incomplete differentiation phenotype *in vitro*, where it is not able to fully differentiate from the fast-growing tachyzoites to the slow growing, persistent bradyzoite form. In order to identify the genomic cause of this differentiation defect we assembled a high-quality genome of the S48 strain using Oxford Nanopore long reads and DNBseq short reads. GC content was used to separate genomic DNA from the mitochondrial and apicoplast genomes, giving rise to 13 complete chromosomes.

SNP calling identified nine possible loss of gene function mutations unique to S48 relative to five other normally differentiating *Toxoplasma* strains. Based on a range of parameters, we selected two genes with unknown function to focus on. Currently, we are using CRISPR-Cas9 to characterise these genes by both tagging and deleting the gene in the cyst forming Type II ME49 strain. Initial characterisation will focus on determining the effect of gene knockouts on parasite differentiation, including cyst formation and bradyzoite gene expression.

BES Ecology 4: Wild Parasitology: Impacts of Infection on Health and Fitness - Sponsor -Xpedite Diagnostics GmbH (Appleton Tower 1)

Chair: Prof Andy Fenton

09:30 (30 mins)

The impact of parasitism within the family

Presenter: **Dr Emma Cunningham**, *Edinburgh University*

E Cunningham[1];

[1] Edinburgh University, UK

Parasites are a ubiquitous component of all animal populations. Some infections may be acute and short-lived but more commonly, individuals experience on-going chronic levels of infection that impact on host life-history traits throughout life. The impact of infection is likely to be particularly acute during reproduction when energetic demands are high for both parents working hard to raise a family and offspring in rapid phases of growth. However, this is also a time when costs may be passed on to different family members, leading to indirect costs of infection on individuals other than the primary host. How this plays out across the family can shape both responses to infection and other key life-history traits across an individual's life. In this talk, I report on seventeen years of data on the impact of parasitism in a long-term study of seabirds, The European Shag. Both novel techniques to measure natural levels of parasitism and experimental manipulations of parasite burden have demonstrated that responses to parasitism are shaped in early life but can impact on different family members in the longer term in very different ways. Family members also differ in their sensitivity to how responses to parasitism play out across different environmental conditions. Quantifying how these ultimately link to breeding success over both the short and the long term is therefore a major part of understanding how predicted environmental shifts may impact both infection dynamics and their impact on host populations.

10:00 (15 mins)

Resource quality and distribution impacts vectors and vector-borne infections in wild wood mice

Presenter: **Mx Agata Delnicka**, *University of Edinburgh*

A Delnicka[1]; RE Bancroft[1]; SG Hillman[1]; M Fonville[2]; H Sprong[2]; JL HallA Fenton[4]; AB Pedersen[1];

[1] University of Edinburgh, UK; [2] National Institute of Public Health and Environment (RIVM), UK; [3] University of Glasgow, UK; [4] University of Liverpool, UK

Introduction: Vector-borne pathogens (VBPs) cause some of the most ubiquitous diseases to humans and animals. Natural and human-impacted environments fluctuate in resource availability and quality, which can have varied impacts on animal populations – for example, by altering host condition, immunity, behavior or demography – and these processes can result in diverse impacts on disease transmission, particularly in wild reservoirs. We used experimental food supplementation of wild wood mice, *Apodemus sylvaticus*, to test how resource quality and distribution impacted host infection by a range of zoonotic VBPs and vectors.

Methods: We carried out longitudinal rodent trapping at two woodland sites in Edinburgh, UK over two years (6-8 months per year). Within each site, we manipulated i) the quality (high vs low) and ii) the distribution (aggregated vs evenly-spread) of supplemented food available to the wood mice. We counted the number of ticks and fleas infecting rodents and collected host blood and tissue samples, which were screened for the presence of nine zoonotic VBPs. We tested how food supplementation quality and distribution affected host infection with VBPs and ectoparasites, using generalised linear mixed effects models.

Results & Discussion: We found that evenly-spread, high-quality food reduced host infection risk with flea-borne bacteria *Bartonella* spp. compared to non-supplemented areas. When we compared high- and low-quality food, we found that high-quality food reduced the infection risk with ticks; this suppressive effect on ticks was enhanced when the high-quality food was evenly distributed. Together, these results highlight how resource quality and distribution can interact to generate taxon-dependent effects of resource availability on VBP infection risk. These results underscore the importance of using experimental approaches to uncover drivers of disease transmission, whilst embracing the heterogeneities present in wild host-parasite systems

10:15 (15 mins)

The potential mechanistic pathways leading from parasite infection to childhood stunting.

Presenter: **Isobel Gabain**, *PhD Student, Royal Veterinary College*

I Gabain[1]*; J Webster*[1]*; AR Ramsteijn*[2]*;*

[1] *Royal Veterinary College, UK;* [2] *University of Aberdeen, UK*

An estimated 149.2 million children under the age of 5 were physically stunted in 2020, defined as falling at least –2 standard deviations below the height-for-age World Health Organization (WHO) Child Growth Standards median. Stunting is a visible indicator of a deficient environment, the consequences of which include child morbidity and mortality, reduced and delayed neurocognitive development, and an increased risk of long-term chronic diseases. The underlying aetiology and pathophysiological mechanisms leading to stunting remain elusive, and therefore few effective treatment and prevention strategies exist. Here, based on available studies, we present potential mechanistic pathways by which parasitic infection of mother and/or infant may lead to childhood stunting. The most well-recognised pathway to stunting is a 'vicious cycle' between deteriorating nutritional status and infection, which is evolving to encompass dysbiosis of the gut, local and systemic inflammation, alongside energetic, hormonal, and metabolic consequences. Anaemia, which is often presented as coexisting alongside stunting, may in fact be contributing. The bidirectional relationship between intestinal parasites and the microbiota in early life, and their combined effects, may also play a key role in stunting. And finally, epigenetic regulation of gene expression may link parasitic infections and poor gut health in early life to stunting. Guided by these plausible mechanisms, future multidisciplinary longitudinal studies and clinical trials should aim to elucidate the most influential factors, and synergies therein, that can lead to stunting, and ultimately towards finding solutions to successfully mitigate against it.

10:30 (15 mins)

Giardiasis and intestinal pathology: Molecular detection and taxon assemblage typing of *Giardia duodenalis* in school-aged children along the shoreline of Lake Malawi, Malawi

Presenter: **John Archer**, *Research Technician, Liverpool School of Tropical Medicine*

J Archer[1]*; L Cunningham*[1]*; A Juhàsz*[1]*; S Jones*[1]*; F Doull*[1]*; SA Kayuni*[2]*; P Makaula*[2]*; B Mainga*[2]*; JE LaCourse*[1]*; J Musaya*[2]*; JR Stothard*[1]*;*

[1] *Liverpool School of Tropical Medicine, UK;* [2] *College of Medicine, Kamuzu University of Health Sciences (KUHeS), UK*

Giardiasis is a waterborne and potentially debilitating intestinal parasitic disease caused by infection with the eukaryotic protozoan *Giardia duodenalis*. Whilst cosmopolitan in distribution, prevalence of human giardiasis can be particularly high in rural areas of low- and middle-income countries (LMICs) lacking adequate water and sanitation hygiene (WASH) infrastructure, including those in sub-Saharan Africa. Of the eight known morphologically identical but genetically distinct *G. duodenalis* taxon assemblages A through H, the majority of human infections are caused by zoonotic assemblages A and B. Differentiation between human infections with *G. duodenalis* assemblages A and B, as well as between single (A or B)- and mixed (A and B)-assemblage infections, is therefore essential to better understand the pathological impact of infection with either, or both, assemblages and thus also for improved disease surveillance and control. Using end-point PCR with subsequent genotyping and phylogenetic analyses, as well as real-time PCR, we assessed the prevalence of human infection with either, or both, *G. duodenalis* assemblages A and B using faecal samples provided by 305 school-aged children situated along the southern shoreline of Lake Malawi, Mangochi District, Malawi; an area where only limited data on the prevalence of human giardiasis is currently available. In addition, pathology data was also collected from all study participants in the form of lateral flow rapid diagnostic tests (RDTs) that detect overt blood and calprotectin in faeces, and questionnaire responses to the questions 'do you currently have abdominal (stomach) pain?' and 'do you currently have loose stool (diarrhoea)?'. Prevalence of *G. duodenalis* infection was 39.3% when using a species-specific 18S diagnostic real-time PCR. When targeting two additional and distinct genetic assemblage-specific loci, 35% of all infections were identified as single *G. duodenalis* assemblage A; 32% were identified as single *G. duodenalis* assemblage B; and 33% were identified as mixed *G. duodenalis* A and B. No infections were identified as *G. duodenalis* assemblages C-H. Whilst there was no association between single infection with *G. duodenalis* assemblage A and any form of pathology, there was a statistically significant and strong positive correlation between single infection with *G. duodenalis* assemblage B and both self-reporting of abdominal pain and self-reporting of diarrhoea. Additionally, there was a statistically

significant and positive correlation between mixed infection with both *G. duodenalis* assemblages A and B and self-reporting of abdominal pain, but no association between mixed infections and any other form of pathology. Our study therefore further highlights the importance of molecular methods that can be used to identify *G. duodenalis* assemblage types and investigate their impact on human intestinal pathology, whilst also reaffirming the need for improved access to WASH infrastructure in rural areas of low- and middle-income countries.

10:45 (15 mins)

A droplet digital PCR (ddPCR) workflow for the detection of helminth and snail host eDNA in water and soil

Presenter: **Dr Christopher McFarland**, *Research Fellow, Queen's University Belfast*

C McFarland[1]*; E McCann*[1]*; P McCann*[1]*; ER Morgan*[1]*; NJ Marks*[1]*; P McVeigh*[1]*; GN Gobert*[1]

[1] *Queen's University Belfast, UK;*

All organis shed DNA into their surrounding environment (eDNA). Recently eDNA has been used for the detection of parasite species and has presented benefits over traditional sampling methodologies. The liver fluke, *Fasciola hepatica*, exhibits a complex lifecycle involving an aquatic snail intermediate host, commonly *Galba truncatula* at temperate latitudes, and a diverse array of mammalian definitive hosts, including agricultural ruminants and humans. Recently, there has also been growing interest in the rumen fluke species, *Calicophoron daubneyi*, in UK agriculture. The external life stages of rumen fluke complete a lifecycle almost superimposable to that of liver fluke and have been shown to use the same intermediate snail host. Despite the importance of the external environment in the fluke lifecycle, traditional diagnostic methods rely on the detection of infection either directly or indirectly from the definitive host. Recent reports of eDNA isolation of both trematode parasites and their intermediate snail hosts have focused on environmental water samples. Despite *G. truncatula* spending considerable periods of time on mud surrounding water bodies, to date the presence of snail or parasite eDNA has not been examined in soil. The use of fully quantitative droplet digital PCR (ddPCR) platfor provides the opportunity for greater sensitivity and reproducibility of eDNA detection. To investigate the use of ddPCR for the detection of parasite and intermediate snail host eDNA, a workflow was developed utilising environmental samples (water and soil) collected from sheep and cattle far in Northern Ireland. Environmental sample collection methodology and DNA extraction was optimised to allow reliable examination of parasite and intermediate host eDNA. Analysis suggests that it is possible to detect parasite and snail intermediate host eDNA from environmental water and soil samples. The isolation of parasite and/or snail eDNA on a farm may provide pre-emptive warning of host infections earlier than current diagnostics, prompting further investigations and guiding anthelmintic treatment strategies.

BES Ecology 5: Host-Parasite Interactions - Sponsored by Bio Molecular Systems Ltd - (Appleton Tower 1)

Chair: Prof Amy Pedersen

11:30 (30 mins)

The transmission modifying effects of parasite coinfections: insights from wild mice

Presenter: **Prof Andy Fenton**, *Professor, University of Liverpool*

A Fenton[1]***;***

[1] *University of Liverpool, UK*

Although we know coinfecting parasites can strongly affect each other within individual hosts, we know little about the consequences of those within-host interactions for the between-host transmission dynamics of those parasites. I will describe results from a wild mammal host population (UK wood mice) and their diverse parasite communities which show, firstly through drug treatment experiments, that the dominant nematode in the system interacts strongly with a coinfecting ‘subordinate’ parasite, suppressing its abundance within individual hosts. Secondly, through spatiotemporal analyses, we provide evidence of localised ‘coinfection-mediated transmission modification’, whereby that within-host interaction suppresses the transmission potential of the subordinate parasite, reducing its force of infection on neighbouring hosts, but only over limited spatial scales. This suggests that the effects of within-host coinfection interactions can ripple out to shape parasite transmission dynamics within local neighbourhoods, potentially altering the spatiotemporal dynamics of those parasites.

12:00 (15 mins)

The epidemiology of periportal fibrosis

Presenter: **Dr Seun Anjorin**, *Post-doctoral Researcher, University of Oxford*

S Anjorin[1]*; B Nabatte*[2]*; S Mpooya*[2]*; CK Opio*[3]*; NB Kabateriene*[2]*; GF Chami*[4]*;*

[1] University of Oxford, Big Data Institute, UK; [2] Vector Control Division, Ministry of Health, Uganda; [3] Department of Medicine, Makerere University, Uganda; [4] Nuffield Department of Population Health, University of Oxford, UK

Background: Intestinal schistosomiasis can cause periportal fibrosis (PPF). If left untreated, it could result in portal hypertension and ultimately death. However, the epidemiology of PPF is poorly understood, especially in settings endemic to *Schistosoma mansoni*

Methods: A cross-sectional study was conducted within the Oxford-Uganda Collaboration and SchistoTrack Prospective Cohort. During baseline assessments in 2022, a total of 1460 households, nested within 38 villages in three rural districts in Uganda, were randomly selected and surveyed. Demographic, socioeconomic, and medical history information were obtained from each member of the households. One child (5-17 years) and an adult (18-90 years) were randomly selected from each household and invited for the clinical surveys. One stool and urine sample were collected from 2836 participants, they were assessed for *S. mansoni* infection using Kato-Katz (KK) microscopy and point-of-care circulating cathodic antigen (POC-CCA). Following the Niamey Protocol, PPF was defined by the highest liver pattern gradings, patterns A and B were coded as normal while patterns C- F were coded as PPF. Multivariable logistic regressions with standard errors clustered at the household level were used with schistosome infection as a key exposure, controlled for demographic, socioeconomic, biomedical covariates.

Findings: PPF prevalence was 12.1% (343/2836) across all study participants, ranging from 4.8% to 18.9% across the three districts. *S. mansoni* was over 43% in all participants, as measured by KK. Adults and male participants had a higher prevalence of PPF across the districts when compared to children and female participants, respectively. Infection indicators, as measured by KK or POC-CCA diagnostics showed no significant relationship with PPF. Each one-year increase in age was found to be associated with a 15.2% increase in likelihood of PPF. Female participants were 31% less likely to have PPF when compared to male participants. Being a fisherman was significantly associated with the likelihood of PPF (87.2% more likely than individuals who were not fishermen). A history of liver diseases, HIV and hepatitis B diagnoses were found to be significantly associated with a higher likelihood of PPF.

Conclusion: These findings suggest that current schistosome infection should not be used as a proxy indicator for severe morbidities associated with schistosomiasis such as PPF. Future research should investigate the contribution of comorbidities and co-infections to PPF development.

12:15 (15 mins)

Prevalence of *Trichomonas vaginalis* and associated risk factors among pregnant women attending antenatal care in government health facilities, Ambo town, Western Oromia in Ethiopia.

Presenter: **Chala Kumsa**, *Ambo University*

C KUMSA[1]*; A Hailu*[2]*;*

[1] Ambo University, Ethiopia; [2] Addis Ababa University, Ethiopia

Trichomonas vaginalis is one of the causes of non-viral sexually transmitted diseases. This parasite is known to adhere to the human vaginal epithelial cells, surviving for years in the typically acidic and hostile vaginal environment that contains a plethora of microbicidal innate immune factors, and is reinforced by the presence of complex commensal bacteria. Pregnancy is known to be one of the most influencing factors of the genital trichomoniasis. The infection can lead to significant complications in pregnancy. It can cause premature rupture of membranes, preterm labor, low birth weight, and post abortion infections.

Objective: To determine the prevalence of *Trichomonas vaginalis* and assess the associated risk factors among pregnant women visiting ANC unit.

Materials and Methods: A cross sectional study design was conducted among 217 pregnant women attending ANC at four Governmental health facilities found in Ambo town. Socio demographic data and *Trichomonas vaginalis* related risk factors were collected using predesigned questionnaires. Vaginal swab sample was collected and wet mount examination and Giemsa staining method was performed.

Result: A total of 217 pregnant women of age ranging 15 to 44 years with mean age 26.06 ± 4.796 (mean ± SD) had participated in this study. From this, 38(17.5%) of them tested positive for *T. vaginalis* by Giemsa staining technique while 31(14.3%) tested positive by wet mount examination. *T. vaginalis* infection was most prevalent among the 25-34 years age groups comprising 23(60.5%) of the 38 positive cases. In relation to occupation, traders were the most infected 7(21.9%). Also, women in the third trimester of pregnancy were observed to be the most infected 16(24.2%). By multivariate analysis, vaginal discharge (AOR = 3.68; 95% CI: 1.5–9, $P < 0.05$) was observed to have significant association with T. vaginalis infection.

Conclusion: The prevalence of *Trichomonas vaginalis* at 17.5% among pregnant women, which was observed in this study, is relatively high. The finding of this study supports the need for improved control activities of *Trichomonas vaginalis* infection in pregnant women to reduce adverse reproductive health outcomes associated with *Trichomoniasis*.

12:30 (15 mins)

Associations of water contact with schistosome infection: A systematic review and meta-analysis

Presenter: **Fabian Reitzug**, *PhD Researcher, Nuffield Department of Population Health, University of Oxford*

F Reitzug[1]*; J Ledien*[1]*; GF Chami*[1]*;*

[1] *Nuffield Department of Population Health, University of Oxford, UK*

Background: Schistosomiasis is a water-borne parasitic disease but the relationship between water contact and the likelihood of schistosome infection remains poorly quantified.

Methods: We conducted a systematic review in accordance with the PRISMA guidelines to estimate the average effect of water contact duration, frequency, and activities on schistosome infection likelihood. We searched Embase, MEDLINE (including PubMed), Global Health, Global Index Medicus, Web of Science, and the Cochrane Central Register of Controlled Trials from inception until May 13, 2022. Observational and interventional studies reporting odds ratios (OR) or hazard ratios (HR) of associations between exposure and schistosome infection were eligible for inclusion. Random-effects meta-analysis with inverse variance weighting was used to calculate pooled ORs and 95% confidence intervals (CIs).

Results: We screened 1,411 studies and included 101 studies representing 192,691 participants across three continents. Included studies mostly reported on the type of water contact activities (69%; 70/101) and current or past history of having any water contact (33%; 33/101). A meta-analysis of 33 studies showed that individuals with water contact were 3.14 times more likely to be infected compared to individuals with no water contact. Subgroup analyses showed that the positive association of water contact with infection was significantly weaker in children compared to studies which included adults and children (OR 1.67; 95% CI: 1.04–2.69 vs. 4.24; 95% CI: 2.59–6.97). An association of water contact with infection was only found in communities with >10% schistosome prevalence. Overall heterogeneity was substantial (I^2=93%) and remained high across all subgroups, except in direct water contact observation studies (I^2 range=44%–98%). We did not find that occupational water contact such as fishing and agriculture (OR 2.57; 95% CI: 1.89–3.51) conferred a significantly higher risk of schistosome infection compared to recreational water contact (OR 2.13; 95% CI: 1.75–2.60) or domestic water contact (OR 1.91; 95% CI: 1.47–2.48). Higher duration or frequency of water contact did not significantly modify infection likelihood. Study quality across analyses was largely moderate or poor.

Conclusions: Any current water contact was robustly associated with schistosome infection status across all age groups and areas with >10% prevalence. More research is needed to understand interactions of water contact with age and gender and their influence on infection likelihood. Our results imply the need for population-wide treatment and prevention strategies in endemic settings as exposure within these communities was not confined to currently prioritised high-risk groups such as fishing populations.

12:45 (15 mins)

Effect of co-habitation on gastrointestinal parasite prevalence and burden in wild and domestic herbivores in Maasai Mara National Reserve, Kenya

Presenter: **Kim van de Wiel**, *PhD Scholar in Biological Sciences, University of Liverpool*

K van de Wiel[1]*; AC Fenton*[1]*; F Kenyon*[2]*; J Bro-Jorgensen*[1]*;*

[1] *University of Liverpool, UK;* [2] *Moredun Research institute, UK*

Parasite transmission between wildlife and livestock is common. Of great importance are ubiquitous gastrointestinal nematodes and coccidia, which are associated with livestock production losses and can interfere with natural ecological processes in wildlife. These parasites are of growing concern, especially in places where livestock-wildlife interactions are increasing, such as the Maasai Mara ecosystem in Kenya. To determine the occurrence of gastrointestinal parasites and cross-species transmission in this region, we quantified nematode egg (*Strongyles spp., Strongyloides spp.*) and coccidia oocyst (*Eimeria spp.*) counts and nematode infective larvae from ~1000 faecal samples of wild and domestic herbivores (>10 kg) across mixed livestock-wildlife and single-occupancy pastures. Through generalised linear models, individual parasite prevalence and intensity were analysed as functions of 'area type' (single vs. mixed occupancy) and 'species type' (livestock vs wildlife) to explore (a) whether wildlife and livestock differed in parasite infection levels, and (b) whether those infection levels for each animal type differed depending on whether they shared pastures with animals of the other type. We generally found wildlife to have significantly higher parasite prevalence and intensities than livestock, likely reflecting the impact of management practices on reducing infection risks in livestock. We found no area type effect on the prevalence of nematodes in both wildlife and livestock. However, animals browsing or grazing on mixed

pastures had significantly higher intensities of nematodes than those on single-occupancy pastures. For coccidia, we found the opposite: prevalence and infection intensities were significantly lower for animals on communal grasslands. These results suggest that livestock-wildlife co-habitation could have community-wide implications for cross-species transmission of parasites. A next step is to identify individual nematode species through next-generation sequencing of the larval nemabiome and use this to develop a multi-host epidemiological model, to determine the directions of transmission for the different parasite species within these wildlife-livestock communities.

Veterinary Parasitology - (Appleton Tower 2) Chair: Dr Adam Hayward

09:30 (30 mins)

Making roundworm data ewe-niversal for all

Presenter: **Dr Fiona Kenyon**, *The Moredun Institute*

F Kenyon[2]; E Geddes[2]; J Duncan[2]; C Morgan-Davies[3]; A McLaren[3]; N Sargison[4]; P Skuce[2]; ER Morgan[1]; L Stubbings[5];

[1] School of Biological Sciences, Queen's University Belfast, UK; [2] Disease Control, Moredun Research Institute, UK; [3] SRUC Hill and Mountain Research Centre, Kirton and Auchtertyre, Crianlarich, UK; [4] Royal (Dick) School of Veterinary Studies, Edinburgh University, UK; [5] Sustainable Control of Parsites in Sheep, UK

Roundworm parasites are a leading cause of lost productivity in grazing livestock. When combined with increasing reports of anthelmintic resistance, these parasites present a serious threat to sustainable sheep farming in the UK. Current advice suggests that regular monitoring of worm challenge (through Faecal Egg Counting (FEC)) and anthelmintic efficacy testing, together with avoidance of 'blanket' or whole group anthelmintic treatments are critical steps in slowing the development of anthelmintic resistance. However, to enable sheep farmers to adopt this approach, there are a number of obstacles, including difficulties in the interpretation of efficacy testing (such as the Faecal Egg Count Reduction Test (FECRT)) which is also expensive and labour intensive. Two recent studies on commercial far (n=26) across the UK have focused on understanding whether a simplified 'drench check' could provide useful information on-farm, while reducing the challenges to uptake compared to a/the FECRT. Pre- and post-treatment faecal samples were analysed for FEC, anthelmintic efficacy and nematode species composition. Results suggested that practical information could be achieved using the simpler 'drench check' protocol. However, while this is useful information for the research community, it needs to be made available to farmers in a more easily understandable format, so they can be empowered to make informed decisions to tackle the proble caused by wor on their farms. We have developed translation tools, through co-design with end-users, to give farmers access to this type of information. One example is the 'FEC Check' app, which graphically visualises FEC results, including basic advice on interpretation including in the context of production-limiting disease. In summary, nematodes and anthelmintic resistance are a combined threat to sustainable livestock farming in the UK and beyond. The people most able to address these challenges are farmers and we have developed resources and tools to enable farmers to make evidence-based decisions regarding the interventions required on their farm.

10:00 (15 mins)

Integrating multi-species swards into parasite management in sheep under climate change

Presenter: **Nicole Henry**, *Queen's University Belfast*

NH Henry[1]*; A Aubry*[2]*; A George*[1]*; C McFarland*[1]*; F lively*[2]*; K Theodoridou*[1]*; ER Morgan*[1]*;*

[1] *Queen's University Belfast, UK;* [2] *Agri-Food Biosciences Institute, Hillsborough, UK*

Background and introduction Gastrointestinal nematodes (GIN) are a significant cause of disease in grazing ruminants causing reduced health and productivity. Anthelmintic resistance is a widespread issue in GIN and a major issue faced by the livestock industry is the necessity to control parasites effectively while reducing chemical anthelmintic use, to ensure continued drug efficacy and to reduce negative environmental consequences. The negative impacts of parasites and the requirement for anthelmintic treatment can be reduced by grazing multi-species swards (MSS), which can lower parasite burdens while enhancing nutrition. Although the effects of individual plant species on parasites, and the impacts of MSS on animal performance have been assessed (e.g., Marley 2003, 2006; Athanasiadou 2004), there is a lack of information on the epidemiological consequences of grazing on MSS and how to design MSS grazing platfor to maximize parasite management benefits. Materials and Methods Parasite infections were compared over 5 months in 3 groups of lambs (n=100, 100, 100) rotationally grazing either perennial ryegrass with white clover (PRG), PRG with white clover additionally enriched with red clover, ribwort plantain and chicory (MSS) or grazing alternately on PRG and MSS (50-50). Nematode faecal egg counts (FEC) monthly, pasture larval counts (PLC) (Molento 2016) at three time points, and end-of-season abomasal worm counts were measured. To evaluate the epidemiological consequences of reductions in parasite burdens through decreased onward infection pressure, FEC data were entered into a mechanistic predictive model of nematode population dynamics (Rose 2015), extended to account for sheep movement between fields (McFarland 2022). The same model was used to simulate different rotation intervals under current and projected future climates across the UK, to maximise impacts of reduced FEC and to evaluate the feasibility of optimising both epidemiological benefits and grass / MSS utilisation. **Results** No significant differences in pasture larval count were observed between the PRG, 50-50 and MSS paddocks ($p>0.05$) but there was a significant difference between the 50-50 group and the PRG group ($p<0.05$) in FEC on the final sampling date and the end of season abomasal worm counts. As FEC was not reduced in the MSS group, modelling did not predict reduced infection pressure on MSS fields. Simulations showed that rotational grazing did not have significant benefits for GIN infection pressure over set-stocking the same area of land, unless re-grazing intervals were extended beyond realistic limits. Climate change scenarios tended towards more rapid development of infective larvae at pasture, and more rapid decline in infectivity, affording opportunities for greater control through rotational grazing provided residence periods were kept below one week. **Conclusions** Although MSS may have offered benefits for performance of grazing lambs under natural GIN infection, these could not be attributed to antiparasitic activity and might be primarily nutritional. Applying modelling alongside data collection can help predict the seasonal risk of transmission of GINs, and these models can be extended to optimise the implementation of this novel combination of control strategies.

10:15 (15 mins)

RNA interference: a functional tool for screening potential vaccine targets in the poultry red mite *Dermanyssus gallinae*

Presenter: **Wan Chen**, *PhD Student, Moredun Research Institute*

W Chen[1]*; JM Sternberg*[2]*; AS Bowman*[2]*; AJ Nisbet*[1]*; S Burgess*

[1] *Moredun Research institute, UK;* [2] *University of Aberdeen, UK*

The Avian haematophagous ectoparasite, the poultry red mite (PRM), *Dermanyssus gallinae*, affects the health and welfare of poultry, bringing substantial economic losses to the layer industry worldwide. Current acaricide-based controls are limited by ineffective application and emerging resistance. A sustainable control method or effective vaccine is therefore urgently needed by the egg laying industry. RNA interference (RNAi) as a gene knock-down tool has now been successfully established for the validation of gene function in *D. gallinae*. In this study, the aspartic protease, Cathepsin D (CatD) from *D. gallinae*, with a likely function in blood meal digestion, was selected for targeted gene knock-down by RNAi. Gene silencing was achieved through the oral delivery of target gene-specific double-stranded RNA (dsRNA) within the PRM blood meal (goose blood) via an *in vitro* feeding device. After 72hrs post-blood meal feeding using female mites, RNA was extracted for qPCR confirmation of CatD gene-knockdown. This confirmed that CatD expression was successfully knocked down by at least 65%. All PR were then re-fed with one more round of target gene-specific dsRNA in order to assess any phenotypic changes in ter of blood meal digestion. The experimental group of PR showed decreased blood digestion by 50% after 2 rounds of target gene-specific dsRNA delivery compared to the lacZ dsRNA treated controls. At the proteomic level, western blotting revealed increased haemoglobin residues and decreased levels of CatD protein in the CatD knock-down group. Previous vaccine trials have demonstrated the potential efficacy of using CatD as a target antigen and this study

demonstrates the potential of using RNAi as a screening tool to identify novel vaccine targets, potentially reducing the need for large animal trials during the target selection phase.

10:30 (15 mins)

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

Presenter: **Dr Nichola Calvani**, *The University of Galway*

*A Vitkauskaite[1]; E McDermott[1]; R Lalor[1]; C De Marco Verissimo[1]; K Thompson[1]; M Dehkordi[1]; H Fearnhead[1]; J Dalton[1]; **N Calvani**[1];*

[1] The University of Galway, Ireland

The helminth parasite *Fasciola hepatica* is a significant cause of animal and human morbidity worldwide. Part of the difficulty in developing new chemotherapeutics and vaccines for the control of fasciolosis lies in our inability to culture and propagate juvenile wor in vitro. Several laboratories maintain *F. hepatica* short-term in simple media, but these are usually for the purpose of collecting excretory/secretory (E/S) products containing molecules important in parasite host interaction, rather than for biological studies. Here we show that the infective stage of the parasite, the newly excysted juvenile (NEJ), exhibit significant growth and development *in vitro* when co-cultured with spheroids derived from HepG2 cells, a human non-tumorigenic liver cell line with high proliferation rates and epithelial-like morphology. We investigated parasite development using antibody probes against two major NEJ proteases, FhCL1 and FhCL3, and by scanning electron microscopy (SEM). Parasites grown in the presence of HepG2 spheroids exhibit not only a rapid increase in size (length and width) but also extensive development of the gut caecum, musculature, and surface sensory system. Parasites were observed regularly interacting with the spheroids, sometimes invading the tissue, and moving between or tangentially to them indicating the importance of tactile stimuli. There was also evidence of parasites 'grazing' on the peripheral cells of the spheroids. We propose that the methodology developed here mimic *in vivo* parasite host liver interactions, greatly improving our ability to investigate and understand *F. hepatica*-host biology with future prospects for the development of new parasite control methods, such as vaccines and anthelmintic drugs.

10:45 (15 mins)

Exploration of the sensitivity to macrocyclic lactones in the canine heartworm (*Dirofilaria immitis*) in Australia using phenotypic and genotypic approaches

Presenter: **Rosemonde Power**, *The University of Sydney*

***R Power**[1]; J Šlapeta[1];*

[1] The University of Sydney, Australia

Canine heartworm disease is a potentially deadly cardiopulmonary disease caused by the mosquito-borne filarial nematode *Dirofilaria immitis*. In Australia, the administration of macrocyclic lactone (ML) drugs has successfully reduced the prevalence of canine heartworm infection. However, the recent re-emergence of canine heartworm in dogs from Queensland, Australia and the identification of ML-resistant isolates in the USA poses an important question of whether ML-resistance has emerged in this parasite in Australia. The aim of this study was to utilise phenotypic and genotypic approaches to examine the sensitivity to ML drugs in *D. immitis* in Australia. To do so, a total of 78 blood samples were collected from 45 dogs in Queensland and New South Wales, Australia across three years (2019 to 2022). We tested for the presence of *D. immitis* infection in these blood samples using a quantitative Modified Knott's test, serology, and real-time PCR targeting *D. immitis* and its associated symbiont *Wolbachia*. A phenotype observed by utilising sequential quantification of microfilariae for 23/45 dogs was coupled with genetic testing of filtered microfilariae for Single Nucleotide Polymorphis (SNPs) previously associated with ML-resistance in isolates from the USA. This presentation will reveal the results of these phenotypic and genotypic tests, and hence reveal the ML-sensitivity of the canine heartwor infecting these Australian dogs.

Vectors and Transmission - Sponsored by Current Research in Parasitology and Vector-Borne Diseases - (Appleton Tower 2)

11:30 (30 mins)

Evolution of Insecticide resistance and efficacy of malaria control in Africa

Presenter: **Prof Charles Wondji**, *Liverpool School of Tropical Medicine*

***C Wondji**[1,2];*

[1] Centre for Research in Infectious Diseases (CRID), Yaoundé, UK; [2] Liverpool School of Tropical Medicine, Liverpool, UK

Malaria control relies on insecticide-based interventions such as insecticide-treated nets or indoor residual spraying. However, increasing resistance to main insecticides in malaria vectors threatens the continued success of these tools. To maximize the effectiveness of insecticide-based interventions, it is crucial to elucidate the genetic basis of resistance and establish its impact on malaria control. Recent studies have revealed a complex genomic evolution of resistance in malaria vectors in Africa which is likely to impact the efficacy of control tools. Target site resistance mechanisms notably knockdown resistance (kdr) markers are spreading extensively in *Anopheles gambiae* populations. There are extensive reports of metabolic resistance to insecticides in major malaria vectors such as *An. gambiae* and *An. funestus* with evidences that it is reducing the efficacy of pyrethroid-based interventions. Transcriptomic and genomic analyses have revealed that cytochrome P450 gene are playing a major role beside other genes including glutathione S-transferases. Whole genome sequencing has detected selective sweep footprints in mosquito populations associated with scale up of insecticide-treated nets. Furthermore, a complex evolution of metabolic resistance has selected evolutionary features including copy number variation (CNV) and structural variations. Moreover, DNA-based markers of metabolic resistance are being detected including in *An. funestus* and *An. gambiae*. These DNA-based markers are allowing to assess the resistance impact on the efficacy of insecticide-treated nets using experimental hut trials. Additionally, resistance impact is now evaluated on epidemiological parameters such as fitness cost and malaria transmission. Improvement in our understanding of molecular basis of resistance will undoubtedly implement suitable control interventions while improving resistance management strategies across Africa.

12:00 (15 mins)

From spillover to persistence: hybridization and schistosomiasis transmission dynamics at the human-animal interface

Presenter: **Dr Anna Borlase**, *Research Fellow, University of Oxford*

A Borlase[1]*; JW Rudge*[2]*; E Leger*[3]*; ND Diouf*[4]*; CB Fall*[5]*; SD Diop*[4]*; S Catalano*[3]*; M Sene-Wade*[6]*; J Webster*[3]*;*

[1] University of Oxford, UK; [2] London School of Hygiene and Tropical Medicine, UK; [3] Royal Veterinary College, University of London, UK; [4] IFSAR Bambey, Universite de Thies, Senegal; [5] Universite Cheikh Anta Diop de Dakar, Senegal; [6] Universite Gaston Berger de Saint Louis, Senegal;

Zoonotic spillover and hybridization of parasites are major emerging public and veterinary health concerns at the interface of infectious disease biology, evolution and control. Schistosomiasis is a neglected tropical disease of global importance caused by parasites of the *Schistosoma* genus, and the *Schistosoma* spp. system within Africa represents a key example of a system where spillover of animal parasites into human populations has enabled formation of hybrids. Combining model-based approaches and analyses of parasitological, molecular and epidemiological data from Northern Senegal, a region with a high prevalence of schistosome hybrids, we aimed to unravel the transmission dynamics of this complex multi-host, multi-parasite system. Using Bayesian methods and by estimating the basic reproduction number (R_0), we evaluate the frequency of zoonotic spillover of *Schistosoma bovis* from livestock, and the potential for onward transmission of hybrid *S. bovis*×*S. haematobium* offspring within human populations. We estimate R_0 of hybrid schistosomes to be greater than the critical threshold of one (1.76; 95% confidence intervals 1.59-1.99), demonstrating the potential for hybridization to facilitate spread and establishment of schistosomiasis beyond its original geographical boundaries. Equally vital to evaluating multi-host systems is the identification of key hosts; we estimate $R_0$for *S. bovis* to be greater than one in cattle (1.43; 95% confidence intervals 1.24-1.85), but not in other ruminants, confirming cattle as the primary zoonotic reservoir. Through longitudinal simulations we also show that where *S. bovis* and *S. haematobium* are co-endemic in livestock and humans respectively, the relative importance of zoonotic transmission is predicted to increase as the disease in humans nears elimination.

12:15 (15 mins)

Functional dissection of the *Leishmania* - sand fly attachment interface

Presenter: **Dr Ryuji Yanase**, *Oxford Brookes University*

R Yanase[1]*; K Pruzinova*[2]*; F Moreira-Leite*[1]*; E Rea*[1]*; J Sadlova*[2]*; B Vojtkova*[2]*; A Taniguchi*[3]*; S Nonaka*[4]*; P Volf*[2]*; JD Sunter*[1]*;*

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Within the sand fly vector, *Leishmania* parasites have two major morphological forms, a motile promastigote and a haptomonad, which is attached to the stomodeal valve through a shortened and modified flagellum. Dissecting haptomonad development and attachment is critical to understanding parasite transmission; however, studies of haptomonads are limited, as this is a technically challenging life cycle form to investigate. To gain an in-depth understanding of the *in vivo* haptomonad cellular architecture and organisation, we combined two volume electron microscopy techniques – serial block face-scanning electron microscopy (SBF-SEM) and serial electron microscopy tomography – generating high resolution 3D models of haptomonads attached to the stomodeal valve. Haptomonads were densely packed around the valve and were attached through the tip of a shortened flagellum. The attachment interface was filled, on the flagellum side, with an electron-dense

plaque that connected to abundant filaments and filament bundles. Next, we generated attached *L. mexicana* haptomonads *in vitro* and confirmed that the fine ultrastructure of these for was comparable to that of haptomonads found *in vivo*. Using comparative proteomic approaches, we identified proteins locating to either the attachment interface or the filaments within the attached flagellum, which we call Kinetoplastid-Insect Attachment Proteins (KIAPs). Importantly, a number of these proteins are present in other kinetoplastid parasites. Deletion analysis using CRISPR/Cas9 compromised *Leishmania* attachment both *in vitro* and in the sand fly, confirming that we have identified critical components of the parasite attachment mechanism. This provides the first molecular insights into a kinetoplastid parasite vector attachment interface, which will underpin our understanding of this crucial interaction and onward transmission.

12:30 (15 mins)

A decade of Trypanosomiasis research in Malawi: is the battle lost or won?

Presenter: **Dr Janelisa Musaya**, *Associate Director, Malawi-Liverpool-Wellcome Trust Clinical Research Programme/Kamuzu University of Health Sciences*

J Musaya[1]*; P NambalaK Kamoto*[2]*; P Chammudzi*[1]*; E Senga*[2]*; J chisi*[2]*;*

[1] *Kamuzu University of Health Sciences (KUHeS), Malawi Liverpool Wellcome Clinical Research Programme (MLW), Malawi;* [2] *Kamuzu University of Health Sciences (KUHeS), UK*

Introduction: African trypanosomiasis is a protozoan disease of Trypanosome origin. It is mainly transmitted by tsetse flies. This disease is a major concern in sub-Saharan Africa, with detrimental effects on both human and animal health and causing significant losses to affected countries. Both Animal African Trypanosomiasis (AAT) and Human African Trypanosomiasis (HAT) are present in Malawi with cases reported in the districts of Rumphi, Nkhotakota and Kasungu where large national parks exist. HAT in Malawi is caused by *Trypanosoma brucei rhodesiense* and is transmitted by the tsetse fly usually *Glossina morsitans* and *Glossina pallidipes.*

For over ten years our NTD group has assessed the presence of trypanosomiasis in the named districts with emphasis on tsetse fly infectivity and human trypanosomiasis prevalence. Here we report a decade's trend of trypanosomiasis in Malawi.

Methods: Since 2012, we conducted xenomonitoring surveys, human active and passive surveillance and hospital archive case retrievals in Rumphi, Nkhotakota, Kasungu and Liwonde.

Xenomonitoring: assessing if the species of tsetse flies that inhabit the nature reserves in these districts harbour *T. b. rhodesiense.* Tsetse flies were collected using Mzee traps, identified and dissected and microscopically examined for parasites. DNA was also extracted and PCR (TBR & SRA) conducted to identify the species.

Human surveillance: communities bordering the nature reserves were surveyed, blood was collected and microscopically checked for parasites. PCR was also done to identify the species.

Mapping: retrospective case finding was done from hospital archives. All villages where the cases came from were located, GPS coordinated taken and spatial maps were drawn.

Results: We have detected 7 species of Trypanosomes in the tsetse fly of Malawi *(T. brucei, T. vivax, T.godfrey, T.simaetsayo, T. simae, T. congolense savanna* and *T.b.rhodesiense*). *T.b.rhodesiense* was detected in all the sites. There was an increase of rHAT in Nkhotakota and Rumphi which is now declining with a total of 18 cases in 2012 and as high as 90 cases in 2019. Almost 70% of HAT cases were men. An outbreak of rHAT was noted from 2019 to 2020. Asymptomatic Human cases were observed in Liwonde but not in the other sites. Rumphi and Nkhotakota showed clustered rHAT cases within a 10km range from the nature reserves.

Conclusion: HAT is still prevalent in Malawi with cases being reported in Rumphi and Nkhotakota. Clustered HAT cases show a possible targeted intervention which is cost effective and area specific. Further study's in AAT is currently underway.

12:45 (15 mins)

Tsetse transmitted trypanosomes: from the skin to a systemic infection

Presenter: **Dr Dorien Mabille**, *Postdoc, University of Antwerp*

D Mabille[1]*; L Dirkx*[1]*; G Caljon*[1]*;*

[1] *University of Antwerp, Belgium*

The African trypanosome species responsible for human sleeping sickness (*Trypanosoma brucei rhodesiense* and *T. b. gambiense*) are transmitted by tsetse flies (*Glossina* sp.). Due to major control efforts, the annual number of reported human cases has declined to about 800, with a roadmap to reach elimination of the gambiense form by 2030. However, the major challenges that remain are the lack of protective vaccines and the occurrence of asymptomatic individuals that sustain the transmission cycle. Moreover, knowledge on the exact immunological basis for the highly efficient trypanosome transmission and asymptomatic infection remains scarce.

Following an infectious bite, inoculated metacyclic parasites rapidly adapt to the skin environment to establish a local infection and to continue a journey to systemic colonization. Making use of the tsetse fly vector, parasite reporter lines for fluorescent detection and *in vivo* bioluminescent imaging, immune-deficient mouse models and immunological profiling of parasite/saliva-exposed cells, we explored the role of innate immune responses in infection establishment and systemic colonization. This led to the discovery of an interesting role for the neutrophil in parasite control and dissemination from the skin microenvironment. Higher parasite loads were observed in the presence of neutrophils and neutrophil antiparasitic functions did not seem to hamper parasite expansion. Despite the armory of recruited anti-pathogen effector functions, parasites escape immune elimination and prominently distribute to tissues such as adipose, spleen and lungs. Parasites adapt to the specific tissue niches, creating specialized microenvironments that contribute to the infection.

The discovery of asymptomatic colonization of the skin and lungs as tissue reservoirs pinpoints future challenges for disease control, but also offers opportunities for the development of novel non-invasive diagnostic tests.

Poster abstracts and titles or Poster abstracts

Poster 1* : Phylogenetic analysis of *Trypanosoma evansi* in cattle with high RoTat1.2VSG gene copy numbers: Haematology and serum biochemistry findings.

Presenter: **Dr Onyinyechukwu Agina**, *Lecturer, University of Nigeria, Nsukka*

OA Agina[1]; S Mohd Rosly[2]; MI Nur Mahiza[3]; M Ajat[3]; M Zamri-Saad[3]; H Hazilawati[3];

[1] University of Nigeria, Nsukka, Nigeria; [2] Malaysian Agricultural Research and Development Institute, Malaysia; [3] Universiti Putra Malasyia, Malaysia

The main aim of the study was to analyse the phylogeny of *Trypanosoma evansi* detected in Malaysian cattle and determine the haemato-biochemical abnormalities associated with natural *T. evansi* infection in cattle with high RoTat1.2VSG gene copy numbers. Blood samples were collected from randomly selected 130 cattle: 90 crossbred Kedah-Kelantan x Brahman cattle and 40 Bali cattle. These cattle were sampled from beef cattle far in Muadzam Pahang and Kemaman, Terengganu Malaysia. Molecular detection and quantitation of RoTat1.2 VSG gene was achieved by real-time quantitative polymerase chain reaction (RT-qPCR) analysis. A curve of dissociation was generated to verify the specificity of the amplifications. The cattle were assigned into two groups namely: cattle with high RoTat1.2VSG gene copy number and clinically healthy cattle. Standard procedures were followed in the haematological and serum biochemistry analyses. A phylogenetic tree was constructed based on the partial RoTat1.2 VSG gene sequences of *T. evansi* and were supplemented with their respective reference sequences from GenBank. The alignment of the gene sequences was performed using ClustalW algorithm. The detection rate of *Trypanosoma evansi* was 4/130 (3.08%;95 CI 1.20–7.64%). Clinical signs observed in the infected cattle include anorexia, weakness, pale mucous membrane, cachexia and ocular discharge. Agar rose gel electrophoresis image showed a 151 bp band for RoTat1.2 VSG gene amplified from the infected cattle blood samples. The number of *Trypanosoma* parasites quantified from the cattle blood samples were between 40,396,41.43 – 65,07798.94 GC/µL. Numerous *T. evansi* were seen in the Giemsa-stained thin blood smears as elongated extracellular protozoan parasites. Furthermore, anisocytosis, poikilocytosis, macrocytes, and numerous echinocytes (artefacts) were evident. The haematological profile of *T. evansi* infected cattle with high RoTat 1.2 VSG include high mean erythrocyte fragility, low PCV, RBC count and haemoglobin concentration with erythrocytes that are larger than normal (macrocytic anaemia). Other abnormalities include high plasma proteins and icteric index, low leukocyte cell with high myelocyte, metamyelocyte and band neutrophil counts, and low monocyte count. Serum biochemistry findings include hyperkalaemia, hypernatraemia, hyperchloridaemia, hyperproteinaemia, low serum aspartate aminotransferase activity, high serum activities of alkaline phosphatase and gamma glutamyl transferase, hypoalbuminemia, hperglobulinemia, low albumin to globulin ratio and hyperbilirubunemia due to high level of unconjugated bilirubin. Serum inorganic phosphate and creatinine levels were high with a low serum urea level. Similarity analysis using nucleotide BLAST (BLASTn) showed that the amplicon sequences of *T. evansi* from this study (MT514513.1-MT514514.1) demonstrated 100% molecular similarit

Poster 2* : Detection And Molecular Analysis of kinesins in Local *Leishmania* in Iraq *L. tropica* and *L. donavani*

Presenter: **Dr Suad Al Kufi**, *Assistance Prof, Nahrain*

S Al Kufi[1]*;*

[1] *Nahrain, Iraq*

The Kinesin KIF13 has been identified as a motor protein which has nuclear localisation specifically at the spindle and spindle poles in some kinetoplastids. Five Kinesin-13 members have been shown in *T. brucei.* TbKIF13-1 is a nuclear protein, TbKIF13-2 and TbKIF13-4 display a flagellar localisation, TbKIF13-3 and TbKIF13-5 are in the cytoplasm. TbKIF13-2 functional analysis in homology proved that the overexpression of TbKIF13-2 reduces flagellum length slightly. Five kinesin-13 family members that belong to the KIF24 subfamily have been identified in the genome of *Leishmania major*, two of them LmjKIN13-1 and LmjKIN13-2 have been characterised. LmjKIN13-1 has a nuclear localisation specifically at the spindle and spindle poles. Kinesin-13 (MCAK/KIF2) members exhibit a microtubules depolymerising activity responsible for their function in mitosis. The present study focused on the KIF13 in local *Leishmania. Sp* in Iraq. Firstly *L. tropica* which responsible for cutaneous leishmaniasis and *L. donavani* responsible for visceral leishmaniasis or kala-azar, the most severe form of leishmaniasis. Initially, PCR technique was used to detect LmxKIF-13 gene, followed by sequence analysis. The study, aimed to identify the function LmKIF-13 in

both *Leishmania*. The kinesin motor protein LmKIF-13 in *L. donovani* and *L. tropica* can be used as therapeutic and potential vaccine candidate against leishmaniasis.

Poster 3* : The interaction of *Schistosoma mansoni* infection with diabetes mellitus and obesity in mice

Presenter: **Dr Alaa Saed Anwer Amer**, *Faculty of medicine Tanta university*

A S. Amer[1]*; A Othman*[1]*; L M. Dawood*[1]*; K A. El-Nouby*[1]*; G N. Gobert*[2]*; D M. Abou Rayia*[1]*;*
[1] *Faculty of Medicine, Tanta University, Egypt;* [2] *Queen's University Belfast, UK*

Background Human schistosomiasis is one of the most prevalent parasitic diseases worldwide. Various host factors can affect the host–parasite interactions, including changes in metabolism, immunological responses, and the genetic background. These changes to the host may lead to the disruption of important parasite functions such as oviposition, worm development and the resulting pathology associated with the infection. In the current study, we sort to determine the parasitological, histopathological, biochemical, and immunological status of the host impacted by *Schistosoma mansoni* infection, concurrent with host metabolic disorders through animal models of streptozotocin-induced diabetes mellitus (DM) and obesity. Aiming to identify the underlying mechanisms of schistosome host interactions leading to the induction of the pathology. Methods The study animals were divided into four groups. Group I is control groups, containing normal, infected, and non-infected DM1, DM2, and obese groups. The mice of the other three groups undergone induction of DM1 (Group II), DM2 (Group III) and obesity (Group IV) before being infected. All mice groups were euthanized in week 8 after cercarial challenge. Mice were subjected to body weight weekly measurement, blood glucose and insulin assessment, parasitological evaluation of tissue egg count and intestinal oogram. Histopathological and immunohistochemical studies were done using anti-glial fibrillary acidic protein (GFAP) and image analysis of Masson's trichrome stained liver sections using Image J (Fiji). Additionally, immunological analysis of TNF-β, IL-5, IL-10, FOXP3 and PTX3 levels besides biochemical study of total lipid profile were evaluated. Results The present study revealed a significant increase in tissue egg output in the obese group compared to the infected control group. The oogram of counted eggs showed prevalence of immature eggs in DM1 group, while DM2 and obese groups showed prevalence of mature eggs. The fibrosis area percentage showed significant increase in DM2 and obese groups while it was decreased in DM1 group in comparison to infected control group. Concerning the immunological parameters, the present results showed significant increase in the levels of TNF-β, IL-5, PTX3 in DM1, DM2 and obese groups in comparison to infected control group, whilst the levels of FOXP3 and IL-10 were increased in the infected groups in comparison to their non-infected controls. Regarding the biochemical study, infected DM1, DM2 and obese groups showed higher blood glucose and lipid profile in comparison to the infected control group. However, these parameters were improved in comparison to their non-infected controls. Conclusions Our study has contributed to the unravelling of the mechanisms of the interaction between schistosome infection and metabolic disorders of the host. Induction of DM2 and obesity increased the body mass, egg count, mature egg percentage, and fibrosis density, while schistosome infection induced changes in the lipid profile and blood glucose levels in infected diabetic and obese groups and impacted favourably insulin levels in obese mice. These differences may aid therapeutic and vaccine studies to better controlling these diseases and improving outcomes in endemic regions. By better understanding the complexities of host–parasite interactions, efforts to reduce the burden of these debilitating diseases can be improved.

Poster 4 : An innovative approach to teaching parasite microscopy on-line

Presenter: **Dr Samuel Boadi**

S Boadi[1]*; PL Chiodini*[1]*;*
[1] *UKHSA, UK*

COVID19 compelled UK NEQAS Parasitology to rethink the delivery of its face-to-face parasite microscopy courses. The challenge was to adapt traditional laboratory sessions to an online environment and cater for an influx of new entrants to the discipline.

The online teaching is divided into three one-hour parts. The first is a presentation on how to go about identifying parasites microscopically. In the second hour, the students practise the techniques taught in the first hour, using digital images provided in a bench manual to aid them in the

process. They are encouraged to have discussions among themselves where it is convenient to do so. The last hour is devoted to reviewing the exercise.

An end of course evaluation of seventy-six faecal course participants produced an average score of 9.45 out of 10 when asked about their likelihood to recommend the event to others. Seventy seven out of 77 thought the learning activities used were effective. In a blood parasite course evaluation, 79 respondents, with an average evaluation score of 8.76 out of 10, said they were likely to recommend the event to others whilst 78 out of 79 also thought the learning activities used were effective. Asked if there was a need for a separate entry-level course, 51.4% said yes and 48.6% said no from the faecal course and from the blood course, 48.9% said yes and 51.1% said no.

Provision of online teaching has allowed many more people to access the course, including those with little experience in parasitology. This affects the depth of teaching provided online, as an attempt is made to cater for the different grades of attendees. This, notwithstanding, the participants' views on the need for a separate course to cater for the needs of new entrants were not decisive. So, further work on the curriculum is being considered to plan for mixed level classes combining both online and face-to-face sessions for the delivery of courses.

Poster 5* : Phytochemical profile and anthelmintic effects of *Laurus nobilis* essential oil against the ovine nematode *Haemonchus contortus* and the murine helminth model *Heligmosomoides polygyrus*

Presenter: **Dr Essia Sebai**, *National School of Veterinary Medicine of Sidi Thabet*

E Sebai*A AbidiH Benyedem[2]; M Dhibil Hammemi[2]; H Akkari*

[1] National School of Veterinary Medicine of Sidi Thabet, Tunisia; [2] Faculty of Sciences of Tunis, Tunis El Manar, Tunisia

Small ruminant production in tropical and temperate countries faced substantial anthelmintic resistance due to the intensive use of commercial anthelmintic drugs. Therefore, alternative treatments including natural bioactive compounds with anthelmintic potential have been investigated looking for its successfully use in the parasite control. In the present study, we describe the chemical profile of *Laurus nobilis* essential oil (EO), the *in vitro* anthelmintic activity of *L. nobilis* EO against *Haemonchus contortus* and its *in vivo* anthelmintic effect against the murine helminth parasite model *Heligmosomoides polygyrus*. Egg hatch assay (EHA) and Adult Worm Motility (AWM) assay were used to assess the *in vitro* anthelmintic activity of *L. nobilis* EO at the concentrations of 0.25; 0.5; 1; 2; 4 and 8 mg/mL against *Haemonchus contortus*. Moreover, *L. nobilis* EO at the concentrations of 300, 600 or 1200 and 2400 mg/kg were evaluated *in vivo* in mice infected with *Heligmosomoides polygyrus*. The *in vivo* anthelmintic efficacy was monitored using faecal egg count reduction (FECR) and total worm count reduction (TWCR). The *in vitro* anthelmintic potential was expressed by an ovicidal effect against *H. contortus* egg hatching with inhibition value of 1.72 mg/mL and 87.5 % of immobility of adult wor after 8 hours of exposure to 4 mg/mL of *L. nobilis* EO. Regarding, the *in vivo* anthelmintic potential, *L. nobilis* (EO) at 2400 mg/kg bw completely eliminated the egg output of *H. polygyrus* after 7 days of oral treatment, together with a 79.2% of reduction in total worm counts. Based on the obtained results, *L. nobilis* EO showed promising *in vitro* and *in vivo* anthelmintic capacities and could be a possible candidate for the control of worm parasites in livestock.

Poster 6 : Utility of the Loop-Mediated Isothermal Amplification Assay for the Diagnosis of Visceral Leishmaniasis from Blood Samples in Ethiopia

Presenter: **Dawit Gebreegziabiher Hagos**, *PhD student at University of Amsterdam, AMC, Mekelle University, Ethiopia*

D Dawit G. Hagos*[1]; YK Kiros[1]; HD Schallig[2]; D Wolday[1];*

[1] Mekelle University, Ethiopia, Ethiopia; [2] University of Amsterdam, The VU, Netherlands

Rapid and accurate visceral leishmaniasis (VL) diagnosis is needed to initiate prompt treatment to reduce morbidity and mortality. Here, we evaluated the performance of loop-mediated isothermal amplification (LAMP) assay for the diagnosis of VL from blood in an endemic area in Ethiopia. LAMP was positive in 117/122 confirmed VL cases and negative in 149/152 controls, resulting in a sensitivity of 95.9% (95% CI: 90.69–98.66) and a specificity of 98.0% (95% CI: 94.34–99.59), respectively. The sensitivity of the LAMP assay was 95.0% (95% CI: 88.61–

98.34) in HIV-negatives and 100% (95% CI: 85.18–100.0) in HIV-positives. Compared with microscopy, LAMP detected 82/87 (94.3%, 95% CI: 87.10–98.11) of the microscopy1 cases and was negative in 11/27 (40.7%, 95% CI: 22.39–61.20) of the microscopy2 cases. Compared with the rK39 serology, LAMP detected 113/120 (94.2%, 95% CI: 88.35–97.62) of the rK391 cases and was negative in 149/154 (96.8%, 95% CI: 92.59–98.94) of the rK392 cases. However, when compared with microscopy only, rK39 detected 83/87 (95.4%, 95% CI: 88.64–98.73) of the microscopy1 cases and negative in only 12/27 (44.4%, 95% CI: 25.48–64.67) of the microscopy– cases. There was an excellent agreement between rK39 and LAMP (Kappa 5 0.91, 95% CI: 0.86–0.96). Furthermore, an algorithm using rK39 followed by LAMP would yield a sensitivity of 99.2% (95%CI: 95.52–99.89) and a specificity of 98.0% (95% CI: 94.34–99.59). The findings demonstrate that LAMP assay is an accurate and rapid molecular assay for VL diagnosis, including in HIV-1 coinfected patients, in an endemic setting.

Poster 7 : Cutaneous leishmaniasis in Guatemala

Presenter: **Yaimie López**, *Tropical Disease Biologist, Universidad del Valle de Guatemala*

Y López[1]; A Casas-Sanchez[2]; E Duran[3]; A Acosta-Serrano[4]; ***RM Mendizabal[1]****;*
[1] Universidad del Valle de Guatemala, Guatemala; [2] Liverpool School of Tropical Medicine, UK; [3] Guatemalan Ministry of Health, Guatemala; [4] Department of Biological Sciences, University of Notre Dame, United States

Cutaneous leishmaniasis (CL) is a parasitic vector-borne disease present in 98 countries. CL is endemic in Northern Guatemala, affecting the poorest population in rural areas. According to the reports of the World Health Organization, in the last 10 years there has been a rising incidence of CL in Guatemala from 28.9 cases/100,000 inhabitants in 2012 to 33.9 cases/100,000 in 2021. Despite the endemicity of the disease, the most important factors that contribute to the transmission of CL are unknown in the country. Our goal was to update vector knowledge by characterizing the sand fly populations and *Leishmania* parasites circulating in Alta Verapaz, a CL endemic region in Guatemala. From March to August 2022, we collected sand flies using light traps in three environments: indoors, outdoors in the animal sheds and in the surrounding forest. The sand fly species were identified using end point PCR targeting the cytochrome C gene followed by Sanger sequencing and screened for *Leishmania* DNA by heat-shock protein 70 gene PCR and sequencing. Using the same methodology on lesion tissue smear material, we identified the species of parasites causing infections in the community. We collected 95 sand flies (79% females) of 10 species, three of which have been previously associated with *Leishmania* transmission: *Nysommyia ylephiletor*, *Bichromomyia olmeca* and *Lutzomyia cruciata*. The highly anthropophilic *Ny. ylpehiletor* was the most common species (30%) and was primarily collected indoors (73.1%). Three female sand flies were positive for *Leishmania* DNA, and one of them (*Ny. ylephiletor)* was captured indoors. *L. guyanensis* parasites were found in both sand flies and patients. In patients we also detected *L. panamensis* and *L. braziliensis* . The average age of the patients was 34 years old, the majority adults (78%) and men (57%), and 40% of the CL lesions were in the lower legs. This is the first report in Guatemala of the presence of *L. guyanensis* and *L. panamensis* in patients and sand flies, and the first characterization of *Leishmania* infections in humans and sand flies that concur in time and geographical area. The capture of highly anthropophilic sand flies indoors, some with *Leishmania* DNA, suggest indoor transmission of CL, but further studies are needed to confirm our results. These findings are key to local health authorities for decision-making on the appropriate prevention measures, such as the use of insecticide treated bed nets.

Poster 8 : Biochemical characterisation and essentiality of proteins involved in myo-inositol metabolism from the parasite *Trypanosoma cruzi*

Presenter: **Veronica Harris**, *PhD student, University of St Andrews*

V Harris[1]*; TK Smith[1];*
[1] University of St Andrews, UK

myo-Inositol is one of the nine naturally occurring inositol stereoisomers. It is ubiquitous amongst eukaryotes and acts as an essential metabolite with roles in signal transduction, membrane formation, and cellular physiology. In the protozoan parasite *Trypanosoma cruzi*—the causative agent of Chagas' disease—*myo*-inositol acts as a precursor to phosphatidylinositol (PI), which is an essential component to membrane lipids. In addition, PI in turn is required for formation of inositol phosphoceramide (IPC), various phosphoinositides, and

glycophosphatidylinositol (GPI)-anchored mucin-type glycoproteins, which coats the parasite's cell-surface allowing the parasite to participate in multiple essential steps in parasite-host interactions. In *T. cruzi, myo*-inositol is proposed to be both *de novo* synthesised as well as scavenged from the environment, however, the proteins involved in both pathways have not been studied in *T. cruzi.* Therefore, the aim of this project is to genetically validate and biochemically characterise the putative inositol-3-phosphate synthase as well as the *myo*-inositol transporter in *T. cruzi*.

Poster 9* : A molecular genetic investigation into the origins of anthelmintic drug resistance in *Ancylostoma caninum* in pet dogs across the USA

Presenter: **Noelle Thundathil**, *Bioinformatics Student, University of Calgary*

N Thundathil[1]*; A Venkatesan*[1]*; E Redman*[1]*; A Morosetti*[1]*; PD Jimenez Castro*[2]*; R Kaplan*[2]*; J Gilleard*[1]*;*
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The canine hookworm, *Ancylostoma caninum*, is a highly pathogenic and the most prevalent intestinal nematode of domestic dogs. Additionally, studies have shown that there was a 47% increase in the prevalence of *A. caninum* between 2012-2018. Management of infections is particularly problematic in greyhounds on breeding far due to the unrestricted access these have to environments that are ideal for hookworm survival. Since greyhounds are exposed to this environment regularly, these are often subject to intense deworming protocols that present a high pressure for drug selection on hookworm populations on these far and racing kennels. Our previous work revealed that *A. caninum* is resistant to multiple anthelmintic drug classes in kennelled greyhounds across the USA and that benzimidazole resistance is associated with mutations at codons 134 and 167 of the isotype-1 B-tubulin gene, the target of this drug class. Both these mutations are now widespread in *A. caninum*, not only from greyhounds, but also from pet dogs in the USA. Based on greyhound treatment history, we hypothesized that resistance originally emerged due to intense drug selection and housing conditions in kennelled greyhounds and subsequently spread to pet dogs via environmental contamination by retired greyhounds being rehomed across the USA. This project tested the specific hypothesis that there has been more intense selection at the *A. caninum* isotype-1 B-tubulin locus in greyhounds than in pet dogs. Deep amplicon sequencing data is already available for the isotype-1 B-tubulin locus, and we undertook additional deep amplicon sequencing of the *cox*-1 and *nad*-1 mitochondrial genes to use as neutral markers to assess the overall genetic diversity of the parasite populations. The *A. caninum* data was taken from 120 pet dog and 50 greyhound hookworm positive faecal samples, and primers were designed to target the 455bp and 394 bp regions of the *cox*-1 and *nad*-1 mitochondrial genes, respectively. Using the generated primer pairs, PCR amplicons were produced and sequenced at depth using the Illumina MiSeq platform. Finally, the DADA2 bioinformatics pipeline was used to generate a table of amplicon sequence variants that allowed for the comparison of genetic diversity between *A. caninum* in these two canine populations. We found that the isotype-1 B-tubulin locus has much lower expected heterozygosity and nucleotide diversity in *A. caninum* from kennelled greyhounds (Hd = 0.36, pi = 0.008) than from pet dogs (Hd = 0.89, pi = 0.017). Additionally, there was a greater departure from neutrality as measured by Tajima's D for *A. caninum* from kennelled greyhounds (-2.70) than pet dogs (-1.85). In contrast, the *cox*-1 and *nad*-1 mitochondrial markers, had similar genetic diversity in *A. caninum* from kennelled greyhounds and pet dogs, respectively. Overall, these results indicate there has been more intense selection at the isotype-1 B-tubulin marker in *A. caninum* infecting greyhounds than pet dogs, which is consistent with the hypothesis of benzimidazole resistance in *A. caninum* in the USA originating in greyhound kennels.

Poster 10 : Social Economic Impact of Onchocerciasis in Ugwuta Local Government Area, Imo State Nigeria

Presenter: **Dr Kamalu Nkiru Anastasia**, *Lecturer, Kingsley Ozumbadiwe University Ogboko Imo State*

K Nkiru Anastasia[1]*;*
[1] *Kingsley Ozumbadiwe University Ogboko Imo State, Nigeria*

The socio-economic impact of the onchocerciasis in Oguta local government Area, Imo State was carried out within three months from December2021 to march 2022. The impact of the disease was studied using questionnaire method, ten communities from the study area were selected using Simple Random Sampling (SRS) and ten questionnaires were evenly distributed by mecting the volunteers randomly among the selected communities which includes: Oguta, Agwa.

Orsu-Ohodo, Egwe, Egbuoma, Awa, Akabor, Mghelle, Ezi-Orsu, and Uzombe and comprise of both male and female gender. The result showed that 64% of the entire population under the study area knows about the disease and they comprises of mostly female (57%) with the highest age bracket of 14-15 (39%). The result shows that the entire population were mostly single (54%) with a higher percentage been self employed (40%) having a monthly earning above N20,000 (66%). Also. despite the greater percentage of singles in the population area, only 42% are the bread winners of their family. a greater number of the entire population under study do not often ger sick (41%) and higher percentage do get drugs from the pharmacy when they get sick (39%) and mostly spends above N1,000 at an average when taking care of themselves when they ger sick onchocerciasis (River blindness) has less socio-economic impact on the entire population of Oguta Local government because large number of the population are self employed and are mostly single. Although only 42% of the population under study are the bread winners of their family. The result also shows that high percentage of the population do not often get sick which also implies that the entire are rarely infected with onchocerciasis. However, onchocerciasis has limited socio-economic impact on Oguta Local government Area. Imo state.

Kerwords:

Ivermectin, Onchocerciasis, Endemic, Lymphatic Filariasis, NDTs. Socio-econome, Vector, Geographical.

Poster 11* : *Toxocara* in your T-bone? Investigating larval contamination of meat and associated public health risks

Presenter: **Sara Healy**, *Postgraduate researcher, University of Surrey*

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Toxocara is a roundworm parasite, with two species of zoonotic importance to humans (*Toxocara canis* and *T. cati*). Dogs, cats and foxes are the definitive hosts for this parasite, with infected animals shedding eggs in their faeces which subsequently contaminate the environment. When other animals, including livestock species, ingest *Toxocara* spp. eggs, the larvae hatch out in the gut and migrate to various parts of the body including meat tissues consumed by humans. If ingested 'rare' or undercooked, infected meat could pose a public health risk with the potential for serious health implications, such as blindness and epilepsy.

In this study we will assess liver and skeletal muscle samples obtained from food animal species naturally exposed to *Toxocara* spp. larvae, in order to explore the prevalence of this parasite in the food chain and assess whether current production practices are sufficient at reducing the risk of *Toxocara* transmission to humans. Chemical and mechanical tissue digestion is used, followed up by microscopic evaluation and larval DNA analysis using RT-PCR in order to confirm species.

Essential training in the required laboratory skills for the project was undertaken at ANSES, Paris. This trip was made possible with the financial assistance of a BSP travel award (2022/23).

Poster 12* : PDI-Trans: a promising target for transmission blocking in malaria

Presenter: **Amelia Ford**, *PhD student, University of Cambridge*

A Ford[1]*; W Gregory*[2]*; AM Blagborough*[2]*;*
[1] *University of Cambridge, UK;* [2] *Department of Pathology, University of Cambridge, UK*

Malaria continues to be an important global challenge, with 241 million cases and 619 thousand deaths in 2021. Though there is a newly licensed anti-malarial vaccine, it has a modest efficacy (30-50%). Resistance markers against frontline antimalarial drugs have been identified in South-East Asia and Africa. More novel antimalarials need to be developed and characterised. Transmission locking interventions (TBI) target the bottleneck sexual stages of the *Plasmodium spp.* either in the human host (gametocytes) or the mosquito vector.

A putative transmission blocking target is PDI-Trans, a protein disulphide isomerase (PDI) expressed on the surface of the male gamete during fertilisation. Homologues of the protein are found in all *Plasmodium* species. Previous work has found that in transgenic lines lacking PDI-Trans, the male gamete showed abnormal fertilisation, a lack of gamete fusion, and no transmission.

Known PDI inhibitors were put through a series of three different screening assays to check for anti-transmission ability, examining: 1) inhibition of recombinant PDI-Trans reductase ability; 2) inhibition in fertilisation 24 hours post gamete activation; and 3) the 'gold standard' standard membrane feeding assay (SMFA), examining establishment of parasitic oocysts within the mosquito midgut.

Repurposed PDI inhibitors were able to successfully inhibit transmission and further development of the sexual stages of *Plasmodium berghei*, with significant (>90%) reduction of transmission to mosquitoes observed when gametocytes are exposed to five specific repurposed PDI-inhibitors that are previously unexamined for anti-malarial efficacy.

Targeting PDI-Trans prevents transmission of malaria from the vertebrate host to the mosquito vector and represents a promising target for novel TBIs.

Poster 13 : Genotyping of *Toxoplasma gondii* in domestic animals from Campeche, Mexico reveals virulent genotypes shared with South America

Presenter: **Dr Heriberto Caballero-Ortega**, *DVM, Instituto Nacional de Pediatria*

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[1] Instituto Nacional de Pediatría, Mexico; [2] Universidad Autónoma de Campeche, Mexico; [3] El Colegio de la Frontera Sur, Mexico; [4] Universidad Nacional Autónoma de México, Mexico

Background. The prevalence of *T. gondii* in México is 43.9% in the human population, being the Neotropical region with the highest rate of antibodies against this parasite. Campeche is one of the South-eastern Mexican states that has favourable climatic conditions for the replication and dissemination of this protozoan. Studies of this zoonotic agent in this state are scarce; thus, identifying the presence of this parasite in sentinel hosts such as dogs (*Canis familiaris*) and free-range chickens (*Gallus gallus*) will allow us to understand its epidemiological dynamics in the study site.

Objective. To identify, isolate and genotype *Toxoplasma gondii* parasites obtained of blood samples and target tissues of stray dogs and free-range chickens from Campeche, México.

Material and methods. Eleven stray dogs and eight free-range chickens from Escárcega and Calakmul, respectively, both municipalities of Campeche, were captured and euthanized to collect blood and tissue samples. The determination of IgG anti-*T. gondii* antibodies in serum samples of dogs was performed by ELISA and western blot. Free-range chickens did not undergo serology. The presence of the parasite DNA was determined by endpoint PCR and qPCR. Parasite isolates were obtained by bioassays in mice, and the genotyping was performed by PCR-RFLP using the panel of 11 typical markers plus 5 PCR-RFLP virulence markers.

Results. Detection of IgG anti-*T. gondii* antibodies in dog sera was 72.7% and 100% by ELISA and western blot, respectively. Six of eleven dogs were positive by endpoint PCR and by qPCR. Two isolates from a dog were obtained, one from the brain and the other from the heart/diaphragm mixture, both resulting in the ToxoDB #116 genotype. Also, two isolates from heart and brain of a free-range chicken were also obtained, being the ToxoDB #38 genotype. Considering the combination of results obtained for the virulence markers, particularly for *ROP*18/*ROP*5, a high virulence is predicted for two isolates and two remain unknown due to the description of a new *ROP5* RFLP pattern.

Conclusions. The use of more than one laboratory technique confor and improves the comprehensive diagnosis of *T. gondii*, proposing new diagnostic approaches in several definitive and intermediate hosts of this parasitosis. ToxoDB #38 and #116 genotypes were found in free-range chickens and stray dogs, respectively. The latter having a previously unreported atypical virulence genotype that could be endemic of this region of México. Campeche state has environmental conditions that favour the *T. gondii* genetic diversity.

Poster 14 : Studies on the biological contaminants (parasites, fungi, and bacteria) of food among food handlers in four higher institutions in Calabar municipal, and the Public health implicationions

Presenter: **Jenavine Onyinye Mbah**, *Lecturer, University of Calabar*

J Onyinye Mbah[1]*; C Osondu Anyanwu*[1]*;*
[1] *University of Calabar, Nigeria*

Food-handlers with poor personal hygiene working in food-service establishments could be potential sources of infection due to pathogenic organisms. The study was carried out to determine the prevalence of biological (bacteria and intestinal parasites) among 419 food-handlers working in the four tertiary institutions in Calabar municipal, cross river state Nigeria. Fingernail contents of both the hands, urine and stool specimens were collected from all the 419 food-handlers. The samples were examined for bacteria and intestinal parasites following standard procedures.

Coagulase-negative staphylococci were the predominant bacteria species (19.33%) isolated from fingernail contents, followed by *Staphylococcus aureus* (13.13%). Bacteria specie isolated from urine samples showed coag neg staph (16.71%) as the most dominance followed by S. aureus (13.60%). Nose bacteria showed S. epidermis dorminance (28.88%). In intestinal parasites detected in the stools of the food-handlers, Thrichuria richuris dorminated (13.60%) followed by *Ascaris* lumbricoides (13.13%). The least infection of parasites is *Schistosoma* mansonia (02.86%) and Blastocyst (02.39%). The distribution of individual pathogens showed that coag neg staph (15.44%) is the leading bacteria among study group while Thrichuria trichuris (05.83%) is leading in parasitic infection.

Prevalence of infection among groups of food handlers recorded hawkers (39.88%) as the most infected followed by restaurant attendants (32.62%), while the least infection is among shop attendants (27.51%).

The findings emphasize the importance of food-handlers as potential sources of infections and suggest health institutions for appropriate hygienic and sanitary control measures.

Key words: Bacteria, Food-handlers, Hygiene, Intestinal Parasitic, foodborn diseases.

Poster 15 : *Echinochasmus* (Trematoda: Echinostomatidae) in Squacco Heron (*Ardeola ralloides*) from the Congo, in the collections of the Harvard University Museum of Comparative Zoology: a first substantiated report of the genus from sub-Saharan Africa

Presenter: **Dr Andrew McCarthy**, *HE Lecturer in Animal Sciences, Canterbury College (East Kent College Group)*

AM McCarthy[1]*;*
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Echinostome trematodes of the genus *Echinochasmus* are, as adults, intestinal parasites of a range of piscivorous birds in the wild in North, South and Central America, Europe, Asia and Africa. Adult wor are also known to infect mammals, including humans. In humans they are known to cause the zoonotic and neglected tropical disease echinostomiasis with individuals becoming infected by the ingestion of the metacercariae usually found in fish second intermediate hosts.

Examination of four adult echinostome specimens, preserved as stained whole mounts, from the collections of Harvard University MCZ confirmed their identity as being of the genus *Echinochasmus* with reference to Kostadinova (2005). The specimens had originally been collected by John H. Sandground of MCZ in 1934 from the small intestine of Squacco Heron *(Ardeola ralloides)* obtained at Pania-Mutombo, a location on the Sankuru River, Kasai, in present day Democratic Republic of Congo (Congo-Kinshasa).The wor are characterized by having twenty two collar spines, and it may be that they are of a species closely related to the only other twenty two collar spined species of the genus so far recorded from Africa, *Echinochasmus mordax* which was found in the intestine of the Great White Pelican (*Pelecanus onocrotalus)* by Looss (1899) in Egypt.

With the exception of records of species of *Echinochasmus* from Egypt, no verified records of the genus from elsewhere in Africa have been found to exist. It is therefore suggested that the specimens examined in the present study provide evidence for the first substantiated record of

the genus *Echinochasmus* from sub-Saharan Africa, and that this study demonstrates the value of specimens from well curated museum collections in providing novel insights into parasite species diversity and geographical distribution.

Acknowledgements

The author gratefully acknowledges Adam J. Baldinger Curator of Invertebrates at Harvard University Museum of Comparative Zoology for arranging access to the *Echinochasmus* specimens.

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Poster 16* : A mixed method approach to explore the clinical biology of *Plasmodium* mixed infections

Presenter: **Nimita Deora**, *PhD Scholar, ICMR-National Institute of Malaria Research, New Delhi*

N Deora[1]*; V Pande*[2]*; A Sinha*[1]*;*

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Coexistence of multiple *Plasmodium* species within the host may stymie malaria elimination. Mixed infections with *P. falciparum* (Pf) & *P. vivax* (Pv) are common & come with multiple challenges. Different species have different characteristics and require different treatments. However, mixed infections with PfPv are treated with ACT (artemisinin-based combination therapy). Although, *P. vivax* can adequately be treated with chloroquine, unnecessary exposing the parasite with ACT might speed up the development of resistance. Further, since the biology of these two parasite species is distinct, it is important to study them individually during mixed infections to see how they behave and interact with each other. This includes studying their growth (asexual and sexual), and response to antimalarial drugs. Evidences indicate that biology of individual species parasite is altered in presence of other but the nature, extent and implications of these remain unveiled.

PfPv mixed-infections might also lead to their co-transmission to mosquitoes with opportunity and possibility of sexual interactions in mosquitoes which might be favoured by drug and shrinking population pressures. Research is reported of understanding the inter-species sexual interactions in rodent *Plasmodium* species (*P. yoleii* and *P. berghei*) but no evidences exist of such studies for PfPv mixed-infections, particularly this cross-species mating behaviour in mosquitoes. Such sexual interactions in the mosquito may give rise to a hybrid, which might go undetected as the current diagnosis of malaria species is capable of independently identifying Pf and Pv and is not targeted to detect their hybrid, if present.

Keeping all the above challenges in mind, the current study reported prevalence & studied the clinical profile and complications of *PfPv* mixed infections from 1030 blood samples collected during June-November 2020 from patients reporting with fever at four different government hospitals in India. Samples were analyzed by microscopy, bivalent RDT and PCR (qPCR & nested). Clinical features and socio-demographic characteristics of patients have been recorded. Mixed *PfPv* infected cases were followed telephonically to identify and study associated complications & recurrences, if any.

Apart from the field study, a systematic review was conducted on 17449 samples screened for *Plasmodium* infection from 39 districts across 13 states of India to study challenges associated with sub-microscopic infections. Analyzed data revealed common flaws in analyzing sub-microscopic mixed *Plasmodium* infections and suggested a more factual analysis of such data. This information would be immensely helpful for the National authorities driving malaria elimination in various countries.

Further this study developed a method to separate Pf & Pv from mixed *PfPv* culture to explore their interactive biology. The experiments include gating of different stages of *Pf* and *Pv* individually and in a mixture of both (in different ratios to mimic the real-life scenario) in flowcytometer. The results of this study which is probably a step towards adding the 'first of its kind' high throughput tool to better understand the comparative/interactive clinical biology of mixed infections. To investigate inter-species fertilization, separation of sex-

specific *Pf* gametocytes was done using genetically modified cell lines and sex-specific drugs (Aphidicolin & Pyronaridine). There are no such lines & drugs are available for *Pv*, hence gametocytes are being purified using MACS. Separated cross-species opposite-sex gametocytes will then cultured in ookinete media to investigate cross-fertilization and zygote formation through flowcytometry.

Poster 17 : Detection and Discrimination of *O. volvulus* and *O. ochengi* from Blackfly Pool DNA using a Novel Polymerase Chain Reaction – Restriction Fragment Length Polymorphism (PCR-RFLP) Technique

Presenter: **Isaac Owusu-Frimpong**, *Principal Technologist, CSIR - Water Research Institute*

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Molecular xeno-monitoring has been a significant technique used to study the impact of vector-borne pathogenic diseases on humans and animals by employing disease surveillance in vector populations. It uses insects carrying pathogen genetic material as a non-invasive surrogate for infection in the human or animal population. In the case of onchocerciasis, the WHO has approved the O150 PCR in blackflies, a molecular xenomonitoring technique, as part of the Onchocerciasis Elimination guideline. However, this technique is laborious and time-consuming, which could be influenced by human errors. Also, the high cost, non-availability, and delay of the ELISA component make the application of the O150 PCR difficult in resource-limited settings. Therefore, this study focused on developing a PCR-RFLP assay to detect and discriminate *O. volvulus* and *O. ochengi* in blackflies. Bioinformatics analysis was employed to identify a unique restriction site within the *COX1* mitochondrial gene sequences of *O. volvulus* and *O. ochengi*. HaeIII, unique to *O. volvulus* only, was identified as the restriction enzyme of choice for the discrimination. Onchocerca-genus primers were designed in the conserved regions of both *O. volvulus* and *O. ochengi* sequences to amplify a 650 bp fragment, which flunks the restriction site. From the conserved sequences, *Onchocerca-COX1* probe was also designed to be used in the magnetic beads capture of *Onchocerca*-DNA from blackflies DNA pool. Assay validation was done with *Onchocerca sp.* sequence data retrieved from the NCBI Genbank. The wet-lab validation of this assay was performed with archived blackflies (*S. damnosum sensu lato*) collected in 2011 from Agbelekeme, an endemic onchocerciasis community. Triplicates of 50 and 100 Blackfly pools were performed separately for heads and bodies. Blackfly pool DNA was extracted, and the Onchocerca-DNA was captured with the *Onchocerca-COX1* probe and magnetic beads. The PCR-RFLP assay was applied to the Onchocerca-captured samples, after which the PCR products were sequenced and analyzed. The restriction site, GG|CC (HaeIII), was unique to only *COX1 O. volvulus* sequences in the NCBI GenBank since they produced 456 bp and 194 bp fragments from the 650 bp PCR product. Of the three 50 blackfly head pools, only 1 (1/3) carried infective *O. ochengi* larval stage. All three 100 blackfly head pools (3/3) carried infective *O. volvulus* larval stage. However, two of the three 100 blackfly body pools (2/3) were infected with *O. ochengi*. Only one of the two infected 50 blackfly body pools carried both *O. volvulus* and *O. ochengi*. The PCR amplicons with the restriction sites showed high homology with *O. volvulus*, whereas the unrestricted amplicons were highly homologous to *O. ochengi* after the DNA sequence analysis. The novel PCR-RFLP assay has demonstrated its effectiveness in detecting and discriminating between *O. volvulus* and *O. ochengi* in blackflies. In addition, with zoonotic onchocerciasis in sight in some parts of the world, this tool will be useful for the early detection of potential zoonotic transmission of the bovine onchocerciasis in the human population, especially in Sub-Sahara Africa.

Poster 18 : Development and application of a new loop-mediated isothermal amplification assay for the diagnosis of schistosomiasis

Presenter: **Isaac Owusu-Frimpong**, *Principal Technologist, CSIR - Water Research Institute*

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As schistosomiasis keeps devastating the world, accurate diagnosis is crucial for disease surveillance and monitoring of treatment. Therefore, applying a fast, accurate, and easy-to-read LAMP assay for diagnosing schistosomiasis would significantly combat the global schistosomiasis burden. In this study, we described the outcome of the newly designed *COX1* species-specific SCH-LAMP assay and the predictability of a laboratory-prepared pH-dependent colorimetric buffer. Archived urine and stool samples from participants in Tomefa, a schistosomiasis endemic community in Ghana, were analyzed with the newly designed species-specific SCH-LAMP assay. The assays' sensitivity and specificity were estimated using bayesian latent class analysis. The positive cases of urogenital and intestinal schistosomiasis detected by microscopy increased from 48.76% and 75% to 52% and 97%, respectively, after applying the LAMP assay. The lowest detection limit was estimated as 0.0122 ng and 1.224 pg for *S. haematobium* and *S. mansoni*, respectively. Test sensitivity and specificity were estimated as 93.7% (88.1% - 97.5%) and 89.8% (84.8% - 93.9%), respectively, for the detection of *S. haematobium* whereas the sensitivity and specificity of *S. mansoni* detection were 83.4% (78.9% - 87.5%) and 90.6% (84.4% - 95.3%), respectively. The newly designed species-specific SCH-LAMP assay has demonstrated its effectiveness in diagnosing schistosomiasis; hence, its applicability in resource-limited settings is unquestionable. In addition, this tool will aid in the accurate evaluation and monitoring of mass drug administration programs.

Poster 19 : NMAS-Seq to Investigate Hybridization in *Schistosoma haematobium*

Presenter: **Dr Oluwaremilekun Ajakaye** , *Lecturer, Adekunle Ajasin University, Akungba Akoko, Ondo state.*

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

As proof-of-concept, we developed a chromosome-wide Nanopore Multiplex Amplicon Sequencing (NMA-Seq) platform using 13 genetic markers of varying phylogenetic strength to generate high-resolution data for studying schistosome genetic diversity. The NMA-Seq multiplexing assay combines dual barcoding and sample pooling of PCR amplicons to generate high depth of coverage per dual barcode using MinION sequencing. Pooled MinION sequences were deconvoluted by aligning to a custom database of all sequence types across the species complex. This methodology was used to screen for schistosome hybrids among 95 parasite isolates obtained through urine filtration or from hatched miracidia collected from two pastoral and two non-pastoral communities in Nigeria. 58 of these isolates were also Sanger sequenced to assess accuracy of the NMA-Seq results. All 95 schistosome isolates resolved as hybrids. Isolates that had been characterized as *S. haematobium* based solely on *mtCOX1* and *rITS* all possessed *S. bovis* alleles at other markers establishing that current markers are insufficiently resolved to infer the population genetic structure within this species complex. All isolates from one non-pastoral site possessed only *S. bovis* mitochondria. Two of these isolates possessed homozygous *S. bovis* alleles at several nuclear markers supporting a model of ongoing hybridization between *S. bovis* and *S. hematobium* in Nigeria. Furthermore, we recorded 99.9% agreement between our Sanger and NMA-Seq data, with a 70% cost reduction for NMA-Seq. Our approach demonstrates the utility and cost-effectiveness of this field-deployable, NMA-Seq strategy to distinguish between *S. haematobium*, *S. bovis*, and hybrids from both species.

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

Poster 20* : Comparing Shell Size and Shape with Canonical Variate Analysis of Sympatric *Biomphalaria* Species within Lake Albert and Lake Victoria, Uganda

Presenter: **Peter Andrus**, *Nottingham University; Life Sciences*

PS Andrus[1]*; JR Stothard*[2]*; NB Kabatereine*[3]*; CM Wade*[1]*;*
[1] Nottingham University; Life Sciences, UK; [2] Liverpool School of Tropical Medicine, UK; [3] Vector Control Division, Ministry of Health, Uganda

The Great African Lakes in Uganda (Lake Albert and Lake Victoria) are known habitats to several sympatric species of *Biomphalaria*, the intermediate snail hosts of the human parasite *Schistosoma mansoni*. Accurate identification of snails by morphology alone, however, can be problematic highlighting a need for robust, on-site identification methods, since only certain species have important roles in parasite transmission. This study investigates the conchological variation within *Biomphalaria* species collected from these two Great East African Lakes. We compared the shell morphologies of *Biomphalaria* species using landmark-based morphometric techniques and were able to distinguish *Biomphalaria* species through canonical variate analysis (CVA) of the umbilical and apertural shell angles. After identification with molecular methods, three *Biomphalaria* species (*B. pfeifferi, B. stanleyi* and *B. sudanica*), with heterogenous occurrences along the shoreline, were identified at Lake Albert (*n*=120) and could be differentiated from one another using a CVA of umbilical and apertural datasets. Conversly, a single *Biomphalaria* species was identified at Lake Victoria (*n*=60; *B. choanomphala*). When snails from both lakes were compared together, a CVA was able to differentiate all four species using the umbilical dataset but not the apertural dataset. Of the *Biomphalaria* species identified, ecological phenotypic variation was only found in *B. choanomphala*, which exhibited two distinct ecological morphotypes. These two *B. choanomphala* morphotypes from Lake Victoria, overlapped upon analysis of the umbilical dataset yet were clearly separated upon analysis of the apertural dataset. Our study demonstrates that landmark-based morphometrics could play a future role in distinguishing sympatric *Biomphalaria* species in Uganda.

Poster 21 : Towards a *Plasmodium* 3D cell atlas

Presenter: **Lilian Patrick Dorner**, *Student assintant, University Clinic Heidelberg, Centre for Infectiology, Parasitology*

L Dorner[1]*;*
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Across the variety of stages in the malaria life cycle, we focus our studies on the formation, egress, and motility of *Plasmodium* parasites within the mosquitoes. After mosquito uptake, *Plasmodium* undergoes rapid differentiation into male and female gametes. These egress from the red blood cells in complex ways to form a zygote. From the zygote a motile ookinete is developed to cross the midgut epithelium and differentiate into an oocyst, within which hundreds to thousands of sporozoites can form. These in turn exit from the protein-delimited cysts and enter salivary glands. To investigate these processes, we explored the use of 3D electron microscopy methods based on chemical fixation followed by serial sectioning and tomography. We have generated whole cell volumes of wild type and genetically modified parasites at different stages including microgametocytes, retorts, ookinetes, and sporozoites. These provide for striking visual representations and novel insights into gene function at a subcellular scale. Our ultimate goal is to provide a full 3D representation of the *Plasmodium* life cycle from multiple malaria parasites as a community resource.

Poster 22 : Reassessing the use of Microscopy-based Techniques in Integrated Zoonotic Parasitic Disease Surveillance

Presenter: **Freddie Freeth**, *School of Veterinary Medicine University of Surrey*

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Introduction: Essential to achieving the 2030 World Health Organization targets related to the elimination of parasitic diseases, schistosomiasis and soil-transmitted helminthiasis, the detection and surveillance of outbreaks is integral to assess and monitor the progress of elimination timelines.

Methods: This study determined the sensitivity and specificity of microscopy-based techniques for soil-transmitted helminths (STH) and schistosomes from field data gathered in the Philippines. In humans, 748 fecal samples were processed using Kato-Katz and formalin ethyl-

acetate concentration technique (FEACT). In animals, 489 fecal samples were processed using modified McMaster, sucrose flotation and simple sedimentation techniques across an animal population consisting of dogs, cats, pigs, water buffalo, and cattle. Across humans and animals, we investigated several intestinal helminth infections such as Ascaris, Trichuris and hookworm and *Schistosoma japonicum*. A Bayesian latent-class analysis Monte-Carlo Markov Chain (MCMC) model was used to evaluate the different diagnostic techniques in the absence of a gold standard.

Results: Our results suggest that Kato-Katz has higher sensitivity in detecting infection than FEACT in humans. Across the animal population, simple sedimentation had the highest sensitivity in the detection of infection across the range of intestinal helminths and *Schistosoma japonicum*.

Discussion: Kato-Katz remains the recommended diagnostic for surveillance in humans, with simple sedimentation showing promise in animal surveillance. However, both techniques have limitations that may lead to untreated populations and persistence of schistosomiasis and soil-transmitted helminthiasis. Results indicate the need for more sensitive techniques to further the control and elimination of these infections to achieve the 2030 WHO targets.

Poster 23 : Identifying host receptors for *Plasmodium falciparum* infected erythrocytes binding to human brain microvascular endothelial cells

Presenter: **Nouhoum Diallo**, *Post graduate student at SBS, University of Edinburgh*

N Diallo[1]*; AJ Rowe*[1]*;*

[1] *Institute of Immunology and Infection Research, University of Edinburgh, UK;*

Background: Sequestration of *Plasmodium falciparum* infected erythrocytes in human brain microvasculature is the hallmark of cerebral malaria. Sequestration is mediated by the molecular interaction between *Plasmodium falciparum* erythrocyte membrane protein 1 family member and receptor expressed on endothelial cells. Severe and cerebral malaria are associated with the expression of *Plasmodium falciparum* erythrocyte membrane protein 1 domain cassettes 8 and 13, but the endothelial receptor for parasites expressing these ligands remain unknown. Previous studies have identified Complement C1Q Binding Protein (C1QBP) as a potential receptor for cytoadhesion of *Plasmodium falciparum* infected erythrocytes on microvascular endothelial cells. However, despite this adhesive interaction being described, it has rarely been investigated and it is unknown whether infected erythrocyte adhesion to C1QBP plays a role in cerebral malaria.

Aims/methods: In this study, we describe the cellular localization of C1QBP as well as other putative receptors on the human brain microvascular endothelial cell line hCMEC/D3 using immunofluorescence assays and fluorescence microscopy.

Results: Resting and TNFa-activated endothelial cells showed intracellular staining for C1QBP, but cell surface staining was not observed. However, after incubation for 2 hours with soluble C1QBP, the endothelial cells did exhibit positive surface membrane expression of C1QBP in both resting and activated conditions. hCMEC/D3 cells also displayed positive surface membrane staining for other known *P. falciparum* adhesion receptors including ICAM1, VCAM1, PECAM1, CSA, EPCR and integrin aV in both conditions. Whereas other putative IE receptors such as CD36, NCAM, fractalkine, thrombospondin, CD62E and CD62P were not detected.

Conclusions: These findings suggest that although C1QBP is not constitutively expressed on the surface of microvascular endothelial cells, it can become associated with the cell surface from human serum or if added exogenously. Future work will examine the concentration of soluble C1QBP in normal plasma and during malaria infection and determine whether human endothelial cell surface associated C1QBP can serve as a receptor for *Plasmodium falciparum* infected erythrocyte cytoadherence.

Poster 24* : The Parasitic infections of the gut in Cambodian Children

Presenter: **Dr Prak Farrilend**, *Pediatrician, Angkor Hospital for Children*

P Farrilend[1]*;*

[1] *Angkor Hospital for Children, Cambodia*

Intestinal parasitic infection is one of the major childhood health proble in developing countries. According to the world health organization, over 270 million pre-school and over 600 million of school children live in areas where the parasites are intensively transmitted [1] Cambodia is the one of country in Asia, under developing country, most of the people living with poor educated about hygiene so parasite infestation is commonly seen in both adult and the children

Objective: Our object is to determine the common parasite in stool in our children, which could be a causing agent of abdominal pain and diarrhea that the other caused has been ruled out and they are coming for consultation and admission in Angkor Hospital for Children.

Method: A retrospective study chart review for 2 years from Jan 2020 to Dec 2022, in a total of 3385 cases but excluded 1610 no result was recorded and other causes of abdominal pain were ruled out. The stool was collected by Angkor Hospital for children's staff and done by laboratories inside the hospital. We focus on the common parasite among the 1775 children who compliant with non-specific abdominal pain under 16 years of age from 4 services: OPD, IPD, ICU, and Surgical.

Result: Among 1775 cases, were compliant with nonspecific abdominal pain with the result of 74% negative of the parasite, and 15% with *Blastocystis*, *Giardia lamblia* 7%, Hookworm 1%, *Entamoeba coli* 1%, *Hymenolepis nana* 1% case and *Enterobius vermicularis* 1% case. Based on our study *Blastocystosis* is commonly seen in non-specific abdominal pain in children under 16 years.

Conclusion: Cambodia is an under-developing country in which most people are having poor education related to hygiene and they are living with low sanitizing conditions in which parasite transmission could occur easily and caused a problem. Anyway, in our study that was shown that 15% is a Blastocystis species which is the common cause of GI proble in children beside of that *Giardia* Lamblia is the second most common of the causing agent, for the other more such. As Hookworm, *Hymenolepis nana*, *Hymenolepis nana*, and Enterobius Vermicularis are uncommon causing agents. Whenever 74% of cases aren't seen of the parasite which is a large amount should do further-more study in order to identify the true cause of abdominal pain in Cambodian Children.

Poster 25 : Comparative biochemical characterisation and inhibitory profiling of cattle tick, human, bovine and mosquito Flavin Adenine Dinucleotide sub-domains

Presenter: **Dr Robert Finn**, *St George's International School of Medicine*

N Cowley[1]*; R Finn*[2]*; O Sparagano*[3]*; AA Pérez de León*[4]*; M Zhang*[1]*;*

[1] Northumbria University, UK; [2] St George's International School of Medicine, UK; [3] City University of Hong Kong, China; [4] United States Department of Agriculture, United States

The Southern Cattle Tick, *Rhipicephalus microplus*, is a significant pest of tropical and sub-tropical regions of the world, who's ectoparasitism by hematophagy results in health detriments to domestic cattle because of anaemia and transmission of erythroparasites. Subsequently, this results in severe economic losses to farmers, many of whom are in developing regions and rely on their cattle for food security. These issues are additionally exacerbated by resistance in tick populations to active ingredients of common pesticides/acaricides. One of the mechanisms underlying resistance is an increased rate of acaricide inactivation/metabolism through upregulation of detoxifying enzymes such as cytochrome P450s (CYPs). In addition to their role in detoxification, CYPs also are essential for many endogenous reactions such as hormone biosynthesis. All CYPs require a single redox partner cytochrome P450 oxidoreductase (POR) to facilitate the supply electrons from nicotinamide adenine dinucleotide phosphate (NADPH) to the enzyme to catalyse the monooxygenation of a substrate. Considering this fact, it is reasoned that inhibition of POR function would consequently shut down all CYP catalysed reactions, resulting in multi-system failure within an organism. One of the first steps necessary to use this approach successfully in the control of acaricide resistance ticks, is determining if there are biochemical differences between host and parasite POR isofor that can be exploited for the development of specific inhibitory agents. Therefore, the aim of this work was to express and biochemically characterise POR from *R. microplus*, as well as the domestic cow, *Bos taurus,* in ter of their kinetic parameters for NADPH and the inhibitory activity of various adenine nucleotide analogues. To extend the value of this study, POR from the malarial mosquito, *Anopheles gambiae*, and from *Homo sapiens* were included. Previous work had indicated reduced expression of a soluble membrane truncated full-length POR from *R. microplus*, therefore a decision was made to express and characterise the conserved flavin adenine dinucleotide (FAD) binding domains from all species, which can accept electrons from NADPH and facilitate transfer to pseudoredox partners such as potassium ferricyanide. All purified FAD binding domains displayed a characteristic flavin oxidoreductase spectrum with absorbance peaks at ~379 nm and ~454 nm. Michaelis-Menten constants for NADPH as a substrate were calculated as 35.98, 62.57, 66.2 and

110.18 µM respectively for RmFAD, BtFAD, AnFAD and HFAD. The turnover, K_{cat}, for NADPH was calculated as 0.1, 0.62, 0.96 and 1.71 sec^{-1} respectively for RmFAD, BtFAD, AnFAD and HhFAD. IC_{50} (Half maximal Inhibitory Concentration) values obtained for the adenosine analogues 2'-AMP and $NADP^+$ were 3.5 mM and 190.4 µM for RM FAD; 4.7 mM and 119.6 µM for BtFAD; 3.58 mM and 149.7 µM for AnFAD and finally, 14.38 mM and 209.5 µM for HFAD. An IC_{50} value for 2', 5'-ADP could only be determined for AnFAD, 5.61 mM. Comparison of the kinetic and inhibitory profiles across all four species identified potential biochemical differences between host and parasite for both *R. microplus - Bos taurus* and *Anopheles gambiae - Homo sapiens* pairings, and that from the adenine analogue inhibitory data, that as expected, *R. microplus* and *Anopheles gambiae* are more similar biochemically to each other than to their respective hosts. These findings, therefore support the potential of POR as a target for the rational design of safer and more potent insecticides/acaricides against parasite populations that have developed metabolic resistance through modification of CYP activity.

Poster 26 : Strongyloidiasis in semi-captive baboons at Knowsley Safari, Prescot, UK

Presenter: **Dr Alexandra Juhasz**, *Dr. Alexandra Juhasz*

*E Spiers[1]; E Tinsley[1]; LJ Cunningham[1]; **A Juhasz**[1]; J Archer[1]; S Jones[1]; B Johnson[2]; J Quayle[2]; J Cracknell[2]; EJ Lacourse[3]; JR Stothard[3];*
[1] Liverpool School of Tropical Medicine, UK; [2] Knowsley Safari, UK; [3] Liverpool School of Tropical Medicine / UoL, UK

As part of their drive through safari experience, Knowsley Safari (KS) offers its visitors a close-up encounter with their colony of olive baboons (*Papio anubis*) from the safety of their vehicles. Exiting vehicles, however, are sometimes contaminated with baboon faeces, posing a small health hazard. Coinciding with an animal welfare check, a coprological survey of baboon stool, both obtained from sleeping areas and cars, was conducted. Faecal material was examined by standard parasitological methods inclusive of: QUIK-CHEK RDT (*Giardia*), Kato-Katz coproscopy (*Trichuris*) and charcoal culture (*Strongyloides*). Across a four day period, a total of 2,662 vehicles were examined with just under 700 stools obtained. Some 11.4% of vehicles were contaminated with faecal material. Overall prevalence of Giardiasis was 37.4%, trichuriasis was 48.0% and strongyloidiasis was 13.7%. Since no faecal cysts of *Giardia* could be seen by microscopy, alongside very low levels of DNA detected by faecal PCR, our RDTs results were judged misleading. Further DNA characterization confirmed the presence of *Trichuris trichiura* and *Strongyloides fuelleborni*. The latter observation represents this species' most northern report of natural transmission. To minimise any public health risk, a future blanket administration of anthelminthic(s) is recommended, with later coprological inspection(s) to ascertain reinfection levels

Poster 27* : Detection of *Toxoplasma gondii* in sylvatic rodents in Poland using molecular and serological methods

Presenter: **Pani Joanna Nowicka**, *PhD student, Medical University of Gdansk*

***J Nowicka**[1]; D Antolová[2]; A Lass[1]; B Biernat[1]; K Baranowicz[1]; A Goll[1]; M Krupińska[1]; B Ferra[1]; A Strachecka[3]; JM Behnke[4]; A Bajer[5]; M Grzybek[1];*
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Rodents are known to be reservoirs of *Toxoplasma gondii* and keep the parasite circulation in the environment. We conducted biomonitoring to assess the role of sylvatic rodents in maintaining *T. gondii* and to analyse the prevalence and seroprevalence of the parasite in seven wild rodent species. Rodents were collected in our open grassland study site located in northeastern Poland and dissected. We collected brain, spleen, blood and serum samples. We applied both molecular (PCR assay, nested-PCR assay) and serological (ELISA and agglutination tests) methods to indicate the best approach for application in the biomonitoring of *T. gondii* in small mammals. We screened samples from 68 individuals using PCR assays and found no *T. gondii* DNA. The agglutination test showed no signal. We found antibodies against *T. gondii* in 5 sera samples out of 56 analysed (seroprevalence = 8.9% [4.4-16.8]). Our results confirm that rodents participate in the life cycle of *T. gondii* as reservoirs of this parasite in the sylvatic environment. However, biomonitoring should be performed with the ELISA tests to search for *T. gondii* antigens, rather than a molecular approach only.

Poster 28 : The Design and Evaluation of a New SYBR-Green Quantitative PCR Assay for the Diagnosis Of Schistosomiasis.

Presenter: **Isaac Owusu-Frimpong**, *Principal Technologist, CSIR - Water Research Institute*

I Owusu-Frimpong[1]*; LB Debrah*[2]*; EJ Tettevi*[1]*; S Armoo*[1]*; NA Kuma*[1]*; YA Ashong*[3]*; FT Aboagye*[1]*; IK Duah*[4]*; B Idun*[1]*; MY Osei-Atweneboana*[1]*;*
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The application of quantitative PCR has been useful in the diagnosis and management of parasitic diseases. In the case of schistosomiasis, different qPCR assays have been developed from different target sequences. The mitochondrial genome has been established to be ideal for the development of a diagnostic assay since it is highly conserved and numerous in a single cell. Moreover, a well-designed SYBR Green qPCR assay is cost-effective compared to a TaqMan probe-based qPCR assay. With schistosomiasis still devastating many developing countries, it is imperative to have a cost-effective diagnostic qPCR assay which sensitive and specific. Therefore, we developed a schistosome species-specific SYBR green qPCR assay to independently target the *COX 1 gene* of *S. haematobium* and *S. mansoni*. This study used previously extracted DNA samples from 200 stool and 150 urine specimens collected from an epidemiological survey conducted at Tomefa, an endemic schistosomiasis community. Schistosome species-specific primers were designed and synthesized from the *COX1 gene* of *S. mansoni* and *S. haematobium* for the SYBR green qPCR assay. The lowest DNA detection limit was estimated as 0.122 pg and 1.216 pg for *S. mansoni* and *S. haematobium*, respectively. The outcome of the diagnostic parameter estimation by the bayesian latent class analysis (BLCA) showed a prevalence of 36.0% and 91.7% for *S. haematobium* and *S. mansoni,* respectively. Test sensitivity and specificity were estimated as 93.7% and 92.3%, respectively, for the detection of *S. haematobium,* whereas the sensitivity and specificity of *S. mansoni* were 82.6% and 90.3%, respectively. The Ct values of the positive diagnostic test ranged from 19.93 to 31.16 for *S. mansoni* and from 27.43 to 37.09 for *S. haematobium*. The correlation between the Ct values and microscopic egg counts for both *S. mansoni* in stool and *S. haematobium* in urine estimated *r* values of 0.365 ($p < 0.01$) and 0.253 ($p < 0.035$), respectively. The developed *COX1* SYBR Green qPCR assay for *S. mansoni* and *S. haematobium* detections presented in this paper has proven effective and could be applicable to epidemiological surveys for treatment monitoring. It is a quick, efficient, and accurate procedure, which can be a good substitute for schistosomiasis qPCR assays that rely on probes.

Poster 29* : Modelling host: parasitic nematode interactions with ovine 'mini-gut' organoids

Presenter: **Hannah Peaty**, *Moredun Research Insitute*

H Peaty[1]*; D Smith*[1]*; NA Mabbott*[2]*; AJ Nisbet*[1]*; TN McNeilly*[1]*; D Price*[1]*;*
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Teladorsagia circumcincta and *Trichostrongylus colubriformis* are two of the most predominant gastrointestinal (GI) nematodes of sheep in temperate regions. Their reported resistance to anthelmintics is increasing so research into new control strategies such as vaccination is vital.

Extracellular vesicles (EVs) are an area of interest for identification of potential vaccine and are released by these parasitic nematodes. However, there are challenges in studying interactions between the host and GI nematodes due to the lack of accessibility of the infection site and the need to rely on infection models which have ethical implications. Recently, ovine GI organoids have been developed which will allow host-parasitic interactions to be studied in a physiologically relevant and host specific *in vitro* cell culture system. The overall aim of the project is to use ovine GI organoids to identify and characterise active components of *T. circumcincta* and *T. colubriformis* EVs that are released during their parasitic life stages. So far, this project has separated EVs from excretory/secretory products of adult *T. circumcincta* and confirmed their presence using transmission electron microscopy and nanoparticle tracking analysis. The samples then underwent protein characterisation using mass spectrometry. Finally, the EVs will be co-cultured with organoids to confirm the uptake of EVs and identify any potential phenotypic changes.

Poster 30 : Defining the molecular determinants required for *Leishmania* life cycle progression and virulence

Presenter: **Dr Eden Ramalho Ferreira**, *Post doctoral research associate, University of York*

ER Ferreira[8]*; RP Neish*[8]*; U Dobramysl*[2]*; J Damasceno*[3]*; K Billington*[4]*; LD Davidson*[5]*; JD Sunter*[6]*; R Wheeler*[2]*; E Gluenz*[7]*; JC Mottram*[8]*;*
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Differentiation between distinct stages is fundamental for the life cycle of intracellular protozoan parasites and for transmission between hosts, requiring stringent spatial and temporal regulation. We applied kinome-wide gene deletion and gene tagging in *Leishmania mexicana* promastigotes to define protein kinases with life cycle transition roles. Phenotyping of pooled gene deletion mutants using bar-seq and projection pursuit clustering revealed functional phenotypic groups of protein kinases involved in differentiation from metacyclic promastigote to amastigote, growth and survival in macrophages and mice, colonisation of the sand fly and motility. This unbiased interrogation of protein kinase function in *Leishmania* allowed targeted investigation of organelle-associated signalling pathways required for successful intracellular parasitism (Baker et al., 2021, Nat Com 12:1244). We are now applying this approach genome-wide in the LeishGEM (http://leishgem.org/) collaborative project. We are using high-throughput reverse genetics to determine protein subcellular localisation of tagged proteins (~2,500) and function through fitness phenotyping of deletion mutants (~8,000). We are also using LOPIT-DC: Localisation of Organelle Proteins by Isotope Tagging after Differential ultraCentrifugation as an additional approach to determine protein location in Leishmania. These high throughput approaches will provide novel insights into the Leishmania-host interaction and will provide new therapeutic targets.

Poster 31 : Visceral leishmaniasis: improved molecular diagnosis using the mini direct on blood PCR Nucleic Acid Lateral Flow Immunoassay (dbPCR-NALFIA)

Presenter: **Dr Henk Schallig**, *Principal Investigator, AMC Medical Research*

H Schallig[1]*; N van Dijk*[2]*; DM Huggins*[3]*; S Menting*[2]*; DG Hagos*[4]*; E Carrillo Gallego*[5]*;*
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Accurate and early diagnosis of Visceral Leishmaniasis (VL) is important to install proper treatment, because of the fatality of the condition and the high toxicity of available treatments. Current diagnostic methods include parasitology and serology (with rK39 dipstick test and direct agglutination test). These methods do have limitations (patient safety or diagnostic accuracy), and molecular testing is proposed to improve diagnosis. Current molecular tools, in particular PCR, have high accuracy for detecting VL, however their complexity and high costs make their use unsuitable for endemic areas with limited resources. Consequently, there is a need for a simple molecular diagnostic test that can be implemented in resource limited setting. We have developed a miniaturized direct-on-blood PCR nucleic acid lateral flow immunoassay (mini-dbPCR-NALFIA) as an innovative, easy-to-use molecular assay for the diagnosis of VL in these particular settings. Unlike other simplified molecular methods, such as LAMP, the mini-dbPCR-NALFIA does not require DNA extraction and utilizes a handheld, portable thermal cycler powered by a solar-charged power pack enabling to perform the test without any laboratory infrastructure. Reading of results is done using a rapid lateral flow strip. In the present study we have conducted a laboratory evaluation on the mini db-PCR-NALFIA to determine its diagnostic accuracy. Patient samples (N=146) with suspected VL were tested using the mini db-PCR-NALFIA and compared to conventional PCR (reference test). Sensitivity and specificity represented the accuracy. Cohen's k determined the degree or agreeableness between the mini db-PCR-NALFIA and other diagnostic tests (PCR and rk39 rapid test). Compared to qPCR, the mini db-PCR-NALFIA for VL had a sensitivity of 95.83% (95% CI, 88.30%-99.13%) and a specificity of 97.22% (95% CI, 90.32% - 99.66%). The agreement between both tests was excellent (k-value: 0.93). The Limit of Detection of the platform is around 10 parasites per microliter of blood (spiked with promastigotes). The VL-mini-db-PCR-NALFIA is now ready for large field evaluations in disease endemic countries.

Poster 32 : Clinical implication of regional *Leishmania* species distribution in Ecuador: a cross-sectional study

Presenter: **Dr Henk Schallig**, *Principal Investigator, AMC Medical Research*

H Schallig[1]*; J Bezemer*[2]*; B Freire*[3]*; H de Vries*[4]*; M Calvopiña*[3]*;*
[1] AMC Medical Research, Netherlands; [2] Fundación Misión Cristiana de Salud - Shell Hospital, Ecuador; [3] Universidad de las Américas, Ecuador; [4] Academic Medical Centre(AMC), Netherlands

Among eight cutaneous leishmaniasis causing *Leishmania* species in Ecuador, *L. guyanensis* and *L. braziliensis* are dominant. Earlier studies on CL species in Ecuador focused on the Pacific areas, included only few patients from the Amazon region, and did not study patient characteristics. The resulting lack of knowledge impairs a region specific diagnosis and therapy for CL possibly leading to treatment delay and patient suffering. Patients were included from January 2019 through June 2021 by private and public primary health care centers and hospitals in the Pacific part of the Pichincha province and in the Amazonian Napo, Pastaza, and Morona Santiago provinces. All patients were subjected to a microscopic smear slide examination of a skin lesion suspected for CL in the participating centers. Patients without *Leishmania* parasite confirmation were excluded. A skin scraping and filter paper imprint sample was taken from the border of the lesion for smear slide microscopy and qPCR. *Leishmania* species was determined by Cytochrome B sequencing in all qPCR positive patients. Additional patient and geographic variables were collected per patient. All calculations were done in SPSS Statistics version 28, considering P <0,05 as statistically significant. Presence of *Leishmania* parasites was confirmed with PCR and/or microscopy in 245 patients who were included for this study. 154 patients (63%) were infected in the subtropical Pacific region and 91 (37%) in the Amazon. Infecting *Leishmania* species could be determined in 135 (73%) patients. *L. guyanensis* was the main CL causing species (93%) in the subtropical Pacific, but more than half of the patients with species determination from the Amazon was either infected by *L. braziliensis* (46%) or *L. lainsoni* (13%). Patients infected in the Pacific region had significantly (P = 0,01) higher concentrations of *Leishmania* DNA in the samples. Median health seeking delay for patients infected in the Amazon was 1 month longer (P <0,01). Lesion type and number of lesions was not significantly different across regions. *L. guyanensis* was the dominant species in CL patients in the Pacific region and health seeking delay was relatively short leading to a low risk of mucosal leishmaniasis (ML). The majority of CL lesions in the Amazon was caused by *L. braziliensis* (causative agent of ML) or *L. lainsoni*, health seeking delay was longer. We recommend future studies of determinants of health seeking delay in CL patients and regional analysis of diagnostic accuracy in Ecuador and neighbor countries Peru and Colombia.

Poster 33* : Investigating the evolution, zoonotic transmission and population structure of the intestinal worm *Ascaris* using genomics approaches

Presenter: **Lauren Woolfe**, *University of Surrey*

L Woolfe[1]*; M Betson*[1]*; AH van Vliet*[1]*;*
[1] University of Surrey, UK

Ascaris is a large soil-transmitted helminth that results in serious morbidity, particularly in lower and middle-income counties. It is a ubiquitous pathogen of both humans (*Ascaris lumbricoides*) and swine (*Ascaris suum*). *Ascaris* affects over 700 million people worldwide, resulting in childhood stunting and developmental delays. It is also the most prevalent nematode in pigs, resulting in significant economic losses. Poor hygiene practices, contact with infected pigs, the use of sewage sludge and inadequate preparation of fruits and vegetables before being consumed, propagate *Ascaris* spread. Cross infections between humans and pigs and hybridisation events between *A. lumbricoides* and *A. suum* have been reported; although, the full evolutionary relationship between the two is still unknown, with continued debate on whether these are in fact the same species.

The World Health Organisation ai to eliminate soil transmitted helminths as a health problem by 2030; however, the threat of treatment resistance is a growing concern. Therefore, it is imperative to elucidate the evolutionary relationships between *A. lumbricoides* and *A. suum*, as well as understand the interplay between humans, pigs, and the environment to help guide and maintain effective control measures in endemic regions.

This project ai to generate new whole-genome data for *Ascaris* samples from different hosts, regions and countries– using Illumina short-read and Oxford Nanopore MinION long-read sequencing. These data will be used to shed light on i) *Ascaris* transmission dynamics ii) evolutionary origin through the identification geographical differences and iii) genome response to selection pressures. We also aim to create a new multi-locus typing scheme to discriminate parasite stains in faecal and environmental samples, and field test this scheme with samples from the UK

and the Philippines.

Poster 34* : New tools for sustainable control of liver fluke in ruminants

Presenter: **Muhammad Abbas**, *University of Surrey, UK*

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Achieving food security is a significant challenge due to the continuing expansion of the global population. Food-producing animals play a significant role in our diet. Food animals such as cattle and sheep can be infected by multiple parasites, which compromise animal health and welfare and cause significant production losses, thus negatively impacting food security. The liver fluke *Fasciola* is a particularly important parasite of ruminants worldwide. This parasite can also transmit to humans, so it has zoonotic importance. In the UK, the liver fluke species *F. hepatica* is endemic in ruminants, costing the cattle industry about £13 to £40 million annually; it reduces net profit by an average of 12% for dairy far and 6% for beef farms. Climate change and porous boundaries directly influence parasite occurrence, making it difficult to control the disease. Understanding parasite genetic diversity, population genetics and multiplicity of infection (MOI), and the number of parasite genotypes in an infected host, can provide insights into infection rates, parasite self/cross mating behaviour, and potential for genetic exchange. Moreover, there is a need for user-friendly genetic markers with good genome coverage for in-depth analysis of *F. hepatica* population structure, and their impact on the emergence and spread of anthelmintic resistance, especially as markers for triclabendazole resistance, has not been identified. The current project ai to develop sensitive and rapid diagnostic tools, based on qPCR and LAMP technology, to screen *F. hepatica* from the faecal samples collected from different far in the UK. Secondly, new whole-genome data will be generated from well-characterized *F. hepatica* isolates from sheep and cattle hosts from different geographical areas. Thirdly, based on the genomic data, high-quality genetic markers will be developed and validated. These markers and the mitochondrial markers will then be used to determine the population genetics structure, MOI, and the potential for genetic exchange among *F. hepatica* populations in sheep and cattle in different locations in the UK. Control of *Fasciola* involves strategic treatment of ruminants with flukicides, guided by "fluke forecasts" based on models using climate data. Therefore, finally, we will integrate genetic data into climate-based models of *F. hepatica* transmission to improve "fluke forecasting."

Poster 35 : A synthetic vaccine against the parasitic worm *Schistosoma mansoni*

Presenter: **Emily Ablett**, *The University of Manchester*

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Schistosomiasis is a parasitic disease that affects over 200 million people worldwide resulting from infection with trematode blood flukes of the genus *Schistosoma.* There are six main human infective species, all of which have a complex life cycle involving aquatic or amphibious snails as intermediate hosts and mammalian definitive hosts. This includes *S. mansoni,* the causative agent of intestinal schistosomiasis which is characterised by inflammatory abdominal symptoms. The current treatment strategies against schistosomiasis involves mass drug administration (MDA) campaigns with the anthelminthic drug Praziquantel and prevention methods including the reduction of snail hosts and 'WaSH' sanitation programmes. However, the effectiveness of these approaches is limited due to the high likelihood of reinfection and the potential for development of drug resistance. This has driven the search for alternative approaches including the production of an effective vaccine. Anti-*Schistosoma* vaccines have the potential to induce long term immunity, as evidence exists of natural resistance in individuals within schistosome endemic areas, linked to a T-helper 2 (Th2) cell-associated response. Although there is currently no effective vaccine against schistosomiasis, several vaccine candidate antigens have been identified and tested, some of which have passed phase 1 clinical trials indicating safety and potential immune responses. However, all these candidates could be improved to reach the Preferred Product Characteristics (PPC) targets of adult worm and egg burden reduction. This could be achieved using an alternative adjuvant, such as a Virus-like particle (VLP) antigen assembly platform. Our VLP system has previously been identified as highly immunogenic against a range of diseases, including against the helminth *Trichuris trichiura,* where our initial data indicates that VLP-based delivery of T cell epitopes is a promising approach towards an effective vaccine. Building on this, we are now investigating the potential of this VLP system for development of an effective vaccine against schistosomiasis, through designed assembly of *S. mansoni* antigens based around a Hepatitis-B core VLP. Three

previously tested *S. mansoni* antigens, *Sm*14, *Sm*GST28 and *Sm*-TSP-2, have been chosen through MHC class II T cell epitope prediction (IEDB), which identified several immunogenic T cell epitopes. Through *in vivo* testing of this multi-component vaccine in a murine schistosomiasis infection model, we aim to interrogate whether VLP-based delivery of these epitopes is successful in generating effective protection against *S. mansoni.*

Poster 36* : Antileishmanial aminopyrazoles: deconvolution of the mode of action by chemical mutagenesis

Presenter: **Rokaya Ahamd**, *PhD student, University of Antwerp*

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Substantial advancements have been made in the discovery of novel antileishmanial leads and clinical candidates by phenotypic evaluation on intramacrophage amastigotes of the visceral *Leishmania* species. Aminopyrazoles have emerged as a promising series and hit-to-lead optimization by the Drugs for Neglected Diseases *initiative* (DND*i*) resulted in compounds with highly potent activity in animal models of leishmaniasis.

Molecular target deconvolution for the most potent aminopyrazoles has proven to be a major challenge because successive drug exposure failed to select for stably resistant phenotypes. Chemical mutagenesis with either ethyl methanesulfonate (EMS) or N-ethyl-N-nitrosourea (ENU) combined with drug selective pressure and whole genome sequencing was used as an alternative approach. From the obtained panel of 28 resistant lines an association between >10-fold resistance and multiple independent heterozygous mutations adjacent to the Zn^{2+} binding site of the zinc finger containing protein LINF_180011100 was discovered. Overexpression of the mutated gene increased resistance up to 10-fold, whereas susceptibility could be restored in mutant lines by transfection of a wildtype copy. Gene editing by CRISPR-Cas9 independently confirmed the contribution of the E and ENU mutations, resulting in H594Y and H594P substitutions respectively, to 10-32-fold resistance exhibited both at the extracellular promastigote and intracellular amastigote stage. Prediction of the molecular function of LINF_180011100 suggests a role in nucleocytoplasmic transport through the nuclear pore complex and cell cycle control.

Collectively, our data provide a sequential validation of LINF_180011100 as a drug target or resistance determinant for several aminopyrazole leads.

Poster 37 : Spatial distribution and epidemiological features of anthroponotic cutaneous leishmaniasis in southwest, Saudi Arabia

Presenter: **Dr Yasser Alraey**, *Assistant professor, King Khalid University*

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Background: Cutaneous leishmaniasis places a major burden on the health authorities in Saudi Arabia. Information on the geographical reach and seasonality of CL in Asir province remains limited. Therefore, this study aimed to investigate the epidemiological features of CL in southwest Saudi Arabia.

Methods: Retrospective data from CL patients in Asir province was collected between 2011 to 2020. Information analysis was performed using R statistic language (version 4.0.5) and the spatial distribution of cases was mapped using QGIS (version 3.20.0). In 2021, 194 geimsa smear samples were collected from CL patients for molecular identification of *Leishmania* strains using PCR-RFLP and PCR-HMR.

Results: A 1565 CL cases were recorded from 2011 to 2020. Children under the age of 13 years were most at risk of contracting CL. CL lesions were primarily located on the face and most cases were reported in the winter and autumn seasons. Geographical expansion of CL between

governates was noted during past ten years. Based on PCR-RFLP and PCR-HMR, 183 patients showed positive amplification of L. tropica and five patients showed positive amplification of *L. major*.

Conclusions: This study describes how the geographical change of CL incidence differs and reveals those people most at of CL infections. Also, it reveals the existence of co-circulation of ACL and ZCL in Asir province. This study highlights the importance of incorporating improved living conditions, school education and public awareness in the development of CL control policies.

Poster 38* : Optimization of gastrointestinal organoids for improved host-parasite interaction studies: the enrichment of rare and specialized host cell types

Presenter: **Will Anderson**, *University of Glasgow*

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Organoids are self-organizing, multicellular cell culture models that are representative of the tissue from which they are derived. The development of these three-dimensional culture syste is accelerating research that seeks to address specific questions tied to human and animal health.

Due to their multicellular composition, organoids represent valuable *in vitro* tools for modelling specific host:parasite interactions. However, some specialized cell types have a very low prevalence in organoids grown under normal growth conditions. Using differential transcriptomics and immunofluorescence microscopy analysis, we demonstrate the optimization of ruminant gastric and intestinal organoid cultures for the enrichment of specialized cell types. We used a single cell transcriptomic dataset of the ovine abomasum to establish cell type-specific markers and found a significant increase in multiple cell markers associated with paneth cells, tuft cells and goblet cells in our differential RNA-seq analysis of bovine and ovine gastric and intestinal organoids grown in differentiation conditions. These findings were supported by immunofluorescence analysis of organoids cultured in growth and differentiation conditions. Using the tuft cell transcription factor POU2F3 as an indicator of tuft cells and Ki67 as a proliferation factor, we found an increase in relative number of tuft cells and a decrease in proliferative stem cells when organoids were cultured in differentiation conditions. To facilitate the counting of cells across lots of organoids and samples, we also developed an imageJ macro for automated cell counting within organoids that represents a useful research tool. Specialized cell types play important roles during parasitic infection. For example, tuft cells are important parasite-sensing cells that trigger the host immune response through the release of IL-25. However, tuft cells are a rare cell type, representing <1% of cells in GI epithelia. This low abundancy makes studying specialized cell types difficult *in vivo*, even with the advance of single cell sequencing technology. Therefore, the ability to enrich for rare cell types in organoid cultures poses a valuable research development for studying their biology and more specifically, for determining the specific roles and responses of rare and specialized cell types during infection with different pathogens of medical and veterinary importance.

Poster 39* : Exploring the cellular targets of anti-leishmanial natural product analogues

Presenter: **Hannah Asiki**, *University of Oxford*

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The dehydrodieugenol family of natural products has specific activity against all *Leishmania* species tested. This prompted synthesis of related analogues, with improved potency comparable to Miltefosine. Elucidating the mechanism of action of these compounds could validate the "goodness" of target and aid drug development efforts. Initial evidence suggested these compounds affect the mitochondrion, however evidence was weak and the protein target(s) are unknown.

We have developed an active analogue with both cross-linking and click capabilities which allows access to various techniques for target identification. As a first approach, we attach a fluorescent azide using *in situ* click to visualise fluorescence localisation of the compound in *L. mexicana*. We show that photo cross-linking using a diazirine on the small molecule is necessary to prevent compound wash-out during the click

protocol. Click-conjugated fluorescence appeared mitochondrial, which we have confirmed using colocalisation with a tagged mitochondrial protein.

These analogues are also a powerful toolkit for photo-affinity protein pull-downs and related techniques. Combining this with parallel approaches, including selecting for drug resistant mutants and transcriptomic analysis of treated cells, will allow us to evaluate protein targets of this family of compounds.

Poster 40 : Draft long-read assembly and annotation of the Chagas disease vector *Rhodnius prolixus*

Presenter: **Antonella Bacigalupo**, *PhD Student, University of Glasgow, SBOHVM*

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Chagas disease is a chronic infection with the protozoan parasite *Trypanosoma cruzi* (Trypanosomatida: Trypanosomatidae), which is transmitted by insect vectors of the subfamily Triatominae (Hemiptera: Reduviidae). American trypanosomiasis continues to be neglected, even among the 'neglected tropical diseases', with no reduction in associated disability-adjusted life years (DALYs) during recent years. In this context, seven years after the publication of the first triatomine species whole genome, only two genomes from other triatomines have been released. Our aim is to enable a resurgence of Chagas disease vector research via sequencing, assembly, and annotation of new triatomine vector species: *Belminus herreri*; *Mepraia spinolai*; *Psammolestes arthuri*; *Rhodnius brethesi* and *Rhodnius ecuadoriensis*. As first step, we sequenced one *R. prolixus* individual by long and short reads, assembled its genome, polished, scaffolded, and annotated it, using as reference the previous assemblies and annotations for *R. prolixus* (GCA_000181055.3; RproC3.5) and *Triatoma rubrofasciata* (Triatoma_chr_assembly; Triatoma_chr_genome). Our new assembly has 1,270 scaffolds and a N50 of 1,466,963 that includes 18,279 genes coding for 18,328 mRNAs with 93,193 CDSs. The benchmarking universal single copy ortholog (BUSCO) gene completeness of our draft assembly reached 98.4% of the hemiptera_odb10 and 97.8% of the insecta_odb10 databases, which are higher than the 96.6% and 95.1% of the current *R. prolixus* reference, and the 98.2% and 97.7% of the *T. rubrofasciata* chromosomal assembly, respectively. We will complement these encouraging results by combining this reference-based annotation with *ab initio* and transcriptome evidence, and we will follow this procedure for the other triatomine species. These genomes will allow for new research on the genomic basis for adaptation in these vectors. This work was supported by Minciencias Convenio 727 DIeI from UR Colombia; ANID - Programa Becas - Doctorado Becas Chile 2019 72200391; Wellcome [204820/Z/16/Z].

Poster 41 : A review of factors influencing over- dispersion of ectoparasites on non- human host species: A lesson for COVID- 19 infections

Presenter: **Prof Zumani Banda**, *Director of Research, ShareWORLD Open University*

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Information regarding factors related to over dispersion of COVID- 19 among humans may be scanty or unknown. However, several studies have documented over dispersion of ectoparasites in non- human host species. This review therefore focuses on analysing factors influencing over- dispersion of ectoparasites on non- human host species with an aim of understanding the spread of COVID- 19 infections among humans. Data was extracted from various data bases on the world- wide web, books and reports. Only articles that covered any combination of over-dispersion with ectoparasites, humans, non- human hosts and COVID- 19 were analysed. Data was synthesized based on the variables of interest. The findings indicate that host diet, age, sex, behaviour, immunity, and physiological status may be responsible for over- dispersion of ectoparasitic species in many host populations. The study further revealed that season and geographical location may also influence the occurrence of ectoparasitic species on different hosts. Understanding how over dispersion works is key to preventing, managing and controlling parasitic and infectious diseases in both human (such as COVID- 19) and non- human host species.

Key words: COVID- 19, ectoparasites, humans, non- human hosts, over- dispersion.

Poster 42* : Two peas in a pod? The roles of REL1 and REL2 in uridine insertion/deletion RNA editing

Presenter: **Laurine Brouck**, *PhD student, University of Edinburgh*

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Uridine insertion/deletion mRNA editing is essential for mitochondrial gene expression in kinetoplastids. This intricate process is directed by small guide RNAs (gRNAs) that provide the genetic information to generate mature, translation-competent mRNAs. Editing involves several macromolecule complexes that anneal gRNA and mRNA, cut the mRNA at specific sites, add or remove uridine nucleotides, and re-ligate the edited mRNA fragments. For each round of editing, this final step is performed by two RNA editing ligases, REL1 and REL2. While REL1 is essential for the survival of the sleeping sickness parasite *Trypanosoma brucei*, REL2 knockdown by RNAi has not produced any detectable phenotype. This is unexpected given that both ligases are active *in vitro* and that strong purifying selection is acting on both ligase genes, as indicated by their low dN/dS ratios. These observations suggest conserved function over evolutionary time. By combining genomics and structure-function analyses, we aim to elucidate the respective roles of REL1 and REL2 in RNA editing. RNA sequencing of editing intermediates in *T. brucei* REL1 knockdown cells revealed the particular importance of this ligase in sealing two types of sites: i) those where editing usually stalls, potentially due to structural barriers, and ii) 'non-canonical' sites, where the mRNA appears to have been cleaved erroneously, suggesting a potential repair function. Moreover, a comparison of substrate requirements using recombinant proteins showed that REL1 is more tolerant than REL2 towards nucleotide mismatches in the gRNA-mRNA anchor duplex. This difference between the two ligases may stem from their highly divergent interdomain region, which is predicted to interact with the RNA substrates. We also generated REL2 null mutants in promastigote *Leishmania mexicana* using CRISPR-Cas9, confirming the non-essential nature of this enzyme across trypanosomatids and providing new opportunities to investigate the function of this ligase. REL1, on the other hand, could only be deleted from the *L. mexicana* genome in the presence of an ectopic REL1 gene copy. In summary, our data confirm functional divergence of the two REL paralogs across trypanosomatid parasites and suggest a structural basis for differences in substrate specificity.

Poster 43* : Initial steps in the study of catalase function and localisation in *Leptomonas seymouri*

Presenter: **Lubomira Chmelova**, *Ph.D. student, University of Ostrava*

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Catalase is a ubiquitous enzyme involved in the protection against reactive oxygen species (ROS). Its main function is the decomposition of hydrogen peroxide to water and oxygen. Hydrogen peroxide is not only a harmful molecule, it is also known to participate in redox signalling and regulation of biological activities by the oxidation of thiolate groups. Therefore, it is important to keep the level of this molecule in the nM range in a cell. This task is accomplished by a set of different enzymes (peroxidases, catalase, peroxiredoxins). Despite its wide distribution, some eukaryotic lineages lack catalase. The catalase-encoding gene is, for instance, absent in species inhabiting anoxic conditions, parasitizing in blood, or photosynthetic eukaryotes with secondary plastids. An interesting pattern of catalase distribution can be found in the family Trypanosomatidae. It has been shown that the gene encoding catalase was acquired three times independently from three different bacterial lineages *via* horizontal gene transfer by the monoxenous Leishmaniinae (from *Brachyspira* spp.), Blastocrithidiinae (from *Snodgrassella* spp.), and Vickermania spp. (from *Acinetobacter* spp.). Subfamily Leishmaniinae is an intriguing example to study catalases of Trypanosomatidae. It unites dixenous (with two hosts in the life cycle), medically important species (*Leishmania sensu lato*) and monoxenous (one host in the life cycle) species. The catalase gene is present in the genomes of monoxenous relatives and was secondarily lost in dixenous species suggesting that presence of this enzyme is incompatible with dixeny. Here we investigated the role of catalase in *Leptomonas seymouri*, a monoxenous trypanosomatid of the subfamily Leishmaniinae. This species is thermotolerant and was often documented in immunocompromised patients or

co-infections with *Leishmania donovani*. We report that *L. seymouri* is amenable to genetic manipulations using conventional and CRISPR/Cas9-mediated approaches by establishing lines with catalase ablation (KO) and add-back (AB). Three investigated cell lines (WT, KO, and AB) were similar in the growth kinetics and morphology, while the cytotoxicity assay revealed an increased resistance to hydrogen peroxide in case of AB compared to WT and KO counterparts (EC_{50}: 1,76; 1,73; 4,07 mM H_2O_2, respectively). The cell line with endogenously tagged catalase was used to study its localization (by IFA and biochemically). We demonstrated that this enzyme has dual localization – glycosomes and cytoplasm – unifying previously reported contradicting results in trypanosomatids. Moreover, the proportion of cytoplasmic catalase increased significantly upon treatment with hydrogen peroxide.

Poster 44 : The molecular basis of heat shock signalling in African trypanosomes

Presenter: **Dr Caroline Dewar**, *Postdoc, Lancaster University*

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The heat shock (HS) response represents a significant virulence factor for African trypanosomes, with disease manifesting in the mammalian host as periodic fever which is sensed by the parasites, requiring adaptation to this stress. Eukaryotic cells typically trigger general protein translation arrest to prevent accumulation of misfolded proteins, whilst increasing the expression of proteins which aid protein folding and degradation.

While a HS response does occur in *Trypanosoma brucei*, the molecular mechanisms mediating this response are not understood. Transcription factors are not present to mediate a global response to the stimulus, and translational arrest is independent of the mechanism found in mammals. Preliminary evidence suggests regulation occurs through a novel post-transcriptional mechanism involving dynamic phosphorylation of unique regulatory RNA binding complexes (Ooi et al., 2020). We aim to combine global quantitative proteomics with phosphoproteomics to kinetically profile trypanosome HS signalling, and examine the role of specific phosphorylation events on post-transcriptional regulators.

We are examining the HS response in both *T. brucei* and the less well characterised *T. congolense*, the leading cause of AAT (Animal African Trypanosomiasis), utilising novel genetic manipulation techniques now possible in this organism. These parasites co-infect the same hosts with exposure to similar evolutionary selective pressures, and it is expected they will show some similarities in their host interactions. We will show data examining the impact of key early phosphorylation events on HS response components TbDHH1, TbPABP2 and TbZC3H11 in survival and progression through the parasite HS response.

Poster 45 : P.I.G & TIBA parasitology samples biobank

Presenter: **Neil Duncan**, *Biobank Manager, University of Edinburgh*

N Duncan[1]*;*

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The P.I.G/TIBA biobank is a unique collection of samples that have been accumulated over a period of over 20 years. These samples are invaluable in the ongoing research into Neglected Tropical Diseases (NTD's) such as Schistosomiasis. The samples comprise Peripheral Blood Mononuclear Cells (PBMC), Plasma, Sera, DNA, RNA, Urine and Stool. The biobank is curated at the University of Edinburgh's Kings Buildings Ashworth Laboratories. The P.I.G/TIBA
biobank is continually growing with samples being collected as part of ongoing studies into NTD's.

Poster 46 : Longitudinal analysis of the impacts of Urogenital Schistosomiasis on the gut microbiota of adolescents in Nigeria

Presenter: **Dr Olumide Ajibola**, *First Technical University*

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The main causative species of urogenital schistosomiasis in sub–Saharan Africa is *Schistosoma haematobium*. The gut microbiome is important for many host physiological processes and helminths have the ability to colonise the same environment or have indirect systemic impacts on the gut, and these interactions may lead to microbial changes. We carried out a longitudinal study of the impacts of *S. haematobium* infection on the gut microbiome of adolescents (11-15 years) in northern Nigeria pre and post praziquantel treatment in order to establish the parasite as a cause of gut microbial changes or determine impact that praziquantel treatment may have. Using 16S sequencing a total of 267 DNA from faecal samples of infected versus uninfected adolescents were amplified following earth microbiome project protocols and sequenced on an Illumina Miseq. We observed that at baseline, the microbiomes of infected versus uninfected adolescents revealed a state of dysbiosis due to increased *Proteobacteria* and decreases in *Firmicutes* and *Cyanobacteria*. We assessed the diversity of the taxa using alpha diversity metrices and observed that using Shannon index we obtained significant differences when we compared infected samples at 3, 9 and 12 months to baseline uninfected controls (P= <0.0001, P=0.0342 and P=0.0003 respectively). Microbial community composition analysis revealed that there were only significant differences at 3, 9 and 12 months (P=0.001, P=0.001, P=0.001 and P=0.001, respectively). Across all time points we also observed significant differences in the differential abundance of the genera at baseline, while the genera at 3 and 6 months resembled the baseline genera changes, the changes at 9 and 12 months were more similar to each other with an increase in *Prevotella*. We also tested for the effects of the drug praziquantel on gut microbiome differences, and we demonstrated that the effects of the infection on the gut was more significant than the drug. Overall, our data suggests that *S. haematobium*, a non-gut resident parasite has indirect interactions with the gut. The bacterial taxa changes we have identified opens up the opportunity to investigate their role in human health, especially in urogenital schistosomiasis endemic communities.

Poster 47* : Effect of repeated anthelmintic exposure on livestock commensal faecal bacteria

Presenter: **Olivia Ingle**, *PhD Student, Queens University Belfast*

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Controlling parasitic helminths is an integral part of ruminant farm management and is highly dependent on using effective anthelmintics. Significant resistance of ruminant gastrointestinal helminths to a broad spectrum of anthelmintics has been well established across the UK, in a time where antimicrobial resistance (AMR) has been declared as one of the biggest challenges to One Health for this generation. Studies in mammals have shown that anthelmintic administration has profound effects on commensal faecal bacteria, particularly in young individuals. Whilst research on parasite-microbiome interactions has increased, very little is known on how anthelmintic treatments may affect ruminant AMR.

We collected faecal samples from individual lambs experiencing their first grazing season residing on two separate pastures to determine whether repeated anthelmintic usage had any effect on A in two experimental treatment groups: 1) Blanket treated dependent on usual treatment schedule (Control group), and 2) targeted-selective-treatment (TST) group. These individual faecal samples were then pooled into their respective pasture group, where bacterial swabs were taken to grow on selective media. We used simple disk diffusion assays using several antibiotic classes to identify resistance. Whilst we determined differing levels of antibiotic resistance in lambs residing on separate pastures; no difference in resistance was observed between the two treatment groups. As local environments are frequently exposed to livestock faeces, through manure fertilizer, and pasture run-offs, further research into livestock management may be of further benefit to combatting A through One Health ideology.

Poster 48 : Queuosine-tRNA modification as a means for gene expression regulation in *Leishmania mexicana*

Presenter: **Dr Julie Kovarova**, *Biology Centre CAS*

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All kinetoplastid parasites including *Leishmania* have polycistronic transcription, hence regulation of gene expression is mediated mostly by post-transcriptional mechanisms. One of the post-transcriptional steps is represented by tRNA modifications that directly regulate translation by modulating codon–anticodon interactions. The Queuosine (Q) modification is found at the wobble position 34 of tRNAs containing the GUN anticodon, leading to the change of GUN into QUN anticodon. Consequently, the efficiency of the translation of NAU and NAC codons by Q-tRNA is symmetrical.

Here, we examine the role of the Q-tRNA modification in *L. mexicana* differentiation and infectivity. Increased abundance of Q-tRNAs in the amastigote stage, when compared to the insect promastigote stage, suggests an important role for this modification in the mammalian-infective stage. The production of Q-tRNAs is catalysed by a highly conserved heterodimeric enzyme, termed tRNA guanine transglycosylase (TGT1/2). To get a deeper insight we employed CRISPR/Cas9 to generate a gene knock-out (KO) for TGT2 subunit in *L. mexicana*, which resultedin the depletion of Q-tRNAs as expected. We did not observe any growth phenotype in TGT2 KO when cultured as promastigotes, or when differentiating promastigotes into amastigotes. Although the TGT2 KO amastigote stage did not show any growth defect in culture, the KO cells exhibited reduced infectivity in macrophages *in vitro* as compared to WT. Most importantly, mice infected with *L. mexicana* TGT2 KO developed significantly smaller lesions than with WT. The decreased infectivity could be a consequence of alteration in the host's immune response. However, the immune analysis of the infected mice showed no difference in the levels of IgG1 and other markers, but a decrease in IgG2a, which corresponds with a lower parasite abundance in the lymph nodes after TGT2 deletion.

In order to explain the observed phenotypes, we performed a proteomic analysis and assessed the abundance of the NAU codons, decoded by Q-tRNAs, in genes encoding the depleted proteins in the KO strain. Overall, TGT2 depletion resulted in a reduction of several proteins, which genes nevertheless mostly did not contain a higher proportion of NAU anticodons. However, a few outstanding hits emerged. Namely, the metalloprotease Gp63, an important virulence factor of leishmania, was significantly reduced in the KO proteome. Interestingly, Gp63 contains a higher proportion of NAU. The arginyl-tRNA synthetase, encoded by a gene with a high proportion of NAU, was also reduced in the TGT2 KO providing a plausible explanation for reduced levels of other proteins, independent of NAU codon frequency.

Here, we conclude that the Q modification is required for *L. mexicana* infectivity. Most likely, the defect is primarily not due to interference with the immune response of the host, but a consequence of altered protein expression in leishmania. Alltogether, the Q modification represents another way how the parasite can regulate the gene expression, and adapt to different hosts and conditions.

Poster 49* : The neglected role of microbes in *Fasciola hepatica*-host interactions: a multi-omic approach

Presenter: **Mae Carpenter**, *Student, Aberystwyth University*

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The diagnosis and control of liver fluke, *Fasciola hepatica* remains a highly researched area due to the dwindling amount of control options. By investigating parasite-host interactions to understand how a parasite can manipulate the microbiome there is potential for new biomarker discovery which represent novel treatment options.

Gallbladder bile represents a so far undiscovered country of uncharacterized microbiota and their relationship to hepatobiliary related diseases, bile was until recently considered a sterile body fluid as it would not provide a suitable growth environment for bacterium, however bacterial flora has been reported in healthy individuals the result being the discovery of enteric bacteria which are able to persist through high concentrations of bile. This study will take a combined multi- omics approach into bile through its meta-taxonomy, genomics and proteomics, with an aim to uncover the nature of helminth infection on bile DNA and how it changes as a result of infections. Meta-proteomics will be used to characterise the protein content F. hepatica secretes into the gallbladder identify protein origins from the host and parasite.

Poster 50 : Sustainable Drugs against NTDs: Lessons learned from investigating the potential use of propolis-based natural products for treating various for of human and animal trypanosomiasis

Presenter: **Dr Godwin Ebiloma**, *Teesside University*

GU Ebiloma[1]*; JO Igoli*[2]*; DG watson*[3]*; HP de Koning*[4]*;*

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Diseases caused by kinetoplastid parasites such as various species of Trypanosomes and *Leishmania* represent some of the Neglected Tropical Diseases (NTDs). However, millions of people, and their domesticated animals, living in endemic regions across the globe are at risk of these NTDs. All of the associated human and veterinary conditions can be disabling or fatal if not adequately treated, and no vaccines are available. Still, drug treatment is hampered by the challenges of drug resistance and toxicity to the mostly very old drugs. We have been researching the potential use of propolis-based natural products as a sustainable treatment option. Propolis is a resinous substance that bees harvest from nearby vegetation to protect themselves naturally from microbial infection. Our interdisciplinary team have collected propolis from various geographical locations across the globe and has tested the crude and purified extracts on various strains of these parasites. Unique new compounds with potent antiparasitic activity, particularly have been identified. Our results show that propolis and some of the phytochemicals isolated from it, have no *in vitro* growth inhibition against mammalian cells, but displayed low EC_{50} against *Trypanosoma* and *Leishmania* species, without a loss of activity against diamidine- and arsenical-resistant or phenanthridine-resistant *T. brucei* strains, or a miltefosine-resistant *L. mexicana* strain. These results provide sufficient scope for further investigations of propolis-derived natural compounds for the rational development of sustainable drugs against human or veterinary diseases caused by these parasites.

Keywords: Propolis; Trypanosome; Leishmania; kinetoplastid; drug discovery; NTDs

Poster 51 : Developing A Multiplex PCR-Based Diagnostic Test For Malaria

Presenter: **Hafsat Alabere**, *Student, Aberystwyth University*

HO Alabere[1]*; K Blinkhorn*[1]*; J Buckle*[1]*; T Bennet*[1]*; R Williams*[1]*; T Brett*[1]*; H Marshall*[1]*; S Kollarik*[1]*; W Jones-Warner*[1]*; JC Hafalla*[2]*; CJ Sutherland*[2]*; J Pachebat*[1]*;*

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Malaria is an endemic disease in most African countries, and it has been implicated as one of the causes of morbidity and mortality worldwide. This is partly due to resistance evolved among the *Plasmodium* species and the difficulty in controlling its vector (Female *Anopheles* mosquitoes). Compared to microscopy and RDT, traditional PCR is highly specific and sensitive. However, it only amplifies one gene locus at a time. Very few assays target multiple loci in the same reaction. Hence, this study ai to develop multiplex PCR assays to amplify various diagnostic sequences in *Plasmodium falciparum* and *Anopheles* species to provide additional information regarding drug and insecticide susceptibility in one test. To date, a highly sensitive and specific multiplex PCR assay targeting the *18S, pfmdr1* and *pfcrt* genes of *Plasmodium falciparum* has been developed using a set of primer combinations amplifying 395bp, 559bp and 145bp of the genes, respectively. Diagnostic sensitivity of the nested multiplex PCR is higher for the *mdr1* gene as the minimum detection limit is 88.04 copies of the *P. falciparum* genome in a reaction of $1/10^5$ dilution. It is proposed that the multiplex PCR has the potential to be a cost-effective, time-reducing, highly specific and sensitive test for malaria diagnosis. Further targets are being incorporated into this assay, and it is planned to apply next-generation sequencing to multiplex PCR amplicons products, which will provide additional data for control and treatment.

Poster 52 : Can larval tapeworms stimulate the immune response of mice to facilitate the reduced progression of melanoma cancer cells?

Presenter: **Dr Tomas Macháček**, *Postdoc, Charles University, Prague*

*M Schreiber[1]; **T Macháček**[1]; M Majer[1]; B Šmídová[1]; V Vajs[1]; O Tolde[1]; D Rösel[1]; J Brábek[1]; P Horák[1];*
[1] Charles University, Prague, Czech Republic

Several studies on mouse models have reported that infection with some helminths can reduce the progression of specific cancer types. However, the critical anti-tumor effector mechanisms remain largely elusive. Here, we present our observations that mice infected with *Mesocestoides corti* or *Taenia crassiceps* dramatically suppress the proliferation and spreading of B16F10 melanoma cancer cells in the peritoneal cavity pre-occupied by the larval tapeworms. To dissect the role of host immunity in this protection, we performed a complex flow cytometry analysis of the sites affected by the tapeworms and melanoma (the peritoneal cavity, liver, and lungs) in ICR and C57BL/6J mice. Additionally, we tested the antigen-specific cytokine production of splenocytes stimulated by either tapeworm or melanoma antigens. While the cytokine profile of splenocytes generally remained unaltered after the restimulation, we detected a massive increase in CD8+ T cells and NK cells in the peritoneal cavity. As these cell types bear a powerful anti-tumor arsenal, their contribution to host protection against melanoma is being further investigated.

Acknowledgment: The study was supported by Czech Science Foundation (21-28946S).

~~Poster 53* : Analysing the impact of sexual recombination on the segregation of virulence genes in African trypanosomes~~

Presenter: ~~**Shannon Massey**~~, ~~*Roslin Institute*~~

~~***S Massey**[1]; J Prendergast[1]; A MacLeodP Steketee[1]; E Paxton[1]; L Morrison[1];*~~
~~*[1] Roslin Institute, University of Edinburgh, UK; [2] University of Glasgow, UK*~~

~~*Trypanosoma brucei* causes severe health and economic burden across sub-Saharan Africa. These extracellular parasites undergo frequent antigenic variation by altering the variant surface glycoprotein (VSG) present on their cell surface, resulting in chronic infections of humans and animals, causing both Human and Animal African trypanosomiasis. Trypanosomes undergo sexual recombination in the salivary gland of the tsetse fly vector, which has the potential to mix up the VSG gene repertoire in trypanosome progeny, but exactly how this occurs is unclear. Understanding how meiotic recombination influences the content of the VSG repertoire could have implications in the field where both human-infective and non-human-infective trypanosomes circulate in the same geographic area. My project ai to sequence the genomes of two parental trypanosomes (TREU 927 and STIB 247) and six hybrid progeny to investigate this further. To date, Nanopore and PacBio HiFi sequencing have been used to generate high quality, high coverage, contiguous parental genomes which have been scaffolded using Illumina Hi-C reads. Chromosome-scale contigs have been assembled, highlighting genome completeness which can be used to improve the current reference genome. Six progeny genomes are currently being sequenced with the aim of studying how the VSG repertoire segregates~~ within the hybrid progeny, providing a better understanding of how virulence genes are propagated in trypanosome populations through meiosis.

Poster 54 : Using eDNA for the detection of sheep helminths in a range of environmental sample types

Presenter: **Emer McCann**, *PhD student, Queen's University Belfast*

***E McCann**[1]; C McFarland[1]; P McCann[1]; P Brophy[2]; GN Gobert[1];*
[1] Queen's University Belfast, UK; [2] Aberystwyth Uinversity, UK

Gastrointestinal helminth and nematode parasites are responsible for considerable economic and welfare burdens in UK agriculture. With the climate changing and UK weather conditions becoming warmer and wetter, the prevalence and distribution of some parasites has increased. Anthelmintic resistance is also increasing to drug classes including benzimidazole, levamisole and ivermectin. As the situation continues to change, livestock parasite management approaches need to be reconsidered in the UK. To achieve this, we need to look towards new improved ways to detect and treat livestock parasites.

All organis shed DNA into their surrounding environment. This “environmental (e)DNA” as a biomarker enables the detection of species diversity and abundance, even for rare species. Isolating parasite eDNA in the environment from multiple matrices offers the opportunity to improve current diagnostic methods at the pasture level. The ability to detect parasite specific biomarkers in the environment has the potential to assess infection risk at the whole-farm scale.

This study will be carried out on sheep far in Northern Ireland utilising grass, soil, and water as environmental sources for the detection of gastrointestinal helminth parasites. Availing of the improved sensitivity and quantitative capabilities of ddPCR technology to improve detection and risk prediction using specific primer-probes. Longitudinal collections of host dung samples will be obtained alongside environmental samples throughout the grazing season. Host dung samples will demonstrate the changes in parasite taxa as grazing progresses, whilst environmental samples will be used as source material for eDNA surveys of parasites. Paired environmental and host sampling of dung will strengthen links between the risk of helminth infection and confirmed infections. Throughout this study, eDNA degradation trials will be carried out to further understand how this parasite biomarker decays in the environment under varying abiotic factors. Understanding these factors will allow us to better model parasite risk prediction. DNA extraction methods for different environmental matrices will be developed and assessed. Improved knowledge of changes in sheep parasite community throughout the grazing season coupled with improved knowledge of parasite distribution at the pasture level, will be used by farmers to guide grazing management and anthelmintic treatments.

Poster 55 : *Trypanosoma carassii*, a model for whole host interaction studies

Presenter: **Sarah Monic**, *Post-doc, University of Cambridge*

S Monic[1]*; E Dóró*[2]*; M Forlenza*[2]*; M Carrington*[1]*;*

[1] *University of Cambridge, Department of Biochemistry, UK;* [2] *Wageningen University and Research Centre, AFI, Netherlands*

Trypanosoma carassii is a freshwater fish parasite that infects a variety of cyprinids (carp family). The prevalence approaches 100%, both in the wild and in fish farms. Here, the procedures for long term culture and transgenesis of *T. carassii* are described as the first step in developing a model to study host-pathogen interaction in zebrafish. We show that *T. carassii* can be genetically modified using approaches developed in *T. brucei* and these have been used to make *T. carassii* cell lines expressing mNeonGreen and Ruby fluorescent protein transgenes driven by either RNA pol II or RNA pol I. The cell lines are currently being used to infect transparent zebrafish larva to allow the tracking of all trypanosomes infecting a host. The response of the fish immune system will be visualised during infections of zebrafish lines with marked immune cell lineages. Together, these experiments will answer long standing questions about tissue tropism, sites of proliferation and extravascularisation, and how the host immune system responds in different tissues.

Poster 56 : Malaria parasites in the intestine – inflammation & intestinal permeability in *Plasmodium chabaudi* AS infected mice

Presenter: **Dr Jason Mooney**, *The University of Edinburgh*

JP Mooney[1]*; SM DonVito*[1]*; R Lim*[1]*; E Riley*[1]*; J Thompson*[1]*;*

[1] *The University of Edinburgh, UK;*

Mild gastrointestinal sympto can be observed during *Plasmodium* spp infection with reports of increased intestinal permeability during *P. falciparum* infection. Malaria-induced enteritis may provide an opportunity for pathogenic intestinal bacteria to breach the intestinal mucosa, resulting in life-threatening bacteraemia. To begin to define intestinal pathology during a mild/resolving malaria infection, C57BL/6J mice were inoculated (i.p.) with recently mosquito-transmitted *P. chabaudi* AS (PcAS). At schizogony, intestinal tissues were collected for qPCR analysis and immunohistochemistry for immune mediators and malaria parasites. Inflammatory proteins were measured in plasma and faeces and intestinal permeability was assessed by measurement of FITC-dextran in plasma 1 hour after oral administration. Parasitaemia peaked at 0.5-3.5% at days 7-9 and resolved by day 14, with mice experiencing significant and transient anaemia but no weight loss. Plasma IFN-ɣ was significantly elevated at day 7, with raised IL-10 concentrations on subsequent days. qRT-PCR of the intestine revealed a significant increase in transcripts for ifng and cxcl10 on days 7 to 11, respectively, along with parasite 18S rRNA. Histological analysis revealed parasites within blood

vessels of both the submucosa and intestinal villi and evidence of mild crypt hyperplasia. In faeces, the inflammatory marker lactoferrin was raised on days 9 and 11. FITC-dextran in plasma (evidence of increased intestinal permeability) was detected on days 9 and 11, and was significantly positively correlated with peripheral parasitemia and faecal lactoferrin. Using a relevant model, we have found that mild, acute malaria infection is associated with intestinal inflammation and increased intestinal permeability. This model can now be used to explore the mechanisms of parasite-induced intestinal inflammation and to assess the impact of increased intestinal permeability on translocation of pathogenic enterobacteria.

Poster 57* : Phylogeny unites apicomplexan and fungal transmembrane proteins in an ancient eukaryotic superfamily

Presenter: **Rachael Murray**, *University of Edinburgh*

R Murray[1]*; J Baranovic*[1]*; E Wallace*[1]*; J Thompson*[1]*;*
[1] *University of Edinburgh, UK*

Eukaryotic pathogens are more closely related to their vertebrate hosts than bacterial pathogens, making drug discovery more challenging. The ideal drug target is essential in the pathogen and lacks homologs in the host, therefore accurately detecting homology is important for drug target identification.

We identified a new family of transmembrane proteins that are found in diverse eukaryotic clades but not in vertebrates. Members of this family include Cysteine Repeat Modular proteins (CRMPs) which are required for host cell invasion in the apicomplexan parasite *Toxoplasma gondii*, and fungal FLC proteins which are required for virulence in diverse fungal pathogens. Our phylogenetic analysis suggests this protein family could be targeted to treat a range of eukaryotic pathogens, however, we have almost no understanding of how these proteins function at the molecular level.

We show that structural predictions of CRMPs and FLCs share a unique arrangement of nine transmembrane helices, and we identify conserved motifs shared by members of this family. Topology predictions show that these proteins have an extracellular N-terminus that is highly variable in domain composition and length, and an unstructured cytosolic C-terminus. Overall, the conserved transmembrane domain coupled to variable extracellular domains is characteristic of transmembrane sensory signalling proteins. Future work will determine if and how these proteins sense and transduce environmental signals.

Poster 58* : Towards improved antileishmanial drug screening models to capture the risk of post-treatment relapse

Presenter: **Yasmine Nicolaes**, *PhD student, University of Antwerp*

L Dirkx[1]*; **Y Nicolaes***[1]*; L Maes*[1]*; G Caljon*[1]*;*
[1] *University of Antwerp, Belgium*

Post-treatment relapse occurs with all known antileishmanial drugs and is of global medical concern. Apart from treatment failure, the scarce collection of existing drugs suffers several drawbacks including toxicity, high cost, parenteral administration requiring hospitalization and the emergence of drug resistance. Moreover, the current R&D pipeline does not capture the risk of relapse, as the ontogeny is poorly understood and currently no efficient prognostic tests exist.

The recent discovery of long-term hematopoietic stem cells (LT-HSC) as a major parasitic niche for drug survival, sheds new light on the underlying mechanisms of disease recurrence. This cellular niche located in the bone marrow serves as a sanctuary where visceral *Leishmania spp.* rapidly adopt a quiescent phenotype. Both the cellular niche and parasite quiescence confer *Leishmania* tolerance to drug exposure. Initialassay development relied on LT-HSC isolation by lineage depletion and fluorescence-activated cell sorting (FACS). This is an arduous and time-consuming process resulting in limited cell yields which restricts its use as an *ex vivo* assay in the lead optimization process. Also with regard to animal ethics, strategies have been explored to reduce the use of mice for bone marrow collection.

Here we describe the optimization of *ex vivo* LT-HSC expansion, a pivotal step towards the establishment of a medium throughput antileishmanial drug screening platform aimed at improving lead selection to reduce the risk of relapse.

Poster 59 : Diffuse reflectance spectroscopy for mosquito surveillance

Presenter: **Dr Mauro Pazmino**, *Postdoctoral researcher, University of Glasgow*

M Pazmino*V Ochoa-GutiérrezH Ferguson[2]; M González- JiménezK WynneF Baldini[2]; D Childs*
[1] University of Glasgow, UK; [2] School of Biodiversity, One Health and Veterinary Medicine, University of Glasgow, UK

Rapid, low cost, high-throughput tools for vector surveillance are urgently needed to develop and optimise new vector control strategies, as vector borne diseases (VBD) are spreading around the globe due to climate change and globalisation, and endemic countries are suffering resurgence of malaria cases following weakening of control tools. Mid-infrared spectroscopy (MIRS) combined with machine learning analysis has shown potential for quick and efficient identification of mosquito species and age groups, which are key traits monitored in VBD surveillance programmes. The main advantages of this optical method are its speed, lack of sample preparation, low cost and established protocols and analysis pipelines. However, current MIRS technology to collect spectra is destructive to the sample and does not allow targeting specific tissues of the mosquito, limiting the identification of other important biological traits such as insecticide resistance. Here, we assessed the use of a non-destructive approach of MIRS for vector surveillance, micro diffuse reflectance spectroscopy (μDRIFT) using mosquito legs to identify species, age and cuticular insecticide resistance within the *Anopheles gambiae* s.l. complex. We measured a total of 344 samples from two species, *An. coluzzii* and *An. gambiae* and two age groups, 3 and 10 days old. Different parts of the mosquito were scanned using μDRIFT to assess their suitability. Legs required significantly less scanning time and showed more spectral consistence compared to other mosquito tissues. Logistic regression was able to identify species (*An. gambiae* and *An. coluzzii)* with an accuracy of 73%. Random forest differentiated the two ages groups with 77% accuracy, and we obtained accuracy of 75% when identifying cuticular insecticide resistance suing support vector machines. Our results highlight the potential of different mosquito tissues and μDRIFT as a tool for biological trait identification on mosquitoes that transmit malaria. By targeting different parts of the mosquito, it opens the possibility to increase MIRS versatility to monitor insecticide resistance and beyond. Our results can guide new ways of identifying mosquito traits which can help the creation of innovative surveillance progra by adapting new technology into mosquito surveillance and control tools.

Poster 60 : Cell cycle synchronization in African trypanosomes

Presenter: **Max Pendlebury**, *Student, Lancaster University*

M Pendlebury*[1]; CG De Graffenried[2]; MU Urbaniak[1];*
[1] Lancaster University, UK; [2] Brown University, United States

Trypanosoma congolense and *Trypanosoma brucei* are vector-transmitted parasites that cause Animal African Trypanosomiasis (AAT) – a livestock wasting disease that results in significant economic losses in sub-Saharan Africa. The regulation of the cell cycle is not fully understood in *T. brucei*, and much less understood in *T. congolense*. Greater understanding of the regulatory mechanisms underpinning the cell cycle could provide novel targets for treatment of African Trypanosomiasis. Study of the trypanosome cell cycle could be improved by utilizing reliable cell synchronization techniques.

The main drawback with synchronization protocols relying on chemical inhibition, such as hydroxyurea synchronization, is the potential for chemically induced artifacts. Centrifugal counter-flow elutriation (CCE) avoids this by separating cells based on sedimentation velocity and has been used to monitor the changes in phosphorylation status of proteins in *T. brucei*. CCE also avoids the drawbacks of flow cytometry cell-sorting methods which are more time consuming and result in a majority non-proliferative population. No synchronization methods have been described for *T. congolense*, and much less is known about the cell cycle, including the timing of nuclear and kinetoplast genome division and the changes in cell morphology.

We show data demonstrating the synchronization of both bloodstream form and procyclic form *T. brucei* by CCE and compare it to our current progress in synchronizing *T. congolense*. We also provide data comparing the synchronization of *T. brucei* and *T. congolense* by both CCE and

hydroxyurea mediated arrest. Success of synchronization is judged by the uniformity of cell size and DNA content within synchronized populations, which is assessed by flow cytometry. Cell lines have also been generated in *T. brucei* with YFP tagged PUF9 and PLK – which have been shown to change

in either abundance or localization throughout the cell cycle. Synchronization can then be assessed by western blots to show changes in abundance, and immunofluorescence microscopy to show changes in localisation. In the future, these will be compared to tagged *T. congolense* cell lines to assess the differences in PLK and PUF9 localisation between the species.

Poster 61 : Diversity and composition of gut protists in young rural Zimbabwean children

Presenter: **Dr Lorraine Pfavayi**, *Postdoc, University of Edinburgh*

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[1] University of Edinburgh, UK; [2] National University of Science and Technology (NUST), Zimbabwe; [3] University of Cambridge, UK; [4] University of Zimbabwe, Harare, Zimbabwe

Background: The human gut microbiome harbours diverse species of archaea, bacteria, fungi, protists and viruses. To date, most gut microbiome studies have focused on bacteria, neglecting other microbial communities. Consequently, less is known about the diversity and abundance of the latter. Here, we aimed to characterise the diversity and composition of protists in the gut of preschool-aged children (PSAC) in rural Zimbabwe relative to host age, sex, and schistosome infection status.

Methods: The gut protist of 113 PSAC (1-5 years) was examined via shotgun metagenomic sequencing and analysed for diversity. Variation in protist abundance with host and environmental factors was analysed by permutational multivariate analysis of variance (PERMANOVA). To investigate how the composition of specific taxa varies across age, sex, nutritional measures and *Schistosoma hematobium* infection status, analysis of the composition of microbiomes (ANCOM) was used.

Results: Eighty protist genera were identified, and the most abundant genera detected was *Blastocystis.* The prevalence of pathogenic protists was comparatively low, with 12.1% and 3.4% of the participants' gut colonised by *E. histolytica* and *Cryptosporidium,* respectively. Of all the independent variables only *S. haematobium* infection showed significant relationship with the structure of the gut protist, being associated with increases in *Peronospora*, *Pseudoperonospora*, *Plasmopara* and *Blastocystis* (FDR= 0.009).

Summary: This study provides data on the prevalence and diversity of the gut protists in young Zimbabwean children with an emphasis on the host factors; age, sex and schistosome infection status. Our results showed no association between the host factors investigated, including anthropometric measures adjusted for age and the intestinal protist composition and structure, but *S. haematobium* infection status was associated with composition of specific taxa. There is a need for more studies determining how pathogenic protist interact with non-pathogenic protist in people exhibiting clinical sympto to inform therapy and nutraceuticals.

Poster 62 : Oligo targeting for profiling acoziborole resistance mutations in *Trypanosoma brucei*

Presenter: **Dr Melanie Ridgway**, *University of Dundee*

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Current drug treatments for human African trypanosomiasis require hospitalisation and disease stage diagnosis by painful lumbar puncture. However, recent phase 2/3 trials for an oral dose of acoziborole showed efficacy against both early and late-stage disease. Benzoxaboroles, including acoziborole, target a subunit of the cleavage and polyadenylation specificity factor complex (CPSF3). We previously developed a simple oligo targeting method for rapid and precision editing of drug targets in otherwise wild type trypanosomatids. Here we report progress in scaling up this approach for saturation mutagenesis of CPSF3. Of >1000 introduced mutations within 5 Å of the predicted binding site of acoziborole in the catalytic pocket of CPSF3, only those encoding Asn^{232}His conferred resistance to 1 or 3 µM acoziborole. Furthermore, none

of the mutations yielded resistance to higher doses of acoziborole selection (9 or 27 µM). This may reflect a restrictive mutational space in the CPSF3 catalytic pocket and demonstrates limited scope for acoziborole resistance conferring mutations within CPSF3.

Poster 63 : Application of SHERLOCK detection for epidemiological surveys of Animal African Trypanosomiases

Presenter: **Dr Brice Rotureau**, *Trypanosome Cell Biology Unit - Institut Pasteur*

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Animal African trypanosomiasis (AAT) is a disease caused by parasites of the genus *Trypanosoma*. After the successful development of a diagnostic test for human African trypanosomiasis, we have adapted the CRISPR-based detection toolkit SHERLOCK (Specific High-sensitivity Enzymatic Reporter unlocking) for trypanosomatid parasites responsible for AAT. SHERLOCK first amplifies nucleic acid using recombinase polymerase amplification (RPA), which is then combined with the Cas13a nuclease for RNA target recognition via specific guides (crRNAs) and a fluorescent reporter linked to a quencher. Target sequence recognition by Cas13a results in promiscuous ribonuclease activity which cleaves the fluorescent reporter, emitting fluorescence used for detection. To test the applicability of this technique in the field, we analysed 360 domestic animal samples (sheep, goat, pig and dog) from two surveys in Cameroon and Côte d'Ivoire. The preliminary results of this pilot study show that the SHERLOCK4AAT method is able to detect and discriminate between trypanosome species involved in multiple infections with a high sensitivity, especially in blood samples. In Côte d'Ivoire, we determined that approximately 60% of the Trypanozoon-positive blood samples collected on free-ranging pigs were co-infected with *T. congolense*, while no *T. vivax* infections were detected. We are now focussing on further improving the sensitivity of the assay and developing a multiplex version for species discrimination in a single test.

Poster 64 : Ablation of *Leishmania mexicana* Ku80 activates ALT pathway necessary for telomere maintenance

Presenter: **Dr Andreu Saura**, *University of Ostrava*

***A Saura**[1]; E Poláková[1]; P Fajkus[2]; J Fajkus[2]; V Yurchenko[1];*

[1] University of Ostrava, Czech Republic; [2] Masaryk University, Czech Republic

Genome stability is guarded by telomere-associated proteins, which play an essential role in protecting the telomere ends from the nuclease activity. Among them, telomerase, Ku70/80, and additional proteins coordinate a fine-tuned mechanism to accomplish this purpose. In contrast, the alternative lengthening of telomeres (ALT) is a homology recombination-dependent pathway presented as an alternative to the canonical telomere maintenance mechanisms. Telomeres of *Leishmania mexicana* get elongated in the absence of Ku proteins. It was proposed that they are maintenance by the ALT pathway. Here we demonstrated that this is indeed the case, as we were able to amplify the 30G-overhang ends targeting specific chromosomes of *L. mexicana* and detect *t*-circles upon ablation of Ku80 in this protist. In addition, we have endogenously tagged the *L. mexicana* Ku80 protein and demonstrated its association with telomeres and interaction with signal transduction intermediates that facilitate DNA repair.

Poster 65 : The Reece Lab On Tour

Presenter: **Dr Petra Schneider**, *Petra Schneider*

***P Schneider**[1]; AJ O'Donnell[1]; J Holland[1]; SE Reece[1];*

[1] University of Edinburgh, Institute of Ecology and Evolution, UK

Public engagement can be amazing! We get to talk about our work, about the cool job we do every day and we get exposed to a different view on the science questions we try to tackle.

In the last 2 years, the Reece Lab turned the idea of going on a science road trip into reality. We took >1000 primary school pupils and their teachers on an adventure to learn about our research.

We've translated our work on malaria parasites, their hosts and vectors, and their biological rhyth into a learning program for primary schools across Scotland, and associated teacher-training. We've travelled around Scotland with a tropical mosquito colony in the back of the car, met the most amazing people, were reminded of how cool being a scientist actually is, and were asked the most interesting and unexpected questions.

Along the way, we learned a lot about designing public engagement, how to improve it, and how to make it sustainable within our research. We'll be sharing some of our experiences with you during the public engagement workshop on Thursday, but please come see our poster too!

Poster 66 : Hell, in Paradise. A portrait of Dengue from Abdulrazak Gurnah's masterpiece

Presenter: **Dr Valeria Silvestri**, *Post Grad Msc Student, MUHAS University of Dar es Salaam*

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Introduction: It is not rare to find in literature at its highest level the powerful representation of human disease. It is the case in the novel Paradise by Abdulrazak Gurnah, which contains a description of a likely *Dengue* outbreak.

Aims and methods: We have analysed the paragraph from the novel emphasizing details that could suggest a Dengue aetiology for the outbreak described among members of the caravan protagonists of the novel.

Early the following day, they set off for Chatu's country. […] They travelled on narrow country paths, beating uphill through lush vegetation. […] Clouds of insects circled their heads. When they stopped to rest, the insects alighted on them and sought out orifices and tender flesh. At the end of their first day in Marungu several of them had fallen ill. They were tormented by mosquitoes in such numbers that in the morning their faces were bloodied and scarred with bites. […] By the third day, the afflicted men were desperately ill and others were showing signs of decline. The worst ones could neither eat nor prevent their bodies from evacuating. Their fellows carried their stinking bodies in turn, ignoring their delirious groans as much as was possible and trying to evade the black blood that oozed out of them. On the steep inclines the men could only move a few feet at a time, dragging their burdens on hands and knees. On the fourth day two of the men died. They buried them quickly and waited an hour while the merchant silently read a sura from the Koran. All of them were now tormented by festering sores, which the insects dug deep into to lay their eggs and draw fresh blood. […] When two more men were found dead on the fifth morning …"

Discussion: Dengue was reported in Tanzania since the fifteenth Century and during the nineteenth century on the Islands of Zanzibar and was endemic at the time and geographic setting where the novel *Paradise* takes place (the actual Republic of Tanzania, during times of colonial invasion). Mosquito vectors are described as "*Clouds of insects circled their heads*" through the lush vegetation, encountered "*early the following day*", likely the day-time beating *Aedes* mosquito, responsible for disease transmission.

The members of the caravan initially exposed to the endemic serotype in coastal regions, could have been infected with a different serotype when traveling into mainland, explaining the rapid onset of sympto due to antibody dependent enhancement. Accordingly, the incubation period for Dengue virus is 4–7 days, followed by a febrile phase and constitutional symptoms, well described in a clear temporal sequence, from "*the end of the first day*" to the "*fifth morning*" as asthenia, anorexia and "*black blood that oozed out of them",* or haemorrhagic manifestations typical of severe disease. The short incubation period, the intensity of sympto reported by the majority of cases and the fatality rate favours the hypothesis of ADE in diagnosis of *Dengue* reinfection during the journey in a region endemic for a different viral serotype, and exclude malaria from differential diagnosis, for which premunition in endemic regions would favour asymptomatic infection.

Conclusions: Notwithstanding the public health burden of Aedes invasive species and of its transmitted arboviruses in Tanzania, there is still nowadays insufficient information on vector dynamics and transmission risk in most parts of the Country. The powerful pictorial view of this endemic condition provided by Gurnah could inspire, through the beauty of art, further research.

Poster 67 : Modification of a novel Whipworm vaccine candidate with a highly immunogenic Tetanus epitope

Presenter: **Jacob Thompson**, *University of Manchester*

J Thompson[1]*; J Derrick*[2]*; KJ Else*[1]*;*
[1] *University of Manchester, UK;* [2] *Lydia Becker Institute of Immunology & Inflammation, UK;*

Trichuriasisis a disease that affects ~465 million people worldwide resulting from infection by the intestinal dwelling parasitic nematode *Trichuris trichiura*, colloquially known as the human whipworm. Whipworm infections can cause colitis, growth retardation, and Trichuris dysentery syndrome (TDS). The current treatment strategies for Trichuriasis and other soil-transmitted helminths promoted by the World Health Organisation (WHO) involve mass drug administration (MDA) campaigns. However, the effectiveness of these MDA campaigns, particularly against *T. trichiura,* is poor. Low drug efficacy has fuelled the search for alternative treatments for Trichuriasis including the development of anti-*T. trichiura* vaccines, which have the potential to provide long lasting immunity. In a recent attempt to synthesize an effective vaccine candidate against whipworms, Zawawi et al genetically fused MCH-II T cell epitopes pertaining to chitin-binding domain-containing proteins (CBDs) and chymotrypsin-like serine proteases (CLSPs) conserved between *Trichuris spp.* to virus-like particles (VLPs). Building upon this vaccine candidate, we fused a Tetanus epitope to these modified VLPs with hopes to improve their immunogenicity and subsequently their effectiveness at eliciting protective immune responses against whipwor *in vivo*. However, using the *Trichuris* mouse model, *T. muris*, we found no significant correlates of protection resulting from vaccination with these VLPs in C57BL/6 mice against a high dose (200 eggs) *T. muris* infection. Therefore, further research and optimization of this VLP based anti-*Trichuris* vaccine candidate is required if it is to progress to clinical trials.

Poster 68* : How can Nanoscale Secondary Ion Mass Spectrometry (NanoSIMS) be used to explore whipworm feeding, and anthelminthic mechanism of action?

Presenter: **Macaulay Turner**, *University of Manchester*

M Turner[1]*; KJ Else*[1]*; KL Moore*[1]*;*
[1] *University of Manchester, UK*

Trichuris trichiura is a major public health concern infecting around half a billion people and causing the loss of around 640,000 Disability Adjusted Life Years. The parasite inhabits the caecum and proximal colon of infected individuals with its anterior end inhabiting an intracellular epithelial cell niche. Currently we do not know what the parasite feeds on, or even its main route of feeding. The parasite has a mouth but lacks a muscular pharynx, arguably making feeding through the mouth unlikely. *Trichuris spp* have a structure termed the bacillary band which runs two thirds of the circumference of its anterior end; the function of the bacillary band has long been debated and has been implicated in feeding.

NanoSI is a high-resolution secondary ion mass spectrometry instrument (beam size can be focused to 50nm) that can be used to image and measure elemental and isotopic distributions in samples at subcellular scale. It has extremely high sensitivity which makes it possible to detect elements at parts per million concentrations depending on the element. Stable isotope probing involves the exposure of a sample to a compound labelled with a stable isotope, then investigating the isotopic enrichment in the sample to infer mechanism of uptake and incorporation.

By exposing the whipworm to isotopically labelled nutrients and then imaging with the NanoSIMS, we aim to show which nutrients the whipworm utilises, and the mechanism of uptake, including the possible involvement of the bacillary band. Ascertaining the feeding mechanism of *Trichuris spp* will help identify novel drug targets. Further, we are also using NanoSI approaches to understand mechanisms of drug uptake and action

Poster 69 : Albendazole efficacy against gastrointestinal nematodes of pigs in Nsukka Local Government Area of Enugu State, Nigeria

Presenter: **Dr Chukwunonso Obi**, *University of Nigeria, Nsukka*

*KI Idika[1]; **CF Obi**[1]; TA Nzeakor[1]; SI Aideyan[1]; GE Aneru[1]; CO Nwosu[1];*
[1] University of Nigeria, Nsukka, Nigeria

Albendazole is the most commonly used anthelmintic in the Nigerian pig industry; however, its continued efficacy is increasingly threatened by emergence of drug-resistant gastrointestinal nematodes (GINs) strains. Thus, the efficacy of albendazole against GINs in pigs was investigated in Nsukka area of Enugu State. Faecal samples were collected per rectum from randomly selected 130 pigs in 13 pig far and examined for GINs. Six out of the 13 pig far were thereafter selected on the basis of no anthelmintic treatment for a 2-month period, from which 10 pigs each were randomly selected and marked for the efficacy of albendazole studies using faecal egg count reduction test (FECRT). Faecal samples were collected from each pig and analysed to determine the pre-treatment FEC prior to albendazole administration. Ten days post-treatment, faecal samples were also collected for post-treatment FEC. Albendazole resistance was confirmed where the FECR percentage was less than 95% and the lower 95% confidence limit was less than 90% but if only one of the two criteria was met, resistance was suspected. GIN prevalence rate of 63.1% was obtained with mixed infection having 74.6% prevalence rate. Albendazole resistance to GINs and trichurids was established in one pig farm but was suspected in two farms. Resistance of strongyle wor to albendazole was suspected in three far but confirmed in one farm while albendazole resistance to ascarids was suspected in five farms. This study revealed varying degrees of efficacy of albendazole against GINs and demonstrated possible presence of albendazole resistance against GIN populations in pigs reared in Nsukka area as well as low efficacy of albendazole against trichurids.

Poster 70 : *Cryptosporidium parvum* dysbiosis of the faecal microbiome of bovine livestock: A computational metagenomic approach

Presenter: **Mumdooh Sabir**, *University of East Anglia*

***M Sabir**[1]; R Low[2]; P PintoA Tsaousis4[4]; GR Hurle[1]; KM Tyler[1]; N Hall[2];*
[1] University of East Anglia, UK; [2] Earlham Institute, UK; [3] University of Kent, UK; [4] University of Edinburgh, UK

Cryptosporidium is a protozoan parasite and is the causative agent of cryptosporidiosis in humans and animals. Sympto of the infection may include abdominal pain, vomiting and diarrhoea or may also be asymptomatic. Agricultural losses globally due to *Cryptosporidium* infection in cattle amount to several billion dollars. The severity of infection depends on many factors, including host immunity. Here, the impact of the gut microbiome on infection was studied. The gut microbiome is the community of bacteria resident in the digestive system. Ruminants including cattle have a stomach with four compartments specialised for pre-gastric digestion. It has previously been demonstrated that specific alterations in the bovine microbiome can facilitate growth of cryptosporidial parasites. Therefore, cryptosporidiosis can lead to long-term dysbiosis of the gut. It has been reported that infections with *C. parvum* are associated with changes in the microbiota as well as a shift in metabolites in the host gut.

Poster 71 : The *Trypanosoma brucei* DNA damage repairome

Presenter: **Dr Monica Zavala Martinez**, *Institut Pasteur*

***M Zavala Martinez**[1]; E McLaughlin[1]; A Dujeancourt-Henry[1]; T Chaze[1]; QG Gianetto[1]; M Matondo[1]; MD Urbaniak[2]; L Glover[1];*
[1] Institut Pasteur, Paris, France; [2] Lancaster University, Biomedical and Life Sciences, UK

Trypanosoma brucei, is an extracellular parasite that has evolved to evade the host's immune system by antigenic variation, facilitated by an extensive variant surface glycoprotein (VSG) repertoire. Antigenic variation principally involves homologous recombination (HR), a complex process that requires both genetic factors and post-translational modifications (PTMs). Several reversible PT (phosphorylation, SUMOylation, ubiquitination, acetylation) are vital for the tight and accurate response to cellular damage but, until recently, only one DNA damage-associated phosphorylation site had been identified in *T. brucei,* that of γH2A. Using an unbiased single-locus biochemical screen, we characterised a double strand break (DSB) at a chromosomal internal region versus a bloodstream form expression site (BES). We detected 6500 phosphorylated sites, including a core set of 211 DSB-responsive phosphosites and found that dephosphorylation predominates at a BES, highlighting a key difference between breaks at these two genomic loci. We identified two additional DSB modifications on the H2A: S113 and

S133, with only T131 and S133 being conserved amongst trypanosomatids, and a novel phosphorylation site at the C terminus of H2B (Tb927.10.10590), S39. Combined, these findings suggest that histone phosphorylation is important to the *T. brucei* DDR, possibly mediating access to associated chromatin. Additionally, we found two phosphosites on RPA1 (Replication Protein A1), S5 and S43, that play a role in efficient DNA repair. We aim to generate a map of repair interactions in trypanosomes and define the 'repairome', which has the potential to provide invaluable insights into the dynamics of VSG switching.

Poster 72 : Tropical Stewart-Treves syndrome: a review of Lymphangiosarcoma occurring in patients affected by chronic lymphatic filariasis

Presenter: **Dr Valeria Silvestri**, *Post Grad Msc Student, MUHAS University of Dar es Salaam*

V Silvestri[1]*; V Mushi*[1]*; MI Mshana*[1]*; W Bonaventura*[2]*; W John*[3]*; A Kalinga*[3]*; B Ngasala*[1]*;*
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Background: Lymphatic filariasis is a parasitic disease caused by filarial nematodes (including *W. bancrofti, B. malayi and B. timori*) , transmitted to humans by mosquito vectors (*Anopheles*, *Aedes*, *Culex*, and *Mansonia)*. One of the clinical manifestations of lymphatic filariasis is chronic limb lymphoedema. Lymphangiosarcoma has been described as a potential complication of chronic lymphedema of various aetiology, including lymphatic filariasis.

Aims: The aim of the study was to describe the occurrence of Stewart-Trevor syndrome in patients with a history of chronic lymphatic filariasis.

Methods: A review of the literature was performed by consulting PubMed, EMBASE, and Scopus databases using the keywords "filariasis" AND "lymphangiosarcoma" OR "Stewart-Treves syndrome". Case reports were considered for inclusion. All available cases reporting the association were included, with no exclusion criteria. Descriptive statistics (mean, standard deviation; median 25th–75th percentile) were used to summarize the continuous variables while frequency and proportions for the categorical variables using the computer software JASP, version 0.14.1.

Results: We retrieved nine cases with the mean age of patients being 45±15.6 years (ranging from 19 - 67 years). The majority (n=six cases) of patients were men. Lymphangiosarcoma was diagnosed in all patients after a long history of chronic lymphatic filariasis, of at least 10 years (mean 19.8±7.4 years).

Lesions (the majority ulcerative) were present from 2 to 24 months before lymphangiosarcoma diagnosis, and were associated with lymphadenopathy in five cases. Pain, anaemia and infection were occasionally reported. Differential diagnosis included Kaposi's sarcoma and lymphangiomatosis; in one case a benign lesion was diagnosed before malignant transformation.

Patients were treated in the majority of cases with major amputation (eight cases) which was above the knee in five cases, below the knee in two cases, in the attempt to achieve eradication of the local disease.

Exitus was reported in two patients, in one for unknown causes and in the other because of the appearance of lung metastasis 3 months after diagnosis.

In patients still alive at report, lung and peritoneal metastasis were reported after 8 years of follow up in one case; four cases were free of metastasis at follow up, but in one case disability secondary to amputation was reported; in one case recurrence of lymphangiosarcoma on the amputation stump occurred.

Conclusions: Lymphangiosarcoma should be considered among differential diagnosis in patients affected by chronic lymphatic filariasis presenting with skin lesions. Lymphatic filariasis patients should be monitored for possible development of lymphangiosarcoma with prompt biopsy of

Poster 73* : Global genetic diversity of the *Plasmodium falciparum* malaria in pregnancy vaccine candidate VAR2CSA DBL2X

Presenter: **Gabrielle Ngwana-Joseph**, *PhD Student, London School of Hygiene & Tropical Medicine*

G Ngwana-Joseph[1]*; S da Silva Santos*[2]*; M Suárez-Mutis*[2]*; D Nolder*[1]*; CJ Sutherland*[1]*; T Clark*[1]*; S Campino*[1]*;*
[1] *London School of Hygiene & Tropical Medicine, UK;* [2] *Instituto Oswaldo Cruz, Brazil*

Malaria infection during pregnancy (MIP) has adverse clinical consequences for both mothers and their unborn child, with no effective vaccine currently available. MIP is caused by *Plasmodium falciparum* infected erythrocytes sequestering to the placenta and massively accumulating. The binding tropism is mediated by VAR2CSA, a parasite-derived protein expressed on the infected erythrocyte surface, which binds to placental chondroitin sulfate A (CSA). Two vaccine candidates under development, PRIMVAC and PAMVAC, target overlapping constructs of the VAR2CSA CSA-binding region, ID1-DBL2X. Clinical data from Phase I trials suggest insufficient cross-reactivity of the candidates against heterologous VAR2CSA variants.

Little is known about the global genetic diversity of the ID1-DBL2X domain, which may impact vaccine efficacy. Here, we analysed >1,200 *P. falciparum* DBL2X sequences spanning 26 countries. 983 DBL2X haplotypes subdivided into four phylogenetic clades. Despite no geographic basis to this clustering, nucleotide diversity was greatest in African populations. The immensely polymorphic nature of DBL2X is principally driven by small insertions and deletions (indels), with increased indel density adjacent to the highly conserved CSA-binding residues. Ongoing work involves the identification of clade-specific consensus sequences to proactively support the development of a polyvalent VAR2CSA vaccine for broadly neutralising protection against MIP.

Poster 74 : Validation of *Dermanyssus gallinae* target genes for use in developing RNA interference (RNAi) mediated gene silencing as a novel tool for parasite control

Presenter: **Naomi Morrison**, *Moredun Research Institute*

N Morrison[1]*; D Price*[1]*; AS Bowman*[2]*; S Burgess*[1]*; AJ Nisbet*[1]*; JM Sternberg*[2]*;*
[1] *Moredun Research Institute, UK;* [2] *University of Aberdeen, UK*

Dermanyssus gallinae (Poultry Red Mite; PRM), a blood feeding ectoparasite, is the most significant parasite to the egg laying industry. PRM feed on poultry blood, causing itchiness, irritability and increased mortality. These detrimental effects lead to decreases in output and quality of poultry eggs, ultimately leading to losses estimated at €230 million per annum for the European egg laying industry. A need for novel control methods is fuelled by changes to legislation for facilities and increasing resistance to chemicals currently relied on for control. Preliminary studies have been performed towards exploring RNA interference (RNAi) mediated gene silencing as a method to validate novel targets in PRM. We have demonstrated that it is possible to selectively silence target genes by feeding dsRNA to adult female mites as part of their blood meal. To further develop RNAi-mediated gene silencing we selected sixteen target genes that perform essential cellular functions in mites and have been previously validated as effective targets in other arthropods. Orthologues of these sequences were identified in *D. gallinae,* and their sequences were confirmed by RT-PCR and Sanger sequencing. dsRNAs for each of the target gene are currently being synthesised and will be assessed for their ability to: i) silence the target gene and ii) impact mite survival and/or fecundity. These initial experiments will provide a base for the future objectives of this project, such as performing a large-scale RNAi screen and developing novel, state of the art delivery methods.

Poster 75* : A mutation in sterol C22 desaturase leading to Amphotericin B resistance in *Leishmania infantum*

Presenter: **Dr Supriya Khanra**, *University of Glasgow*

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Visceral Leishmaniasis is a neglected tropical disease, caused by parasitic protozoa of the *Leishmania* genus. In recent years, the polyene amphotericin B (AmB) has emerged as a treatment of choice against the disease. The drug acts by binding ergosterol in the parasite membrane, leading to cell lysis. *L. Infantum* promastigotes were selected for resistance to AmB. Sterol analysis of the resistant line identified a loss of ergosterol and an increase in 5,7, 24(28)-ergostatrienol. Genome sequence analysis revealed mutations in sterol C22 desaturase which converts 5,7, 24(28)-ergostatrienol to ergostatetraenol. The mutation comprises a 21 base pair deletion corresponding to a 7 amino-acid hydrophobic patch at the periphery of the enzyme. Over-expression of this mutant allele in WT parasites also yielded amphotericin B resistance while over-expression of the WT allele in the mutant cell line restored sensitivity. Moreover, the resistant parasites retained virulence in mice and the resistant line was not cleared by amphotericin B whereas WT were in treated mice. The data indicate that amphotericin B resistance in *Leishmania infantum* can come about through changes to the sterol pathway, as previously indicated in other amphotericin B resistance *Leishmania* lines where mutations to different enzymes in the sterol pathway were noted. Interestingly, the *L. infantum* AmB resistant line described here is hypersensitise to nitric oxide inducing agents and also to pentamidine, as has been described for other AmB resistant lines, offering a potential route to treatment of resistant cases should their emergence become problematic in the field.

Poster 76 : The evaluation of host antibody response to *Ixodes ricinus* as an indicator of exposure and disease risk

Presenter: **Haya Alkharaz**, *PhD student, University of Aberdeen*

H Alkharraz[1]; P Wilhelmsson[2]; PE Lindgren[2]; A Bowman[1]; J Sternberg[1];
[1] University of Aberdeen, UK; [2] Linköping University, Sweden

Antibodies against tick salivary protein antigens are potential markers of tick exposure. In a systematic review of *Ixodes spp*. salivary proteins and host-response, we selected calreticulin as a candidate antigen with which to study the antibody response to *I. ricinus* bite. The aim was to determine if human antibody responses to calreticulin detect exposure to ticks, and whether bioinformatic prediction may be used to develop a peptide-based immunoassay.

I. ricinus calreticulin (Genbank ID: AAR29958.1) was expressed as a recombinant protein (rCT). Linear B-cell epitopes were predicted using IEDB tools and non-linear epitopes using Ellipro and Discotope. Putative cross-reacting B-cell epitopes were screened using protein BLAST search. Overlapping 15amino acid oligopeptides were synthesized to cover predicted diagnostic epitopic regions. Sera were obtained from human subjects with confirmed tick bite within 48h of exposure and also at 3 month follow up. Negative control sera were obtained from a commercial biobank.

Overall subjects exhibited significantly increased IgG and IgM responses to rCT compared to negative control after a recent tick bite in ELISA studies; IgG responses were further enhanced three months after a tick bite. Immunoblots confirmed IgG and IgM reactivity against *I. ricinus* salivary gland proteins and rCT, with calreticulin being the most dominant and consistent antigen in crude salivary gland extracts as confirmed by LC-MS/MS. Bioinformatic analysis of *I. ricinus* rCT identified two putative B-cell epitopic regions. After eliminating peptide sequences that exhibited potential cross reactivity against host proteins and those of other haematophagous taxa, nine oligopeptides (15-mer) were synthesized. The performance of the individual and pooled oligopeptides is being compared to that of rCT in the optimization of a tick bite immunoassay.

A platform for a tick (*I. ricinus*) bite immunoassay was established using ELISA and the specificity of the response confirmed by western blot. This assay refines previous work in individuals challenged with *I. scapularis* and offers the potential for monitoring tick exposure at the individual and population level in both high-risk groups and the wider population.

Poster 77 : Characterising Male Germ Cell-Associated Kinase Orthologues in the Kinetoplastid Cell Cycle

Presenter: **Olubukola Owolodun**, *University of Glasgow*

O Owolodun[1]*; HM De la Torre*[1]*; R McCulloch*[1]*; TC Hammarton*[1]*;*
[1] *Institute of Infection, Immunity and Inflammation, University of Glasgow, UK*

The male germ cell-associated kinase (MAK) is known to play roles in meiosis, mitosis and ciliary length regulation in mammalian cells. However, very little is known about the MAK orthologues in kinetoplastids. Our study shows that TbMAK in bloodstream form *Trypanosoma brucei* is tightly regulated and essential in the cell cycle during late mitosis or early cytokinesis, as RNAi depletion or overexpression of TbMAK causes growth arrest together with an increase in cells with two nuclei (N) and two kinetoplasts (K), and later polyploid cells with abnormal NK complements, leading to eventual cell death. In contrast, in *Leishmania mexicana* promastigotes, LmxMAK appears to be non-essential or redundant since CRISPR Δ*LmxMAK* null mutants are viable in culture. TbMAK and LmxMAK show differential subcellular localisations, evidenced by CRISPR tagging of both proteins with mNeonGreen. While TbMAK:mNG mainly localises in between the nucleus and kinetoplast, possibly to the basal bodies and Golgi in bloodstream form *T. brucei,* LmxMAK:mNG is expressed in the flagellum and cytoplasm. To investigate whether LmxMAK plays any role in flagellum biogenesis or function, we have tagged the flagellar protein SMP1 in our null mutants and flagellum analysis is in progress. We are also setting up XL-BioID to investigate TbMAK and LmxMAK-interacting proteomes to understand the differences in MAK function in these related kinetoplastids.

Poster 78* : Uncovering Echinococcosis Farm Infection risk in Italy

Presenter: **Dr Joaquin Prada**, *University of Surrey*

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Introduction: Cystic echinococcosis (CE) is a zoonotic disease caused by the cestode *Echinococcus granulosus sensu lato* (*s.l.*), affecting canids and ruminants. It is endemic in central-southern and insular Italy, with sheep, goats, cattle, and water buffalo being the most commonly infected livestock species. In this study, we aimed to investigate the spatial distribution of CE in livestock and estimate the prevalence of infection on far in central-southern and insular Italy.

Methods: A Stochastic Partial Differential Equations (SPDE) model was used to analyse animal samples collected from far of different livestock species between 2019-2021. Samples were inspected for *E. granulosus s.l.* cysts through routine surveillance in abattoirs by post-mortem visual examination, palpation, and incision of target organs. The geographic location of the farm of origin was recorded for each sample.

Results: We analysed 3141 animal samples from 2878 farms. The overall CE prevalence at the farm level was estimated to be 46.0%, with sheep far having the highest prevalence (78.3%), followed by cattle far (36.5%), water buffalo far (23.5%), and goat far (28.6%). The spatial model showed a high clustering of infected cattle far in Sardinia and Sicily regions, while sheep far in the Salerno province (Campania region) had the highest prevalence of infection.

Discussion: Our study highlights the need for improved surveillance and control progra in endemic areas of Italy. The findings can be used to identify CE hot spots and develop targeted intervention strategies for the prevention and control of CE in livestock populations.

Poster 79 : Genome plasticity, an essential adaptive mechanism that drives drug resistance in *Leishmania* spp

Presenter: **Edubiel Alpizar**, *University of Durham*

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Discovering how small molecules function to kill *Leishmania* parasites can result in the identification of new "chemically validated" targets. By generating promastigote stage parasites resistant to the clinical antileishmanials amphotericin B and miltefosine we have, via whole-genome sequencing coupled with other metabolomic and lipidomic approaches, identified mutations associated with drug resistance *in vivo*. Implementing this pipeline has also allowed us to interrogate other structurally distinct antileishmanial candidates whose mode of action (MoA)

was previously unknown, including for validation of the IPC synthase as a target of the orphan drug clemastine-fumarate. This MoA was supported by reduced drug susceptibility and accumulation of lipid species in a sphingolipid-deficient mutant *L. major,* generated via gene knockout using homologous recombination. To begin to understand how these parasites survive in the absence of 'essential' sphinglopids we further explored the genome of this historic LCB2 (loss of the catalytic subunit of serine palmitoyltransferase) knockout cell line which demonstrated a complete loss of sphingolipid biosynthesis. While this mutant remained viable and infective in mice, whole genome sequencing revealed a number of SNPs and structural changes such as CNVs and gene deletions, including of a putative ABC3A sterol transporter. Importantly, simultaneous deletion of this ABC3A gene facilitated LCB2-targeted knockout in the model *L. mexicana,* suggesting a compensatory effect. We are currently expanding the tools employed in this pipeline to characterise a series of new molecules with activity against *Leishmania* and *T. cruzi*. Whole genome sequencing supported with other analytical tools have provided essential insights into gene function and proven useful in revealing mutations and other structural changes that play a role in promoting resistance in *Leishmania* spp. Furthermore, we emphasize the necessity to re-examine the many other historical *Leishmania* spp knockout lines where genes, such as LCB2, were previously deemed non-essential.

Poster 80* : Distribution of intermediate snail hosts and their infection status with *Schistosoma* spp.: Lake Malawi, Mangochi District

Presenter: **John Archer**, *Research Technician, Liverpool School of Tropical Medicine*

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The HUGS (Hybridisation in UroGenital Schistosomiasis) study is a 4-year Wellcome Trust funded collaboration between the Liverpool School of Tropical Medicine and the Malawi-Liverpool-Wellcome Trust Clinical Research Centre. This project incorporates several environmental surveys spaced at intervals of around three months to assess the abundance of intermediate snail hosts and their infection status of *Schistosoma* spp. Environmental surveys were conducted at sites in Mangochi District, Malawi. Collecting sites incorporate locations on the shores of Lake Malawi as well as locations in surrounding water bodies and the Shire River as it feeds into the lake. The HUGS project is primarily aimed at identifying hybrids of *Schistosoma haematobium*-group trematodes and as part of this project the distribution of the *Bulinus* spp. snails that act as intermediate hosts for these parasites is investigated. However, due to a recent outbreak of intestinal schistosomiasis in Mangochi District the *Biomphalaria* spp. intermediate hosts of *Schistosoma mansoni* are also under investigation. Here we report the findings of recent surveys in relation to the distribution of *Bulinus* spp. and *Biomphalaria* spp. in Mangochi District, Malawi and their status with either patent or pre-patent infections. We also report the methodology and preliminary results behind a novel and high-throughput high resolution melt-curve (HRM) real-time PCR assay designed to rapidly identify *S. haematobium*-group hybrids.

Poster 81* : Is it a Fluke or is it Reproducible? Understanding Isolate Variation in *Fasciola hepatica*

Presenter: **Olugbenga Samuel Babatunde**, *PhD Student, Aberystwyth University, UK*

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Fascioliasis is a disease caused by *Fasciola hepatica*, which infects livestock and humans and thus poses a substantial threat to food security and human health. The control and management of *F. hepatica* depend solely on triclabendazole (TCBZ) in the absence of vaccines. The over-

dependence on TCBZ for the control and management of fascioliasis has led to the establishment of TCBZ-resistant *F. hepatica*. Further complications arise in tracking resistance through limited diagnostics to differentiate between resistant and susceptible liver fluke. Recent work has identified a major locus and the gene content likely conferring TCBZ resistance. Hence, there is a need to further confirm potential TCBZ resistance targets, particularly at the protein level. Therefore, the current study ai to utilize an in-depth proteomic approach to confirm the protein profiles from isolates of *F. hepatica* varying in their TCBZ susceptibility. Specifically, proteomic profiles are generated from somatic cells, extracellular vesicles (EVs), and EV-depleted excretory-secretory products. Four different isolates of *F. hepatica* have been utilized, two TCBZ susceptible (Aberystwyth and Italian) and two TCBZ resistant (Penrith and Kilmarnock). Initially, somatic proteins have been extracted from the four *F. hepatica* isolates and fractioned via 1-D SDS PAGE prior to mass spectrometry (GeLC) or a combination of size exclusion chromatography (SEC) prior to 1-D SDS PAGE and mass spectrometry (SEC-GeLC) to provide in-depth somatic proteome profile to date. Results from this work aim to support the future confirmation of TCBZ targets confirming resistance. In addition, it will provide further insights into diagnostics for improved control and management of fascioliasis.

Poster 82* : The impact of nutrition and anthelminthic treatment on the gut microbiome in a lab-to-wild model

Presenter: **Rowan Bancroft**, *PhD student, University of Edinburgh*

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The mammalian gastrointestinal tract is a rich ecosystem and focal-point for a wealth of interactions; it is the largest site of the host immune system and diet-derived nutrient absorption, whilst also a preferential niche for helminth parasites and host to the community of microorganis known as the gut microbiota. Whilst host-nutrition is a key source of intestinal microbiota it also plays a fundamental role in the development of an effective immune response, highlighted by immunodeficiency and increased susceptibility to infection in the malnourished. Moreover, intestinal helminth infections can impair nutrient absorption and compromised immunity can lead to reduced anthelmintic efficacy. To date, the relationship between nutrition, helminth immunity and the gut microbiome has been studied in controlled laboratory models focusing on alterations of specific macro- or micro-nutrients. However, these are unlikely to truly represent findings in natural populations where there is more genetic, ecological, and behavioural variation that can determine exposure and susceptibility to infection, as well as the occurrence of co-infections alongside fluctuations in resource availability due to seasonal shifts which can impact the gut microbiome composition and diversity. We used a high-quality, nutrient-rich diet to experimentally supplement both wild and wild-derived, lab-reared wood mice (*ApodeMus sylvaticus)* and measured anthelmintic treatment efficacy and resistance to infection with the gastrointestinal nematode *Heligmosomoides polygyrus.* Previously, we have shown that in both settings, wood mice given this supplemented diet, were more resistant to *H. polygyrus* infection, cleared adult wor more efficiently after treatment and had higher general and parasite-specific immune responses. Here, we expand upon these findings with gut microbiome data from the same study, where we highlight key differences in the diversity and composition of the microbiome between the lab and wild, during infection and determine how supplemented nutrition impacts this – beginning to unravel the mechanisms driving nutrition-induced *H. polygyrus* resistance.

Poster 83* : Network Representation of Host-Pathogen Interactomes With Machine Learning For *Eimeria* Vaccine Development

Presenter: **Roman Baptista**, *Royal Veterinary College*

R Baptista[1]*; V Marungan-Hernandez*[1]*; K Yang*[2]*; P Gomes*[2]*; D Blake*[1]*; F Tomley*[1]*; L Toni*[2]*; X Dong*[1]*;*

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Eimeria tenella is an intracellular apicomplexan parasite which can infect the chicken with absolute host specificity, causing a haemorrhagic variant of enteric coccidiosis. This disease can cause diarrhoea, blood loss, malnutrition, and increased risk of secondary infection, with a deleterious effect on egg and meat production. The financial impact of *E. tenella* is estimated at ~£10.4 billion/year worldwide and is predicted to

rise as poultry becomes the primary sustainable food source for a growing global human population. The established method of rotating anticoccidial drug regimens has led to widespread resistance to all existing chemoprophylactics, prompting consumer and legislative pressure in both the US and EU to move towards vaccine-based interventions. Identification of subunit vaccine candidates has focused on leveraging *Eimeria* proteins to generate effective immune responses, promising higher efficiency and lower cost-per-animal compared to current live-oocyst vaccines which require the full *in vivo* infective process to occur.

This project seeks to generate an *in silico*, graph-based network (GBN) representation of the host-pathogen protein interactome to streamline candidate prioritisation in vaccine development. Mapped with RNAseq gene expression profiles generated from an infection time course experiment, interactome GBNs have been curated that comprise 7,430 unique proteins and ~200,000 connections for the host, and 1,675 proteins with ~40,000 connections for the pathogen.

This network is being exploited in a variety of ways. Firstly, as a platform for the visualisation and analysis of multi-omics data, including longitudinal expression changes that provides an understanding of the localisation of differentially expressed genes, as well as the foundation for a graph-based deep learning dynamic model of gene expression profiles across time. Secondly, it provides network-specific measures such as node degree and centrality which determine changes in connectivity and importance of groups of nodes, pinpointing candidates for disruption with drug or vaccine-based interventions. Thirdly, embedding of the complete network can be used to highlight nodes which experience temporal changes in local neighbourhood structure, representing pathways or cellular compartments normally affected by the infective process for consideration in treatment design.

A mixture of binding prediction algorith along with curated interaction data are currently being employed to produce a novel, joint host-pathogen interactome for *E. tenella* infections. The joint network will allow us to highlight interfacing hotspots between organis with vaccine target potential, supplemented by the VACCEED machine learning framework for target prioritisation based on a reverse vaccinology approach. The aforementioned deep learning dynamic model will be developed in tandem with this joint network, to be used for *Echinococcus multilocularis* simulation of potential interventions and later validated using downstream wet-lab techniques. The application of this pipeline will be tested and fine-tuned with RNASeq datasets collected from in-house chicken vaccine trials.

Poster 84 : Low-cost, point-of-care blood-based nucleic acid test for schistosome infections

Presenter: **Rory Barnes**, *University of Glasgow*

R Barnes

[1] *University of Glasgow, UK*

Schistosomiasis is primarily endemic to rural, low-resource areas with inadequate water and sanitation infrastructure. Currently available diagnostics include microscopy, rapid CCA (antigen) tests and PCR assays. They either lack sensitivity or are prohibitively costly and complex to be used as point-of-care devices in these settings, which limits their impact on timely interventions. In this project, we aim to address the trade-off between diagnostic sensitivity, cost, and complexity by integrating highly sensitive nucleic-acid amplification assays into a single, low-cost lateral-flow device. We have developed loop-mediated isothermal amplification (LAMP) assays that detect the two *Schistosoma* species that are most prevalent on the continent of Africa, where over 85% of global Schistosomiasis cases occur. We modified LAMP primers for *S. haematobium* and *S. mansoni* with labels to enable binding of amplicons to gold nanoparticles conjugated to antibodies, which are then immobilised on the streptavidin-coated test line of a lateral flow strip to indicate a positive result. The limit of detection (LoD) within 30 minutes for the *S. haematobium* and *S. mansoni* assays were 2000 and 20,000 copies of target sequence, respectively. These preliminary results provide proof-of-concept that these assays are able to detect these *Schistosoma* species using LAMP assays combined with a lateral-flow readout. Next, we will investigate methods to improve the sensitivity of these tests, their ability to detect cell-free parasite DNA in serum, and multiplex the assays into an integrated, simple to use 'one-pot' assay.

Poster 85 : Living with parasites: exploring tolerance of infection to reduce the impact of gastrointestinal nematodes on sheep

Presenter: **Phoebe Beal**, *Moredun Research Institute*

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Productivity loss caused by gastrointestinal nematode infection is a major problem in the livestock industry. Resistance to anthelmintic drugs is rampant, therefore, new, sustainable methods of control are needed. There are two strategies an individual can use to manage infection - resistance, and tolerance. Resistance reduces the parasite burden with more resistant individuals having lower nematode burdens, but this is often at the cost of productivity. Tolerance is the maintenance of health, or productivity, despite increasing parasite burden. Tolerance has been researched in plant science for over a century, with many plants bred for their ability to be tolerant to a range of adverse conditions, such as disease, salinity, and drought. However, whilst breeding for resistance is common practice in livestock, tolerance has been neglected in livestock research. In my PhD, I will be looking at tolerance of nematode infections in domestic sheep, including its genetic basis, the role of the immune system, and the effect of nutrition. Over my first year, I have been and will continue to analyse data from two sources. Preliminary results show individual and sire variation in performance traits, measured as body weight and average daily gain. Going forward, we will be investigating the involvement of various antibodies, such as IgA and IgG, and cytokines, such as IL-4 and IL-17, in tolerance to gastrointestinal nematodes. In the future, we will be looking at whether nutrition can promote tolerance through a rotational grazing trial.

Poster 86* : Development of an Assay to Measure Thiol Groups in the *Cryptosporidium* Oocyst Wall

Presenter: **Flora Caldwell**, *Masters Student, University of Dundee*

FC Caldwell[1]*; S Seizova*[1]*; B Colon*[1]*; MC Pawlowic*[1]*;*
[1] *Wellcome Centre for Anti-Infectives Research, Division of Biological Chemistry and Drug Discovery, University of Dundee, Dundee, UK*

Cryptosporidium is an apicomplexan parasite and the causative agent of the diarrhoeal disease cryptosporidiosis. The parasite is waterborne and transmitted faecal-orally as an oocyst. The oocyst wall provides environmental resistance, protecting the infectious sporozoites within. The oocyst is resistant to common disinfectants (including chlorination) due to its hardy wall. It has been suggested that the cysteine-rich *Cryptosporidium* Oocyst Wall Proteins (COWPs) form a meshwork of disulfide bonds in the inner wall. We hypothesise that this meshwork provides the oocyst its structural strength, however it is currently unclear what catalyses the formation of these disulfide bonds.

Sulfhydryl Oxidases (SOX), a highly conserved enzyme family, generally catalyse the induction of disulfide bonds, helping form higher order protein structures. A *Cryptosporidium* SOX, identified by a proteome from the Pawlowic Lab, has been shown to be highly expressed and enriched in the oocyst wall.

I hypothesise that SOX is involved in the formation of disulfide bonds in the *Cryptosporidium* oocyst wall, is important to crosslinking the COWPs and such contributing to the strength of the oocyst wall. Using a transgenic strain of *Cryptosporidium* lacking the SOX gene, I show that fewer disulfide bonds are formed in the oocyst wall by a novel thiol assay, utilising thiol reactive probes.

Poster 87 : Does *P. falciparum* develop faster in mosquitoes with higher ageing rate?

Presenter: **Ivan Casas Gomez-Uribarri**, *University of Glasgow*

I Casas Gomez-Uribarri[1]*; M Pazmiño Betancourth*[1]*; FO Okumu*[2]*; SA Babayan*[1]*; F Baldini*[1]
[1] *University of Glasgow, UK;* [2] *Ifakara Health Institute, Tanzania*

According to life history theory, parasites that can adjust their investment into growth or transmission stages in response to environmental conditions should have greater fitness all else being equal. Temperature affects both mosquito lifespan and *Plasmodium*'s extrinsic incubation period (EIP). Understanding how the temperature effects on the ageing rate of malaria mosquitoes impacts the EIP of *Plasmodium* is crucial to produce good estimates of malaria risk, particularly in the context of climate change.

Our experiments involve rearing mosquitoes in environmental chambers with fluctuating temperatures that mimic real-world conditions. We will measure the impact of temperature on mosquito ageing rate (using survival analysis) and the time to salivary gland invasion by sporozoites

(using molecular analysis of regularly sampled mosquitoes). We will use generalised linear models to describe the relationship between temperature, mosquito lifespan, and EIP. Additionally, we will monitor sporozoite expectoration in the filter papers used for sugar feeding to explore their potential as a non-destructive EIP analysis method.

Our experimental pipeline also includes using mid-infrared spectroscopy (MIRS) to analyse the cuticle of all mosquitoes. MIRS spectra are influenced by the chemical composition of the samples, and spectroscopic methods have been used in the past to distinguish mosquitoes of different species or age groups. These methods require no refrigeration chain, reagents, or sample preparation, and offer much higher throughput than molecular methods. We will generate a heterogeneous dataset with spectra from mosquitoes of different species, age, ageing rate, and infection status. We will train machine learning algorith on these data to predict these characteristics of mosquitoes, including infection status, to ultimately develop a new surveillance tool to determine malaria transmission dynamics in mosquito populations.

Poster 88* : Dissecting heterogeneous host-*Toxoplasma gondii* interactions

Presenter: **Praveena Chandrasegaran**, *PhD Student, University of Edinburgh*

P Chandrasegaran[1]*; B Shi*[2]*; A Gossner*[3]*; M Hassan*[2]*;*

[1] *University of Edinburgh, UK;* [2] *The Royal (Dick) School of Veterinary Studies and the Roslin Institute, The University of Edinburgh, UK;* [3] *Roslin Institute, UK*

Infection with intracellular pathogens, such as *Toxoplasma gondii*, is a single cell problem where pathogen encounters with a population of same cell type can simultaneously produce responses that are both beneficial or detrimental in a host. Heterogenous host-pathogen encounters can also result in pathogen subsets with different phenotypic properties in the same host. Therefore, only when we transcend the population level averages of cellular responses to pathogens will we make significant progress in infectious disease biology. *Toxoplasma* interaction with immune cells can simultaneously produce distinct infection outcomes in the same host (actively invaded, phagocytosed, uninfected-injected and uninfected bystander cell). Current knowledge on *Toxoplasma*-host cell interaction is mostly based on averaged host cell and/or parasite responses from a bulk cell population. Here, we used single cell RNA sequencing (scRNA-seq) and bulk RNA sequencing to investigate the transcriptional profiles that underpin heterogenous *Toxoplasma* interaction with human peripheral blood mononuclear cells (PBMCs). From the scRNA-seq, we observed that macrophages and dendritic cells are not only preferentially infected by *Toxoplasma*, but also are the main cells that transcriptionally respond to the parasite in human blood. From the bulk RNA-sequencing, we observed heterogeneity in the transcriptional profiles of actively invaded and phagocytosed cells. We also report genes that are differentially expressed in these infection outcomes in human monocytic cells. Using differentially expressed genes from the bulk RNA-seq data as reference panel for the scRNA-seq data, we observed that macrophages and dendritic cells mainly express genes that are differentially expressed in cells infected via phagocytosis. This study provides important insight into the transcriptional profiles of immune cells at a single cell level and their different infection outcomes as well as opening new avenues to investigate the role of genes in disparate infection outcomes.

Poster 89 : Thermal proteome profiling to identify drug targets of antimalarials

Presenter: **Dr Victoriano Corpas Lopez**, *Postdoctoral Research Assistant, University of Dundee*

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Early identification of a compound's molecular target can greatly benefit the drug discovery process. Thermal proteome profiling or TPP is a powerful, unbiased chemical proteomics tool to identify binding partners of active compounds and can help deconvolute the mechanism of action of novel drugs identified by phenotypic screening. The Mode of Action (MoA) group has developed TPP in kinetoplastids and *Plasmodium falciparum*. In this work we will show how this technique was optimised and successfully used in the identification of drug targets of antimalarials and will discuss other applications of TPP, including the study of protein interactions through the analysis of protein melting curves.

Poster 90 : Dual reporter *L. major* strain for *in vitro* and *in vivo* investigations of cutaneous leishmaniasis

Presenter: **Jodie Dixon**, *Research Trainee, University of York*

J Dixon[1]*; K Van Bocxlaer*[1]*;*
[1] *University of York, UK*

Cutaneous leishmaniasis (CL) is a neglected tropical disease causing a range of skin lesions resulting in life long scarring and thus, discrimination and stigmatisation in poorer communities. Unlike visceral leishmaniasis CL is not fatal and with no human vaccine available, disease control relies on chemotherapeutics; mostly of which are sub-optimal, mounting drug resistance and toxicity. With limited advances in drug research and development (R&D) for CL, better understanding of parasite activity in the host and interactions with compounds is needed to be able to follow infection and accelerate the drug discovery pipeline. Here we justify the development of dual reporter bioluminescent (red-shifted firefly luciferase) and fluorescent (mCherry) virulent strains of Leishmania, allowing for a wide range of possibilities for both *in vitro* and *in vivo* investigations. A 1696bp of PpyRE9h firefly luciferase coding region was PCR amplified from pTRIX2-LucNeon vector, as well as a 755bp mCherry coding region amplified from pGL1894 expression vector. Fragments were inserted into pLEXSY-hyg 2.1 (Jenabioscience, Jena, Germany) vector using a High fidelity DNA assembly kit (New England Biolabs). We then engineered a transgenic *L. major* friedlin strain to express the bioluminescent and fluorescent proteins. In an *in vitro* model, parasites infected THP-1 cells to quantify infectivity and validate comparability to wild type strains, and to assess activity of relevant drug compounds when added to infected cells. Validation is ongoing, yet this dual reporter may be a promising tool to investigate drug activity both *in vitro* and in experimental models of CL.

Poster 91 : Using spatial transcriptomics to explore the gastric infection of *Ostertagia ostertagi*

Presenter: **Dr Marc Faber**, *Moredun Research Institute*

MN Faber[1]*; A Chapuis*[1]*; K Hildersley*[1]*; J Moore*[1]*; D Smith*[1]*; LJ Morrison*[2]*; NA Mabbott*[2]*; AJ Nisbet*[1]*; TN McNeilly*[1]*;*
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Gastro-intestinal nematodes (GIN) infect millions of cattle globally and represent a major constraint to efficient livestock production. *Ostertagia ostertagi* has a direct life cycle with a pre-patent period of around 18-21 days. After ingestion, infective third stage larvae (iL3) penetrate the gastric glands in the abomasum (gastric stomach) where they develop into fourth stage larvae (L4) before emerging into the lumen at around 10-14 days post-infection to become sexually mature adults. Infection causes significant pathology, particularly when L4 larvae emerge from the gastric gland, causing hyperplasia of the gastric glands, epithelial cytolysis and loss of parietal cells, resulting in elevated abomasal pH and impaired protein metabolism. The mechanisms by which *O. ostertagi* modulates the gastric epithelial function after invasion are poorly understood, due to the inaccessibility of the abomasum for *in vivo* temporal analysis of early host-pathogen interactions. Bulk and single-cell RNA-seq lack the spatial resolution to identify cellular responses in, and surrounding, an infected gland. In this study, day 10 and day 21 post-infection abomasal tissues sections were used to investigate differences in the epithelial transcriptome of infected glands and glands proximal and distal to infected glands. Using a custom bovine RNA probe panel designed for the Nanostring GeoMx® spatial transcriptomic platform, we identified a local loss of parietal cell transcripts and increased mucin gene expression in infected glands, with the effect extending to neighbouring uninfected glands, but absent in distal glands. In summary, using this approach we were able to identify the localised effect of nematode cellular modulation of the host interface at an unprecedented level of spatial resolution.

Poster 92 : Alternative splicing in *Plasmodium*: improved identification of stage-specific transcript isofor during parasites' sexual development and validation

Presenter: **Isabelle Fitzmaurice-O'Neill**, *University of Glasgow*

IA Fitzmaurice-O'Neill[1]*; Z Rolande De Laurent*[1]*; O Janha*[1]*; LV Carruthers*[1]*; A TobinD Beraldi*[1]*; K Modrzynska*[1]*;*
[1] *University of Glasgow, Institute of Infection, Immunity & Inflammation, UK;* [2] *University of Glasgow, UK*

Plasmodium has a complex life cycle with multiple life stages generated from the relatively compact ~20 Mb genome. Interestingly, despite the general tendency towards the reduction of the genome size, many *Plasmodium* genes contain introns. Thus, it is highly possible that alternative splicing (AS) contributes to the generation of different life stages and expands the protein landscape of the parasites. While multiple transcript isofor have previously been observed, very few studies investigate this phenomenon systematically. This is partially due to poor performance of default AS analysis software packages when faced with the specificity of the *Plasmodium* genome.

We have generated a custom algorithm based on intron/exon junction counts. Initial validation was carried out by generating the list of junctions affected by a previously identified splicing inhibitor (TCMDC-135051), independently defining differentially expressed and differentially spliced transcripts and validating the predicted mechanism of drug action. Using the same approach, we were able to map differential splicing between different life stages of *P. berghei:* asexual, male/female gametocyte and ookinete. We have identified several stage-specific transcript isofor involved in sexual differentiation, DNA replication or cell signalling.

To validate the biological significance of these events, we have generated parasite lines expressing different GFP-tagged isofor of a conserved *Plasmodium* protein involved in sexual differentiation. Md2 contains two exons interspaced with an intron. Md2 RNA-sequencing data shows differential splicing between male and female gametocytes. We have shown that both GFP tagged isofor can be translated and result in different protein localisation and parasitic phenotypes. RNA-sequencing analysis confirmed that correct splicing of the gene is required for male sex determination and absence of either isoform results in lines able to produce female gametocytes only. In summary, we have confirmed differential splicing is a key feature of *Plasmodium* biology and plays key role in the life cycle progression.

Poster 93 : How *Leishmania mexicana* control their motility?

Presenter: **Dr Cecile Fort**, *Oxford University*

C Fort[1]*;*

[1] *Oxford University, UK*

Leishmania are major human pathogens that, like all trypanosomatid parasites, use their single flagellum for vital roles including motility, surface attachment and environmental sensation. Normal motility is necessary for *Leishmania* to establish infection of the sandfly vector however comparatively little is known about how they control their motility to navigate the sandfly gut. We previously showed that differences between the proximal and distal flagellum, specifically in the outer dynein ar and dependent on a paralogous pair of docking complex heterodimers, are conserved between *Trypanosoma brucei* and *Leishmania* and are important for controlling flagellum beat type. We asked how common proximal-distal asymmetry was, if it always involves the outer dynein ar and if it is always docking complex-dependent. To address this, we used TrypTag to identify 25 proximal and 26 distal flagellum-specific proteins, and subjected the ~30 with a *Leishmania* ortholog to analysis. Each was endogenously tagged with a fluorescent protein, to ask if their asymmetry was similar to *T. brucei*, identifying 9 with a conserved proximal localisation and 9 with a conserved distal localisation. To test if their asymmetric position was dependent on the proximal and distal docking complex, we used a combinatorial protein tagging and gene deletion strategy. This showed that the localisation of 3/9 proximal and 4/9 distal are docking complex-dependant, indicating that there are at least two mechanisms generating asymmetry in the flagellum. In deletion mutants of each proximal/distal-specific protein, electron microscopy showed only very small changes in electron density. Therefore, to address whether asymmetries involved the outer dynein ar we tested if proximal/distal-specific proteins were dependent on the outer dynein arm motor proteins. This showed that 4 proteins are outer dynein arm-associated in some way. Our work shows that there is complex proximal/distal adaptation of axoneme molecular composition through at least two mechanisms, and particularly involving the outer dynein arms. In the future, we will test our panel of mutants for defects in swimming and flagellum beating to fully map, genome-wide, the contribution of proximal/distal-specific flagellar proteins to flagellum beat control.

Poster 94* : Track & trace: using dual-colour imaging to visualise transport in the beating flagella of *Leishmania mexicana*

Presenter: **Sophie Gray**, *University of Oxford*

S Gray[1]*; R Wheeler*[1]*;*

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Intraflagellar transport (IFT) is the bi-directional transport of proteins on IFT trains necessary for building flagella, including in *Leishmania mexicana*. *L. mexicana* promastigotes possess a 9+2 motile flagellum which is vital for normal life cycle progression. Since IFT discovery nearly three decades ago, IFT train movement has essentially exclusively been analysed in cells with flagella immobilised either mechanically or by mutations.

To visualise IFT train movement in actively beating flagella, we developed high framerate dual-colour fluorescence microscopy, using a triple mNeonGreen-tagged IFT protein (3×mNG::IFT172) and either red light phase contrast or mCherry-tagged flagellar proteins. IFT is visible in the green channel and the flagellum can be traced using the red channel, allowing simultaneous capture of flagellar bending and IFT train movement. Using this microscopy approach, we measured anterograde and retrograde IFT train speed in free moving flagella undergoing different beat types, in comparison to mechanically immobilised flagella.

IFT trains may experience steric hinderance from other axoneme-associated complexes and presumably compete for ATP with the motor proteins driving axoneme beating. Therefore, we also measured IFT train speeds in distal docking complex (dDC) 1, dDC2, dynein light chain 1, outer dynein arm beta, radial spoke protein 4/6 and paralysed flagella 16 deletion mutants with defective beats.

We showed that mechanical immobilisation of a motile flagellum to observe IFT puts it in a non-physiological state, resulting in slower trains with increased intermittent stalling than in free flagella. IFT train speed is independent of beat type- suggesting chemotaxis-based beat switching is not associated with flagellum length changes, although mechanical flagellum trapping may be. IFT train speed is also increased in mutants with defective flagellar motility. Together this data reveals the importance of visualising IFT in a free moving flagellum.

Poster 95* : Non-natural myristate analogues: Synthesis and their potent, selective activity upon bloodstream *T. b. brucei*

Presenter: **Rachel Humann**, *St Andrews University*

R Humann[1]*;*

[1] *St Andrews University, UK*

Inadequate and antiqued drugs for treating sleeping sickness, a neglected tropical disease caused by the protozoan *Trypanosoma brucei* (*T. brucei*), remains a persistent problem across many developing countries. One chemotherapeutic target, the N-myristoyltransferase (NMT) has received significant attention in recent years and has been validated as drug target against *T. brucei;* ablating the NMT gene led to cell death and is thus essential for parasite survival. By synthesizing trypanocidal myristate analogues, it is hoped that these structural mimics will be taken up and utilised by *T. brucei* NMT, leading to the interference and disruption of their downstream metabolic pathways. Additionally, fatty acid elongation and repurposing is likely to be disrupted upon the treatment of non-natural analogues, potentially leading to toxic effects. This research mainly describes the chemical synthesis of myristate analogues based on the 14:0 fatty acid chain with some compounds showing EC_{50} values of <10 µM in the presence of 10 % foetal bovine serum (FBS) and significantly lower EC_{50} values in FBS depleted environments. Initial analysis of free fatty acid and phospholipid species via techniques has highlighted significant differences in the abundances of certain species. Tandem MS/ can be used to identify the specific changes in individual phospholipid species. This analysis is complemented by general non-specific metabolomics on whole cell samples to further identify biological pathways affected by these compounds. To directly determine the extent at which the NMT enzyme is affected or inhibited, TbNMT protein was expressed and purified to allow thermal shift assays and secondary peptide activity assays to be carried out. The synthesis and use of bi-functional probes for drug localisation studies is also explored in this research in the hopes of further elucidating drug localisation and thus potential target identities.

Poster 96* : Counting *Cryptosporidium*: A simple and novel drug assay

Presenter: **Georgina Hurle**, *University of East Anglia*

GR Hurle[1]*; D Steverding*[1]*; KM Tyler*[1]*;*
[1] *University of East Anglia, UK*

Cryptosporidiosis is one of the leading causes of diarrhoeal death in children under 5 globally, as well as causing severe disease in immunosuppressed individuals, such as those with HIV/ AIDs. It also causes severe illness in cattle, having a significant impact on yields. Currently, parasite cultivation methods are limited to co-culture with cell lines that cannot sustain infection and are high maintenance, or the use of infection models such as mice or gnotobiotic piglets. The lack of a cultivation system has compounded into a lack of treatment for those at risk of severe disease, alongside a lack of knowledge on parasite basic biology. Currently, there are no licensed drugs against Cryptosporidiosis in the UK, and a limited number of treatments licensed in cattle. Nitazoxanide is currently the only drug licensed for use in humans in the United States. Our study takes advantage of the human oesophageal squamous cell carcinoma line COLO-680N, which has previously been reported as able to support continuous co-culture of the parasite. This system was adapted in order to develop a working *in vitro* drug assay for *C. parvum*. After infection using freshly excysted sporozoites, parasite growth and development were initially assayed microscopically by counting the free swimming and motile merozoite for which were released at fixed time points after infection. We tested two drugs in our cultivation system: Paromomycin and Nitazoxanide and the licensed natural product Excential Alliin Plus©. We found significant reductions (p=>0.0001) in merozoite output at 120 hours post-infection and an overall suppression in parasite growth over time with all three compounds. An Alamar Blue assay was evaluated as a potential high-throughput screening method, based on evaluating host protection against infection. Alamar Blue is a colorimetric and fluorescent dye which is metabolised by living cells. We were able to record a decrease in Alamar Blue output from COLO-680N cells as the infection inoculum increased, indicating this method could be a viable option to test the effects drugs have both on the parasite, as well as measuring toxicity against host cells. In the future it is anticipated that the adoption of a luciferase expressing parasite into the high throughput assay system may provide an alternative screening method against transfected strains of *Cryptosporidium* spp.

Poster 97 : Defining the spatial interactome of VSG-Exclusion Proteins 1 and 2 using TurboID in African Trypanosomes

Presenter: **Dr Lianne Lansink**, *University of York*

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African trypanosomes are 'masters of disguise'. They rely on a vast genetic repertoire (>2,600 genes and pseudogenes) encoding from their variant surface glycoprotein (VSG) to undergo antigenic variation and successfully evade their host immune response. Their ability to express a single *VSG* at any given time, *monogenic expression*, is imperative for successful antigenic variation; yet mechanisms governing this complex process are not fully understood in any eukaryote. In *Trypanosoma brucei* bloodstream-form, the single active-*VSG* is transcribed by RNA-Polymerase I within the expression-site body (ESB). VSG-exclusion-2 (VEX2) accumulates at the ESB and binds VEX1 at the *Spliced-Leader* (SL) locuson another chromosome; other *VSGs* are excluded from this sub-nuclear 'expression-factory' (PMIDs: 31289266; 33432154). The VEX proteins, particularly VEX2, are critical to sustain *VSG* monogenic expression, however the mechanism remains mysterious. To dissect VEX1/VEX2 function(s) and the sub-nuclear context in which these proteins operate, we sought to define their spatial interactome using proximity labelling combined with LC-MS/ analysis. We generated cell lines where VEX1 or VEX2 were fused with TurboID. In the case of VEX2, because it is an unusually large protein (>200 kDa; 1 megadalton in native conditions), TurboID was placed at either the N or C- terminus to increase spatial resolution. Super resolution microscopy revealed that all the TurboID-protein fusions localised to the expected sub-nuclear compartments; upon biotin addition, highly compartmentalised biotinylation could be achieved. Further, following LC-MS/ analysis, the identification of known VEX-interactors (e.g. CAF-1 subunits) as well as a collection of proteins known to reside within the ESB or in close spatial proximity, validated the approach. Interestingly, we found several additional proteins of known and unknown function enriched in either or both VEX2-TurboID datasets. We are currently validating whether these proteins directly interact with VEX2. Moreover, phylogenetic analysis suggests that VEX2 is a rather divergent form of Senataxin (SETX), an RNA:DNA helicase involved in transcription and splicing regulation in mammals, as well as resolving R-Loops at transcription termination sites. We are currently exploring whether VEX2 displays similar substrate preference and functions in trypanosomes. Interestingly, we found some potential interactors in our proximity labelling data that are consistent

with SETX-related functions. Overall, we hope that this study will improve our understanding of the molecular mechanisms underpinning allelic exclusion.

Poster 98 : Ruminating Over EVs: Microbiome Manipulation Through Rumen Fluke Extracellular Vesicles

Presenter: **Jacob Leonard**, *Student, Aberystwyth University*

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Recent work has demonstrated a substantial contribution of parasite-mediated changes in the ruminant gut microbiota following investigation into the rumen fluke, *Calicophoron daubneyi*, within an *in vitro* rumen model. Within this setting, extracellular vesicles (EV) were identified as vital components in shaping bacterial communities within the host rumen, yet the direct effects are not fully understood. At present, EV release from helminths has only been demonstrated *in vitro* within helminth maintenance media. Thus, confirming EV release *in vivo* must be a priority. To assess this, EV populations were purified from 1) infected rumen fluid with a 4-hour incubation containing rumen fluke at 37°C, 2) infected rumen fluid with no worm culture, 3) uninfected rumen fluid, and 4) DMEM with a 4-hour incubation containing rumen fluke at 37°C representing an *in vitro* model. All samples were then centrifuged at 15,000 ×*g*, and filtered through 5 µm, 0.4 µm and 0.2 µm PTFE membrane filters. Following size exclusion chromatography (SEC) for the purification of rumen EVs, TEM has demonstrated the identification of small likely bacterial EVs in addition to larger EVs, which have only been identified within rumen fluke containing rumen fluids thus likely representing rumen fluke EVs. To confirm the larger EVs as *C. daubneyi* specific EVs, gold labelling TEM utilising fluke specific antibodies (Anti-FhGST-S1) known to bind to the surface of fluke EVs, will be used to identify EVs more accurately secreted into both *in vitro* rumen simulation and infected rumen fluid coupled with a meta-proteomics approach. In addition, utilising SEC purified *C. daubneyi* EVs in an optical density assay has identified antimicrobial and bacteriostatic activity. EVs derived from *C. daubneyi* cultured in DMEM, were observed to be more effective at suppressing *Escherichia coli* and *Bacillus megaterium* within these optical density assays when compared to EVs purified from *in vitro* rumen fluid cultivars. Future optical density assays aim to target additional rumen relevant microbes including *Prevotella, Ruminococcus* and *Staphylococcus*. Once these interactions are understood and characterised, novel approaches to control involving the interaction with the ruminant microbiome may be investigated.

Poster 99* : Bottling It All Up: Using parasite population biology to identify susceptibility pathways in visceral leishmaniasis

Presenter: **Ciara Loughrey**, *University of York*

C Loughrey[1]*; P Kaye*[1]*; J Mottram*[1]*; J Carnielli*[2]*; H Ashwin*[1]*; N Brown*[1]*;*
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Leishmania donovani causes systemic multi-organ disease known as visceral leishmaniasis. However, little is understood about the mechanisms controlling parasite dissemination, survival and growth within and between different host tissues. Specifically, it is not known where or when parasite populations are reduced by immunological 'obstacles'; understanding this process could enable us to identify new methods to block dissemination and limit clinical disease.

CRISPR-cas9 genome editing has recently been adapted for use in *Leishmania* parasites, and used to insert genetic barcodes analysis via high-throughput sequencing, a technique termed Bar-seq. Sequence Tag-based Analysis of Microbial Population Dynamics (STAMP) has been used to determine within-host microbial pathogen dissemination patterns by comparing barcode frequencies between initial input and different host tissues at a later time-point, to calculate founder population sizes (a measure of bottleneck stringency) and genetic distance between individual populations (a measure of connectivity).

We have developed and optimised a protocol to combine these two techniques to build a library of 102 *L. donovani* lines, each containing a unique barcode. A subset of these lines (n=10) were evaluated for their growth characteristics *in vitro* as promastigotes and their ability to infect

murine bone marrow-derived macrophages was also assessed. These studies showed comparable growth characteristics in the lines tested. Parasite location within LAMP1+ parasitophorous vacuoles was also confirmed using immunofluorescence confocal microscopy.

A pilot *in vivo* study was then performed by infecting BALB/c mice with 10 barcoded lines to demonstrate proof-of-concept for our methodology of barcode abundance assessment via high-throughput Illumina sequencing of PCR products from infected tissues. A second *in vivo* study in B6.CD45.1 mice compared PCR amplification at various time-points post-infection. Based on the results of these studies, we conclude that our approach will be suitable for analysing spatial and temporal bottlenecks at different stages of infection using STAMP.

Additionally, we will utilise genetic relatedness analysis to build a network model to describe the connectivity of parasite populations within the host. These *in vivo* results will then inform further *in vitro* studies to examine the underlying mechanisms.

Poster 100* : Pulmonary migration of *Trichobilharzia szidati* (Schistosomatidae) and its potential immunomodulatory effect against allergic asthma in mice

Presenter: **Martin Majer**, *PhD student, Charles University, Prague*

M Majer[1]*; B Šmídová*[1]*; A Revalová*[1]*; P Horák*[1]*; T Macháček*[1]*;*
[1] *Charles University, Prague, Czech Republic*

Schistosomula of *T. szidati* migrate through the lungs of their hosts, ducks (definitive hosts) or mice (accidental hosts). Interestingly, the migration initiates only mild pulmonary inflammation in mice, suggesting host-parasite interactions preventing inflammation and, subsequently, tissue damage. We assume that this is mediated by active immunomodulation by the parasite. Therefore, we tested the protective effect of the infection on the progression of ovalbumin (OVA) induced asthma. As was observed in flow cytometry and histology analyses, *T. szidati* infection reduced the number of eosinophils and other leukocytes in the lungs of asthmatic mice. The eosinophilia diminution was also confirmed in bronchoalveolar lavage, where it was accompanied by decreased levels of IL-4 and IL-5 cytokines. Moreover, qPCR analysis of the lungs revealed that *T. szidati* infection increased expression of regulatory cytokine *Il10* and downregulated *Chil3* and *Arg1*, markers of M2 macrophages, compared to the asthma-only group. On the other hand, splenocytes restimulated with OVA produced higher levels of cytokines (IFNγ, IL-4, IL-5, IL-10) in the *T. szidati*-asthma group, and co-cultivation with OVA and parasite antigens even boosted the cytokine production. Seemingly, the regulatory effect induced by the infection is only local (i.e., restricted to the lungs). In summary, the invasion of the lungs by *T. szidati* alleviates the progression of asthma, probably by induction of the regulatory milieu and downregulation of the Th2/M2 pathway directly in the lungs.

Acknowledgment: The project was funded by Charles University Grant Agency (580120).

Poster 101 : Using genome-wide quantitative fitness profiling to identify the molecules responsible for detecting the oligopeptide differentiation signal in bloodstream *Trypanosoma brucei*

Presenter: **Dr Kirsty McWilliam**, *University of Edinburgh*

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[1] *University of Edinburgh, UK;* [2] *University of Nottingham, UK*

African trypanosomes are the causative agent of sleeping sickness in humans and nagana in livestock in Sub Saharan Africa. Within the infected mammalian host, *T. brucei* undergoes a density-dependent developmental transition from proliferative 'slender' cells, that establish infection, to cell-cell arrested 'stumpy' cells that are competent for transmission into the tsetse fly vector. Recent studies have established that this differentiation is triggered in response to an increasing concentration of extracellular oligopeptides, that are themselves generated by the release of parasite-derived peptidases into their environment. These oligopeptides are received by the parasite surface transporter *Tb*GPR89. Our previous screen to identify the components of the slender to stumpy quorum sensing (QS) signalling pathway used cell permeable molecules that bypassed the oligopeptide signal reception steps. Thus, the molecules that detect the oligopeptide differentiation signal and activate the previously characterised components of the signalling pathway are not known.

To identify these molecules we have recently used DRiF-Seq to perform a genome-wide quantitative fitness profiling in response to the physiological oligopeptide differentiation signal in a pleomorphic cell line capable of differentiation. We have identified a number of genes whose RNAi confers decreased sensitivity to BHI-derived oligopeptides and have validated these *in vitro* using independently generated inducible RNAi cell lines. We will next ask if the knock down of these novel regulators of oligopeptide sensitivity also creates defects in the QS signalling pathway. To do this, we will use cell cycle analysis and expression of the stumpy specific marker protein PAD1 to assess stumpy formation upon exposure to oligopeptides *in vitro* and during an infection *in vivo.*

I will present the outcomes of the DRiF-Seq screen and our current progress validating the identified molecules.

Poster 102* : Exploring the potential of synthetic sulphonated polymers as candidates for adjunct therapies for malaria

Presenter: **Rebecca Mobley**, *Keele University*

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Malaria continues to cause health and economic concerns despite control efforts, highlighting the urgent need for new therapeutics. Previously, heparin was evaluated as an adjunct treatment for malaria but was discontinued due to the health risks associated with its anticoagulant activity. The antiplasmodial activity of heparin has been attributed to the negatively charged sulphate groups within its structure, although how these directly affect *Plasmodium* and the host erythrocytes is not well understood.

To tackle the anticoagulant risk of heparin, a library of synthetic sulphonated polymers were evaluated as alternatives. Their antiplasmodial activity and rate of kill (RoK) was determined using luciferase bioluminescence assays. Potent antiplasmodial activity was found within this library and was demonstrated in two distinct strains of *Plasmodium falciparum* (NF54^{luc} and Dd2^{luc}), with some having more potent antiplasmodial activity than heparin. Microscopy investigations and RoK investigations suggest the blocking of erythrocyte invasion and/or egress, with fast action. These stages are a unique target and offer promise for the development of drugs with low resistance potential. The anticoagulant properties of lead compounds was assessed using Prothrombin time (PT) and activated partial thromboplastin time (aPTT) assays. These compounds demonstrated no anticoagulant activity in the PT assay and some levels of anticoagulant activity was observed in the aPTT assay, lower than that of heparin. This study explores the benefits and risk of utilizing synthetic sulphonated compounds as an adjunct therapy in combination with current treatments of malaria.

Poster 103 : Validation of a quantitative ribo-profiling approach for the study of *LeishmaniaLeishmaniaLeishmania* translational regulation

Presenter: **Dr Ana Maria Murta Santi**, *PostDoc, Institut Pasteur*

AM M Santi[1]*; P Pescher*[1]*; GF Spath*[1]*;*
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Protozoan parasites of genus *Leishmania* show a remarkable level of phenotypic diversity with respect to clinical symptoms, disease outcome, and drug susceptibility, which challenges disease management. Such diversity is surprising given the largely constitutive gene expression in these parasites that lack classical, promoter-driven control, raising essential questions on how *Leishmania* adapts and evolves in response to environmental change. Using an experimental evolution approach, we recently uncovered a series of regulatory mechanisms that govern *Leishmania* fitness gain in culture, including frequent gene dosage changes caused by the parasite's intrinsic genome instability, and compensatory post-transcriptional responses. We further correlated fitness gain to changes in snoRNA abundance and the modifications they guide on ribosomal (r) RNA. These data suggest the presence of fitness-adapted ribosomes that may support parasite adaptability through

translational regulation, potentially filtering harmful from useful gene dosage effects. To investigate this hypothesis, we explore here the adaptive changes in mRNA translatability by profiling active ribosomes using the RiboLace method (Immagina Biotechnology). We first evaluated the quantitative use of this method by developing *L. donovani* transgenic parasites that express low and high levels of GFP (respectively termed as LdGFP_L and LdGFP_H) based on changes in the Kozak sequences that affect mRNA/ribosome interaction. Flow cytometry analysis indeed showed a 4-fold reduced mean fluorescence intensity in LdGFP_L compared to LdGFP_H parasites. Using the RiboLace kit, we isolated active ribosomes from both parasite strains and revealed by RTqPCR a 2.74-fold lower recovery of GFP mRNA in LdGFP_L compared to LdGFP_H parasites, thus validating the applicability of RiboLace to quantify differences in translatability. We are currently applying this method to assess the role of translational control and the presence of fitness-adapted ribosomes in *Leishmania* during adaptation to *in vitro* culture.

Poster 104* : The first study of Parasitic feather mites (Acari: Acariformes) on some common birds of Pakistan, with an association of Keratinophilic fungus *in vitro* growth.

Presenter: **Dr Saima Naz**, *Assistant Professor, University of Sindh*

S Naz[1]*; S Khaskheli*[1]*; AA Ujjan*[1]*;*

[1] *University of Sindh, Pakistan;*

Birds' feathers are a rich source of keratin found in the ecosystem and can be considered an important source for the growth of fungi feeding on keratin. The fungus on feathers is grown abundantly on most terrestrial birds and causes serious damage to the feathers hence affecting flight, preening can damage the barbs and barbules. In the present study, it was observed for the first time that a bird carries a high burden of ectoparasites mainly including mites and fungus on them. The study was designed to explore the variety of parasitic feather mites, also their interaction with the keratinophilic fungus. for this purpose, the mites from the common bird's species were collected and identified as *Falculifer rostratus* Buchholz, 1869, *Hyperaspidacarus tridentatus* Atyo and Smith, 1983, *Freyana anatina* (Koch, 1844), *Freyana ferinae* sp.n., *Trouessartia corvina* (Koch, 1940), *Proctophylloides turdoides* sp.n., *Pterolichus francolinae* sp.n., *Bychovskiata subcharaderii* Dubinin, 1951 and a species of the genus *Avenzoara* sp. were collected from *Columba livia, Streptopelia segalensis, Anas platyrhynchos, Anas crecca, Aythya ferina, Corvus splendens, Turdoides striatus, Francolinus pondicerianus, Tringa glareola, Himantopus himantopus,* and *Egretta garzetta* from various parts of the province of Sindh during 2021-2022. The keratinophilic fungus was grown in SDA medium under standard laboratory protocols on some common bird's feathers and four genera of fungi were identified as *Alternaria* sp., *Paeceliomyces* sp., *Microsporum* sp., *Trichophyton* sp., and *Aspergillus niger* Michelli, 1729 were reported for the first time from Pakistan.

Poster 105 : The impact of mosquito resources on malaria parasite development and consequences for transmission

Presenter: **Catherine Oke**, *University of Edinburgh*

C Oke[1]*; SE Reece*[1]*;*

[1] *University of Edinburgh, Institute of Ecology and Evolution, UK*

Malaria parasites (*Plasmodium* spp.) have a complex lifecycle, where transmission between human hosts relies on passage through female *Anopheles* mosquitoes. Parasites rely on resources from their mosquito host to develop, and there is evidence that an additional blood meal can speed up growth. However, how parasites respond to poorly-resourced mosquitoes is unknown. Using *P. chabaudi*-infected mosquitoes we test how an additional blood meal and varying fructose only diets influence oocyst growth and sporozoite burden over the course of parasite development. Our data suggest that well-resourced mosquitoes allow parasites to grow quicker and produce more sporozoites, and poorly-resourced mosquitoes cause constraints on oocyst productivity. As mosquitoes with higher sporozoite burdens are more likely to initiate infection in a vertebrate host, understanding the factors which can benefit or constrain parasite development is highly important for

understanding transmission potential. Furthermore, as vector control tools are altering mosquito genotypes and phenotypes, including resource use, understanding how mosquito resources shape transmission is particularly timely.

Poster 106* : Investigating the conservation, function, and vaccine potential of PfEMP1 DBLepsilon and DBLzeta domains

Presenter: **Brian Omondi**, *PhD student, University of Edinburgh*

BR Omondi[1]*; PM Sharp*[2]*; JA Rowe*[1]*;*

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Background: *Plasmodium falciparum* erythrocyte membrane protein 1 (PfEMP1) is a parasite-derived infected erythrocyte surface antigen that facilitates adhesion and immune evasion. The PfEMP1-encoding *var* gene family is unique to the subgenus *Laverania*, the closest relatives of *P. falciparum*. Modular in structure, PfEMP1 comprises of duffy-binding like (DBL) domains and cysteine-rich interdomain regions (CIDR). Of interest, PfEMP1 DBLε and DBLζ domains bind to serum proteins such as non-immune immunoglobulin M (IgM) and α2-macroglobulin (α2M). These interactions have been linked to both severe and pregnancy-associated malaria. We hypothesise that the serum binding PfEMP1 DBLε and DBLζ domains are potential targets for disease intervention and thus there is need to examine their conservation, function, and vaccine potential.

Methods: We have analysed the Pf3k Normalised varDB (714 single clones) and PlasmoDB databases to understand the patterns of PfEMP1 domain conservation across the global *P. falciparum* population, and the *Laverania*, respectively. We have also selected some African parasite isolates, recently culture-adapted for rosetting and IgM binding, and characterised their predominant PfEMP1 variants.

Results: With 319 hits (BLAST alignments) at 80% amino acid (aa) identity, the IgM and α2M-binding TM284VAR1 DBLζ2 domain was conserved in approximately 45% of the Pf3k Normalised varDB *P. falciparum* isolates. This supersedes the conservation of the chondroitin sulphate A (CSA)-binding 3D7VAR2CSA DBLpam2 domain (210 hits at 80% aa identity, 29% of isolates) which is currently under vaccine development to protect against pregnancy-associated malaria. Other IgM-binding domains such as 3D7VAR2CSA DBLεpam5 (692 hits, 95% of isolates), IT4VAR1CSA DBLε5 (400 hits, 55% of isolates) and HB3VAR06 DBLζ2 (87 hits, 12% of isolates) also had good levels of conservation. Domains involved in other adhesion phenotypes (rosetting, CSA, endothelial protein C receptor (EPCR), intercellular cell adhesion molecule 1 (ICAM1) or cluster of differentiation 36 (CD36)) did not rival this level of conservation. Moreover, the majority of the domains within the hypervariable PfEMP1 family are conserved in less than 5% of the parasites at a similar cut off. Analysis of other species of *Laverania* (PlasmoDB) revealed a homologue of an IgM-binding domain, IT4VAR60 DBLε12, in the gorilla parasite, *P. praefalciparum*, from which *P. falciparum* originated (PPRFG01_1151300, 95% aa identity), while a homologue of TM284VAR1 DBLζ2 was found in the chimpanzee parasite *P. reichenowi* (PRG01_0043100, 83% aa identity). There was also evidence of full-length conservation of IT4VAR60, TM284VAR1 and VAR2CSA within the *Laverania*. Taken together, these analyses show that the PfEMP1 serum-binding phenotype is well-conserved and has an ancient origin. It is plausible that

Poster 107 : Protein turnover as a key determinant in *LeishmaniaLeishmaniaLeishmania donovani* parasite stage differentiation

Presenter: **Pascale Pescher**, *Engineer, Institut Pasteur*

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Leishmania survival and pathogenicity depends on the parasite's capacity to adapt to different host environments through stage differentiation of promastigotes within the sand fly, and of amastigotes inside mammalian host cells. *Leishmania* stage-specific expression occurs in the absence of classical transcriptional regulation, raising the question on alternative regulatory mechanisms. We investigated these mechanisms applying RNAseq, label-free quantitative proteomics and phosphoproteomics approaches on hamster-purified amastigotes and corresponding, culture-

derived promastigotes. Comparison of the stage-specific transcriptomes and proteomes revealed a three times higher dynamic range for protein compared to RNA abundance suggesting that translational and post-translational mechanisms may outweigh RNA turnover in regulating stage differentiation. We next investigated protein turnover by applying label-free quantitative proteomic on both amastigotes and promastigotes in presence or absence of the irreversible, proteasomal inhibitor lactacystin. Inhibitor-treated amastigotes were viable but failed to convert into promastigotes in culture, revealing an essential role of protein degradation in *Leishmania* development. We identified 180 proteins (fold change 2, adj. p-value < 0.01) as proteasomal targets during the amastigote-to-promastigote transition, which represent putative differentiation factors. Applied on promastigotes, lactacystin treatment rescued 289 proteins from degradation (fold change 2, adj. p-value < 0.01) but neither affected parasite morphology nor proliferation. Interestingly, we observed stabilization of amastigote-specific proteins in lactacystin-treated promastigotes (and vice versa) suggesting a role of proteasomal degradation in regulating stage-specific protein abundance. Surprisingly, 18 proteins (fold change 2, adj. p-value < 0.01) were stabilized in both stages, including 11 proteins that were only identified in lactacystin treated parasites, thus uncovering a set of proteins that undergo constitutive degraded in our experimental system. Our data identified respectively 6 and 11 protein kinases that were rescued from degradation in treated amastigotes and promastigotes, suggesting differential protein kinase turnover as a regulatory switch in parasite development. Finally, we investigated the pathways controlled by protein kinase activities during differentiation using label-free, quantitative phospho-proteomics analysis of splenic amastigotes and culture-derived promastigotes. We identified 7095 phosphopeptides in promastigotes and 2080 in amastigotes of which 6128 (61%) are exclusive to one stage or the other. Twenty five proteins with exclusive stage-specific phosphorylation were linked to proteasomal protein degradation, including 3 proteasomal subunits, 5 ubiquitin transferases, 5 ubiquitin ligases, 1 ubiquitin-conjugating enzyme, 1 ubiquitin-activating enzyme and 10 ubiquitin hydrolases. In conclusion, our results link stage-specific, proteasomal degradation of protein kinases to parasite differentiation, and vice versa link stage-specific protein kinase activities to differential phosphorylation of proteasomal components. This reciprocal relationship likely establishes a proteasome/kinome regulatory network that controls *Leishmania* stage differentiation and confir both the kinome and the proteasome as interesting targets for anti-parasitic intervention.

Poster 108 : A recently-established facility for studies of *Plasmodium* spp. transmission to mosquitoes in London

Presenter: **Harry Pollard**, *Higher Scientific Officer, London School of Hygiene and Tropical Medicine*

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The Malaria Transmission Facility was established in December 2020 at the London School of Hygiene and Tropical Medicine (LSHTM), supported by a Biomedical Resources grant from the Wellcome Trust. The facility provides access to malaria parasite transmission for both research groups within LSHTM and external collaborators both in the UK and wider afield. Our specialist team works with these collaborators to design and execute studies relating to the transmission of *Plasmodium* parasites. The facility boasts a recent history of successful transmission experiments through vigorous optimisation of methods, with control feeds of *Plasmodium falciparum* NF54 to *Anopheles stephensi* carried out in 2023 averaging an infection prevalence of 82% and an average infection intensity of 19 oocysts per infected midgut. Experiments can be designed to interrogate various stages of the malaria transmission cycle using *Plasmodium* gametocytes grown *in vitro* in the laboratory or collected directly from clinical samples received in the UK HSA Malaria Reference Laboratory and fed to insectary-reared *Anopheles* mosquitoes via artificial membrane-feeding. Depending on the study, different *Anopheles* strains can be set up and maintained to meet the demands of a particular scientific question. A variety of experimental end points can be analysed, which includes but is not limited to imaging of ookinetes, prevalence and intensity of oocysts in the midgut lining and sporozoite positivity and intensity within the salivary glands of an infectious mosquito. The facility is currently supporting studies of the impact of knock out (including conditional KO) transgenic *P. falciparum* lines on transmission capabilities, insecticides exposure on malaria transmission, xeno-monitoring of parasite prevalence in non-vector blood-feeding insects and parasite resistance and its impact on transmission. The malaria transmission facility welcomes wider collaboration with any interested parties and opportunities are eagerly sought to work on transmission-related research with UK and international collaborators. Data from some of our current projects will be presented, as well as a summary of experimental approaches available in the Facility.

Poster 109* : A geospatial analysis of local intermediate snail host distributions provides insight into intestinal and urogenital schistosomiasis within under-sampled areas of Lake Malawi

Presenter: **Amber Reed**, *PhD student in Statistics and epidemiology, Lancaster University*

AL Reed[1]*; C Jewell*[1]*; JR Stothard*[2]*; C Fronterre*[1]*; MC Stanton*[2]*; SA Kayuni*[2]*; M Alharbi*[2]*;*
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Along the southern shoreline of Lake Malawi, autochthonous transmission of intestinal and urogenital disease can occur. However, the underlying distribution(s) of intermediate snails is only partially known from previously sampled sentinel locations. It is known that the distribution of snails are often focal and patchy due their dependency on the habitat which varies greatly between species and indirectly affected by different types of environmental conditions. To model and interpolate snail distributions, a secondary geospatial data analysis of existing malacological survey data and a set of environmental data measured along the shoreline was undertaken. Data on snail abundance collected at focal sites along the lakeshore were fitted using a Bayesian Poisson latent Gaussian process model. By smoothing the abundance estimates out along the shoreline and using extracted environmental covariate data for all predicted locations, this method allowed us to estimate the abundance of snails that might be observed at any of the intervening points dependent on covariate data, together with a measure of uncertainty engendered by the inherent inability to observe snail abundance at all points. Separate models were fitted to the number of snails observed at our study sites for each species (viz. *Biomphalaria* and *Bulinus*). Our adopted model, used a combination of two-dimensional (2D) and one dimensional (1D) mapping to allow us to predict along the shoreline. Our interpolations identified certain areas of interest for each snail species dependent on environmental conditions, respectively which helps refine future geospatial sampling frames. Furthermore, we have shown substantive heterogeneities in snail distributions along the lake which, in turn, provide insight into local dynamics of schistosomiasis transmission.

Poster 110* : What's the point of point-of-care? Using haematological testing in the field to assess parasitic coinfections in children

Presenter: **Sarah Rollason**, *PhD student, Cardiff University*

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The study of tropical parasites such as malaria and schistosomes, along with their associated diseases, frequently necessitates working in areas where these infections are endemic. This means that fieldwork takes place in rural settings with limited access to laboratory resources. In parasitic coinfection, one of the ways parasites interact is by their effects on the host immune system. Methods of quantifying immune cell parameters in the field may be useful in investigating coinfected populations. The effects of schistosome coinfection on malaria is unclear, with previous studies showing conflicting results. Greater understanding of the underlying immune processes in coinfection may assist in unpicking these seemingly contradictory results, and point-of-care testing offers an alternative to transport of samples for laboratory analysis. In this work we utilise haemoglobin and white cell counting in a rural field site in Malawi to assist in the assessment of children with malaria, schistosomes or coinfection. We found point-of-care assessment of these haematological parameters to be user-friendly and feasible for rapid screening of a study population. In this group with high prevalence of both malaria and schistosomes we found differences in blood parameters between singly and coinfected participants, as well as some individuals with markedly abnormal white cell counts requiring follow up from local clinicians. Point-of-care testing under field conditions is not without its challenges, but also carries opportunities for both research and clinical use.

Poster 111 : Global human Health is a One-health, Sustainability and Equality problem altogether - raising awareness and promoting actions

Presenter: **Dr Ilaria Russo**, *Keele University*

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Climate has already changed quite significantly in several locations of our planet. Data are not only unconfutable but also overwhelming. Ecosyste are either breaking or migrating with resulting in new challenges to the living organisms, as we know them. New pathogens are emerging or jumping to previously uncommonly infected species and arthropod disease-vectors have demonstrated an elevated adaptability to environment changes.

Our lab is building awareness of the current situation and the astonishingly level of public disinterest. This is aggravated by the total unpreparedness for the events that are displaying and coming into the near future. It see almost if humanity has learned very little from the recent pandemic other than shortcutting and/or speeding-up clinical trials.

All together climate will directly put in great danger our health independently from the wealth of each country. In addition the indirect impacts on our health will be exponentially far higher encompassing food security (plants, marine/lake-habitats, livestock), Housing safety, Human displacements for natural disasters, A and unsustainable economy.

Our assessment of the level preparedness show significant gaps of void in our ability to monitor risks and contain/counteract emergency situations. While the governments are hopefully working to contain emissions and toward a more sustainable and equitable life, we feel compelled to educate to, promote and put in place actions in protection of Global Health.

In order to accomplish this, we are building a surveillance and preparedness network that encompass the main life domains, human, animals and plants looking at pathogens and insects variations associated to climate and environment changes.

You are all invited and welcomed to help and/or join us either with a constructive discussion or a direct involvement, whatever you prefer.

Poster 112 : Histidine rich protein II, from malaria diagnostic marker to strong proinflammatory and risk factor for severe malaria outcomes

Presenter: **Dr Ilaria Russo**, *Keele University*

MC Musasa[1]*; WD Wichrij*[2]*; GD Goldberg*[3]*; I Russo*[1]*;*

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Malaria is a parasitic disease that prevails in the world's poorest regions severely hampering socio-economic development. In humans, it is caused by five protozoan species belonging to the genus *Plasmodium* of which *P. falciparum* accounts for 99% of all malaria-associated deaths. Strong correlations have been established between high concentrations of Histidine-rich protein II (HRPII), produced by *P. falciparum*, and higher mortality and severity of malaria outcomes, including cerebral malaria.

The neuropathogenesis of cerebral malaria remains unclear and it presents as a multifactorial pathogenesis depending from the patient status and multiple parasitic virulence mechanisms. Recent data identify HRPII as one of the critical virulence factors for the onset of cerebral malaria as its ability to disrupt the Blood-Brain Barrier by itself. The suggested pathogenic mechanism includes activating the NF-kB pathways and the inflammasome in brain endothelial cells (even in the absence of other parasite factors, or immune cells, or astrocytes) with the release of hIL1β. These events then trigger the loss of integrity of the BBB, both in *in vitro* model syste and *in vivo*. It remains largely unknown how these cellular responses are mediated by HRPII binding to the cell and which HRPII-binding molecules are required to trigger this response. In fact, HRPII has shown high affinity towards some glycosaminoglycans, divalent cations, heme, and others, all of which could play binding, structural or cofactorial role. This study ai to investigate the early brain endothelial response of HRPII through the analysis of gene activation/repression using RNAseq data and provide a better understanding of the metabolite patterns related to HRPII-mediated effects.

The disruption of the BBB due to HRPII at concentrations comparable to its serum levels during infection has been previously shown using directly HRPII-producing parasites or purified HRPII either from parasites and parasite-conditioned media or from *E. coli* recombinant expression. Using RNA-seq and *in-vitro* BBB model system, we have investigated the HRPII-triggered transcriptional changes in endothelial cells. We used HRPII protein either produced and purified from standard *E. coli* BL21(DE3) or a BL21(DE3)-derived mutant lacking lipopolysaccharide endotoxin, called Clear-coli® (Lucigen). This comparison aimed to rule out any contribution to the cellular responses of traces of endotoxin eventually carried over during purification from *E. coli* and bound to HRPII, possibly via the repetitive glycan polymer, commonly referred to as O antigen. An extensive LPS-removal step using Triton-X114 was performed in both purifications, and the purified protein was negative at the LAL-test. We have then analysed the cellular transcriptional responses over three time points, 0, 3h, and 12h. By including gene responses to LPS only, we have also analysed the differences between endotoxin and HRPII activation of the endothelial cells.

The RNA was extracted from eight conditions in quadruplicate, and the libraries were prepared, assessed for high quality, and then sequenced using an Illumina HiSeq. On average, a sequence depth of 26.9 ± 0.8 million reads per sample was obtained and about 99% mapped to the human genome. The average length-mapped of ~255bp was higher than expected (~200bp), and the sequencing depth per library achieved in this experiment was sufficient for downstream differential expression analyses. Our analysis of the RNAseq data revealed that HRPII purified from Clear coli® did not trigger the activation of the innate immune response, supporting the hypothesis that endothelial response to HRPII requires costimulatory molecul

Poster 113 : A comparison between the heat shock responses of *T. brucei* and *T. congolense*

Presenter: **Abbey Taylor**, *Masters by Research Student, Lancaster University*

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Periods of host fever can be used as a strategy to prevent infections, by killing temperature sensitive bacteria and viruses. *Trypanosoma brucei* and *Trypanosoma congolense* are vector borne parasites causing Animal African Trypanosomiasis (AAT), a disease currently lacking effective drug-treatments. AAT affects cattle and causes extensive economic hardship in sub-Saharan Africa. Sympto of AAT include periods of fever as high as 41°C, eliciting a heat shock response in the parasites to allow survival in the host. Eukaryotic cells respond to heat shock by triggering a global translational arrest and upregulating heat shock proteins. This response appears to be conserved by trypanosomes however the mechanisms used differ.

At 41°C, bloodstream form *T. brucei* display a heat shock response, with a decrease in polysomes and global translation and an increase in the number of P-bodies (containing DHH1, SCD6, XRNA1, PABP2) and HS stress granules (containing PABP1, eIF3E1 to E4) (Kramer et al., 2008, Kramer et al.,2013). It has also recently been shown that heat shock in *T. brucei* results in altered phosphorylation of the post-transcriptional heat shock regulatory complex MKT1-ZC3H11-DHH1 (Ooi et al., 2020). Whilst *T. brucei* is a well-studied model organism, little work has been done on the close relative *T. congolense*. As *T. congolense* is a close relative of *T. brucei* and they co-infect the same hosts, it is expected they will show similarities in host interactions.

We will interrogate and compare survival mechanisms of *T. brucei* and *T. congolense* by characterising its heat shock response, in hopes of paving the way for the discovery of novel drug targets. Data will be shown that demonstrates the similarities and differences found so far between the *T. brucei* and *T. congolense* heat shock response, including re-localisation and abundance of heat shock proteins, growth recovery and cell cycle analysis. This data represents some of the first investigations into specific pathways involved in the *T. congolense* heat shock response.

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Poster 114 : Identification of interferon stimulated genes that control *Toxoplasma* in pig cells

Presenter: **Dr Marzuq Ungogo**, *University of Edinburgh*

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Toxoplasma gondii is a zoonotic parasite that infects warm-blooded animals. Toxoplasmosis is estimated to cost the UK livestock industry over $15 million annually. In pigs, acute *Toxoplasma* infection causes severe morbidity and mortality, while chronic infection suppresses immunity and presents significant risk to human foodborne infection. In vertebrate hosts, Interferons (IFNs) control *Toxoplasma* pathogenesis by inducing the transcription of hundreds of interferon-stimulated genes (ISGs). Important insights into IFNg-induced *anti-Toxoplasma* responses have been gained from studies in mice and human cells, but such information cannot fully apply to pig cells. Therefore, there is need to investigate IFNg-induced *anti-Toxoplasma* responses in pigs, given the huge health, production and zoonotic implications of the disease in this host. In preliminary overexpression screens of ISGs, we tested the impact of 34 porcine ISGs with functionally validated homologs in mice and humans shown to affect *Toxoplasma* infection.

We found four ISGs (*RIPK1, IPF2, CXCl12* and *IRF1*), that inhibited parasite growth in both Neonatal Swine Kidney (NSK) cells and Intestinal Porcine Enterocytes (IPEC-J2). Conversely, the overexpression of ISGs *DUSP6* and *CASP10* enhanced *Toxoplasma* growth in both NSK and IPEC-J2 cell lines. In addition, twenty other ISGs were found to affect *Toxoplasma* growth in cell type-specific manner. Following on this, we have developed the first ISG-knockout and ISG-expression libraries for the pig containing 2,966 unique ISG candidates. Together with fluorescently-tagged parasites, we are using these ISG libraries to systematically screen and identify how ISGs control *Toxoplasma* in porcine macrophages. This will be followed by functional characterization of ISG hits to identify counteracting *Toxoplasma* genes.

Poster 115 : Characterising *Cryptosporidium* invasion proteins and developing a workflow to investigate their potential as vaccine candidates against cryptosporidiosis

Presenter: **Abigail Webb**, *PhD Student, Aberystwyth University*

AL Webb[1]*; RM Morphew*[1]*; J Hamilton*[1]*; C Evans*[2]*; J King*[2]*; JA Pachebat*[1]*;*
[1] *Aberystwyth University, UK;* [2] *Wales Veterinary Science Centre, UK*

The protozoal parasite, *Cryptosporidium*, is responsible for the gastroenteric disease, cryptosporidiosis. Within the livestock sector, this disease is of great health, welfare, and economic importance, where high morbidity levels can be found, and fatal outcomes are particularly common for neonatal ruminants. The zoonotic pathogen, *C. parvum*, is the most commonly identified aetiological agent of cryptosporidiosis in cattle and sheep. Despite the veterinary importance of this parasite for livestock, treatment options are limited, and a vaccine is currently not available for either animals or humans. Parasitic proteins, considered to be involved in the attachment and invasion process of the parasite to host cells have been previously suggested as potential vaccine candidates against the disease, but many areas of this research question are lacking. Such proteins include: Cpa135, CP2, CP15 and P23. This research ai to develop the understanding of the DNA and amino acid sequences responsible for these proteins, along with their structure, allowing work to begin into the potential of these biomolecules. Accordingly, this research describes a bioinformatic analysis of DNA and protein sequences from *C. parvum* isolates and from isolates of other *Cryptosporidium* species. Another process that will allow for the consideration of these proteins as vaccine candidates, is being able to produce recombinant proteins to work on identifying whether these proteins produce a protective immune response for the host. This investigation started with bioinformatic analysis to explore sequence conservation of these invasion proteins between both *C. parvum* isolates and from isolates of other *Cryptosporidium* species. From faecal samples, DNA was extracted, and the specific regions of interest were amplified and purified. Cloning of bacterial cells to introduce the DNA regions of interest was performed. This is so the proteins can be incorporated into an expression plasmid to perform downstream analysis of the protective immune response they may provide to the host. Being able to identify and work with

these proteins, and their coding sequences, both bioinformatically and *in vitro*, will allow for a developed understanding into the interaction between the parasite and the host and how this interaction can provide prophylactic benefit for targeted hosts against a parasitic disease.

Poster 116 : Development of multiplex PCR to enhance the detection of *Cryptosporidium* species of veterinary concern in livestock

Presenter: **Abhijit Nikam**, *Student, Aberystwyth University*

A Nikam[1]*; S Balogun*[1]*; AL Webb*[1]*; O Polak*[1]*; Z Krupa*[1]*; B Lesetedi*[1]*; M Buerdsell*[1]*; B Davies*[1]*; C Evans*[2]*; J King*[2]*; J Alexander*[1]*; JA Pachebat*[1]*;*
[1] Aberystwyth University, UK;[3] Wales Veterinary Science Centre, UK

Neonatal diarrhoea is caused by the protozoan parasite *Cryptosporidium* species. Young animals are particularly vulnerable to the parasite, and they might experience severe diarrhoea and a high mortality rate, which results in large economic losses from mortality, anorexia, and stunted growth. Several *Cryptosporidium* species are known to infect neonates, with different pathogenicity. The diagnostic identification of oocysts under a microscope is not very reliable because oocysts from different *Cryptosporidium* species share similar morphology. Molecular detection involves PCR followed by restriction fragment length polymorphism (RFLP) or gene sequencing and is expensive and time-consuming. Most investigations have only been done on a small quantity of positive clinical samples and are either genus wide or target a specific species making it challenging to accurately determine the incidence of *Cryptosporidium* species present in animals and to specifically identify the species causing disease. To address this issue, a multiplex PCR assay is being developed and optimised to identify several *Cryptosporidium* species in sheep and cattle faecal samples, with the aim of characterising PCR amplicons by nanopore sequencing. With regard to current sequencing techniques, this improvised method for mixed infection diagnosis saves time and money, as the only reliable way of identification of mixed infection samples with high specificity and sensitivity.

Poster 117 : Assessing ChatGPT's utility in systematic reviews: Covid-19 seroprevalence case study act

Presenter: **Hankun Chen**, *University of Edinburgh*

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Introduction: With the advancement of Artificial Intelligence (AI), it may soon be possible to utilize such technologies to assist scientific research endeavours. One such software is ChatGPT, which has been programmed to acquire humanized intuition and high working efficiency that warrant further enquiries into its utility. Extracting data from publications is a time-consuming process in meta-analyses. Combining the humanized thinking of ChatGPT with data extraction could revolutionize the work process for those in this field. The aim of the present study was to assess the proficiency of ChatGPT to simplify data extraction to conduct a meta-analysis addressing the seroprevalence of SARS-CoV-2 on the African continent.

Methodology: Three versions of a "Task list" were created for ChatGPT to memorize, with the aim of establishing an assembly line approach.

Results: Extracting data based on providing only the paper title in the query was unsuccessful, and even after improving the "Task list" the data extracted by ChatGPT did not match the original text.

Conclusion: Based on the aforementioned results two reasons for the failure of extraction were identified: 1) the limited database of ChatGPT restricts its searching ability; 2) providing only the paper title does not provide enough information for searching. ChatGPT is then compared to the classical data extracting tool, and showing that this AI still needs to be improved.

Poster 118 : Population dynamics of adult *Schistosoma mansoni* wor before and after deworming treatment

Presenter: **Thomas Arme**, *University of Glasgow*

TM Arme[1]*; C McMurray*[1]*; J Clark*[1]*; B Lukubye*[2]*; M Arinaitwe*[3]*; A Nankasi*[3]*; WS Sands*[1]*; CL Faust*[1]*; PH Lamberton*[1]*;*
[1] University of Glasgow, UK; [2] Emory University, Uganda; [3] Vector Control Division, Ministry of Health, Uganda

Schistosomiasis is a waterborne parasitic disease caused by infection with dioecious trematodes of the genus *Schistosoma* (family: Schistosomatidae). The wor form mating pairs and live in the venous system of their vertebrate host *in copula*, releasing eggs that are excreted in the urine or stool. Treatment with the anthelminthic praziquantel targets and kills adult worms. However, evidence suggests that clearance of adult wor is not absolute, and any juvenile wor remain unaffected. It is not fully known how the adult worm population is affected by praziquantel and if eggs being excreted post-treatment are from wor surviving treatment, or from surviving juveniles or new infections that have now matured and started to produce eggs. Understanding these dynamics is particularly important in areas where transmission remains high despite multiple rounds of annual treatment (*i.e.,* persistent hotspots). It is impossible to differentiate between these two using standard diagnostics, as the wor are not directly accessible. However, larvae may be hatched from collected eggs and genotyped to infer parental lineages and to determine if the eggs are from new or surviving worms. *Schistosoma mansoni* is endemic across the lakeshore communities of Lake Victoria, Uganda, and causes intestinal schistosomiasis. Miracidia larvae of *S. mansoni* were collected from infected primary school-aged children in a persistent hotspot in Uganda pre-treatment and weekly post-treatment for up to six months. Up to 1000 miracidia were collected from 20 children pre-treatment and up to 300 a week from children post-treatment. Microsatellite data (a total of 17 *loci*), analysed using Geneious and Colony, are being used to genotype miracidia pre and post-treatment to i) identify all of the genotypes of adult wor in the effective breeding population prior to treatment, and ii) evaluate the changes in the composition of adult worm mating pairs and survival after treatment. Genotyping of pre-treatment larvae has begun for eight individuals (Early predicted number of worm pairs in individuals ranging from 28 to 65) and this preliminary data on aim 1 will be presented and discussed in the context of aim 2.

Poster 119 : Development of a mobile molecular lab (mLab) for the xenomonitoring of African trypanosomiasis

Presenter: **Dr Lucas Cunningham**, *LSTM*

LJ Cunningham[1]*; SJ Torr*[1]*; I Saldanha*[1]*; S Dunkley*[1]*; A Hope*[1]*;*
[1]*Liverpool School of Tropical Medicine, UK*

Background: Current field diagnostics for human African trypanosomiasis (HAT) were developed prior to the advent of molecular methods and although pragmatic are not suitable as monitoring tools checking for resurgence in near-elimination or post-elimination settings. As control of HAT intensifies and success is made the community has found that it lacks the diagnostics required to continue to accurately monitor the conditions on the ground. The goal of this project was to develop a fully mobile molecular laboratory (mLab) that can be used to screen the vector of African trypanosomiasis for the disease in low resource settings. These low resource settings would include locations with unreliable or non-existent electrical power supply, no dedicated laboratory and minimal access to postal or delivery service. As such the laboratory would have to be compact, be capable of supplying its own power and to operate for extended periods without re-supply. Key hurdles to be overcome include:

1) Suitable qPCR assays that are not reliant on a cold chain
2) Field friendly DNA isolation method that is rapid and simple but comparable to current tsetse DNA extraction methods
3) Trial the novel qPCR and DNA isolation methods in-country

Methods: A HRM qPCR assay was adapted from a nested-ITS PCR to screen field-tsetse for trypanosomes, with each major tsetse species identified based on their unique melt-peak signature. To complement the ITS qPCR, a HAT specific qPCR was also included to provide the mobile laboratory the ability to discriminate between the sub-species of *T. brucei s.l.* and identify *T. b. gambiense* positive flies. This gives the mLab an important tool to help it monitor a post-elimination gambian-HAT foci. To negate the need for a cold chain to store reagents all qPCR assays were adapted to a dry-format, allowing for transportation and storage at room temperature.

Parallel to the new qPCR assays a DNA isolation method was developed utilising magnetic beads, allowing for rapid and simple DNA extraction and same-day qPCR. To confirm successful DNA isolation a novel internal control qPCR was developed which targeted the obligate symbiont *Wigglesworthia glossinidia* to confirm successful DNA isolation.

These methods were then introduced to field staff and trialled at the Trypa-NO! research field laboratory in Arua (N.W. Uganda). As part of the project field staff underwent an intensive 2 week training course to introduce them to the qPCR assays and the new hardware.

Results and Conclusions: The field-friendly mobile molecular laboratory were successfully introduced to the field site and has been running as a trial for ~3 months, in that time it has successfully processed 320 samples. The new DNA isolation method has consistently performed well, confirmed by the amplification of the *W. glossinidia* marker. The mLab has also successfully detected several trypanosome species markers in the locally caught tsetse population.

Poster 120 : A CRISPR-Cas9 knockout screen identifies interferon-induced regulators of *Toxoplasma gondii*

Presenter: **Dr Anton Gossner**, *University of Edinburgh*

A Gossner[1]*; A Raper*[2]*; M Hassan*[2]*;*

[1] *University of Edinburgh, UK;* [2] *Roslin Institute, Universilty of Edinburgh, UK*

A third of the world's human population is thought to be chronically infected with *Toxoplasma gondii* despite the robust immune response to the parasite in immune competent individuals. As with many intracellular pathogens interferon gamma (IFNγ) production has been shown to be central to the immune response to *Toxoplasma* and control of infection of this protozoan parasite, which is able to infect almost all warmblooded vertebrates. Murine studies of innate immunity to *Toxoplasma* have identified pathways involved in the IFNγ response to parasite infection. Despite this the response in humans is less defined and there are fundamental differences between the human and murine innate immune systems, such as TLR11 and TLR12, both involved in resistance to the parasite in mice are absent in humans. Despite these differences IFNγ has a major role in the induction of cell-autonomous responses by the human innate immune system, through activation of a large transcriptional sequence of events resulting in the heightened expression of interferon stimulated genes (ISGs). Among the many ISGs expressed by IFNγ stimulated cells, GTPases such as guanylatebinding proteins (GBPs) and immunity-related GTPases (IRGs) have been shown to be important in the immune response to *Toxoplasma*. While the role of IFNγ, a type II IFN, has been extensively studied and shown to be important in immunity against Toxoplasma, other IFNs are less well studied. IFNβ, a type I IFN, has been shown to restrict *Toxoplasma* growth in both mouse and human macrophages. Mice lacking the functional Interferon alpha and beta receptor subunit 1 (IFNAR1), a type I IFN receptor, have increased susceptibility to *Toxoplasma* infection. While there is activation of overlapping pathways by type I and II IFNs there are also specific type I and II ISGs, resulting in the activation of different signalling pathways. A potential role for type I IFNs in response to the parasite was investigated using a type I IFN inducible ISGKnockout library. Several genes restricting *Toxoplasma* growth in type I IFN stimulated THP-1 cells, a human monocytic cell line, were identified. These IFN inducible genes included sorting nexin-5 (SNX5), coagulation factor II receptor-like 2 (F2RL2), myc-associated factor X (MAX) and small RNA Binding Exonuclease Protection Factor La (SSB), all previously unknown restrictors of *Toxoplasma* growth. To characterise their mode of action we generated CRISPR knockout THP1 cell clones for each gene. These THP1 KO cells will be used to functionally characterise how the selected ISGs restrict *Toxoplasma* growth in THP-1 cells in a type I IFN-induced response.

Poster 121 : First record and molecular characterisation of two *Gnathia* species (Crustacea, Isopoda, Gnathiidae) from Philippine coral reefs

Presenter: **Prof Kerry Hadfield**, *Associate Professor, North-West University*

K Hadfield[1]*; **MO Shodipo***[2]*; PC Sikkel*[3]*; NJ Smit*[1]*;*

[1] *North-West University, South Africa;* [2] *Silliman University, Philippines;* [3] *University of Miami, United States*

Gnathiid isopods are marine crustacean ectoparasites that do not permanently live on their fish hosts, being parasitic only in their larval stages. As gnathiids have been reported as one of the more common marine ectoparasites in coral reef habitats, it is unsurprising that more than a third of *Gnathia* species have either been originally described or reported from the Central Indo-Pacific. While the Philippines sits within the region of highest marine biodiversity in the world, the coral triangle, no gnathiid species have been identified or described from that region. Here we present the first records of two gnathiid species collected from the Visayas, central Philippines: *Gnathia malaysiensis* Müller, 1993, previously described from Malaysia, and *G. camuripenis* Tanaka, 2004, previously described from southern Japan. This study provided detailed morphological redescriptions, drawings and scanning electron microscope images as well as the first molecular characterisation of both species.

Poster 122 : First records of the branchial fish parasitic genus *Mothocya Costa*, in Hope, 1851 (Crustacea, Isopoda, Cymothoidae) from Nigeria

Presenter: **Prof Kerry Hadfield**, *Associate Professor, North-West University*

*K Hadfield[1]; **S van der Wal**[2]; NJ Smit[1]; NL Bruce[1]; B Olaosebikan[3];*
[1] North-West University, South Africa; [2] Ludwig Maximilians University, Germany; [3] Federal College of Freshwater Fisheries Technology, Nigeria

The isopod fauna of tropical and subtropical West Africa remains one of the least documented regions of the world and the family Cymothoidae Leach, 1818 is no exception. With a mere eleven publications on the family in this region since 1920, it is unsurprising that the predominately branchial attaching genus *Mothocya Costa*, in Hope, 1851, one of the better-known genera of the family, remains very poorly known from this region. The first record of *Mothocya* from West Africa was *Mothocya longicopa* Bruce, 1986. Recently, another two species have been added to this list. *Mothocya andoni* van der Wal, Smit, Bruce, Olaosebikan & Hadfield, 2021 and *Mothocya powelli* van der Wal, Smit, Bruce, Olaosebikan & Hadfield, 2021 were recorded for the first time from Nigerian brackish waters on *Monodactylus sebae* (Perciformes: Monodactylidae). Most cymothoids are distributed in marine environments, but several species have also been recorded from brackish and freshwater environments. In Africa, there are only two possible records of Cymothoidae in freshwater, thus making these records more noteworthy. Furthermore, *Mothocya andoni* from the Andoni River is tolerant of marine and brackish water (similar to its host) and *M. powelli* is from the Bonny River; both of which could potentially be useful in investigating the polluted waters in the Andoni and Bonny river systems.

Poster 123* : Exploiting susceptible and resistant rodent syste to uncover immune signatures underlying *L. donovani* symptomatic infection

Presenter: **Paul Jenkins**, *PhD Candidate, Institut Pasteur*

***P Jenkins**[1]; P Pescher[1]; G Spaeth[1];*
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Leishmania (*L.*) resists macrophage cytolytic activities and exploits these cells as hosts for intracellular proliferation. Chronic infection in humans can be either asymptomatic or symptomatic, causing devastating immuno-pathologies. The host determinants and immune mechanisms underlying this dichotomy are largely unknown, even though pathways controlling the infection could inform on urgently needed, immune-therapeutic interventions. C57BL/6 mice are reported to control *L. donovani* infection conversely to hamsters that develop progressive and lethal visceral leishmaniasis. Here we assess the role of the macrophage response between these two rodents in defining systemic infection. We first demonstrated by *in vitro* infection that only hamster but not mouse peritoneal macrophages allow robust proliferation of *L. donovani* parasites, thus correlating their permissivity with disease outcome. We further investigated the underlying macrophage responses by transcript profiling. Following the establishment and validation of a protocol for the bulk production of hamster bone marrow-derived macrophages (BMDMs) that allowed robust parasite growth, we conducted a comparative RNAseq analysis in hamster and mouse BMD to decipher host-specific transcript signatures in response to infection with *L. donovani* metacyclic-enriched promastigotes. Total mRNA was extracted from BMD 24 hours and 3 days after infection and submitted to RNA sequencing. Our analysis revealed rodent-specific expression changes, with hamster and mouse BMD respectively showing changes in abundance for 1941 and 511 transcripts (fold change 1.5, adjusted p-value < 0.05) at 24 hours, and 3320 and 273 transcripts at day 3 post-infection. The transcript profiles correlated with host factors previously linked to permissivity, including increased expression in hamster BMD of arginase 1 known to promote parasite infection, and increased expression in mouse BMD of NF-kappa B pathway members known to trigger anti-parasitic activities. Our comparative experimental system allowed us to firmly correlate the differences in parasite survival to differences in the rodent macrophage responses, suggesting that the initial parasite-host cell interaction can define the trajectory towards either acute symptomatic or silent chronic *L. donovani* infection.

Poster 124* : Detection and genetic characterization of tick-borne encephalitis virus from sylvatic rodents from north-eastern Poland

Presenter: **Martyna Krupińska**, *PhD student, Medical University of Gdansk*

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[1] Medical University of Gdańsk, Poland; [2] University of Helsinki, Finland; [3] University of Warsaw, Poland; [4] University of Nottingham, UK

Tick-borne encephalitis virus (TBEV) is a flavivirus widespread in Baltic countries, Russia and Asia, capable of causing infection of the central nervous system, in humans and in vertebrate reservoirs. Morbidity and mortality vary greatly depending on the strain of the virus. Three TBEV subtypes are known - European (TBEV-Eur), Siberian (TBEV-Sib), and Far-Eastern (TBEV-FE) differing in clinical course and outcome. In Poland, 200-300 TBE cases are registered annually, of which, on average, 70% of reported cases come from north-eastern Poland – a region considered to be highly endemic. Although, up to this day, only the TBEV-Eur subtype was detected in Poland, all three subtypes of TBEV were detected for the first time in Estonia and Latvia. Rodents are recognized as one of the most important mammalian reservoir hosts of TBEV and are considered good indicators of TBEV circulation, carrying persistent latent infections.

Overall, 270 rodents from three ecologically similar study sites in north-eastern Poland were captured. Blood samples from the heart were tested for the presence of antibodies against TBEV using an immunofluorescence assay. Rodent brains were collected during the section, RNA was isolated and TBEV detection was carried out in samples using RT-nested PCR. TBEV-positive samples were subsequently sequenced and subjected to phylogenetic analysis.

We detected RNA of TBEV in 37 out of 270 brain samples with an overall prevalence of 13.7% (95%CL 10.5-17.5). The IgG antibodies were detected in 18 of 270 (6.7%) blood samples. Interestingly, 9 of 37 rodents with detected TBEV RNA in brain tissue had anti-TBEV antibodies and 9 antibody-positive rodents did not have detectable levels of TBEV RNA in the brain. 10 complete TBEV sequences were obtained. All detected TBEV strains belong to the European subtype, forming 2 clusters.

To the best of our knowledge, we report the first complete genomic sequences of TBEV strains in Poland. Our study brings novel data for understanding TBEV circulation in rodent populations. We believe that biomonitoring of rodents against zoonotic diseases is the best way to predict peak years and high-risk sites, helping preventing human cases of TBE and thereby contributing significantly to the public health.

Poster 125* : A situational analysis of Neglected Tropical Diseases in Zimbabwe

Presenter: **Gabrielle Thompson**, -

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Neglected Tropical Diseases (NTDs) disproportionately affect marginalized and impoverished communities and hinder a country's socio-economic progress. NTDs are closely related to Sustainable Development Goals (SDGs), particularly those related to health and poverty reduction. The ultimate goal is to completely eliminate NTDs, which can be accomplished by executing a comprehensive master plan. The objective of this situational analysis was to perform a literature review that reports the occurrence of various NTDs in Zimbabwe and the measures available for controlling them. A systematic search was conducted in PubMed, Google Scholar, web of science, WHO reports, and grey literature using certain literature terms. Results from individual studies providing an overall prevalence of the NTDs are reported. The prevalence of some NTDs was available from population-based studies conducted in different districts. Zimbabwe is burdened with several NTDs of public health significance, and the routine health information system in Zimbabwe only captures cases that are attended at health facilities. Therefore, there is insufficient knowledge about the prevalence and distribution of most of the suspected NTDs in Zimbabwe. NTDs can be controlled and eliminated through the implementation of five evidence-based strategies, including preventive chemotherapy, innovative and intensified disease management, vector control, veterinary public health measures for zoonotic diseases, and clean water, sanitation, and hygiene facilities. The importance of the masterplan was to outline a comprehensive approach for managing NTDs, including strategies for prevention, treatment, and control. The analysis highlights the need for more research to be conducted on NTDs in Zimbabwe and calls for the implementation of the comprehensive masterplan.

Poster 126 : Nemabiome metabarcoding shows varying levels of genetic diversity in anthelmintic-resistant gastrointestinal nematodes

Presenter: **Dr Osama Zahid**, *The Roslin Institute, Universilty of Edinburgh*

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Gastrointestinal nematodes (GINs) pose a significant threat to the livestock industry. Faecal egg count reduction tests (FECRTs) are commonly used to detect anthelmintic resistance of GINs. However, they do not provide information about the dynamics of resistant GIN species. In this study, we conducted a molecular analysis of GIN populations from pre- and post-treatment samples collected from 18 sheep far in southeast England. We collected faecal samples from three groups (10 lambs each) at the time of treatment with recommended doses of ivermectin, levamisole, and a combination of both; and at 14 days post-treatment. Eggs from the samples were hatched for DNA extraction, followed by nemabiome metabarcoding and next-generation Illumina sequencing. The results showed that anthelmintic resistance was widespread, and different GIN species showed different levels of resistance to the tested drugs. The post-treatment results showed a dominance of *Teladorsagia circumcincta* and *Trichostrongylus colubriformis*, indicating the most anthelmintic resistance in these species. Our findings provide insights into the diversity of resistant GIN species and may aid in determining the most suitable drugs or combinations for individual farms. Moreover, the study provides a strong foundation for understanding the diversity present in resistant GIN species and the genomic basis for anthelmintic resistance.

Poster 127 : *Plasmodium berghei* histone deacetylase 1 (HDA1) plays important, dual, sex specific roles in gametocyte maturation and viability

Presenter: **Dr Scott Millar**, *University of Glasgow*

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In order for *Plasmodium* species to transmit to a mosquito vector they must transition from an asexual parasite of the erythrocytic cycle into a sexual male or female gametocyte. Epigenetically regulated expression of *ap2-g*, a member of the AP2 family of transcription factors was identified as orchestrating this developmental change, guiding the parasite through gametocytogenesis. Although some epigenetic actors controlling *ap2-g* expression and thus commitment have been identified the full extent of epigenetic regulation remains unclear. Histone Deacetylase 1 (*hda1*) has previously been shown to be upregulated in *P. falciparum* following upregulation of *ap2-g*, suggesting a role in gametocyte development or function.

To investigate the role of HDA1 in both the sexual and asexual life cycle of the rodent malaria species *Plasmodium berghei*, we generated *hda1*$^{-}$ knockout and *hda1::gfp* tagged lines. Analysis of the knockout by flow cytometry revealed a complex phenotype of slow growth and altered sex ratio. Further investigations using imaging flow cytometry revealed a defect in male exflagellation, which resulted in an absence of ookinetes in the knockout. Transcriptomic and chromatin accessibility studies on sorted male, female and schizont *hda1*$^{-}$ populations identified an upregulation of variant gene family members. Analysis of the *hda1::gfp* line revealed a punctate nuclear HDA1 signal distinct from the DAPI and H3K9me3 signal, suggesting a role in chromatin regulation. In summary, HDA1 plays a key role in gametocyte commitment and emergence.

Poster 128 : Effect of parasitic *Cryptosporidium* on the gut-microbiome of bovine livestock: A computational metagenomic approach

Presenter: **Mumdooh Sabir**, *University of East Anglia*

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[1] *University of East Anglia, UK;* [2] *Earlham Institute, UK;* [3] *University of Kent, UK*

This study investigated the impact of *Cryptosporidium* infection on the gut microbiome of cattle. Cryptosporidiosis is a parasitic disease that can lead to significant economic losses amounting to billions of dollars worldwide. Using a bioinformatic pipeline based on shotgun metagenomic sequencing, we found that infection with *Cryptosporidium* spp. is associated with dysbiosis, a pronounced reduction in bacterial diversity in the gut microbiome of infected hosts. We also observe a positive correlation between the relative abundance of and potential pathogens of the genus fusobacterium in the microbiota. The study highlights the importance of understanding the relationship between *Cryptosporidium* infection and gut microbiome alterations and how the gut microbiome differs between infected and uninfected hosts.

Poster 129 : The occurrence of *Toxoplasma gondii* in the red fox (*Vulpes vulpes*) population in the Pomerania Voivodeship, northern Poland

Presenter: **Dr Anna Lass**, *Medical University of Gdansk*

A Lass[1]*; K Baranowicz*[1]*; A Świątalska*[2]*; B Biernat*[1]*;*
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Toxoplasma gondii is a worldwide distributed protozoan parasite that can infect humans as well as all warm-blooded animals. Infection can be acquired mainly via the oral route through consumption of raw meat of infected animals containing cysts filled with parasites and through the ingestion of oocysts that can be present in environmental matrices. Toxoplasmosis is one of the most prevalent parasitic infections in humans; higher seroprevalence is found in Latin America, parts of Eastern/Central Europe, the Middle East, parts of southeast Asia, and Africa. In Poland, according to serological investigations, about 50% of human population can be infected with this parasite. *Toxoplasma* infections are also frequently reported in livestock. However, there is currently a gap in the knowledge about the occurrence of the parasite in wildlife. Red fox (*Vulpes vulpes*) is one of the most abundant species of wild carnivores in Europe. As predators and scavengers, foxes represent a potentially sensitive indicator of the circulation of T. gondii in environments where humans co-exist. The main aim of this study was to estimate the prevalence of *T. gondii* in the red fox population living in the area of northern Poland. Foxes hunted in two sites of Pomorskie Voivodeship: Rzucewo (Puck County and community) and Pażęce (Kartuzy County, Stężyca community) during hunting season 2022 were investigated. Fox carcasses were dissected at the veterinary hygiene facility in Gdańsk and samples of brain and diaphragm muscles were collected from a total of 179 animals (96 from Rzucewo and 83 from Pażęce). Prior to DNA extraction, the tissue samples were prepared using ten freeze-thaw cycles (using a -70oC freezer and a water bath) to destroy the tissue cysts and improve the efficiency of DNA extraction. Afterwards, DNA extraction was performed using a commercial DNeasy Blood & Tissue Kit, Qiagen (Germany) according to the manufacturer's instructions. For the specific detection of *Toxoplasma gondii* DNA, real-time PCR was performed with the use of a pair of primers (ToxB-41F, ToxB-169R) targeting a 129-bp fragment of the 35-fold repetitive B1 gene and the fluorescent-labelled TaqMan probe (ToxB 69P). Out of a total of 179 examined red foxes, *Toxoplasma* DNA was detected in 12 (6.7 %, +/- 95% CL 3.3- 12.9) foxes, 4 (4.2 %, +/- 95% CL 0.9-13.6) in Rzucewo and 8 (9.6 %, +/- 95% CL 4.2-19.8) in Pażęce. Positive results were obtained in 7 diaphragm muscles and 5 brain samples. However, simultaneous positive samples from the brain and diaphragm muscles in the same individual were not detected. The infection in red foxes presumably is the result of eating infected prey or infected carcasses left on the hunting grounds after the evisceration of shot animals. In addition, contamination of the surroundings with *T. gondii* oocysts and their possible transfer to other animals are also probable. Studies are to be continued taking into account a greater number of foxes and the area of other voivodships.

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Poster 130* : WHAT IS A SCHISTOSOMIASIS HOTSPOT? A critical review of published literature

Presenter: **Rivka Lim**, *University of Edinburgh*

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Accurate identification of disease hotspots is essential for effective control and reducing the global burden of infectious diseases. Inconsistent use of the term "hotspot" in schistosomiasis research poses a significant challenge to collating informative study outputs for the evaluation and modification of effective control and/or elimination strategies. This review presents a comprehensive analysis of the evolution of the term "hotspot" in schistosomiasis research, the influence of different studies on its usage, and proposes standardised definitions to address the inconsistencies found in the literature.

Using a qualitative analysis, we assessed the World Health Organization's (WHO) new preliminary definition of a persistent hotspot, which refers to regions where prevalence persists despite control measures and could hinder progress to reaching the WHO goal of eliminating schistosomiasis as a public health problem by 2030. We provide a detailed overview of the WHO definition's key features, where potential limitations were identified by examining each of the criteria individually and measuring how restrictive or permissive they are to hotspot identification. Modifications were proposed to add clarity and ensure that regions requiring additional treatment due to persistence post-treatment are not missed in future control programs.

This review contributes to ongoing efforts to improve disease control and elimination strategies by providing a better understanding of the term "hotspot" and provides a framework for future operational research and implementation.

Poster 131 : A 2A peptide-based system for *in situ* gene tagging that preserves cis-acting regulatory elements in trypanosomatids

Presenter: **Dr Calvin Tiengwe**, *Imperial College London*

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Conventional approaches for *in situ* gene tagging in trypanosomes replace endogenous untranslated regions (UTRs). Such replacements alter normal gene expression levels since regulatory elements within the endogenous UTR are disrupted. Using a self-cleaving viral 2A peptide, we provide a new system for epitope-tagging that retains native UTRs. The system uses CRISPR/Cas9 to knock-in PCR-generated 2A peptide cassettes containing a drug selectable marker and a fluorescent tag flanked by ~40 bp homology ar to the target site. We validate our system by fusing mNeonGreen (mNG) or mScarlet (mSc) to three proteins: ESAG3 (E3), cytosolic Hsp70, and ESAG7 (E7). PCR and whole genome sequencing showed single-copy in-frame integration events only at the target loci, with full length endogenous UTRs intact. We show localisation of mNG-tagged E3, and N- and C- terminal tagging of cytosolic Hsp70 with mNG and mSc, respectively. By tagging one subunit (E7) of the transferrin receptor, an essential protein for iron uptake and survival, we demonstrate that our approach maintains normal expression, regulation in response to an external stimulus iron, and does not interfere with transferrin binding. Comparative western blot analyses of all 2A-tagged proteins generated in this work provide evidence for high cleavage efficiency of the 2A peptide derived from *Thosea asigna* in bloodstream trypanosomes. Our 2A tagging system is applicable to all Kinetoplastids amenable to CRISPR/Cas9 gene editing which rely on polycistronic transcription and is useful for studying post-transcriptional and post-translational regulation of a given gene within the framework of a single experiment.

Poster 132* : Diagnostics of dogs in the Pomeranian Voivodeship for *Echinococcus multilocularis* infection

Presenter: **Emilia Zalugowicz**, *Department of Tropical Medicine and Parasitology*

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Echinococcus multilocularis is the most dangerous parasite in the northern hemisphere and a causative agent of alveolar echinococcosis (AE), a disease fatal if unproperly treated. Infection in humans is initiated by the ingestion of *E. multilocularis* eggs passed in stool by definitive hosts. Transmission of *E. multilocularis* occurs predominantly in a sylvatic cycle with wild canids, mainly foxes, as definitive hosts. In some areas, however, domestic dogs may play the same role in a synanthropic cycle.

Dog ownership is the most clearly established risk factor for acquiring human AE. In some regions of Poland, it may facilitate the transmission of the parasite to humans, thus, increasing the incidence of human AE. Dogs seem to contribute to the occurrence of human AE in China, while in Europe, their involvement is less clear. Research conducted in non-endemic areas of Poland in the early 2000s revealed no infected dogs or cats.

The aim of the study was to establish the role of dogs in *E. multilocularis* transmission in non-endemic Pomerania (northern Poland). In 2022, stool samples from 75 dogs of private owners and 228 dogs from eight shelters (303 samples total) were examined using nested PCR, semi-nested PCR and real-time PCR. The sensitivity of these methods was assessed. Positive samples were sequenced for confirmation.

The genetic material of the tapeworm was detected by more than one diagnostic method in seven samples. The highest sensitivity was achieved in nested PCR, followed by semi-nested PCR, and real-time PCR. Our preliminary results indicate that some AE cases in the studied area may be dog-related, but the incidence of *E. multilocularis* in dogs suggests low risk to humans.

Poster 133* : What are the drivers of enteric neuropathy in experimental Chagas disease?

Presenter: **Harry Langston**, *London School of Hygiene and Tropical Medicine*

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Chagas disease, caused by the protozoan parasite *T. cruzi*, affects ~7 million people worldwide. Around 30-40% of those infected develop chronic cardiac or gastrointestinal (GI) sequelae. GI disease is associated with a high morbidity, but the mechanisms responsible for the underlying enteric agangliosis and megasyndromes are largely unknown. The leading hypothesis for enteric nervous system pathology has been collateral damage induced by reactive nitrogen species, synthesised by iNOS-expressing myeloid cells during the acute phase immune response. This hypothesis has been questioned since bioluminescence imaging led to the discovery of chronic parasite reservoirs in the colon, which suggests an ongoing role for the infection in sustaining pathogenesis. Recent work has shown that C3H/HeN mice infected with TcI-JR parasites develop GI dysperistalsis, which is a common symptom of human digestive Chagas disease. This project ai to provide insight into the mechanisms responsible for enteric neuropathy in this mouse model. We conducted a histopathological analysis of colon tissue and found a significant increase in cellular infiltration in the smooth muscle of chronic Digestive Chagas Disease (DCD) mice, but not when a less pathogenic *T. cruzi* strain (TcVI-CLBR) was used. This inflammation was hyperfocal to the GI smooth muscle, adjacent to the adipose rich mesentery in the proximal colon. There was also evidence of focal fibrosis, high iNOS expression and neuronal damage. In ongoing *in vivo* experiments, we are investigating the effects of anti-parasitic and immunomodulatory treatments on the initiation of gut dysfunction in the DCD model, which occurs between 2 and 3 weeks post-infection. Benznidazole treatment suppressed the parasite burden below the limit of detection and prevented the initiation of gut dysfunction. Elimination of CD8+ T cells (which are critical for parasite control) by anti-CD8 immunotherapy increased the parasite burden by 19 fold and had no impact on the gut dysfunction phenotype. Interestingly, treatment with the broad immune suppressant cyclophosphamide completely reversed the GI peristalsis defect, even though the parasite load increased by 15 fold.

The results suggest an antagonistic interaction between parasites and immune action is key to the initiation of gut dysperistalsis, but one where CD8+ T cells are not critical pathological effectors. These results are generating insights into the early host-parasite interactions that drive digestive Chagas disease pathogenesis.

Poster 134 : Spatially resolved single cell transcriptomics reveal a critical role for γδ T cells in the control of skin inflammation and subcutaneous adipose wasting during chronic *Trypanosoma brucei* infection

Presenter: **Dr Matthew Sinton**, *University of Glasgow*

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African trypanosomes colonise the skin in a process critical for disease transmission, but skin responses to infection remain unexplored. Here, combining spatial and single cell transcriptomics, we investigated local immune responses of the skin in a murine model of infection. Our results reveal an unexpected crosstalk between subcutaneous adipocytes and gamma-delta ($\gamma\delta$) T cells during infection. During chronic infection, we detected an expansion of IL-17-producing Vg6^{+} $\gamma\delta$ T cells in the infected murine skin compared with naïve controls. *Echinococcus multilocularis* cell-cell communication analyses suggest that adipocytes trigger Vg6^{+} cell activation *via Cd40, Il6, Il10,* and *Tnfsf18* signalling, indicating a role for adipocytes in controlling T cell activation locally. *In vivo*, the infected skin of Vg4/6$^{-/-}$ mice displays more inflammation compared with infected wild type controls, correlating with an elevated capacity of dermal CD8^{+} T cells to produce IFNγ, independently of dermal T_H1 CD4^{+} T cells. Intriguingly, the Vg4/6$^{-/-}$ mice do not experience subcutaneous adipose tissue wasting to the same extent as the FVB/N controls, indicating that Vg4/6 $\gamma\delta$ T cells might also control this process. Based on these observations, we propose a model whereby adipocytes and Vg4/6 $\gamma\delta$ T cells act concertedly in the skin to limit CD8^{+} T cell-mediated inflammatory responses, imposing an immunological barrier for parasite transmission. These studies shed light onto the mechanisms of $\gamma\delta$ T cells-mediated immunity in the skin in the context of African trypanosome infection, as well as a potentially novel role of adipocytes as regulators of skin immunity during chronic infection.

Poster 135* : The *Leishmania mexicana* cell cycle: Patterns of organelle duplication and segregation revealed by 3D electron microscopy and their implications for parasite biology

Presenter: **Molly Hair**, *Oxford Brookes University*

M Hair; *R Wheeler*[2]*; RY Yanase*[1]*; S Vaughan*[3]*; JD Sunter*[3]*;*

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The cell cycle of *Leishmania* spp. has previously been studied using fluorescence light microscopy combined with thin section transmission electron microscopy. Yet, little is still known about the intrinsic spatial organisation of organelles within the cell body and their manner and pattern of duplication and inheritance. Using an advanced volume EM approach - serial block face scanning electron microscopy, we have produced a three-dimensional spatial and quantitative overview of the *L. mexicana* cell cycle. This has generated important insights into organelle positioning, division and inheritance. Firstly, during the cell cycle for all organelles there is a general increase in number and volume. Interestingly, at earlier time points in the cell cycle the volume of the glycosomes and acidocalcisomes remained constant yet there was an increase in organelle number. This suggests these organelles are able to divide in addition to being generated de novo by the endoplasmic reticulum. Secondly, *Leishmania* undergoes closed mitosis and we demonstrate that during late mitosis nuclear pores are no longer present on the nuclear bridge. The nuclear bridge is therefore a specialised region of the nuclear envelope, with the absence of nuclear pores likely required for accurate nuclear envelope resolution. Overall, our data provides a detailed spatiotemporal framework for the *Leishmania* cell cycle, which is fundamental to our understanding of the cell division processes required to produce daughter cells in the image of the mother.

Poster 136* : CEP43 – a protein with unexpected and divergent functions in the assembly and stability of the trypanosome flagellum

Presenter: **Aro Nugawela**, *Lancaster University*

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The *T. brucei* flagellum contains both a canonical 9+2 eukaryotic axoneme and extra-axonemal paraflagellar rod (PFR). In most flagellated eukaryotes, flagellum assembly depends upon intraflagellar transport (IFT), a bidirectional transport system that transports cargo along axonemal microtubules. A conserved protein complex consisting of CEP43, CEP19 and RABL2B localised at the basal body facilitates anterograde (i.e. base to tip) IFT. However, we have previously demonstrated the primary impact of *Tb*CEP43^{RNAi} depletion is on PFR assembly, with minimal impact on axoneme formation. To determine whether the entire CEP43/CEP19/RABL2B complex is involved in PFR rather than axonemal assembly in *T. brucei*, we assessed the roles of *Tb*RABL2B and *Tb*CEP19. Our data demonstrates that depletion of *Tb*RABL2B and *Tb*CEP19results in flagellum phenotypes indicative of a generalised IFT defect; mirroring published short-flagellum phenotypes of IFT-mutants, rather than a specific failure in PFR assembly. This develops our understanding of the unexpected divergence of CEP43 function in trypanosomes within a protein complex that is otherwise functionally conserved in facilitating IFT.

To further investigate *Tb*CEP43 function we studied the temporal appearance of PFR abnormalities following induction of *Tb*CEP43^{RNAi} using immunofluorescence, transmission and scanning electron microscopy. Our investigations indicate that in addition to failing to assemble a coherent PFR structure in newly forming flagella, we observe disruptions in PFR uniformity in flagella that were assembled prior to induction of *Tb*CEP43^{RNAi}, as well as an unusual rudimentary polymerisation of PFR material. These results are, as far as we are aware, the first evidence for the integrity of the highly ordered PFR structure being affected after its construction has been completed. In addition, live cell imaging of *Tb*CEP43^{RNAi} induced cells expressing a fluorescently tagged IFT protein, revealed an unexpected IFT phenotype; characterized by a reduction of IFT processivity and aberrant switching between anterograde and retrograde transport along the length of the flagellum. Our studies raise intriguing questions on how *Tb*CEP43 influences PFR assembly and stability in the *T. brucei* flagellum, and the relationship between IFT-dysregulation and PFR maintenance.

Poster 137 : Environmental sensing and metabolism and growth control by trypanosome QIQ1

Presenter: **Dr Anna Trenaman**, *University of Dundee*

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[1] University of Dundee, UK; [2] University of Edinburgh, UK; [3] London School of Hygiene and Tropical Medicine, UK

Bacterial pathogens, and some eukaryotic parasites, moderate their growth and density, and this can extend the timeframe of infection in their hosts. A quorum-sensing pathway has been described in bloodstream African trypanosomes, for example, whereby morphological 'stumpy' differentiation can be triggered by oligopeptides or cyclic AMP analogues. Here, we describe a distinct environmental sensing mechanism in African trypanosomes. A genome-scale loss-of-function genetic screen revealed a small cohort of knockdowns that increase trypanosome growth, several with domains implicated in calcium-signalling. The dominant hit (Tb927.8.6870) encodes multiple putative calmodulin binding IQ-domains, and we name this protein QIQ1 for Quintuple IQ-domain protein 1, also reflecting the 'quick' growth phenotype observed following knockdown. QIQ1 is localized to the parasite flagellum, while the competitive advantage displayed by *qiq1*-null trypanosomes at high cell density is abolished in the presence of calcium chelators. We show that *qiq1*-null parasites achieve higher maximum cell density both *in vitro* and *in vivo*; in culture and in a mouse model, respectively. Notably, *qiq1*-nulls maintained morphological differentiation and expressed a 'stumpy' cell marker *in vivo*. Proteomic analysis revealed a specific deficit in mitochondrial ATP-synthase reprogramming at high cell density in the absence of QIQ1. We conclude that African trypanosomes employ multiple mechanisms to modulate growth. We suggest that QIQ1 and other proteins identified here constitute components of a calcium-signalling pathway that reduces growth rate at high density, facilitating energy efficiency in resource-poor environments.

Poster 138* : The hematopoietic stem cell as a parasitological niche responsible for antileishmanial treatment failure

Presenter: **Laura Dirkx**, *PhD student, University of Antwerp*

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Amongst the parasitic diseases, visceral leishmaniasis (VL) belongs to one of the deadliest, yet most neglected diseases of the world. Treatment options are scarce, have many limitations and post-treatment relapse is common while there is no effective test-of-cure.

Recently, long-term hematopoietic stem cells (LT-HSC) in the bone marrow were identified as a sanctuary niche where parasites can survive drug treatment by transitioning through a quiescent state, serving as source of systemic parasite spread and relapse. A vast number of parasites reside within this hospitable niche that is characterized by low oxidative burst levels and a unique transcriptional signature, named StemLeish, significantly overlapping with human VL and HIV co-infected blood transcriptomes. Silencing of the various StemLeish genes pinpointed a pivotal role of Cxcr4 in shaping the LT-HSC niche.

Parasites that transitioned through quiescence displayed an increased cellular infectivity and high transmission capacity through the *Lutzomyia longipalpis* sand fly vector, emphasizing the risk of propagation of enhanced phenotypes following post-treatment relapse. Transcriptional profiling of quiescent parasites revealed a novel set of markers and potential drivers, several with predicted involvement as regulators of cell cycle progression and of gene expression at various levels.

Collectively, this work delivers unprecedented insights regarding post-treatment relapse during VL, providing novel biomarkers and drug targets for both host-directed and anti-parasite therapeutics targeting differential genes in quiescent amastigotes and in the hematopoietic niche.

Poster 139 : Malaria parasite development is rhythmic and is synchronised with host feeding-fasting rhythms: How? Why? Huh?

Presenter: **Aidan O'Donnell**, *University of Edinburgh*

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Research into the role of daily rhyth in infections is gaining traction because explaining the regulatory mechanisms and fitness consequences of biological rhyth exhibited by parasites and hosts offers new avenues to treat infections. Malaria (*Plasmodium*) parasites exhibit ~24h developmental rhyth during replication in the mammalian host's blood and during transmission to insect vectors. The survival and transmission of malaria parasites is determined by whether these developmental rhyth are synchronised to the host's circadian rhythms, but how periodicity in these parasite traits is generated and maintained during infection is poorly understood. We address this using rodent malaria (*Plasmodium chabaudi*) infections of wild type (WT) and arrhythmic clock mutant (*Per1/2* double knock out) mice. We compare parasite and host rhyth in WT mice kept in LD and DD, with rhyth observed in mutant mice in DD. Second, we use the mutant mice and a restricted feeding regime to decouple host rhyth in feeding from body temperature and locomotor activity and examine the consequences for parasite rhythms. Finally, we apply a 'phase-shift' to parasites and track the parasite schedule for over ten cycles to determine how they resynchronise with host rhythms. We show that (i) parasite rhyth match the phase of the host's feeding-fasting rhythm and not the phase of rhyth in activity or body temperature; (ii) the timing of the parasite replication cycle is independent of the canonical 'core' host clock (i.e. transcription translation feedback loop); (iii) following perturbation, parasites reschedule to regain synchrony with the timing of the host's rhythm within 7 replication cycles and achieve this by speeding up the replication rhythm by 2-3 hours per cycle. We discuss how it is beneficial for parasites to be in synchronization with their host's feeding-fasting rhyth and plasticity in their development duration facilitates this synchrony by enabling parasites to make small daily changes to their schedule when necessary.

Poster 140 : Structure of the PfRCR complex which bridges the malaria parasite and erythrocyte during invasion

Presenter: **Dr Brendan Farrell**, *Postdoctoral Research Associate, University of Oxford*

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Invasion of erythrocytes by *Plasmodium falciparum*, the deadliest human malaria parasite, is a tightly coordinated process involving many host-pathogen interactions. Among these is the essential interaction between PfRH5 and basigin on the red blood cell surface. PfRH5 is a member of the five-component PfPCRCR complex also containing PfCyRPA, PfRIPR, PfCSS and PfPTRAMP. Each of these five conserved proteins are essential to erythrocyte invasion, and each raises invasion-blocking antibodies or nanobodies, making them the leading blood stage malaria vaccine candidates. Despite its essential role, the molecular mechanism by which the PfPCRCR complex acts remains unclear. To gain insights, we determined the structure of the PfRCR complex comprising PfRH5, PfCyRPA and PfRIPR using cryo-EM. This reveals the structure of PfRIPR for the first time, showing that it is composed of a novel multi-domain core attached flexibly to an elongated tail. We find that PfRH5 is largely structurally unchanged when part of the PfRCR complex, and that a rigid disulphide-locked PfRH5 mediates efficient erythrocyte invasion, suggesting that it does not undergo large conformational changes. Additionally, we show that the parasite surface complex PfCSS-PfPTRAMP binds to the tail of PfRIPR, the target of anti-PfRIPR growth neutralising antibodies. Together with composite modelling of the PfRCR complex on the erythrocyte surface, these data indicate that the PfRCR complex acts to bridge the parasite and erythrocyte membranes, with a modular PfRIPR anchoring the complex to the parasite surface through its tail, presenting its structured core bound to PfCyRPA and PfRH5 such that they can engage with erythrocyte receptors. These findings provide novel insight into erythrocyte invasion and open the way to new approaches in rational vaccine design.

Poster 141* : Genetic validation of the function of PfEMP1 in *Plasmodium falciparum* rosette formation using CRISPR-Cas9 genome editing

Presenter: **Stanley Otoboh**, *PhD researcher, University of Ediburgh*

SE Otoboh[1]*; HM Abkallo*[1]*; J Jungels*[1]*; JA Rowe*[1]*;*
[1] *Institute of Immunology & Infection Research, University of Edinburgh, UK*

Rosetting, the binding of *P. falciparum* infected erythrocytes to uninfected erythrocytes to form clusters (rosettes), is thought to contribute to severe malaria pathology. This adhesion phenotype is mediated through the adhesive properties of *P. falciparum* erythrocyte membrane protein 1 (PfEMP1), encoded by the *var* gene family (~60 distinct copies per parasite genome, expressed in a mutually exclusive manner). The N-terminal domain (NTS-DBLα) of PfEMP1 has been implicated as the functional erythrocyte binding domain. However, the role of PfEMP1 in adhesion in live infected erythrocytes has not been studied by reverse genetics, mainly due to the difficulty in genetically manipulating *P. falciparum*. Here, we use CRISPR-Cas9 genome editing to generate a population of parasites expressing a single *var* gene and to add epitope tags to PfEMP1. We also investigated whether the PfEMP1 variant "IT4var60" is sufficient to mediate the rosetting phenotype in *P. falciparum*. Our results reveal that a *var* gene co-expressed with a drug resistance gene via a 2A peptide can be inducibly and exclusively expressed under drug pressure. We have also shown that the IT4var60 PfEMP1 variant is sufficient to mediate rosetting in *P. falciparum*, as rosetting was completely abolished in IT4var60-knockout transgenic parasites. We further reveal that specific residues within the DBLα domain of IT4var60 PfEMP1 variant may be critical in *P. falciparum* rosette formation. Thus, our results suggest potential targets for the design and development of anti-rosetting interventions, and provide a general strategy for reverse genetic studies of PfEMP1.

Poster 142 : mt-LAF3 is a pseudouridine synthase ortholog required for mitochondrial rRNA and mRNA gene expression in *Trypanosoma brucei*

Presenter: **Dr Suzanne McDermott**, *Acting Assistant Professor, Seattle Childrens Research Institute*

S McDermott[1]*; V Pham*[2]*; I Lewis*[2]*; M Tracy*[2]*; K Stuart*[1]*;*
[1] *Seattle Childrens Research Institute and University of Washington, United States;* [2] *Seattle Childrens Research Institute, United States*

Trypanosoma brucei and related kinetoplastid parasites possess unique RNA processing pathways, including in their mitochondria, that regulate metabolism and development. Altering RNA composition or conformation through nucleotide modifications is one such pathway, and modifications including pseudouridine regulate RNA fate and function in many organisms. We surveyed pseudouridine synthase (PUS) orthologs in trypanosomatids, with a particular interest in mitochondrial enzymes due to their potential importance for mitochondrial function and metabolism. *T. brucei* mt-LAF3 is an ortholog of human and yeast mitochondrial PUS enzymes, and a mitoribosome assembly factor, but structural studies differ in their conclusion as to whether it has PUS catalytic activity. Here, we generated *T. brucei* cells that are conditionally null for mt-LAF3 and showed that mt-LAF3 loss is lethal and disrupts mitochondrial membrane potential (ΔΨm). Addition of a mutant gamma-ATP synthase allele to the conditionally null cells permitted ΔΨm maintenance and cell survival, allowing us to assess primary effects on mitochondrial RNAs. As expected, these studies showed that loss of mt-LAF3 dramatically decreases levels of mitochondrial 12S and 9S rRNAs. Notably, we also observed decreases in mitochondrial mRNA levels, including differential effects on edited vs. pre-edited mRNAs, indicating that mt-LAF3 is required for mitochondrial rRNA and mRNA processing, including of edited transcripts. To assess the importance of PUS catalytic activity in mt-LAF3 we mutated a conserved aspartate that is necessary for catalysis in other PUS enzymes and showed it is not essential for cell growth, or maintenance of ΔΨm and mitochondrial RNA levels. Together, these results indicate that mt-LAF3 is required for normal expression of mitochondrial mRNAs in addition to rRNAs, but that PUS catalytic activity is not required for these functions. Instead, our work, combined with previous structural studies, suggests that *T. brucei* mt-LAF3 acts as a mitochondrial RNA-stabilizing scaffold.

Poster 143 : The ATAD2/Abo1/Yta7 homologue, Bromodomain Factor 7, is essential for macrophage infection by *Leishmania mexicana*

Presenter: **Dr Nathaniel Jones**, *Research Fellow in Drug Discovery, University of York*

NG Jones[1]*; AJ Wilkinson*[1]*; J Mottram*[1]*;*
[1] *University of York, UK*

To facilitate processes such as transcription and replication, eukaryotes have evolved factors able to disassemble nucleosomes so that they do not present obstructions on the chromatin. The conserved Type II AAA+ ATPases ATAD2/Abo1/Yta7 can remove nucleosomes from chromatin and perform such a function. Exemplified by Yta7 the H3 N-terminal tail is recognised by a crown of atypical bromodomains, before the homohexamer of the ATPase removes the H3 monomer from the nucleosome, resulting in its dissociation. *Leishmania* Bromodomain Factor 7 was identified as a potential homologue of these factors, based on its domain organisation. Alphafold modelling of the BDF7 bromodomain suggests it does not contain the canonical binding pocket to accommodate acetylated lysine residues. Live-cell imaging of mNeonGreen::BDF7 revealed it is a nuclear protein in *L. mexicana*. To explore BDF7 function a null mutant was generated in promastigote stage *L. mexicana* using the T7/Cas9 system. The null mutant strain exhibited normal growth in culture but lost viability when transitioned to amastigote differentiation conditions, a phenotype that was restored when an add-back BDF7 allele was integrated using the pRIB vector. The BDF7-null mutant could not establish productive infections in murine bone marrow-derived macrophages. RNA-seq was used to assess the BDF7-null strain under metacyclic enriching conditions and indicated a dysregulated transcriptome that may leave the cell unprepared the cell for infection. Many histone genes were differentially expressed as were protein components of the ribosome. XL-BioID was performed to identify proteins in spatial proximity to BDF7, using CRK9 as an organellar control; defining 82 proteins as significantly enriched. The GO ter describing these proteins indicate the enrichment of factors involved in the regulation of gene expression and factors also involved in ribosome biogenesis. Ongoing work seeks to identify the genomic loci, if any, at which BDF7 is enriched.

Poster 144 : Competition among variants is predictable and controls the antigenic variation dynamics of African trypanosomes

Presenter: **Dr Douglas O Escrivani** , *University of Dundee*

D O Escrivani[1]*; VS Scheidt*[2]*; M Tinti*[1]*; J Faria*[3]*; D Horn*[1]*;*
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African trypanosomes employ antigenic variation of variant surface glycoproteins (VSGs) to evade mammalian host immune responses, transcribing one telomeric VSG expression-site at a time, and exploiting a reservoir of (sub)telomeric VSG templates to switch the active VSG. It has been known for over fifty years that, *in vivo*, new VSGs emerge from a reservoir of (sub)telomeric VSG templates in a predictable order in *Trypanosoma brucei*, and differential activation frequencies are now known to contribute to the hierarchy. Switching of approximately 0.01% of dividing cells to many new VSGs, in the absence of post-switching competition, suggests that VSGs are deployed in a highly profligate manner, however. Here, we report that switched trypanosomes do indeed compete, in a highly predictable manner that is dependent upon the activated VSG. We induced VSG gene recombination and switching in *in vitro* culture using CRISPR-Cas9 nuclease to target the active VSG. VSG dynamics were subsequently assessed using RNA-seq, independent of host immune selection. Although trypanosomes activated VSGs from repressed expression-sites at relatively higher frequencies, cells that activated minichromosomal VSGs displayed a competitive advantage and came to dominate the population. Furthermore, the advantage correlated with VSG length. Differential growth was also associated with wider transcriptome differences, affecting transcripts involved in nucleolar function, translation, and energy metabolism. We conclude that both VSG template location and VSG length contribute to post-switching antigenic variation dynamics in African trypanosomes. Competition among variants likely prolongs immune evasion with a limited set of antigens.

Poster 145 : *Leishmania (Viannia) braziliensis* long non-coding RNAs are enrolled in parasite fitness and interact with proteins in a structure-dependent manner

Presenter: **Caroline Ricce Espada**, *Postdoctoral researcher, University of York*

CR Espada[1]*; RM Magalhães*[1]*; JC Quilles*[1]*; L de Almeida*[1]*; TP Defina*[1]*; MJ Plevin*[2]*; PB Walrad*[2]*; C Anthon*[3]*; J Gorodkin*[3]*; AK Cruz*[1]*;*
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During its life cycle *Leishmania* parasites alternate between the phlebotomine vector and the mammalian host facing dramatic environmental changes which requires a rapid shift in gene expression to survive. Recently, the Cruz laboratory sequenced the transcriptomes of the three main life stages of *Leishmania braziliensis*, the main causative agent of tegumentary leishmaniasis in Brazil. These revealed differences not only in the expression of messenger RNAs but also of non-coding RNAs (ncRNAs). This observation raised the hypothesis that these ncRNAs could play a role in gene expression regulation in these different morphologies. To test this hypothesis, we selected 10 differentially expressed long (>200nt) presumably intergenic ncRNAs (lncRNAs) as targets. Using CRISPR/Cas9 we successfully generated knockout of 6 lncRNAs and tested their fitness in experiments mimicking key steps of the *Leishmania* life cycle. Four of these mutants presented significant differences in fitness compared to the parental line. For *ΔlncRNA45* and *ΔlncRNA66* a reduction in growth rates was observed for procyclic promastigotes in culture. For *ΔlncRNA52* a reduction in metacyclogenesis rates was observed. For *ΔlncRNA31* a reduction in axenic amastigotes duplication rate as well as a reduction in lesion sizes in BALB/c mice was observed. The existence and size of these four targets, precise start and end sequences as well as the presence or absence of Spliced Leader RNA (SL-RNA) and poly(A) tail were confirmed by northern blotting and circularization assays. While *lncRNA45*, *lncRNA66* and *lncRNA52* presented poly(A) tails of variable sizes, only *lncRNA52* presented the SL-RNA sequence at the 5' UTR. No poly(A) tail nor SL sequence were detected for *lncRNA31*. Mutations that can cause loss of secondary RNA structure were predicted *Echinococcus multilocularis* for *lncRNA45* and *lncRNA66* based on conservation. Pulldown assays using the aptamer S1m were performed using both the original and the mutated sequences of *lncRNA45*. These revealed distinct protein profiles interacting with WT vs mutant transcripts suggesting the secondary structure is essential for these lncRNA activity in *L. braziliensis*. We obtained the add back lines of these lncRNAs and they will be compared with knockout and parental fitness, to check if the parental phenotype is restored.
For *lncRNA45* and *lncRNA66* we also generated add back lines with mutated sequence to evaluate if the loss of secondary structure can impair the fitness recovery which would strengthen the hypothesis that these structures are essential for ncRNA activity. Our results show that lncRNAs exist and are implicit in the regulation of biological processes in *L. braziliensis* parasites.

Poster 146 : Structural basis for IL-33 recognition and its antagonism by the hookworm effector HpARI

Presenter: **Dr Abhishek Jamwal**, *Post Doctoral Fellow, University of Oxford*

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[1] *Department of Biochemistry, University of Oxford, Oxford, United Kingdom, UK;* [2] *Division of Cell Signalling and Immunology, University of Dundee, Dundee, UK*

Interleukin 33 (IL-33) plays a major role in inflammation, allergy, and host defence against parasitic hookworms. As a result, *Heligmosomoides polygyrus* (Hp) is armed with a potent effector called Alarmin Release Inhibitor (HpARI) which contributes to suppression of protective immune responses in its host by antagonizing IL-33. As a side effect, recombinant HpARI administration also reduces host asthma and allergy. Here we present the first crystal structure of HpARI bound to mouse IL-33. HpARI is a CCP domain containing protein and the structure reveals that that it contacts IL-33 primarily through second and third CCP modules. In particular, the binding site on IL33 occupied by a large loop from the third CCP domain of HpARI overlaps with the binding site for the IL-33 receptor, ST2. Therefore, this structure reveals how HpARI prevents the IL-33-ST2 interaction, reducing host innate defences. It also provides a structural framework for rational design of inhibitors against IL-33 for treating certain inflammatory conditions and diseases.

Poster 147 : Deep mutational resistance profiling of an anti-trypanosomal proteasome inhibitor

Presenter: **Simone Altmann**, *University of Dundee*

S Altmann[1]*; M Tinti*[1]*; M De Rycker*[1]*; M Thomas*[1]*; C Mendoza Martinez*[1]*; J Saini*[1]*; D Horn*[1]*;*
[1] *University of Dundee, UK*

We recently reported the development of oligo targeting for profiling drug resistance mutations in the parasitic trypanosomatids. This simple, and Cas9-independent, method allows for rapid and precise editing in otherwise wild type trypanosomatids. Improving our understanding of mutations associated with drug resistance is a priority given that several new anti-trypanosomal drugs, with known targets, are currently in clinical development. Accordingly, we have scaled-up oligo-targeting for deep mutational scanning and have applied the approach to the *Trypanosoma brucei* proteasome, using a promising proteasome inhibitor (EC_{50} 4 nM). Using cryo-EM structural data, we identified 20 proteasome β5 subunit residues within 5 Å of the drug-binding pocket. A set of codon-mismatched oligonucleotides was used for site saturation mutagenesis at these sites and to generate a pooled library of 1280 *T. brucei* mutants. Amplicon sequencing was used to validate library complexity and for codon variant scoring following drug selection, which revealed >100 distinct resistance conferring base-edits. The digital data was used to derive virtual dose-response curves for >45 distinct amino acid edits. We are assembling a panel of mutants for validation and are investigating structure-activity relationships using cryo-EM structural data and computational modelling. We hope to provide unprecedented insights into proteasome-inhibitor interactions, to facilitate assessment of resistance potential, and to improve prospects for future drug design.

Poster 148 : Safety and preliminary protective efficacy of immunisation with genetically attenuated Pf mei2 (GA2) malaria parasites in healthy Dutch volunteers

Presenter: **Olivia Lamers**, *Promovendus, Leiden University Medical Center*

OA Lamers[1]*; JP Koopman*[1]*; GV Roozen*[1]*; JJ Janse*[1]*; SV Chevalley-Maurel, S.C.*[1]*; FJ Geurten*[1]*; HM de Bes-Roeleveld*[1]*; ED Colstrup*[1]*; E Wessels*[1]*; GJ van Gemert*[2]*; M van de Vegte-Bolmer*[2]*; W Graumans*[2]*; TR Stoter*[2]*; EL Houlder*[1]*; RA Murugan*[1]*; CJ Janse*[1]*; BM Franke-Fayard*[1]*; MB McCall*[2]*; M Roestenberg*[1]*;*
[1] *Leiden University Medical Centre, Netherlands;* [2] *Radboudumc, Netherlands*

Introduction: Given the recent resurgence of malaria, an effective vaccine is needed now more than ever. The only vaccine candidates that have achieved >90% protection in clinical trials consist of live attenuated sporozoites. Attenuation can be achieved by radiation, chemoprophylaxis or genetic modification. Irradiated parasites cause few side effects and abort development 1-2 days after hepatocyte invasion, yet have a lower potency than chemo-attenuated parasites. Depending on the pharmacological intervention, chemo-attenuated parasites can persist longer in the host, which may improve immunogenicity. The advantage of genetic attenuation is that parasites can be engineered to arrest at a desired timepoint, combining biosafety with increased potency. We previously tested an early-arresting (EA) genetically attenuated *Plasmodium falciparum (Pf)* parasite (GAP) that interrupts development 2-3 days into the liver stage. While the EA-GAP

GA1 (*PfNF54 slarp b9*) was safe and well-tolerated, its protective efficacy in malaria-naïve Dutch participants was lower than desired. We therefore created GA2 (*PfNF54 mei2*), a late-arresting (LA) GAP that aborts development just before the end of the liver stage (after 6-7 days). We hypothesized that by prolonging exposure and broadening the variety of antigens presented to the immune system, immunogenicity and therefore protective efficacy would be increased.

Methods: We conducted a phase 1/2a partially double-blind placebo-controlled clinical trial, assessing the safety, tolerability and preliminary protective efficacy of GA2. Forty-three healthy male and female, non-pregnant, malaria-naïve volunteers, aged 18-35, participated in the study. The primary endpoints were the frequency and severity of adverse events (AE) and parasitaemia, assessed by 18s qPCR. Stage A was a dose-escalation study: participants were exposed once to 15 or 50 GA2-infected mosquito bites (MB). In stage B, GA2 was compared to its predecessor GA1 and a placebo. Participants were exposed to 50 GA2-, GA1-infected or uninfected MB, three times at 28-day intervals. Three weeks later, all participants underwent controlled human malaria infection (CHMI) by means of 5 MB infected with the unattenuated homologous *Pf* isolate (3D7).

Results: The GA2 parasite was found to be safe and well-tolerated and the frequency and severity of AEs was low and comparable across groups. No breakthrough infections occurred following GA2-administration at any dose. Immunisation with GA2 resulted in 89% protection against homologous CHMI: 8 out of 9 participants remained qPCR negative until the end of the trial. GA1 immunisation achieved a protective efficacy of 13%: 7 out of 8 participants developed parasitaemia following CHMI. Immunisation with uninfected MB offered no protection. GA2- and GA1-immunised participants exhibited a significant increase in anti-*Pf* circumsporozoite protein

Poster 149* : Reticulocyte Binding-like Proteins as potential vaccine targets for *Plasmodium knowlesi* and *Plasmodium vivax*

Presenter: **Sophia DonVito**, *PhD Candidate, London School of Hygiene and Tropical Medicine*

SM DonVito[1]*; M Hart*[2]*; RM Moon*[1]*;*

[1] *London School of Hygiene and Tropical Medicine, UK;* [2] *The Royal Veterinary College, UK*

Plasmodium vivax is the most widespread species of malaria in humans globally and is increasingly understood to cause clinical disease in endemic areas of Southeast Asia and South America. However, vaccine development is hindered by the lack of a long-term culture system. The closely related zoonotic parasite, *Plasmodium knowlesi*, has been adapted to culture in human erythrocytes, offering an *in vitro* model which shares many common invasion pathways with *P. vivax.* High efficiency CRISPR/Cas9 genome editing in this system allows us to study *P. vivax* blood-stage vaccine targets via orthologue replacement, as well as improving understanding of *P. knowlesi* invasion biology in its own right. Previous work in this system demonstrated sequential roles for two essential *P. knowlesi* invasion proteins, with normocyte binding protein Xa (NBPXa) required for erythrocyte deformation followed by Duffy binding protein α (PkDBPα) binding DARC to trigger a Ca^{2+} flux and merozoite reorientation. These proteins are part of larger families essential to invasion shared across *Plasmodium spp.*: the reticulocyte binding-like/reticulocyte binding homologous proteins (RBLs/RHs, including NBPXa), and the Duffy binding/erythrocyte binding-like proteins (EBLs, including PkDBPα). *P. vivax* is believed to follow a similar stepwise invasion process, using a repertoire of RBLs and the homologous PvDBP. Both parasite species lack functional redundancy for DBP-DARC binding in human infection, so PvDBP is a prime vaccine target. However, increasing reports of *P. vivax* infections in $Duffy^{neg}$ individuals raise concerns around monovalent DBP-based vaccines, so other essential blood-stage antigens must be urgently explored for use alongside (or instead of) DBP. In this work, we demonstrate that NBPXa-targeting antibodies inhibit *P. knowlesi* growth in culture, and combined inhibition with DBP shows potential synergistic activity. Additionally, we have generated a transgenic *P. knowlesi* line which enables conditional NBPXa complementation. This allows us to examine the role of gene copy number on invasion efficacy, how polymorphis affect antibody mediated inhibition, and the role of the various *P. vivax* RBLs. For example, the mechanism of reticulocyte restriction in *P. vivax* has not been confirmed, but PvRBP2b and 2a have each been highlighted as potential mediators. This transgenic line could help to study these proteins during invasion and identify specific PvRBLs to prioritise for combined vaccine approaches.

Poster 150* : Preclinical evaluation of a novel nucleoside analogue for the treatment of animal trypanosomiasis

Presenter: **Kayhan Ilbeigi**, *PhD Student, University of Antwerp*

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[1] University of Antwerp, Belgium; [2] Institute of Tropical Medicine Antwerp, Belgium; [3] Sciensano, Belgium; [4] Janssen Pharmaceutical Companies of Johnson & Johnson, Belgium; [5] University of Glasgow, UK; [6] Ghent University, Belgium

Animal trypanosomiasis (AT) is a widespread disease caused by *Trypanosoma spp.* and has a devastating effect on animal husbandry all over the world due to the scarcity of efficient drugs and development of drug resistance, hence emphasizing the need for novel treatment options. Following previous identification of 3'-deoxytubercidin as a highly potent trypanocide with curative activity in mouse models of both stage-1 and stage-2 Human African Trypanosomiasis (HAT), we now present a comprehensive preclinical evaluation of new 6-amino substituted tubercidin analogues with promising activity against a broad range of AT species. Potent hits were identified *in vitro* across all important AT species, *i.e. T. b. brucei*, sensitive and isometamidium (ISM)-resistant *T. congolense*, *T. vivax*, *T. evansi* (type A and B) and *T. equiperdum*. Selected 'hits' were further tested for *in vitro* metabolic stability (using bovine, horse and piglet liver microsomes), *in vivo* mouse models for each AT species, genotoxicity assays and mode-of-action studies (*i.e.* genome-wide RNA interference library screening, metabolomics). Analogue **3** was highly active in *T. vivax*, *T. congolense*, *T. equiperdum*, *T. evansi* and *T. brucei* curative mouse models. Furthermore, there was no indication of *in vitro* genotoxicity as confirmed by Vitotox®, the micronucleus and the comet assays. Mode-of-action studies for **3** revealed that the P1 nucleoside transporter and adenosine kinase are involved in drug uptake and activation, respectively. Given the preferred target product profile for a broad-spectrum drug against AT, analogue **3** represents a promising 'lead' candidate for treatment of animal trypanosomiasis, regardless of the causative species.

Poster 152 : Molecular genetic profiling of freshwater snails and schistosomes in Malawi

Presenter: **Mohammad Alharbi**, *LSTM*

M Al-Harbi[1]*;*

[1] LSTM, UK

My research project implemented molecular methods of snail and schistosome identification in a novel attempt to better understand schistosomiasis transmission in Malawi. I investigated several factors that each may play important roles within and the country settings. We conducted three malacological surveys measuring key environmental variables, mapping snail and parasite populations and thereafter using molecular characterisation methods, for example, by PCR-RFLP, DNA sequencing, microsatellite markers and phylogenetic tree construction methods.

Novel snail distribution maps of various planorbid snail species, with focus upon *Biomphalaria* and *Bulinus*, were presented. We also noted the presence of *Lymnaea*, conducting basic DNA typing. Most importantly, the spread of *Biomphalaria pfeifferi*, a keystone intermediate host for *Schistosoma mansoni*, between 2017 and 2019, was confirmed. Assembled molecular evidence pointed towards a recent populational founder effect for this species as it exhibited low genetic diversity at all loci inspected. By real-time PCR, the prevelance of *S. mansoni* within *Bi. pfeifferi* was 18% and underpins the newly appreciated environmental risk of intestinal schistosomiasis transmission within Lake Malawi.

Similarly, DNA evidence confirmed the presence of *Bulinus globosus* but newly reported the presence of *Bulinus africanus* and two, as of yet, poorly known species within the *Bu. africanus* species group. These can now be better recognised and more easily detected with a PCR-RFLP assay of the *cox*1 using double digestion with HaeIII and SacI restriction enzymes. By real-time PCR the prevalence of *Schistosoma haematobium* within snails was 31 %. Furthermore, characterisation of available miracidia, using a combination of mitochondrial and nuclear loci, from locally infected children identified the presence of novel schistosome hybrids of *S. haematobium- mattheei* as well as characterisd group IV *S. mansoni*.

The Information we presented is of direct use to national schistosomiasis control planning by providing up-to-date information on snail and schistosomes distributions in Malawi. Moreover, updated info provide help to clarify previously cryptic aspects within the snail-schistosome relationship.

Poster 153* : The protein Kinase Ataxia-Telangiectasia and RAD3-related (ATR) is an important player to guarantee the genome integrity in *Leishmania* major

Presenter: **Dr Gabriel Lamak Almeida da Silva**, *University of Glasgow*

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[1] University of Glasgow, UK; [2] University of Sao Paulo, UK

The protein kinase Ataxia-Telangiectasia and Rad3-related (ATR) is a master regulator of the eukaryotic response to DNA injuries that is activated in response to the accumulation of single stranded DNA (ssDNA) and orchestrates checkpoint activation, cell cycle arrest, replication fork stabilization/restart, control of origin firing and telomeric stabilization providing genome maintenance and stability. However, little is known about ATR kinase functions in an organism with a remarkable plastic genome such as *Leishmania*. Using CRISPR/Cas9 editing tool we were able to generate cells expressing a N'terminal tagging (^{myc}ATR) that reveals the presence of the kinase at nuclear and kinetoplast. The deletion of ATR C'terminal region (^{myc}ATR$^{\Delta C}$), where the kinase domains are predicted, see to affect the protein location, expression and/or stability. Those mutant ATR cells showed to be sensitive to replication stress: accumulating ssDNA, DNA damage markers (γH2A), and a disrupted cell cycle. The Marker Frequency Analysis (MFA-seq) showed that in ^{myc}ATR$^{\Delta C}$ the replication activation on the main origin is not affected with or without replication stress. However, under stress, those cells showed a significantly decrease of sub-telomeric replication signal. Those results suggest that ATR is important to genome maintenance guarantying the proper replication process after stress.

Poster 157 : Parasite Street Science: Addressing Transmission of Trypanosomiasis Through Street Theatre

Presenter: **Hannah Bialic**, *Public Engagement Manager, Wellcome Centre for Integrative Parasitology*

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Human African Trypanosomiasis, or African sleeping sickness, is caused by the parasite *Trypanosoma brucei* which is transmitted by the Tsetse fly. Public engagement about this disease and education regarding the methods of control and elimination of the insect vector has proved lacking in endemic regions. Mistrust in research in sub-Saharan African regions also poses a barrier to intervention methods. This mistrust can often be traced to the ebola outbreaks of the past decade and can lead to a hostile environment. Beliefs, fears, and unsubstantiated rumours such that healthcare workers and researchers were responsible for disease spread, have resulted in the injury and death of scientific and medical field workers. Trust in scientists is crucial in working toward the World Health Organization's target of eliminating sleeping sickness transmission by 2030. Our project, Parasite Street Science, has addressed these barriers through a unique method of public engagement: street theatre. The project provides a model for stirring the public's interest in science that is readily transportable and accessible across geographical and language boundaries. The performance was developed by a diverse team of Malawian and Scottish scientists, performers, and health officials, and was performed in Glasgow and Malawi at football matches, which has brought an important educational component to underserved communities but also demonstrates the value of and enhances the trust in, scientific research. This unique engagement project, and the methodology behind its development, have provided a platform for future artistic and theatrical endeavours that can be adapted to other vactor driven diseases – such as malaria and leishmaniasis. Our process and outcomes now serve as a toolkit to support the development of future researcher/artist collaborations for future interactive performances, resulting in a strong legacy for the project for years to come.

Poster 158 : Prevalence of Intestinal Parasites and associated risk factors among Inflammatory Bowel Disease suspected patients in Tigray Regional State, Northern Ethiopia

Presenter: **Merhawi Alemu Birhanu**, *Lecturer, and Academic Researcher, Adigrat University*

M Birhanu[1]*;*
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Background: Intestinal parasite infections are important public health concerns globally. Besides, some Intestinal parasite infections aggravate symptoms, have a clinical similarity, and considered differential diagnosis with Inflammatory Bowel Disease. So, the prevalence of intestinal parasites and associated risk factors among Inflammatory Bowel Disease suspected patients were determined.

Objective: To determine the prevalence of intestinal parasites and associated risk factors among Inflammatory Bowel Disease suspected patients attending in Ayder Comprehensive Specialized Hospital, and Hiwot Private Clinic, Mekelle, Tigray, Ethiopia, 2020.

Methods: A cross-sectional study was conducted among individuals who were Inflammatory Bowel Disease suspected patients in the Ayder Comprehensive Specialized Hospital, and Hiwot Private Clinic from February 01, 2019 to July 30, 2020. Descriptive statistics were computed to summarize data and result was presented using tables. Association between different variables with outcome was analyzed using Bivariate and multivariate logistic regressions. The p-value less than 0.05 were considered as statistically significant.

Results: A total of 297 Inflammatory Bowel Disease suspected patients were included. Of these, 54.9% were males. The overall prevalence of intestinal parasites was 127 (42.76%). *Entamoeba histolytica/dispar* 76 (25.58%), and *Giaardia lamblia* 32 (10.77%) were the most predominantly identified parasites. Participants with untrimmed fingernail (AOR =2.4 95% CI =1.3-4.3, *p* =0.002), eating unwashed vegetables (AOR=2.3, 95%, CI: 1.2-4.3, *p* =0.011), and family size of greater than five (AOR=1.7, 95% CI= 1.029-2.881, *p* = 0.039) were found to be independent predictors of intestinal parasites.

Conclusion: Overall prevalence of intestinal parasites infection was 127 (42.76%). *Entamoeba histolytica/dispar* was the most prevalent parasites. Family size of greater than five, eating unwashed vegetables, and untrimmed fingernail were found to be statistically associated with infection of intestinal parasites. Therefore, health care providers should screen and treat Inflammatory Bowel Disease suspected patients for intestinal parasites in order to ensure good diagnosis, and treatment.

Keywords: Crohn's disease, Inflammatory Bowel Disease, Intestinal parasite infections, and Ulcerative colitis, Mekelle, Ethiopia.

Poster 159 : African trypanosomes secrete a cocktail of VSG family antigens

Presenter: **Gustavo Bravo Ruiz**, *University of Dundee*

G Bravo Ruiz[1]*; M Tinti*[1]*; D Horn*[1]*;*
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Variant surface glycoproteins (VSGs) coat bloodstream-form African trypanosomes and underpin antigenic variation and immune evasion. For this, trypanosomes have a vast repertoire of sub-telomeric *VSG* genes, and they periodically switch the expressed, and RNA polymerase I transcribed, telomeric *VSG*. However, there is also a subset of genes, belonging to the wider *VSG* family, with distinct roles, in transferrin uptake and innate immunity, for example. A bioinformatics assessment revealed >50 chromosome-internally located *VSG* family genes in *Trypanosoma brucei,* typically located at the sites where polycistronic transcription converges. We also observed a similar distribution of VSG family genes in human-infective *T. b. gambiense.* Using transcriptomics and polymerase-selective RNA-seq, we confirmed that *VSG* family genes are transcribed by RNA polymerase II (pol-II). Quantitative proteomics revealed that VSG family proteins are typically expressed in the bloodstream stage, but not in the insect mid-gut stage. Although predicted to be structurally similar to VSGs, typically incorporating signal peptides, many VSG family proteins appear to lack GPI-anchor signals, suggesting that they may be secreted. Secretome data derived using quantitative (direct data-independent acquisition) proteomics revealed that many VSG family proteins are indeed secreted, along with many digestive enzymes. We suggest that bloodstream-stage specific secretion of a cocktail of VSG family antigens plays a role in modulating the host immune response. We are now incorporating signal-peptide compatible epitope-tags to further monitor the fate of a VSG and several VSG family proteins following secretion.

Poster 161 : Stars and Drugs, a classic combination: Stellate Amoebae vs Killer compounds

Presenter: **Richard Childs Hunt**, *PhD Student, London School of hygiene and tropical medicine*

R Childs Hunt[1]*; R Mooney*[2]*; CJ Sutherland*[1]*; FL Henriquez-Mui*[2]*; D Nolder*[1]*;*

[1] *London School of Hygiene and Tropical Medicine, UK;* [2] *University of the West of Scotland, UK*

Acanthamoeba is a genus of free-living amoebae that opportunistically infect humans and can cause several diseases, most common of which is, the sight threatening *Acanthamoeba* Keratitis (AK).

In AK, the cornea is invaded by *Acanthamoebae* and unless quickly treated, a slow, painful destruction of the corneal stroma follows, as the amoebae use it for a food source. AK is often diagnosed late, and is refractory to treatment due to the chemically resistant cyst stage of *Acanthamoeba* and the challenging nature of drug bioavailability in the corneal environment.

The cure rate of the most effective treatment (PHMB 0.02%) is poor (84% after a year of treatment) and average resolution time is very long at an average of 5 months (Papa *et al.* 2020) and in the majority of cases there is some degree of sight loss. Treatment outcome is highly variable, and the treatment often results in adverse effects that can result in treatment halts. The need for improved therapeutics is clear.

Several different isolates of *Acanthamoeba* with different genotypes and/or presenting different morphotypes were exposed to a panel of currently used and experimental therapeutic compounds, to investigate the therapeutic response of genetically and morphologically distinct *Acanthamoebae*. The amoebae tested include clinical isolates from AK cases sent to the Diagnostic Parasitology Laboratory at LSHTM and Type-culture collection cultures from ATCC and CCAP.

Poster 162 : Life cycle stages and molecular phylogeny of *Hepatozoon fitzsimonsi* (Dias 1953) (Adeleorina: Hepatozoidae) in tortoises *Stigmochelys pardalis* (Cryptodira: Testudinidae) and ticks of the genus *Amblyomma* (Acari: Ixodidae) from South Africa

Presenter: **Prof Courtney Cook**, *Associate professor, North-West University*

LS Mofokeng[1]*; NJ Smit*[1]*; CA Cook*[1]*;*

[1] *Water Research Group, Unit for Environmental Sciences and Management, North-West University, Potchefstroom, South Africa*

Haemogregarines (Apicomplexa: Adeleorina) are commonly encountered haemoprotozoan parasites of reptiles, of which, the genus *Hepatozoon* appears to be the most prevalent. Species display a heteroxenous life cycle, requiring a vertebrate and invertebrate host. Based on relationships of haemogregarine genera inferred using the 18S rRNA gene, the genus *Hepatozoon* remains paraphyletic, which lead to a new genus being erected *Bartazoon* Karadjian et al., 2015, with solely haematophagous insects acting as vectors. *Hepatozoon fitzsimonsi* was one of the species proposed to be a member of *Bartazoon*. However, past research done on *H. fitzsimonsi* observed a close association with tortoises and ticks, observing what appeared to be sporogonic stages in ticks collected from tortoises. Recently, two molecular screening studies, identified the presence of *H. fitzsimonsi* in ticks from tortoises in Kenya and South Africa. These findings in mind, the present study aimed to revisit the potential of ticks as vectors for *H. fitzsimonsi* in tortoises, by (i) collecting blood/tissue and ticks from tortoises, (ii) screening both microscopically for the presence of blood, merogonic, and sporogonic stages respectively, and (iii) molecularly characterising these stages using fragments of the 18S rRNA gene to determine if they are that of *H. fitzsimonsi*.

A total of 14 tortoises were collected, including nine individuals of *Kinixys* spp. and five *Stigmochelys pardalis*. Ten of the 14 (71%) tortoises were infested with ticks belonging to three species of *Amblyomma*. As *Kinixys* spp. are known to harbour both *H. fitzsimonsi* and *Hemolivia parvula* concurrently, three of the *S. pardalis* were selected, two of these showing a peripheral blood infection with *H. fitzsimonsi*. Impression slides from ticks showed sporogonic stages within the haemocoel, molecularly comparing to *H. fitzsimonsi*. Furthermore, merogonic stages were observed in the liver of one *S. pardalis* infected with *H. fitzsimonsi*.

The present study thus provides further support for ticks acting as the vectors of *H. fitzsimonsi* based on observation of its developmental stages in tortoises as well as the invertebrate host (*Amblyomma* spp.), with these stages linked molecularly. This would be the second haemogregarine of tortoises for which ticks are the vector, *Hemolivia mauritanica* being the first, and the first species of *Hepatozoon* infecting chelonians for

which ticks have been identified as a definitive host. This will hopefully encourage further research into chelonian *Hepatozoon*, a research area that remains largely neglected at present.

Poster 163 : A systematic screen of extracellular host:parasite protein interactions to identify new immunomodulatory pathways

Presenter: **Dr Cecile Crosnier**, *Research Fellow, Department of Biology, University of York*

*K Lee[1]; N Muller-Sienerth[2]; J Shilts[2]; GJ Wright[3]; **C Crosnier**[3];*
[1] Department of Biology, University of York, UK; [2] Wellcome Sanger Institute, UK; [3] YBRI and Department of Biology, University of York, UK

To establish chronic infections, parasites must develop efficient strategies to escape destruction by their host's immune system. One of these evasion mechanisms is to trigger signalling through cell surface and secreted proteins to subvert the host immune response. Identifying the parasite proteins generating these modulatory signals and the host immune receptors with which they interact could not only provide new targets for vaccine development against parasitic diseases, but also new therapeutic leads in the control of allergic and auto-immune conditions. To identify new immunomodulatory pathways, we have recently performed a systematic protein:protein interaction study between a library of 750 human surface receptors representing the membrane-tethered repertoire of leukocytes, and extracellular protein libraries from two major parasites able to establish chronic infection, *Schistosoma mansoni* and *Plasmodium falciparum*. New host:parasite interactions were identified, which are currently being characterised biochemically and functionally. These resources provide a new platform to identify extracellular host:parasite interactions.

Poster 164 : Hybridization in UroGenital Schistosomiasis (HUGS): A novel real-time PCR assay, with high resolution melt profiling, useful for the detection of hybrid schistosomes in Malawi

Presenter: **Dr Lucas Cunningham**, *Liverpool School of Tropical Medicine*

***LJ Cunningham**[1]; SA Kayuni[2]; P Makaula[2]; B mainga[2]; G Namacha[2]; D Lally[2]; D Kapira[2]; P Chammudzi[32]; S Jones[1]; S Rollason[1]; AL Reed[3]; J Archer[1]; A Juhasz[1]; EJ Lacourse[1]; J Musaya[2]; JR Stothard[1];*
[1] Liverpool School of Tropical Medicine, UK; [2] Malawi Liverpool Wellcome Trust Programme of Clinical Tropical Research, UK; [3] Lancaster University, UK

Introduction: The ability of schistosomes to form inter-species mating pairs with resultant hybrid or introgressed offspring has been previously described. Such studies have relied upon sequence analysis of nuclear genome (nDNA) and/or the mitochondrial genome (mDNA) loci. Although highly accurate, sequence analysis of large numbers of targeted specimens is prohibitive, being both time-consuming and overtly costly. We have developed a rapid, low-cost two-tube real-time (rt)PCR multiplex assays, with high resolution melt profiling, screening both mDNA and nDNA loci. We have conducted a large-scale examination of recently collected material from two communities in Nsanje and Mangochi Districts.

Methods: Species-specific primers producing unique high-resolution melt-peaks were designed for mDNA gene targets (tRNA Lys, ND4 and ND6) for six schistosome species (*S. mattheei, S. currassoni, S. bovis, S. haematobium, S. mansoni* and *S. margrebowei*). To compliment the mDNA qPCR, a single-plex nDNA rtPCR targeting a 168bp variable region of the ITS2 gene was also developed. Comparison of the specific melt-peaks produced by each assay can be used to distinguish individuals of different species and determine if they have any mixed species parental signatures, especially maternal mitochondrial carriage.

Preliminary results: A combined total of 1,012 urine filter samples obtained from ~2,400 people were screened using the mDNA and nDNA qPCR assays. This resulted in the identification of 77 putative mixed infections and/or hybrid cases, equating to an overall prevalence of 7.3%. Furthermore, our assays identified gross under-reporting of mixed species infections with ectopic *S. mansoni* (n=95) versus microscopy (n=6), including 18 individuals with markers for triple-species infections.

Conclusion: With new assays, our ability to screen natural populations of schistosomes for introgressed for expands. Our study indicates the presence of various hybrid schistosomes capable of infecting local communities of endemic areas of Malawi in sub-Saharan Africa. Further analysis of individual eggs on FTA cards is ongoing to clarify the distinctions between mixed infections versus introgressed genotypes. We also aim to complement these qPCR assays with development of discriminatory sex-specific loci to better interrogate the directional basis of hybridisation between male (ZW) and female (ZZ) genders.

Poster 165 : Assessing the potential of nucleoside transporters to deliver drugs against the various *Trypanosoma* species responsible for African animal trypanosomiasis

Presenter: **Prof Harry De Koning**, *University of Glasgow*

*MA Ungogo[1]; M Aldfer[2]; M Natto[1]; K Ilbeigi[3]; JI Asseri[1]; G Caljon[3]; R Burchmore[1]; S Van Calenbergh[4]; **HP De Koning**[1];*
[1] University of Glasgow, UK; [2] Institute of Infection, Immunity and Inflammation, University of Glasgow, United Kingdom, UK; [3] University of Antwerp, Belgium, UK; [4] Ghent University, Belgium

Animal African trypanosomiasis is a complex disease of many different domestic animals, multiple trypanosome species and multiple modes of transmission, as well as vast geographical areas from African and the Middle East to South America and Asia. To control this disease, there is only a handful of old drugs. However, not only has resistance been reported to these drugs, there is an innate difference in sensitivity to them in the various species, in part linked to differences in transporters such as TbAT1/P2 and TbAQP2, which are absent from the *T. congolense* and *T. vivax* genomes but responsible for the uptake of diamidine and melaminophenyl arsenical drugs in *brucei*-group trypanosomes. Yet, at point of care, it is usually not known which (single or multiple) species of trypanosome have infected the animal, often leading to sub-optimal treatment. A single treatment active against all relevant *Trypanosoma* species and tolerated by the various hosts, would be a genuine breakthrough. Our groups have been working towards a nucleoside drug treatment with that desirable profile. One potential bottleneck would differences in nucleoside transporters, which could cause some nucleosides to be excluded from specific species, making them insensitive? We have thus developed a bespoke *Leishmania mexicana* cell line ('SUPKO') from which the NT1.1/NT1.2 locus and the NT2 gene were deleted, creating a null background for the heterologous expression of *Trypanosoma* nucleoside transporters. We have used this system to express *T. congolense* and *T. vivax* transporters of the ENT family and found that TcoAT1 and TvxNT3 are broad specificity nucleoside transporters and that their expression sensitises this *Leishmania* cell line to specific nucleoside analogues. Detailed characterisation using [^{3}H]-adenosine showed these carriers are high affinity, with Km values of 0.42 and 1.41 µM for TcoAT1 and TvxNT3, respectively. The interactions these transporters make with adenosine showed close similarity to the those of the *T. brucei* P1 transporters and we identified 'allowed' modifications that did not impede uptake through any of these carriers.

Poster 166 : Developmental differentiation of trypanosomatid parasites into biofilm colonies in honeybees: main morphological changes and functional gains *in vitro* and *in vivo*

Presenter: **Dr Luis Miguel de Pablos Torró**, *Senior Lecturer, Universidad de Granada*

*J Carreira de Paula[1]; T Gómez Moracho[1]; P García Olmedo[1]; M Buendía Abad[2]; M Higes[2]; A Osuna[1]; **LM de Pablos Torró**[1];*
[1] University of Granada, Spain; [2] CIAPA IRIAF, Spain

Biofil are defined as a structured community of microbial cells firmly attached to a surface and embedded in a matrix composed of Extracellular Polymeric Substances (EPSs). EPSs are composed by polysaccharides, nucleic acids, proteins, lipids and other biomolecules. This lifestyle allows cells to survive in hostile environments, but also to colonize new niches by dispersal of microorganis from the microbial clusters. In this work, we demonstrated that the honeybee-infective trypanosomatid parasite *Lotmaria passim* promastigote for are capable to secrete EPSs and differentiate into sessile biofilm colonies composed by surface-attached haptomonad parasite forms. This developmental differentiation is shown in experimental infections of honeybees and is also shown in vitro. Microscopic analysis showed that EPSs are structured in long fibers formed by monomeric structures of spherulites and extracellular vesicles over the surface of haptomonad cells. Proteomic comparison with dixenous trypanosomatid parasites showed that 58 out of 244 proteins are specific to *L. passim* EPSs with several being related with biofilm formation in

prokaryotic organisms. Moreover, and in comparison to dixenous trypanosomatid parasites such as *Trypanosoma cruzi* or *Leishmania major*, we demonstrated that EPSs are necessary and partially sufficient to provide and increased resistance to osmotic and temperature stresses in *L. passim*. This results demonstrate that EPS and biofilm formation are necessary for survival, resilience and dispersal of monoxenous trypanosomatid parasites in nature.

Poster 167 : High throughput single-cell genome sequencing gives insights into the generation and evolution of mosaic aneuploidy in *Leishmania donovani*

Presenter: **Dr Malgorzata Anna Domagalska**, *Institute of Tropical Medicine*

M Domagalska[1]*; G Negreira*[1]*; P Monsieurs*[1]*; JC Dujardin*[1]*;*
[1] *Institute of Tropical Medicine, Antwerp, Belgium*

Aneuploidy is a ubiquitous feature of *Leishmania* and represents an adaptive mechanism, modulating gene expression and possibly impacting phenotypes. Aneuploidy may vary within single parasites in clonal populations, a phenomenon termed mosaic aneuploidy (MA), with important evolutionary and functional implications which remains under-explored. In previous work (1), we applied and validated a high throughput single-cell genome sequencing (SCGS) method to study for the first time the extent and dynamics of whole karyotype heterogeneity in two *Leishmania donovani* clonal populations representing different stages of MA evolution in vitro. We found that drastic changes in karyotypes quickly emerge in a population stemming from an almost euploid (except for chr 31) founder cell -here further called euploid-. This possibly involves polyploidization/hybridization at an early stage of population expansion, followed by assorted ploidy reduction. During further stages of expansion, MA increases by moderate and gradual karyotypic alterations, affecting a defined subset of chromosomes. To gain insights into the first molecular events during MA emergence and identify potential drivers, we set up new series of experiments (unpublished): we derived 20 subclones (euploid or highly aneuploid) from one *L. donovani* strain and characterized the independent evolution of 8 of those clones over 10 passages *in vitro* (~70 generations). With longitudinal bulk genome sequencing, we revealed a process of convergent evolution where subclones starting with different founder karyotypes developed similar aneuploidy profiles in short time periods (4-5 passages). Moreover, a longitudinal SCGS monitoring of one of these euploid subclones revealed that mosaic aneuploidy is already detectable – to a minor extent – at stages as early as 21 generations after cloning, with ~21 karyotypes identified between 720 cells. At this stage, 95% of the sequenced parasites still had the euploid karyotype. However, at generation 36, different sub-populations were identified with distinct highly aneuploidy karyotypes, with one of these subpopulations further outgrowing the group of parasites with the euploid profile at generation 46, suggesting a fitness gain in this highly aneuploidy group. The longitudinal SCGS also revealed that the rate at chromosome copy number change increase exponentially over time, suggesting that maintenance in culture is accompanied by increase in genome instability, promoting higher karyotype heterogeneity in fewer generations. Lastly, a longitudinal single-cell RNA sequencing of this same clone revealed candidate genes that might be involved in this increased genome instability. These results indicate that the mechanisms governing mosaic aneuploidy in *Leishmania* can be differentially modulated to increase or constrain the diversification of karyotypes and provide a first glance at which molecular factors can act in this regulation.

Reference 1. Negreira GH, Mon

Poster 169* : Detection of *Echinococcus multilocularis* metacestodes of Asian origin in human samples from Warmia-Masuria (north-eastern Poland)

Presenter: **Pawe G Adysz**, *Medical University of Gdańsk*

P G Adysz[1]; A Lass[1];
[1] Medical University of Gdansk, Poland

Recently reported presence of Asian-genotype *Echinococcus multilocularis* tapeworms in Eastern European red foxes prompted the question of metacestode descent in the human population. In this study, a Maximum Likelihood tree based on partial sequences of *E. multilocularis* mitochondrial genes *cox1*, *cob* and *nad2* coupled with hierarchical clustering analysis of EmsB microsatellite profiles revealed

Asian ancestry in two samples from alveolar echinococcosis patients living in Warmia-Masuria, north-eastern Poland, implying that the increasing red fox synanthropy facilitates infiltration of genotypes to date associated only with the sylvatic cycle into the human population.

Poster 170 : Prevalence of *Leptospira spp.* among sylvatic rodents in NE Poland

Presenter: **Aleksander Goll**, *Student, Medical University of Gdansk*

A Goll[1]*; M Grzybek*[1]*; M Krupińska*[1]*; J Nowicka*[1]*;*
[1] *Medical University of Gdańsk, Poland*

This study aimed to analyse the prevalence of *Leptospira* in sylvatic rodents in northeastern Poland. Animals were trapped in Masurian Lakes District between 2020 and 2022 using wooden live traps with seeds, peanut butter, and fruits as a lure. Trapped rodents belonging to *Apodemus*, *Myodes*, *Microtus* and *Mus* species, were culled with overexposure to anaesthetics and a parasitological section was performed. Genomic DNA from rodent spleens was isolated and PCR was applied. We confirmed the presence of *Leptospira* DNA in 4 out of 305 rodents with 1,31% prevalence.

Our results confirm that rodents may take part in the circulation of Leptospira. Further biomonitoring is necessary to assess different intrinsic and extrinsic factors that might influence *Leptospira* prevalence in rodents. This will help to indicate and predict possible places of outbreaks that can lead to severe infections in humans, livestock and companion animals.

Poster 171* :Assessing recombination in *Trypanosoma cruzi* from Colombian Attalea pal using Genome-Wide Locus Sequencing Typing

Presenter: **Clara Gyhrs**, *University of Glasgow*

C Gyhrs[1]*; M Llewellyn*[2]*;*
[1] *University of Glasgow, UK;* [2] *Institute of Biodiversity, Animal Health and comparative Medicine, University of Glasgow, UK*

Chagas Disease is a systemic and chronic disease caused by infection with kinoplast *Trypanosoma cruzi*. *T. cruzi* is divided into 6 strains, and variation between these has been linked to variations in clinical presentations. With limited knowledge on diversity of certain DTUs, frequency of mixed infections, and hybrids in the wild, high resolution and low-cost genetic profiling methods are essential to understand disease associations and other important *T. cruzi* traits. Here we use a culture-free amplicon sequencing approach with a genome-wide locus sequence type panel to detect variation in four metapopulations of *Trypanosoma cruzi*, from triatomine vector *Rhodnius prolixus* in four *Attalea butyracea* pal in Colombia. All samples cluster with the diverse *T. cruzi* subpopulation, TcI, with evidence for gene flow between populations (F_{ST} = 0.065). We illustrate possible evidence for distinct mechanisms of reproduction to occur simultaneously in a metapopulation of *T. cruzi*.

Poster 172 : New insights into *Trichomonas* - Bacteria Interactions through comparative Genomics, Transcriptomics and Biochemistry

Presenter: **Adam Hart**, *PhD Student, Newcastle University*

AJ Hart[1]*; J Biboy*[1]*; J Gray*[1]*; W Vollmer*[1]*; RP Hirt*[1]*;*
[1] *Newcastle University, UK*

The *Trichomonas* genus represents a diverse group of parasitic protozoans which can infect a range of animal species (including birds and mammals) with a well-established zoonotic potential. Species include *Trichomonas vaginalis*, which is a Human STI, and *Trichomonas gallinae*, which infects birds, primarily Columbiformes.

They reside at mucosal surfaces of their host, which includes a complex microbiota. *Trichomonas* species have been described as able to damage host tissue and induce inflammatory host responses. Notably, infections of Human and birds by *Trichomonas* species are also

associated with changes in the microbiota taxonomic composition. In Humans, change associated *with T. vaginalis* infection of the female urogenital tract are considered to lead to a dysbiotic microbiota that can also contribute to disease states, which are characterised by excessive inflammation and increased susceptibility to other pathogens, such as HIV.

However, interactions between *Trichomonas* and the members of the microbiota are still poorly understood at the molecular and cellular level.

This work ai to gain new insights into *Trichomonas*-Bacterial interactions through integrating microbiological, biochemistry/enzymology, comparative genomic and transcriptomic approaches.

Using *Trichomonas gallinae* as a model we present evidence that *Trichomonas* species, including *T. vaginalis*, have acquired a repertoire of genes encoding enzymes capable of interacting with the bacterial cell wall which have their transcripts significantly modulated in the presence of the bacterial *Escherichia coli*.

These tools can potentially allow *Trichomonas* to out-compete their neighbouring bacteria and/ or liberate molecules that can promote *Trichomonas'* growth. These findings bring new insights into *Trichomonas*-Bacterial interactions and how these evolutionarily conserved interactions can potentially influence the zoonotic ability of *Trichomonas*.

Poster 173 : Non-natural myristate analogues: Synthesis and their potent, selective activity upon bloodstream *T. b. brucei*

Presenter: **Rachel Humann**, *St Andrews University*

R Humann[1]*;*

[1] *St Andrews University, UK*

Inadequate and antiqued drugs for treating sleeping sickness, a neglected tropical disease caused by the protozoan *Trypanosoma brucei* (*T. brucei*), remains a persistent problem across many developing countries. One chemotherapeutic target, the N-myristoyltransferase (NMT) has received significant attention in recent years and has been validated as drug target against *T. brucei;* ablating the NMT gene led to cell death and is thus essential for parasite survival. By synthesizing trypanocidal myristate analogues, it is hoped that these structural mimics will be taken up and utilised by *T. brucei* NMT, leading to the interference and disruption of their downstream metabolic pathways. Additionally, fatty acid elongation and repurposing is likely to be disrupted upon the treatment of non-natural analogues, potentially leading to toxic effects. This research mainly describes the chemical synthesis of myristate analogues based on the 14:0 fatty acid chain with some compounds showing EC_{50} values of <10 µM in the presence of 10 % foetal bovine serum (FBS) and significantly lower EC_{50} values in FBS depleted environments. Initial analysis of free fatty acid and phospholipid species via techniques has highlighted significant differences in the abundances of certain species. Tandem MS/ can be used to identify the specific changes in individual phospholipid species. This analysis is complemented by general non-specific metabolomics on whole cell samples to further identify biological pathways affected by these compounds. To directly determine the extent at which the NMT enzyme is affected or inhibited, TbNMT protein was expressed and purified to allow thermal shift assays and secondary peptide activity assays to be carried out. The synthesis and use of bi-functional probes for drug localisation studies is also explored in this research in the hopes of further elucidating drug localisation and thus potential target identities.

Poster 175* : Hybridization of urogenital schistosomiasis (HUGS) study in Malawi: Preliminary parasitological findings of the Baseline Human survey on *Schistosoma haematobium* hybrid infections in Nsanje and Mangochi districts.

Presenter: **Dr Sekeleghe Kayuni**, *Postdoc, HUGS project, Malawi-Liverpool-Wellcome Trust*

SA Kayuni[1]*; P Makaula*[1]*; G Namacha*[1]*; D Lally*[1]*; D Kapira*[1]*; P Chammudzi*[1]*; S Jones*[2]*; S Rollason*[2]*; AL Reed*[3]*; J Archer*[2]*; L Cunningham*[2]*; A Juhasz*[2]*; EJ Lacourse*[2]*; J Musaya*[1]*; JR Stothard*[2]*;*

[1] *Malawi-Liverpool-Wellcome Trust Clinical Research Programme, Malawi;* [2] *Liverpool School of Tropical Medicine, UK;* [3] *Lancaster University, UK*

Introduction: The discovery of novel *Schistosoma haematobium* hybrids co-infecting school-aged children in Nsanje and Mangochi districts in Malawi exposes critical knowledge gaps in the control of schistosomiasis. Hybridization in urogenital schistosomiasis (HUGS) is a longitudinal population study aimed at investigating transmission biology and epidemiological impact of *Schistosoma haematobium*-hybrids in Malawi.

A baseline survey was conducted to determine the prevalence of urogenital schistosomiasis in two communities of Nsanje and Mangochi districts and if the proportion of *S. haematobium* hybrid co-infections are uniform across the communities.

Methods: We conducted a baseline survey in July 2022. Households were selected randomly in 8 villages around Mthawira Primary school in Nsanje district and 3 villages around Samama Primary school in Mangochi district to attain a sample size of 2,400. Individual questionnaires were administered to enrolled participants, eliciting information on their health, education, socioeconomic status, water contact and available livestock.

Urine samples were collected to tests for schistosomiasis using point-of-care CCA and urine filtration. All positive individuals were treated with Praziquantel (PZQ), according to the Ministry of Health treatment guidelines in addition, all people in the 2 study communities were offered PZQ as part of preventive chemotherapy recommended by WHO.

Preliminary results: 348 participants (32.7%) had *S. haematobium* eggs in their urine samples in Nsanje district, with 617 (49.8%) in Mangochi district. 8.2% participants had high infection intensity (50+ *S. haematobium* eggs eggs) in Nsanje, while 16.4% were in Mangochi.

The high infection intensity in Nsanje was observed more in older children and adults, 7-15 and 26-36 years' age-group, as compared to younger children in Mangochi, thus 1-6 and 7-15 years' age-groups. 1.0% participants had positive POC-CCA in Nsanje indicative of possible intestinal *S. mansoni* co-infection, and 12.4% in Mangochi district.

Further data and molecular analyses to determine the level of the hybrid infections in the survey population are currently in progress and preliminary findings to be presented subsequently.

Conclusions: Preliminary results indicate significant burden of urogenital *S. haematobium* infections among survey participants in the 2 districts.

Further studies include follow-up surveys of hybrid co-infections in the study population and assessment for increased morbidity from genital schistosomiasis in hybrid co-infected participants in the study districts.

Poster 176* : Winners vs. Losers - comparative transcriptomic analysis of *Schistosoma mansoni* mature and immature eggs from gut and liver

Presenter: **Lukas Konecný**, *PhD student, Charles University, Prague*

L Konecný[1]*; K Peterková*[1]*; M Sombetzki*[2]*; J Dvořák*[3]*;*
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The eggs of the blood fluke *Schistosoma mansoni* are the main cause of the clinical manifestations of chronic schistosomiasis. It is important to note, however, that only the egg "losers" trapped in the host tissues, especially in the liver, are responsible for these manifestations. After laying, the egg "winners", on the other hand, manage to attach to the endothelium of the mesenteric vein, and after a period of development, induce the growth of a small granuloma which facilitates their passage through the intestinal wall to gut lumen. "Losers" carried with a blood stream to non-specific tissues also undergo full development and induce a big granuloma formation, but their life ends there. Although these trapped eggs represent a dead end in the parasite life cycle, vast majority of transcriptomic, proteomic and secretomic studies attempting to describe the biology of the *S. mansoni* eggs have studied these liver-trapped "losers" instead of gut-attached "winners". Thus, the fundamental question is if and possibly how the gene expression of the egg is affected by the surrounding tissues. In our study, we isolated *S. mansoni* eggs from the liver and intestinal tissues of experimentally infected mice, divided these eggs into mature and immature and compared their transcriptomic profiles. In addition, we evaluated viability of these eggs via hatching assays. Our results clearly show that gene expression in *S. mansoni* eggs is

critically dependent on tissue localization. In addition to the crucial differences in expression between eggs derived from the two tissues, the expression profiles of liver-derived eggs are very similar regardless of their developmental stage, whereas gut-derived eggs show remarkable changes during their maturation. Among most differentially expressed genes of interest between these tissues are mitochondrial genes, which together with proteases and protease inhibitors, are substantially more active in intestinal eggs. In stark contrast, IPSE/alpha-1, Omega-1, as well as the majority of micro-exon genes (MEGs), which are often proposed as the primary egg immunomodulators, are, in fact, restricted to liver losers. We argue that such differential expression of many important groups of molecules directly reflects the environment in which the egg is located. While in the case of the gut eggs, the up-regulated molecules probably represent the tools for successful passage to the external environment, in the case of the liver-trapped eggs, the high expression of immunomodulatory molecules may serve as a bait for the immune system for the benefit of the intestinal eggs.

Poster 177* : Transmission strategies of model trematodes from first intermediate snail hosts

Presenter: **Petra Kundid**, *PhD student, Biology Centre CAS*

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Trematodes represent a large, entirely parasitic group of helminths distributed in aquatic ecosyste worldwide that infect a wide range of definitive and second intermediate hosts, but the first intermediate host is almost always a mollusk. A number of transmission strategies have evolved among trematode species to enhance changes in completing their life cycle, including the synchronized emergence of large numbers of larvae, the cercariae, from snail hosts. In addition, trematodes can affect the reproduction, growth, shell shape, and locomotory activity of the snail by diverting its energy resources to better meet the needs of the parasite, which can lead to a reduced lifespan of infected snails. The main ai of the present study were: i) to monitor cercarial emergence patterns and daily output rates from snails of three model species with different life cycle characteristics, and ii) to investigate the effect of trematode infection on snail host longevity. Emergence of cercariae was monitored over main day-time intervals (sunrise, day, sunset and night) for three consecutive days under natural light/dark and controlled temperature conditions. The effect of trematode infections on snail survival was studied under laboratory conditions using naturally infected and putatively uninfected control snails. Snails with trematode infections were sampled in 2021 and 2022 in four lakes in the Czech Republic. The eye fluke, *Tylodelphys clavata*, showed a nocturnal pattern of emergence, peaking at sunset, while there were marked differences in output rates between seasons. The mean daily emergence rate was 207 cercariae snail^{-1}day^{-1} in August and 1,469 cercariae snail^{-1}day^{-1} in September. This trematode uses three hosts: snail, fish and bird. The emergence of *Sanguinicola inermis* peaked at night, and the daily emission rate was 4,205 cercariae snail^{-1}day^{-1}. *Sanguinicola inermis* has a two-host life cycle, with cercariae emerging from the snail to infect the fish definitive host. Emergence of *Plagiorchis* sp. occurred during periods of low or total light deficiency (sunrise, sunset, and peaked at night), while it was nearly arrested during daytime. This trematode species had a mean daily cercarial output rate of 4,029 cercariae snail^{-1}day^{-1}. *Plagiorchis* sp. exhibits the lowest host specificity of the species studied, using a wide range of second intermediate and definitive hosts. Laboratory survival experiments showed that snails infected with *Plagiorchis* sp. had a 45 % shorter life span (7.3 days) than uninfected control snails (16.1 days). While it is commonly reported that cercarial emergence is triggered by high light intensity, the three investigated trematode species showed the highest emergence during periods of low or no light intensity. The clear peaks in cercarial emergence correspond to the periods of the highest activity of the most common next hosts of *T. clavata* (perch) and *S. inermis* (carp). In lake Medard (*T. clavata* sampling site) most abundant fish species were whitefish, bleak and roach, while perch was the most abundant benthic species. In lake Otakar (*S. inermis* sampling site), carps make the majority of the fish population. Because *Plagiorchis* sp. is generalist, the cercarial emergence is probably not coordinated with the activity of a particular host, but rather with avoiding high predatory pressure during daytime. Although it is beneficial for parasites to keep their hosts alive as long as possible, they can induce significant damage by shortening the host's lifespan by nearly half. However, due to the long co-evolution of both organisms, trematodes are well adapted to compensate for countable losses via numerous sophisticated transmission strategies, such as the timing of cercarial emergence with the next host's occurrence.

Poster 178 : Protein Kinase A is essential for fidelity of mitosis in sporozoites and controls malaria parasite transmission

Presenter: **Dominika Kwecka**, *PhD student, University of Edinburgh*

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During its complex life-cycle, the malaria parasite experiences several atypical mitosis events where nuclear and cellular division are uncoupled. In the mosquito vector a single parasite undergoes 10-11 rounds of mitosis to generate thousands of sporozoites that are essential to establish infection in the mammalian host. Currently, the signalling pathways mediating sporozoite development remain poorly understood. Here we investigated the role of the regulatory subunit of Protein Kinase A (PKAr), the chief effector of cAMP. Using the DiCre-loxP conditional excision system we deleted PKAr exclusively in mosquito stages revealing its essential role for sporogony. We found that during sporogony, parasites lacking PKAr can correctly complete parts of the cell-cycle including DNA replication and nuclear division. However nuclear migration to the centre of the budding sporozoite cell is completely blocked. Consequently, parasites fail to egress from the mosquito midgut resulting in a complete block of transmission to the mammalian host. Currently, we are performing high-resolution microscopy studies to investigate whether fine-tuning of PKA signalling is essential for microtubule and kinetochore dynamics which could underpin correct placement of nuclei. Our study demonstrates that cAMP signalling plays a vital role in *Plasmodium* mitosis, and illuminates molecular mechanisms that regulate this critical step for parasite transmission.

Poster 179* : Zinc-based regulation of the trypanosomatid ZIP transporters

Presenter: **Teresa Leão**, *Instituto de Investigação e Inovação em Saúde - i3S*

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Transition metals, such as zinc, are crucial micronutrients to trypanosomatids, yet deleterious if in excess. To thrive and cause disease, it is imperative that pathogens rapidly sense host-induced changes in metal bioavailability and translate these into adaptive responses.

In *Leishmania,* the expression of the high-affinity zinc transporter *Li*ZIP3 (Carvalho et al, *Mol. Microbiol*, 2015) is tightly regulated by zinc. It has been proposed that a short-lived negative regulatory and zinc-responsive RNA-binding protein interacts with a *cis*-acting element in the ZIP3 3'UTR, but this factor has not been identified. Here we show that the transcript levels of the *Trypanosoma brucei* ortholog ZIP proteins (*Tb*ZIPs) increase when parasites are cultured in zinc-limiting conditions (with the zinc chelator TPEN), this being reverted by addition of the metal. The fact that parallel assays with an iron chelator (DFO), Fe(II) and Fe(III) show no effect on *Tb*ZIP transcript levels indicates that the modulation of these transporters is zinc-specific. Additionally, analysis of mutated *Tb*ZIP 3'UTRs allowed the mapping of a zinc-sensing element to a 75-bp fragment.

To gather more insights on *T. brucei* ZIP transporter regulation, we generated a reporter strain where a Neo resistance gene is under the control of a *Tb*ZIP 3'UTR. The resulting Neo-reporter was confirmed to respond to zinc limiting and surplus conditions as expected. This strain was subjected to a genome-scale RNAi (RIT-seq) screen where gain of fitness under Neo pressure should identify zinc-responsive *trans*-acting negative regulators. *Tb*ZIPs are themselves strong hits in this screen, indicating that the Neo-reporter is upregulated in zinc-limiting conditions that arise due to TbZIP knockdown. Furthermore, we identified a nuclear RNA-binding protein with several zinc knuckle motifs and a putative nuclease domain. We suggest that this is a conserved mediator of the trypanosomatid response to environmental zinc availability.

Poster 180 : Molecular identification of *Ascaris spp*. infecting humans and animals in Caraga Region, Philippines based on ribosomal DNA profiles

Presenter: **Kennesa Klariz Llanes**, *Graduate Student, University of the Philippines Los Baños*

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Ascaris lumbricoides and *Ascaris suum* are two of the most widespread intestinal helminths affecting the health of humans and pigs respectively worldwide. The two species of *Ascaris* are morphologically indistinguishable when diagnosed through microscopy, but artificial infection studies and molecular epidemiological investigations from other countries indicate that *A. lumbricoides* can infect pigs and vice versa. To date, there is a dearth of similar studies that have been published in the Philippines. Hence, this study aimed to investigate the molecular identities of *Ascaris* spp. from humans and animals, i.e. dogs, cats, pigs, and water buffalo collected from the Philippines.

Stool samples from humans and animals were collected from eight municipalities in Caraga region in a household-based survey. The samples were processed using Kato-Katz technique for the human samples and sedimentation and flotation techniques for the animal samples and were examined through microscopy for the presence of *Ascaris* spp. ova. Samples were then subjected to PCR-linked RFLP (Restriction Fragment Length Polymorphism) analysis of the nuclear ITS1 region which distinguishes *A. lumbricoides* and *A. suum* upon distinctive RFLP profiles. While most of the samples exhibit the characteristic pattern of *Ascaris* species expected for the host, the occurrence of *A. suum* genotype in 19 (2.6 %) human-derived samples and 3 (2.3 %) *A. lumbricoides* genotype in pig- derived samples and of 43 (5.0 %) samples with both *A.lumbricoides/A.suum* patterns indicate possible cross transmission and hybridization events. Furthermore, the detection of *Ascaris* spp. in other animals suggests that these animals may also contribute to environmental contamination and transmission of *Ascaris* spp. to humas. The zoonotic potential of *Ascaris* should not be overlooked and must be considered in planning for more efficient control and treatment programs.

Poster 181 : Bsep/Abcb11 knockout ameliorates *Schistosoma mansoni* liver pathology by reducing parasite fecundity

Presenter: **Dr Tomas Macháček**, *Postdoc, Charles University, Prague*

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Infection with *Schistosoma mansoni* is one of the worldwide leading causes of liver fibrosis and portal hypertension. Here we examined the disease outcome in mice lacking the bile salt export pump (*Bsep/Abcb11* KO mice; further referred to as “BSEP KO”). BSEP is a transporter localized on the hepatocyte canalicular membranes where it facilitates biliary excretion of bile acids. BSEP KO mice accumulate polyhydroxylated bile acids that protect them from the development of cholestatic liver injury (including inflammation and fibrosis). Therefore, we infected WT and BSEP KO mice with *S. mansoni* and examined them eight weeks later. Specifically, we evaluated effects on liver histology, serum biochemistry, the gene expression profile of (pro-)inflammatory cytokines and fibrotic markers, and the hepatic collagen content. Also, the host immune response was analyzed by flow cytometry. The infected BSEP KO mice showed significantly less hepatic inflammation and tendentially less fibrosis than WT controls. Despite elevated ALT, AST, and AP levels in infected BSEP KO mice, inflammatory cells such as M2 macrophages and Mac-2/galectin-3+ cells were reduced in these animals. Accordingly, mRNA-expression levels of anti-inflammatory *Il4* and *Il13* were increased in infected BSEP KO mice. Furthermore, they exhibited decreased hepatic egg load and parasite fecundity, affecting the worm reproduction rate. These findings may, at least in part, be attributed to elevated serum bile acid levels and hence lower blood pH in infected BSEP KO mice. We conclude that the loss of BSEP and the resulting changes in bile acid composition and blood pH reduce parasite fecundity, thus attenuating the development of *S. mansoni*-induced hepatic inflammation and fibrosis.

Poster 182 : Decoding redox stress responses in African trypanosomes

Presenter: **Enock Mararo**, *PhD student, University of Edinburgh*

E Mararo
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Trypanosomes are parasites that cause diseases in humans and animals throughout Sub-Saharan Africa. The main cause of livestock trypanosomiasis are *Trypanosoma congolense* and *T. vivax*. With no available vaccine and few chemotherapeutic options, new chemotherapies are needed to improve agricultural production in endemic areas. Most understanding of trypanosome biology comes from the human-infective subspecies of *Trypanosoma brucei* with only a few studies on *T. congolense*. This study aimed to compare redox balance pathways in *T. brucei* and *T. congolense* using multi-omics approaches to identify candidate genes/pathways required for parasite survival in response to oxidative stress. Preliminary data show that two trypanosome species exhibit differential sensitivity to a nitric oxide donor (SNAP) and LPS-stimulated macrophages. Metabolomics revealed that the oxidative stress response pathways – pentose phosphate pathway and ketoacid production pathways – differ between trypanosome species, necessitating further investigation. These findings point to differences in the ability of the two species to respond to oxidative stress, which has implications for host-trypanosome infection dynamics. Future research will aim to characterize candidate pathways/genes essential for oxidative stress response in both species and their impact on macrophage function *in vitro*.

Poster 183 : The tandem zinc-fingers of RNA helicase-associated KH2F1 differentially impact the editing of distinct mitochondrial transcripts in *Trypanosoma brucei*.

Presenter: **Dr Suzanne McDermott**, *Acting Assistant Professor, Seattle Childrens Research Institute*

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The generation of functional mRNAs encoding respiratory complex components in trypanosome mitochondria involves U-insertion/deletion (U-indel) RNA editing. Editing is developmentally regulated between mammalian bloodstream form and insect procyclic form *T. brucei*, correlating with the differential utilization of glycolysis and oxidative phosphorylation between the forms. However, the mechanisms underlying this regulation are only just beginning to be understood. The editing process requires the coordinated actions of several multiprotein complexes. One such complex is the RNA Editing Helicase 2 Complex (REH2C) that contains the DEAH-box RNA Editing Helicase 2 (KREH2), and its partner proteins, KREH2-associated zinc-finger protein (KH2F1), and KH2F2. Of these, KREH2 and KH2F1, are essential for growth and are required to achieve full and accurate editing in procyclic form *T. brucei*, and KREH2 is essential for growth and editing in bloodstream form. Here we report the generation of bloodstream form *T. brucei* cells that are conditionally null for KH2F1 and show for the first time that KH2F1 is also essential for bloodstream form growth and editing. KH2F1 contains eight tandem zinc-fingers, and we assessed the importance of each of the zinc fingers via mutagenesis. Surprisingly, we show that the individual zinc fingers have different effects on bloodstream form growth, and on the abundances of edited transcripts whose editing is developmentally regulated. Together these data suggest that the KH2F1 zinc fingers may direct specific substrates to KREH2, including those whose editing differs between mammalian bloodstream form and insect procyclic form *T. brucei*, thereby playing a key role in the developmental regulation of editing across the parasite life cycle.

Poster 184* : Novel antimicrobials produced by *Streptomyces coelicolor* when challenged by *Aspergilli* species under modified nutrient-deplete conditions

Presenter: **Ruth Nair**, *Ruth Nair*

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Background: There is an ever-increasing need to identify and produce novel antimicrobials and anti-parasitic compounds. Public Health England estimates 10 million deaths globally by the year 2050 if the current trend of rampant antimicrobial resistance is left unchecked. Co-culturing *Streptomyce*s with competing microorganis is a viable method to identify novel antimicrobial and anti-parasitic compounds.

Objective: This study ai to identify novel medically useful compounds by co-culturing *S. coelicolor* with *A. flavus and A. parasiticus*.

Methods: Wild-type (WT) and mutant (M1146, M1152, M1154) *S. coelicolor* strains were co-cultured with *A. flavus* and *A. parasiticus* under nutrient-deplete conditions to induce secretion of secondary metabolites by *S. coelicolor*.

Results: Pronounced anti-fungal activity was observed in original minimal media developed for this study. *Aspergillus* radial growth was reduced, with the mean diameter of colonies decreased by ~12 mm when co-cultured with all strains of *S. coelicolor.* Zones of inhibition for *Aspergillus* sporulation around a seeded *Streptomyces* colony was 31 ± 1.5 mm and 22 ± 6.7 mm when co-cultured with the *S. coelicolor* strains M1152 and M1146 respectively, a significant increase compared to when co-cultured with WT *S. coelicolor* (4 ± 0.6 mm; $P < 0.0001$, $n = 3$). Quantitation by florescence microscopy indicate that *S. coelicolor* suppress *Aspergillus* growth by inhibiting fungal hyphae proliferation. SEM images show penetration of *Aspergilli* biofilm and damage to hyphal structures by *Streptomyces* strains. Extracts from co-culture conditions which suppress *Aspergillus* growth inhibit ESKAPE pathogens. Analyses of these extracts by HPLC- have identified known compounds with anti-microbial activity, along with novel compounds with predicted anti-microbial activity and anti-parasitic activity. More than 4000 novel secondary metabolites were observed in ethyl acetate extracts of co-cultured supernatants. RNA sequencing of co-cultured *Streptomyces* mutants is underway to study expression of known and novel bacterial cryptic gene clusters.

Conclusions: The data argue that *Streptomyces* species competing with *Aspergillus* under specialized nutrient-deplete conditions secrete secondary metabolites with broad range anti-microbial, anti-parasitic and insecticidal activity. *S. coelicolor* mutant, M1152, exhibits significantly increased antimicrobial activity in co-cultured conditions as compared to WT or other mutants tested in this study.

Poster 185 : Adult Male Poultry Red Mites (*Dermanyssus gallinae*) are haematophagous

Presenter: **Dr Francesca Nunn**, *postdoc, Moredun Research Institute*

F Nunn[1]*; E Ramos*[1]*; K Bartley*[1]*; AJ Nisbet*[1]*;*
[1] *Moredun Research institute, UK*

Poultry red mites (PRM) are blood feeding ectoparasites that live off-host, only seeking a bird from which to rapidly engorge a bloodmeal every few days. Despite being a major agricultural pest species [1] relatively little is known about the mite's fundamental biology [2]. Adult females, deutonymphs and protonymphs are known to feed but adult males are thought not to blood-feed or to only feed rarely [3].

Recently, we described using Baudruche membrane in an *in vitro* device to feed adult female PRM [4] using goose blood as a food source, leading to improved, reproducible feeding rates and the device has since been optimised for feeding both haematophagous nymph stages. Here we describe using the device to establish if adult male PRM will feed on blood in vitro.

Mixed stages of PRM were collected on farm and stored at 5°C for two weeks before being stored in an environmental chamber at 20°C and 75% relative humidity (RH) for seven days, to allow digestion of their last blood meal. Nine replicate *in vitro* feeding chambers, containing 50 adult female PRM and 10-25 adult male PRM and 9 replicate *in vitro* feeding chambers, each containing 10-25 adult male PRM only, were fed heparinised goose blood for three hours. Following feeding, mites were sorted into "fed" and "unfed" groups. Fed mites (which are easily identified by the presence of fresh blood inside the mites) were monitored for 8 days. Moulting during this period would identify any male mites that were mis-identified originally as deutonymphs (deutonymphs and adult males are similar in gross morphology). Any male PRM which had failed to feed were placed into 100% ethanol for later microscopic confirmation of sex. After 8 days, fed males were also placed in 100% ethanol for lifestage confirmation using microscopy. Male PRM were confirmed by identification of a holoventral shield, which is distinct from the undeveloped epigynal shield in nymphs and developed epigynal shield in adult females, and the genital opening which is absent in other lifestages [2].

In those replicates where male PRM were fed in the presence of female PRM, a total of 48 confirmed males fed in the *in vitro* feeding devices (39% of the total number of males used). Where males were fed in the absence of female PRM, a total of 65 confirmed males fed in the *in vitro* feeding devices (44% of the total number of males used). We have therefore definitively demonstrated that adult male PRM are indeed haematophagous and do not require the presence of co-feeding female PRM to stimulate their feeding behaviour.

1.Flochay et al 2017. 2. Pritchard et al 2015. 3. Chauve et al 1998 4. Nunn et al 2020

Poster 186 : The role of point-of-care ultrasound in the assessment of schistosomiasis-induced liver fibrosis: a systematic scoping review

Presenter: **Eloise Ockenden**, *1st Year PhD Student, University of Oxford*

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Background. Abdominal ultrasound imaging is an important method of hepatic schistosomiasis diagnosis and staging. Several ultrasound staging syste have been proposed, all attempting to standardise periportal fibrosis (PPF) diagnosis. This review ai to establish the role of ultrasound in the diagnosis and staging of PPF, and to map the evolution of ultrasound staging syste over time, focusing on validation and reproducibility.

Methods. A systematic search was undertaken on 21/12/2022 (protocol registered at https://osf.io/jrcmn), considering multiple databases with no restriction on publication date. Case reports, systematic reviews and meta-analyses, and studies exclusively using elastography or Doppler imaging were excluded. Variables relevant to the ai of the review were extracted from included studies, for example the staging system, ultrasound technology used and validation methods. The PRISMA-ScR guidelines were followed to inform the structure of the review.

Results. 223 studies were eligible for extraction: the initial search yielded 575 unique articles, 169 of which were screened out with titles and abstracts, and 183 of which were excluded at full-text screening. Most studies were conducted after the year 2000 and were located in Brazil, Egypt and Sudan. The staging syste used in the studies took on three forms: feature-based, measurement-based and image pattern-based. The Niamey protocol, a measurement and image pattern-based system, was the most used among the staging systems, despite being the most recently proposed (1996). Of the studies using the Niamey protocol, most only used the image patterns element. Where ultrasound technology was specified, studies conducted before 2000 overwhelmingly used linear array transducers, with studies after 2000 more likely to use convex transducers. Few studies detailed their methods of validation or referenced reproducibility of the staging system that was used.

Conclusions. The exclusive use of the image patterns in many studies, the increasing number of studies involving ultrasound staging of PPF over time, and the movement in ultrasound technology used since 2000 all indicate a need for an update to the Niamey protocol.

Poster 187* : Using Imaging Flow Cytometry to automate cell cycle analysis and define G2 phase in *Leishmania mexicana* promastigotes

Presenter: **Sulochana Omwenga**, *PhD student, University of Glasgow*

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Leishmania mexicana, a parasitic protist, is spread by sand flies and causes cutaneous leishmaniasis in humans and animals. Key to being able to complete its digenetic life cycle and to cause disease in its hosts, is the ability of the parasite to proliferate. It has a complex cell division cycle characterised by the ordered replication of several single-copy organelles, accompanied by cell cycle stage-dependent morphological changes. Understanding this complex process may provide insight into new therapeutic targets. Currently, cell cycle analysis is primarily carried out via flow cytometry, to assess the distribution of cells throughout the cycle, or using microscopy to understand the finer details of cell cycle presentation, such as proportions of cells undergoing mitosis. Both of these methods have limitations, with flow cytometry unable to provide specific cell cycle stage information, and microscopy being time-consuming and labour-intensive.

Here, we present a comprehensive analysis of the *L. mexicana* promastigote cell cycle in live cells using imaging flow cytometry. IFC boasts the high-throughput and quantitative analysis of flow cytometry with the visual and spatial information of microscopy. While IFC has previously been used to study host-parasite interactions of *Leishmania*, as well as morphology and viability, its application for cell cycle analysis is limited to mammalian cells (Terrazas et al. (2015) J Immunol Methods, 423, 93-98; Dandugumula et al. (2022) Pathogens, 23;11(9):952; Blasi et al. (2016) Nature, 7, 10256). Using Vybrant™ DyeCycle™ Orange (DCO), we can simultaneously quantify and visualise DNA in live cells. After developing gating and masking strategies, automated analysis of cell cycle distribution was achieved, providing information on the quantity of DNA within a cell, the number of nuclei and kinetoplasts and the length and width of the cell body.

However, DNA staining and morphology alone are insufficient for precise cell cycle stage identification, with late S-phase, G2, and early mitotic cells having similar morphological presentations. Therefore, tagging of the spindle-associated kinesin, LmxKINF, was carried out. By automatically determining the cell cycle-dependent localisation of KINF (single spot, rod shaped or two spots) we were able to improve the resolution of post-S phase cell cycle stage identification, and for the first time, define the proportion and duration of *L. mexicana* cells in G2.

Poster 188 : tRNA anticodon stem length variations are critical for stop codon reassignment

Presenter: **Dr Zdenek Paris**, *Biology Centre CAS, Institute of Parasitology*

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Transfer RNA (tRNA) delivers a specific amino acid residue to ribosomes. Its anticodon pairs with the complementary mRNA codon according to the universal genetic code, which defines translation termination by three stop codons. Remarkably, some protists have reassigned all three stop codons to sense codons, neglecting this fundamental principle. Performing an across- the-genome analysis of in-frame stops in 7,259 predicted protein-coding genes of the newly isolated trypanosomatid *Blastocrithidia nonstop* we show that their distribution is not random and their representation diminishes with increasing protein abundance. Robust comparisons with other trypanosomatids revealed specific features at the end of the coding regions defining UAA as the only functional termination codon. We demonstrate that while novel tRNAs^{Glu} fully cognate to UAG and UAA evolved to reassign these two stops, recoding of UGA followed an unprecedented path via shortening the anticodon stem-loop of tRNA$^{Trp}_{CCA}$ from 5 to 4 base pairs (bp). While tRNA^{Trp} with a canonical 5-bp long stem-loop recognizes UGG as dictated by the genetic code, its shortened version efficiently incorporates tryptophan also into in-frame UGA to allow translation to continue. Mimicking this radical evolutionary twist by engineering and overexpressing both stem loop variants of tRNA$^{Trp}_{CCA}$ from *B. nonstop*, *Trypanosoma brucei* and *Saccharomyces cerevisiae* in the latter two species, we recorded a significantly higher readthrough for all 4-bp stem-loop variants. The phenomenon is specific for tRNA^{Trp}, since decoding by other two *S. cerevisiae* tRNAs near-cognate to UGA (tRNA^{Cys} and tRNA^{Arg}) was unaffected by alterations in their stem-loop length. Furthermore, we demonstrate that a specific mutation in the release factor 1 (eRF1) of *B. nonstop* specifically restricts UGA recognition and thus robustly potentiates the UGA recoding to tryptophan. Revealing that the same strategy has also been independently adopted by the ciliate *Condylostoma magnum*, we propose that these two key alterations co-evolved synergistically. Altogether, we have defined a novel and universal mechanism underlying the stop codon recognition by specific variants of tRNAs^{Trp} in combination with mutated eRF1, which has been exploited in unrelated eukaryotes with reassigned stop codons.

Poster 189 : Challenges of accessing treatment for cutaneous leishmaniasis in Brazil, Ethiopia and Sri Lanka

Presenter: **Prof Helen Price**, *Keele University*

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ECLIPSE is a five-year applied healthcare programme which ai to improve the patient journey and reduce stigma for people living with cutaneous leishmaniasis (CL) in the most underserved communities in Brazil, Ethiopia and Sri Lanka. ECLIPSE brings together leishmaniasis expertise in an international, cross-cultural, multidisciplinary team of over 60 researchers, including anthropologists, parasitologists, clinicians from different medical specialties, psychologists, disease specialist and public health researchers. We have used a range of qualitative and quantitative methods to gain in-depth understanding of people, communities and healthcare professionals' experiences and views on the effects

of CL on the daily lives of those affected, the barriers to seeking healthcare, obtaining accurate, early diagnosis and receiving effective treatment.

Here we present findings on the many challenges to accessing diagnosis and treatments for CL in our field sites, within the context of the COVID-19 pandemic and the conflict in Tigray, Ethiopia. Multiple injections of pentavalent antimonials remain the most common biomedical treatment for CL and are predominantly delivered in tertiary-level healthcare facilities. There are multiple barriers to accesssing healthcare for CL, including lack of awareness of the disease (in both community members and healthcare professionals), the need to travel long distances, cost, fear of side-effects, loss of earnings during treatment and caring responsibilities. Self-treatment and the use of traditional remedies are very common, particularly in sites where there is poor access to biomedical treatment. We discuss recommendations to improve the patient journey for people living with this highly neglected disease.

Poster 190 : Identifying protein subcellular localization in *Leishmania* using spatial proteomics

Presenter: **Dr Eden Ramalho Ferreira**, *Post doctoral research associate, University of York*

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Leishmania is a flagellated kinetoplastid protozoan and causative agent of Leishmaniasis, a neglected tropical disease with 0.7 to 1 million new cases each year. To understand the biology of *Leishmania* parasite, information on subcellular localization of proteins is key to making testable predictions about protein function, complex formation, organelle composition and cellular architecture. For this purpose, we are using Localisation of Organelle Proteins by Isotope Tagging after Differential ultracentrifugation (LOPIT-DC). To obtain suitable cell lysates for LOPIT-DC cytoplasmic membrane needs to be ruptured but organelles and compartments should be preserved. In this sense, we successfully obtained cell lysates in eleven fractions by mechanical disruption using nitrogen cavitation associated with differential ultracentrifugation. To test the fractionation effectiveness, we submitted the samples to western blotting using antibodies for proteins known to be localized in different compartments which demonstrated we could successfully separate organelles by weight/volume. Next, fractions from four independent experiments were labelled with TMT-11plex reagents for relative quantification and analysed by LC- using an Orbitrap Fusion Tribrid mass spectrometer with multi-notch MS^3 acquisition to minimise chimeric interference. Mass spectrometry analysis and database searching of the *Leishmania mexicana* promastigotes resulted in the identification and relative per-fraction quantification of 3782 proteins common in all four experiments. Using the resulting set of abundance data across all eleven fractions in the four experiments, we employed a Gaussian Mixture model to infer the localisation of previously un-annotated proteins. We trained this model on known protein localisation annotations in *Trypanosoma brucei* (tryptag.org) carried over to *L. mexicana* using gene orthology and thresholding by a sequence similarity of at least 30%. We then added known ribosome, flagellar pocket and Golgi apparatus to the list of *L. mexicana* marker proteins (from TriTrypDB). Dimensionality reduction using t-Stochastic Neighbourhood Embedding (t-SNE) and hierarchical density-based clustering (HDBSCAN) showed that we could effectively visualize protein clusters according to the organelle and sub-organelle compartments. This will provide a comprehensive understanding of the proteomic organization, function and evolution including adaptive interaction with host and assumptions on protein-protein interaction within organelles.

Poster 191 : Consistent detection of *Trypanosoma brucei* but not *T. congolense* DNA in faeces of experimentally-infected cattle

Presenter: **Isabel Saldanha**, *Research Associate & PhD Student, Liverpool School of Tropical Medicine*

I Saldanha[1]*; M Betson*[2]*; KR Matthews*[3]*; E Paxton*[4]*; C Vrettou*[4]*; LJ Morrison*[4]*; SJ Torr*[1]*; LJ Cunningham*[1]*;*

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Species of *Trypanosoma* transmitted by the tsetse fly (*Glossina*) vector are responsible for important medical and veterinary diseases across sub-Saharan Africa. Although advances have been made in the control of human African trypanosomiasis (HAT), animal African

trypanosomiasis (AAT) remains a disease of significant economic burden and livestock mortality in sub-Saharan Africa. Current AAT surveillance tools suffer from poor sensitivity and specificity, with serological methods also requiring animal restraint and blood collection by trained personnel. Faecal sampling is an attractive potential option for more accessible sample collection and screening. This study set out to determine whether it is possible to detect DNA of AAT aetiological agents (*T. brucei* and *T. congolense* savannah) in the faeces of experimentally-infected cattle.

Five male Holstein-Friesian calves of post-weaning age were inoculated with *T. brucei* AnTat 1.1 and the infection course was followed for a total of 68 days. A total of 146 faecal samples (12 pre-inoculation, 134 post-inoculation) and 148 blood samples (10 pre-inoculation, 138 post-inoculation) were collected. In a parallel study, six male Holstein-Friesian calves were inoculated with *T. congolense savannah* IL3000 and infection course followed for 66 days. A total of 151 faecal samples (6 pre-inoculation, 145 post-inoculation) and 167 blood samples (12 pre-inoculation, 155 post-inoculation) were collected. All samples were screened using *Trypanosoma* species-specific PCR assays and novel probe-based qPCR assays targeting *Trypanozoon*-specific and *T. congolense savannah*-specific repeat regions in kinetoplast minicircle DNA respectively.

T. brucei target DNA was successfully detected in 85% (n=114) of post-inoculation faecal samples by qPCR and 50% (n=67) by PCR. *T. brucei* target DNA was detected in faecal samples collected between four days post-inoculation (dpi) to 66 dpi by both qPCR and PCR. Amplification of target DNA was confirmed by Sanger sequencing of PCR products, which revealed significant homology to the target sequence. *T. brucei* DNA was detected in 100% (n=138) of post-inoculation blood samples by qPCR. Linear regression analysis revealed a weak yet statistically significant positive relationship ($p=0.0354$, $R^2=0.06$) between Cq values obtained from matched *T. brucei* blood and faecal samples. However, *T. congolense* target DNA was detected in just 3.4% (n=5) of post-inoculation faecal samples by qPCR and none by PCR. *T. congolense* DNA was detected in 100% (n=155) of post-inoculation blood samples.

These results confirm, for the first time, the ability to consistently detect *T. brucei* DNA from the faeces of infected cattle. By contrast, *T. congolense* DNA could not be reliably detected in faeces, despite the respective qPCR assays having the same approximate limit of detection. This finding may be explained by the differences in *Trypanosoma* species tissue distribution; with *T. brucei* capable of tissue invasion whilst *T. congolense* remains largely restricted to the blood circulatory system. Whilst these findings show the potential of using faeces as an easily-accessible sample to screen for active *T. brucei* infection, blood sampling is still required to reliably detect *T. congolense* in cattle. Future research should focus on refining this novel diagnostic method in field and wildlife samples to broaden *T. brucei* surveillance.

Poster 192* : In-trap DNA contamination: tsetse sampling and screening methods can lead to biased estimates of *Trypanosoma brucei* infection

Presenter: **Isabel Saldanha**, *Research Associate & PhD Student, Liverpool School of Tropical Medicine*

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Tsetse flies (*Glossina sp.*) are vectors of subspecies of *Trypanosoma brucei* which cause human African trypanosomiasis (HAT). Catching tsetse and screening them for the presence of *T. brucei* is an important method of HAT surveillance. Classically, individual tsetse were dissected and subjected to microscopic analysis to identify trypanosomes if present. However, in the 'genomics age' such techniques are being replaced by molecular xenomonitoring – screening vectors for genetic targets indicative of pathogen presence. Assays using a range of *T. brucei* genomic targets have been developed for this purpose, including the 10,000-copy *T. brucei* tandem repeat (TBR) region. The use of such highly sensitive targets in an end-point assay such as TBR-PCR can lead to difficulties in differentiating true biological infection from the mere presence of a DNA marker. These sensitive methods are also vulnerable to DNA cross-contamination. Such contamination may occur at capture, when live tsetse are held in a small trap cage for a day or more. Several xenomonitoring studies that have used TBR-PCR have reported higher-than-expected proportions (5% or more) of *T. brucei*-positive flies. Therefore, this study set out to investigate whether it is possible for *T. brucei* infected tsetse to contaminate uninfected tsetse with *T. brucei* DNA when housed in the same trap.

A total of 140 teneral *G. morsitans morsitans* were fed a bloodmeal spiked with *T. brucei brucei* TSW 196 and maintained in solitary cells. TBR-qPCR screening of tsetse faecal samples collected 9-14 days post inoculation (dpi) were used to determine individual fly infection status. At

19dpi, 48 infected flies (IFs) and 96 (not inoculated) uninfected flies (UFs) were placed in plastic bottles similar to the cages used with traps. The numbers of IFs and UFs in the bottles was varied according to four classes of treatment. The four treatments comprised IF:UF in the ratios: (T1) 9:3, (T2) 6:6 (T3) 1:11 and (T4) 0:12. Each treatment was replicated three times (A, B and C). After 24-hour incubation, all flies that had ingested an infectious bloodmeal (n=110) were dissected and microscopically analysed to determine infection status. All tsetse samples (n=206) then underwent DNA extraction and screening using TBR-PCR and TBR-qPCR.

Microscopy revealed that 100% (n=48) of IFs selected for experiments had developed mature midgut infection. Furthermore, screening of these IFs by TBR-qPCR revealed Cq values between 14.46-21.57 (mean=17.74, SD=0.7458) in 100% of flies. However, UFs also contained TBR target DNA, the quantity of which varied according to the proportion of infected flies within the trap. For T1 and T2, 100% of UFs had detectable DNA with mean Cqs of 26.72 (±0.7498 SD) and 29.84 (±1.375 SD) respectively. For T3, 91% UFs had detectable DNA with a mean Cq of 33.70 (±1.352 SD). Low-level amplification was detected in

Poster 193* : Exploring actinomycetes natural products to identify potential multi-target inhibitors against *Leishmania donovani*

Presenter: **Satyendra Singh**, *Research Scholar, Central University of Rajasthan*

S Singh[1]*; VK Prajapati*[2]*;*

[1] *Central University of Rajasthan, India;* [2] *Central University of Punjab, India*

The neglected tropical disease visceral leishmaniasis (VL) disproportionately affects impoverished populations in the Indian, African, and South American subcontinent. However, the increasing resistance and the toxicity to antimonials, miltefosine, and amphotericin B underscores the pressing need to develop a safe and effective anti-leishmanial drug. To address this, we conducted a study and screened 6519 secondary metabolites from an actinomycetes source against five key proteins involved in the metabolic pathway of *Leishmania donovani* using three sequential docking protocols (HTVS, SP, and XP). These proteins included adenine phosphoribosyltransferase (PDB ID: 1QB7), trypanothione reductase (PDB ID: 2JK6), N-myristoyl transferase (PDB ID: 2WUU), pteridine reductase (PDB ID: 2XOX), and MAP kinase (PDB ID: 4QNY). The study predicted the binding energy of the top ligands using the MM-GBSA module of the Schrödinger suite, and SP and XP docking modes identified 55 multi-targeted ligands against *L. donovani*. The top 18 ligands with good-binding affinity and binding-free energy for four of the targeted proteins (compared to miltefosine, paromomycin, and a reference ligand for each target selected as positive control) were selected using MM-GBSA analysis. Finally, molecular dynamics simulation trajectory analysis (RMSD, RMSF, SASA, Rg, and H-bondong), post-MD-binding-free energy (MM-PBSA), and principal component analysis (PCA) identified three ligands (Adenosine pentaphosphate, Atetra P, and GDP-4-keto-6-deoxymannose) that met the screening parameters and were considered potential drug candidates to combat *L. donovani* parasites.

Poster 194 : Sulfadoxine-pyrimethamine drug resistance markers hint malaria drug policy shift in India

Presenter: **Dr Abhinav Sinha**, *Scientist, ICMR-National Institute of Malaria Research*

A Sinha[1]*; S Kar*[1]*; L Kori*[1]*; C Chauhan*[1]*; CP Yadav*[1]*;*

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India is on track of malaria elimination by 2030 but emerging resistance to its first line antimalarials is one of the major roadblocks. Two instances of rapid development, spread & selection of drug resistant mutant parasites have already been evidenced (Chloroquine in whole of India & Artesunate+Sulfadoxine-Pyrimethamine (AS+SP) in India's north-eastern states). Looking at these rapid changes in SP drug resistance conferring mutation profile of *P. falciparum*, it becomes evident to systematically monitor the validated mutations in Pfdhfr & Pfdhps genes across India along with the AS+SP therapeutic efficacy studies. However, unfortunately, no systematic & robust countrywide surveillance has been reported for these parameters. Therefore, we are presenting here the first exhaustive systematic review & data synthesis on the prevalence of WHO-validated SP-resistance markers in *P. falciparum* across India from 2008 to date. This systematic review covers published reports from the major databases including PubMed®, Web of Science™, Scopus®, Embase® & Google Scholar & presents a chronology of

reported events of interest across India. A total of 37 publications that had data collected between 2008 & 2018 were included in the analysis. *Pfdhfr* mutation data were obtained from 3438-3801 samples & that for *Pfdhps* from 2891-3596 samples. The *Pf*DHFR double mutants were the most prevalent (55%) overall. The overall prevalence of triple & quadruple mutations was 7% & 6%, respectively. The most common *Pf*DHPS mutation is A437G with rising & near-100% prevalence in some states. For *Pf*DHFR/*Pf*DHPS quintuple & sextuple mutations, despite a low overall prevalence, some states had a prevalence of >30%. This study gains importance in light of the flags raised towards emerging 'artemisinin-resistance' in eastern India & brings forward the SP-resistance hot-spots & emphasizes critical gaps, challenges & suggests malaria genetic surveillance till malaria is successfully eliminated. The key question is whether the time has come for a change from AS+SP to AL in the rest of India? The evidence for the risks needs to be weighed but taking the risk of continuing AS+SP, particularly in the light of rising flags of artemisinin-resistance might be counterproductive & the decision needs to be made sooner rather than later.

Poster 195 : Invasive zebra mussel, *Dreissena polymorpha*, as an efficient diluent of *Diplostomum cercariae (Digenea)*

Presenter: **Dr Miroslava Soldanova**, *Biology Centre, Czech Academy of Sciences*

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The zebra mussel (*Dreissena polymorpha*) is an invasive bivalve native to the Ponto-Caspian region that has established introduced populations throughout Europe and North America over the past 200 years. Its high colonization, reproduction and filtration capabilities, combined with the rapid formation of dense population colonies make *Dreissena polymorpha* one of the most successful invaders affecting the structure and function of freshwater ecosyste with serious ecological, environmental, and economic consequences. In contrast to these negative impacts, however, zebra mussels can also be beneficial to native biota by diluting parasites whose larval stages occur in large numbers in the environment, such as trematode cercariae released from their first molluscan intermediate host. While many organisms, including bivalves, have been shown to reduce parasite density and thus transmission and disease risk in the next hosts in their life cycle, the dilution effect of zebra mussels on trematode cercariae still see to be overlooked.

The aim of this study was to investigate the non-host *D. polymorpha* (i.e., a dead-end host for the parasite) as an efficient diluent of trematode cercariae of *Diplostomum mergi* emerging from their snail hosts, *Radix* spp. (Lymnaeidae). Eye flukes of the genus *Diplostomum* are widespread pathogens of the second intermediate fish host, causing eye cataracts, impaired vision and fish mortality due to decreased food intake and increased susceptibility to predation. Therefore, it is imperative to understand the potential impact of non-host-pathogen associations on disease dynamics in nature.

All model organis were sampled in July 2022 in the artificial lakes Most and Medard in northern Bohemia (Czech Republic). The ability of zebra mussels to dilute *D. mergi* cercariae was tested in the laboratory using mussel individuals of similar size under controlled light conditions at two temperatures (18°C and 22°C) reflecting the average water temperature of colder (spring and autumn) and warmer months (summer). At each temperature, 20 mussels were allowed to filter 115 cercariae freshly emerged from naturally infected snails for 30 minutes. Despite the relatively high variability among mussel individuals, the presence of *D. polymorpha* significantly decreased the density of *D. mergi* cercariae by nearly half at each temperature. Moreover, the number of cercariae diluted by mussels was significantly higher at 22°C (47%) than at 18°C (40%), suggesting a seasonal/temperature-dependent removal rate of the parasites.

Our results provide additional evidence that bivalves may play an important ecological role in trematode population dynamics by efficiently diluting their free-living stages, thus limiting their transmission success. Because parasites are recognized as essential components of food webs that affect ecosystem structure and functioning, our results highlight the need for further research to examine intimate non-host-parasite interactions in detail, which will improve our understanding of ecosystem processes when both non-host and pathogen are considered.

Poster 196* : Identification of FDA-approved drugs with triple targeting mode of action for the treatment of Monkeypox:a high throughput virtual screening study

Presenter: **Varshita Srivastava**, *Central University of Rajasthan*

V Srivastava[1]*; D Prusty*[1]*;*
[1] *Central University of Rajasthan, India*

According to the Center for Disease Control and Prevention, as of August 23, 94 countries had confirmed 42,954 Monkeypox Virus cases. As specific monkeypox drugs are not yet developed, the treatment depends on repurposed FDA-approved drugs. According to a recent study, the Monkeypox outbreak is caused by a strain with a unique mutation, raising the likelihood that the virus will develop resistance to current drugs by acquiring mutations in the targets of currently used drugs. The probability of multiple mutations in two or more drug targets at a time is always low than mutation in a single drug target. Therefore, we identified 15 triple-targeting FDA-approved drugs that can inhibit three viral targets, including topoisomerase1, p37, and thymidylate kinase, using high throughput virtual screening approach. Further, the molecular dynamics simulation analysis of the top hits such as Naldemedine and Saquinavir with their respective targets reveals the formation of stable conformational changes of the ligand-protein complexes inside the dynamic biological environment. We suggest further research on these triple-targeting molecules to develop an effective therapy for the currently spreading Monkeypox.

Poster 197 : Avian schistosomes: *Bilharziella polonica* cercariae in Knowsley Safari, Prescot, United Kingdom, with notes on other trematodes implicated in human cercarial dermatitis

Presenter: **Prof Russell Stothard**, *Medical Parasitologist, Liverpool School of Tropical Medicine*

R Stothard[1]*; A Juhàsz*[1]*; S Jones*[1]*; LJ Cunningham*[1]*; EJ Lacourse*[1]*;*
[1] *Liverpool School of Tropical Medicine, UK*

As part of surveillance of snail-borne trematodiasis in Knowsley Safari (KS), Prescot, United Kingdom, a collection was made in July 2021 of various planorbid (n = 173) and lymnaeid (n = 218) snails. These were taken from 15 purposely selected freshwater habitats. In the laboratory emergent trematode cercariae, often from single snails, were identified by morphology with a sub-set, of those most accessible, later characterised by cytochrome oxidase subunit 1 (*cox*1) DNA barcoding. Two schistosomatid cercariae were of special note in the context of human cercarial dermatitis (HCD), *Bilharziella polonica* emergent from *Planorbarius corneus* and *Trichobilharzia spp.* emergent from *Ampullacaena balthica*. The former schistosomatid was last reported in the United Kingdom over 50 years ago. From *cox*1 analyses, the latter likely consisted of two taxa, *Trichobilharzia anseri*, a first report in the United Kingdom, and a hitherto unnamed genetic lineage having some affiliation with *Trichobilharzia longicauda*. The chronobiology of emergent cercariae from *P. corneus* was assessed, with the vertical swimming rate of *B. polonica* measured. We provide a brief risk appraisal of HCD for public activities typically undertaken within KS educational and recreational programmes.

Poster 198 : A First for Parasite Conservation in South Africa: The Case of Threatened Freshwater Fishes in The Cape Fold region

Presenter: **Dr Marliese Truter**, *Postdoctoral Research Fellow, North-West University*

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Species conservation is no novel concept, however, in recent years the conservation of parasitic species has received considerable attention. A global initiative has caught momentum to include this highly diverse group of organis present in almost all potential hosts across the globe. At first deemed an unconventional idea, it became apparent that parasitic species contribute considerably to the biomass of ecosyste and its functioning, rendering them essential candidates for application in the conservation of ecosystems. At least 50% of South Africa's ~118 freshwater fish species are threatened and only a fraction of parasitic data is known for all of these species, which was last assessed in the early 1980s. Since then, a considerable increase in parasitological studies came to light, and a number of alien and invasive fishes with their co-introduced and potential co-invasive parasitic species have been translocated across freshwater systems. The present project ai to facilitate a first initiative in South Africa to assess freshwater fish parasite diversity and communities from six National Freshwater Ecosystem Priority Areas

that are greatly underrepresented in historic and current biodiversity data. The first eight fishes of conservation concern and their associated parasite communities are discussed with a way forward for utilising these unique parasites as a conservation tool.

Poster 199* : Dynamic protein ubiquitination by USP1 is essential for malaria transmission

Presenter: **Neelakshi Varma**, *University of Edinburgh*

N Varma[1]*; EJ Marr*[2]*; L Lemgruber; N Philip*[2]*;*

[1] *University of Edinburgh, UK;* [2] *Institute for Immunology and Infection Research, University of Edinburgh., UK;* [3] *University of Glasgow, UK*

Transmission of malaria parasites from the vertebrate host to mosquito vector requires specialised precursor cells also known as gametocytes. Gametocytes are sexually dimorphic where both male and female cells have unique properties to initiate the sexual life cycle in the mosquito. Within seconds of experiencing the mosquito environment male gametocytes commence a complex signalling cascade resulting in three rounds of DNA replication, endomitosis and axoneme assembly to release eight flagellated gametes in 10-15 minutes, which will fertilise with female gametes to form diploid zygotes. How the male gametocyte is capable of such astonishing speed of mitosis is an intriguing question. Light and electron microscopy have revealed, in comparison to the female, the male contains a significantly larger nucleus, understandably to accommodate the 8n genome. Moreover, the male nucleus appears to have an unusual chromatin organisation which is large and loosely stained. We have discovered this unique chromatin topology is regulated by a key post-translation modification, ubiquitination. Ubiquitination of proteins acts as signals that have multiple effects from targeting substrate proteins for degradation to modulating their function, localisation and activity. Reversible ubiquitination is catalysed by the action of both writers (E3-ligases) and erasers (deubiquitinases). We have found an essential role for a deubiquitinase, USP1 during two crucial stages of sexual development, resulting in a complete block in parasite transmission to the mosquito. In the absence of USP1, male gametocytes show abnormal chromatin compaction and, in response to the mosquito environment, fail to replicate their DNA. Curiously, the activity of USP1 is not restricted to male gametocyte DNA or nuclear homeostasis. Although USP1 deficient females are capable of fertilization, the fertilized cells do not undergo subsequent meiotic DNA replication implying USP1 activity orchestrates DNA replication dynamics in both sexes. Our proteomic studies reveal that USP1 interacts with the DNA replication and chromatin modification machinery and we are now in the process of identifying the molecular effectors of USP1. Currently we are building a clearer picture of how USP1-mediated dynamic protein ubiquitination regulates the unique biology of *Plasmodium* gametocytes, which could lead to new transmission blocking strategies.

Poster 200 : Detecting *Trypanosoma brucei* daughter cell asymmetries genome-wide using an accessible TrypTag python toolkit

Presenter: **Dr Richard Wheeler**, *University of Oxford*

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TrypTag has great potential as a disruptive resource for discovery biology. It comprises 65,474 images containing 4,584,261 cells across 12,459 cell lines which map subcellular protein localisation genome-wide in the *Trypanosoma brucei* parasite procyclic form. TrypTag is the first genome-wide protein subcellular localisation dataset for a parasite, for a flagellate and for a eukaryote outside of animal/fungi. This makes it a powerful resource for understanding trypanosomatid pathogen biology, evolution of parasitism, flagellar biology and eukaryotic diversity. However, to do so, effective data access is needed. Here, we describe a python module for seamless access to TrypTag data. It automatically handles fetching and caching localisation and microscopy data, massively simplifying access. A localisation search is simply "result = tryptag.localisation_search('paraflagellar rod')", while loading a field of view of microscopy data is "images = tryptag.open_field('Tb927.7.1920', 'n')". All data is loaded directly from the permanent public Zenodo data depositions supporting our 2023 TrypTag main paper. This easy data access is supported by trypanosome-specific image analysis tools based on our previous work. *Trypanosoma brucei* famously has asymmetries in division, even during normal proliferation, where single copy organelles which have duplicated ready for division have differing protein compositions. However fundamental questions remain unanswered, for example are the two daughter nuclei identical or is there an ‘old’ and a ‘new’ nucleus with differing protein composition? This is extremely difficult to identify using any information other than protein localisation. As an example of the power of this python toolkit, we demonstrate a quantitative and statistically-supported high-throughput search for such

asymmetrically distributed proteins – all in <50 lines of code. This is just one example of the enormous number of possible analyses, exploiting the highly organised nature of the *T. brucei* cell, that this python module enables.

Poster 201 : RNA editing in non-model kinetoplastids

Presenter: **Prof Vyacheslav Yurchenko**, *Professor, University of Ostrava*

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The kinetoplastids are unicellular flagellates that derive their name from the 'kinetoplast', a region within their single mitochondrion harboring its organellar genome of high DNA content, called kinetoplast (k) DNA. Some protein products of this mitochondrial genome are encoded as cryptogenes; their transcripts require editing to generate an open reading frame. This happens through RNA editing, whereby small regulatory guide (g)RNAs direct the proper insertion and deletion of one or more uridines at each editing site within specific transcript regions. An accurate perspective of the kDNA expansion and evolution of their unique uridine insertion/deletion editing across kinetoplastids has been difficult to achieve. Here, we resolved the kDNA structure and editing patterns in the early-branching kinetoplastid *Trypanoplasma borreli* and compare them with those of the well-studied trypanosomatids. We find that its kDNA consists of circular molecules of about 42 kb that harbor the rRNA and protein-coding genes, and 17 different contigs of approximately 70 kb carrying an average of 23 putative gRNA loci per contig. These contigs may be linear molecules, as they contain repetitive termini. Our analysis uncovered a putative gRNA population with unique length and sequence parameters that is massive relative to the editing needs of this parasite. We validated or determined the sequence identity of four edited mRNAs, including one coding for ATP synthase 6 that was previously thought to be missing. We utilized computational methods to show that the *T. borreli* transcriptome includes a substantial number of transcripts with inconsistent editing patterns, apparently products of non-canonical editing. This species utilizes the most extensive uridine deletion compared to other studied kinetoplastids to enforce amino acid conservation of cryptogene products, although insertions still remain more frequent. Finally, in three tested mitochondrial transcriptomes of kinetoplastids, uridine deletions are more common in the raw mitochondrial reads than aligned to the fully edited, translationally competent mRNAs. We conclude that the organization of kDNA across known kinetoplastids represents variations on partitioned coding and repetitive regions of circular molecules encoding mRNAs and rRNAs, while gRNA loci are positioned on a highly unstable population of molecules that differ in relative abundance across strains. Likewise, while all kinetoplastids possess conserved machinery performing RNA editing of the uridine insertion/deletion type, its output parameters are species-specific.

Poster 202* : Spatiotemporal patterns of cutaneous leishmaniasis in the district Dera Ismail Khan, Khyber Pakhtunkhwa, Pakistan

Presenter: **Dr Kiran Afshan**, *Assistant Professor, Quaid-i-Azam University, Islamabad*

K Afshan*A Mansoor*[1]*; S Firasat*[1]*;*

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Background: Leishmaniasis is a parasitic infection that affects people in tropical and temperate regions. It is spread by the biting of sand flies, particularly *PhelotoMus* and *Lutzomyia*. Sindh, Punjab, Baluchistan, and Khyber Pakhtunkhwa (KP) have higher rates of Cutaneous *Leishmania* (CL). Environmental variables influencing CL endemic locations in Pakistan are not understood clearly. The current study was aimed to determine the prevalence of cutaneous *Leishmania* and to develop risk map for predicting CL distribution in Khyber Pakhtunkhwa, Pakistan.

Material and Methods: A total of 1135 clinically verified subsequent cases of cutaneous leishmaniasis from January 2019 to March 2022 were included in this investigation. Using fine needle aspiration, the diagnosis was validated. Environmental and clinical data from DHQ D.I.K and other local CL centers were collected. By mapping the data using ArcGIS version 10.8 and Google Earth Pro version 7.3.0, the spatiotemporal prevalence of CL infection was examined.

Results: Cutaneous leishmaniasis was recorded highest 65% among individuals aged under 30 years. The male cases were high 61.7% than females 38.3%. Early and late lesions were classified from less than two months to more than a year, highest lesion duration between 1-2

months was 57.1%. A total of 1204 lesions, out of these 76.1% individuals fall in category of single lesion while 23.9% fall in multiple lesions. Most of the lesions seen on exposed body areas such as lower extremity was 34.2%, face 30.7%, upper extremity 29.1% and a least number of mixed lesions was 6.1%. Lesions were of nodular and ulcer type. In spatiotemporal analysis, a total of 989 CL cases were recorded in all villages. A choropleth map showed that Tehsil-wise increased incidence of CL at D.I.K with reported cases 63% followed by Paharpur 10%, Paroa 7%, Kulachi 6% and Daraban 2%. An elevation map of the average incidence of CL plotted on DEM, showed that high altitudes have a lower prevalence of CL. The future epidemic threats of CL infection were predicted by IDW map and D.I.K, Kulachi and Paharpur were CL risk areas. The high cluster counties according to the cluster and outliers study were D.I.K and Paharpur ranging from 42750.0 m (z-score= 1.8, P=0.071397) to 51300.0 m (z-score= 1.4, P=0.142513) and Kulachi, Daraban and Paroa ranging from 17100.0 m (z-score= 1.0, P=0.273971), 25650.0 m (z-score= 1.4, P=0.150079) and 34200.0 m (z-score= 0.9, P=0.318580) respectively, were the high and low outlier villages.

Conclusion: Leishmaniasis is prevalence was high among the local population, but a temporal increasing pattern was seen in the tehsil D.I.K. and Paharpur, which suggests a potential danger for the spread of CL. For disease prevention and management at the individual and community levels, the area needs to get the right attention.

Poster 203* : Companion animals as reservoirs and sentinels for zoonotic helminths in selected rural communities in Caraga Region, the Philippines

Presenter: **Allen Jethro Alonte**, *Student, Institute of Biological Sciences, University of the Philippines Los Banos*

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The growing population of companion animals as well as their close interaction with humans contributes to zoonotic transmission and persisting endemicity of intestinal helminths in poor and developing countries, such as the Philippines. Thus, this research focused on the role of dogs and cats as reservoirs and sentinels of intestinal helminths in selected communities in Mindanao, the Philippines. To our knowledge, this study is the first to report intestinal parasitism in dogs and cats in the region, practically serving as baseline data providing a foundation for research-based policy recommendations for responsible animal ownership and integrated control and elimination of parasitic diseases in endemic rural communities. A total of 135 dog and 33 cat fecal samples from 120 households were collected and processed using the simple sedimentation and modified McMaster techniques as well as multiplex real-time PCR. Surveys were also conducted to document animal care practices of companion animal owners. The results of the study showed high prevalence of diverse helminth species and evidence of multiple infection among companion animals in the selected study sites, confirming that they are important reservoir hosts of intestinal helminths. Among these, hookwor were found to have the highest prevalence with rates reaching up to 70.6% followed by *Toxocara* spp. at 33.3%. Furthermore, results of the multiplex real-time PCR allowed species identification of the zoonotic helminths showing possible cross transmission of parasites to non-native host. As seen in the case of *N. americanus*, where it was detected among dogs although it is widely known that humans are its definitive host, *T. canis*, an ascarid of dog, was also seen in cat; while *T. cati*, an ascarid of cat, was seen in dog. Findings of the study also suggested low awareness of owners in the role of animals in the continuing endemicity of intestinal helminths. This is evident in the animal care practices of the owner. The findings of the study showed the importance of dogs and cats as reservoirs and sentinels for a wide range of intestinal parasites, suggesting their major role in the zoonotic transmission of intestinal parasites. It also highlights the need for veterinary public health measures in the country to address the gaps in intestinal helminth control. The status of intestinal helminthiasis in companion animals stresses the need for an integrated approach to accelerate control and elimination of intestinal helminthiasis in the Philippines.

Poster 204 : *Leishmania braziliensis* Protein arginine methyltransferases 1 and 3 are mutually dependent for activity

Presenter: **Dr Pegine Walrad**, *University of York*

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Gene expression is carefully controlled in response to environmental stimuli in *Leishmania*. Post-translational modifications (PTMs) have important roles in regulating functions of proteins that carefully control gene expression. Arginine methylation is one such PTM, catalysed by protein arginine methyltransferases (PRMTs). We biochemically characterised PRMT homologs in *Leishmania braziliensis* : PRMT1, 3, 5, 6 and 7. We recombinantly expressed and purified each of these enzymes and assayed against substrates rich in arginine residues to identify intrinsic methyltransferase activity. *Lbr*PRMT5 and 7 demonstrate strongest activity on an RBP16$^{102\text{-}119}$ peptide and *Lbr*PRMT7 has broader substrate activity. K_m data measured confirm *Lbr*PRMT7 has highest affinity for the RBP16-derived peptide. *Lbr*PRMT6 demonstrated no activity at all against the substrates tested. Individually, *Lbr*PRMT1 and 3 were inactive against all the peptide substrates tested. Gel filtration data clearly show *Lbr*PRMT1:3 form a hetero-tetrameric complex in solution. This complex was confirmed and characterised via cryo-EM and incubating peptide substrates with *Lbr*PRMT1 and 3 together demonstrates strong methyltransferase activity. Methyltransferase assays with catalytically dead double E loop mutants of *Lbr*PRMT1 and 3 demonstrate *Lbr*PRMT1 is the active component of the complex. Finally, we assess activities of the *Lbr*PRMTs at different temperatures. We show that temperature affects each *Lbr*PRMT in a substrate-specific manner. Combined, our data indicate that *Lbr*PRMT1:3 has a very similar paradigm to *Tb*PRMT1^{ENZ}:1^{PRO}, however the retention of a complete double E loop in *Lbr*PRMT3 raises questions about the activity of the *Lbr*PRMT1:3 complex. Therefore, *Lbr*PRMT3 retaining the second glutamate of the double E loop may catalyse an alternative target or serve an unknown function. Moreover, our temperature assay data provides insight into the activities of *Lbr*PRMTs that suggests biologically-relevant, as yet uncharacterised layers of regulation.

Poster 205* : Novel morphological data of *Allopodocotyle pedicellata* (Stossich, 1887) Pritchard 1966 (Platyhelminthes: Digenea) from *Sparus aurata Linnaeus*, 1758 (Teleostei) off the Algerian coast; the first record

Presenter: **Fatima Zohra Zedam**, *Phd student, USTHB*

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Our Study, based at the Laboratory Biodiversity and Environment ai to significantly increase the number of digeneans species known from Algeria. As part of a continuing effort to explore the diversity of Digenea flatworms' parasites of fishes off Algeria, 120specimens of *Sparus aurata* Linnaeus, 1758 collected off Algeria, southwestern Mediterranean. The digestive tube was carefully examined for the presence of parasitic Digeneans. *Allopodocotyle pedicellata* Stossich, 1887 belongs to the family *Opecoelidae* Ozaki, 1925, a taxonomic study of this species based on morphological data. Our specimens were described here and compared morphologically and metrically to the species description by Bartoli et al, 1989. *Allopodocotyle pedicellata* Stossich, 1887 was found for the first found in the intestine of the type host S. aurata from Trieste, Adriatic Sea, we found it in the same host species for the first records species off the Algeria coast. Keywords: Platyhelminthes, Digenea, Opecoelidae, Allopodocotyle pedicellata, Sparus aurata. Mediterranean.

Poster 206 : TUSK: A ubiquitin hydrolase complex modulating surface protein abundance in trypanosomes

Presenter: **Prof Mark Field**, *Professor, University of Dundee*

MC Field[1];

[1] University of Dundee, UK

Control of protein levels is vital to cellular homeostasis, for maintaining a steady state, to coordinate changes during differentiation and other roles. In African trypanosomes surface proteins contribute to immune evasion, drug sensitivity and environmental sensing. The trypanosome surface is dominated by the GPI-anchored variant surface glycoprotein, but additional GPI-anchored and trans-membrane domain proteins are present with known roles as nutrient receptors and signal transducers. The evolutionarily conserved deubiquitinase orthologs of Usp7 and Vdu1

in trypanosomes modulate abundance of many surface proteins, including the invariant surface glycoproteins, which have roles in immune evasion and drug sensitivity. Here we identify multiple trypanosome Skp1 paralogs and specifically a divergent paralog SkpZ. Affinity isolation and LCMS indicates that SkpZ for a heterotrimeric complex with TbUsp7 and TbTpr86, a tetratricopeptide-repeat protein. Silencing SkpZ decreases TbUsp7 and TbTpr86 abundance, confirming a direct association. Further, SkpZ knockdown decreases the abundance of multiple trans-membrane domain (TMD) proteins but increases GPI- anchored surface protein levels. Hence, a heterotrimeric complex of TbTpr86, TbUsp7 and SkpZ (TUSK) regulates expression levels of a significant cohort of trypanosome surface proteins mediating coordination between TMD and GPI-anchored protein expression levels.

Poster 207 : Histone divergence in *Trypanosoma brucei* results in unique alterations to nucleosome structure

Presenter: **Gauri Deak**, *PhD Student, University of Edinburgh*

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Trypanosoma brucei is a parasitic kinetoplastid that causes severe disease in both humans and livestock animals. Contrary to model eukaryotes, histone sequences in *T. brucei* are highly divergent. However, the structural and functional consequences of their variation on chromatin-based processes are unknown. We determined the cryo-EM structure of the *T. brucei* nucleosome core particle (NCP). Intriguingly, the histone fold architecture of the NCP is mostly conserved but specific sequence alterations lead to distinct DNA and protein interaction interfaces. The *T. brucei* NCP is unstable and has weakened binding to entry/exit nucleosome DNA but exhibits a novel compensation mechanism via its H2A-H2B dimer interface. The acidic patch in *T. brucei* has altered topology and is refractory to known binders, indicating that critical chromatin interactions may be altered in this parasite. Phylogenetic analysis and modelling reveal that a majority of our findings are also conserved in other pathogenic Kinetoplastids, opening avenues for multi-target drug discovery. Overall, our results provide a detailed molecular basis for understanding Kinetoplastid chromatin structure and regulation at the mononucleosome level.

Poster 208 : From genome to function: The first global genomic analysis of *P. malariae* parasites provides evidence of pyrimethamine resistance

Presenter: **Dr Amy Ibrahim**, *London School of Hygiene and Tropical Medicine*

A Ibrahim[2]*; F Mohring*[2]*; E Manko*[2]*; D Van Schalkwyk*[2]*; J Phelan*[2]*; D Nolder*[2]*; S Borrmann*[4]*; SM Di Santi*[1]*; F Nosten*[3]*; CJ Sutherland*[2]*; RM Moon*[2]*; T Clark*[2]*; S Campino*[2]*;*
[1] *University of Sao Paulo, UK;* [2] *London School of Hygiene and Tropical Medicine, UK;* [3] *Shoklo Malaria Research Unit, Thailand;* [4] *University of Tübingen, UK*

Malaria caused by *Plasmodium malariae* is a neglected tropical disease with a wide geographic distribution, contributing up to 40% of cases in regions of Africa and South America. This parasite species has unique biological traits, including a quartan life cycle, an ability to persist in the blood causing chronic infections, and associations with severe disease including nephropathologies and anaemia. These unique features may underly the observed reduced parasite drug response and drive malaria recurrence, representing a challenge for disease elimination. To provide insights into *P. malariae* biology, we explore the genomic diversity of this parasite across 4 endemic regions, spanning 25 countries. Through selective whole genome amplification, we obtain whole genome sequence data for 235 clinical isolates of *P. malariae* and identify 1,288,675 genome-wide SNPs, filtered to 143,201 high-quality SNPs within the core genome. We determine population structure, demonstrating a clear separation in parasites from Africa and Asia, and shared ancestry between South American and African parasites. We identify signals of selection in genes associated with the human immune response (pmmsp3) in addition to orthologs of genes associated with artemisinin-induced latency (pmeIF2a). We identify mutations within orthologs of genes associated with drug susceptibility, including amino acid substitutions in the DHFR-TS, CRT, MDR2, K13, CORONIN, PI4K, MRP1 and UBP1 proteins. We investigate the DHFR protein further and find mutations which align with known resistance mutations in the *P. falciparum* ortholog and use this as a candidate for further functional analysis. Due to the lack of a stable culture method for *P. malariae*, we use orthologue replacement in *P. knowlesi* parasites at the DHFR locus to investigate pyrimethamine susceptibility in the *P. malariae* genotypes described. We demonstrate a *P. malariae* genotype at the DHFR locus which reduces

pyrimethamine susceptibility in comparison to the *P. knowlesi* genotype, in addition to known control phenotypes validating the approach for drug susceptibility assays. Our study provides the first evaluation of *P. malariae* population structure and creates a valuable resource for elucidating important insights into the biology and pathogenesis of this neglected pathogen.

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Maps and Venues

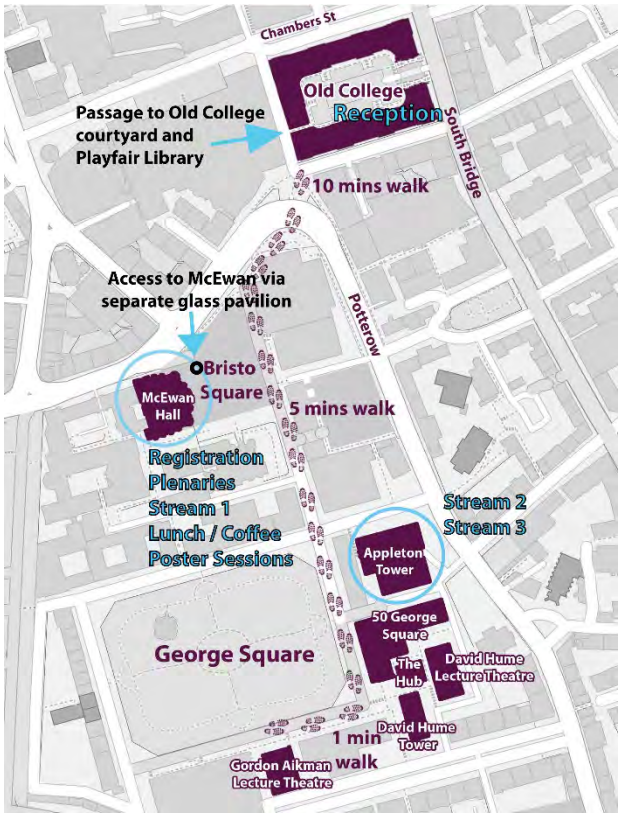
Registration on all days: **McEwan Hall** (Map 1). Access to McEwan Hall is via the round glass pavilion on Bristo Square.

Reception Tuesday: **The Playfair Library** is located in the **Old College** (5 mins walk from McEwan Hall). Enter the courtyard through a passage from the west (Map 1).

Meeting Venues are:

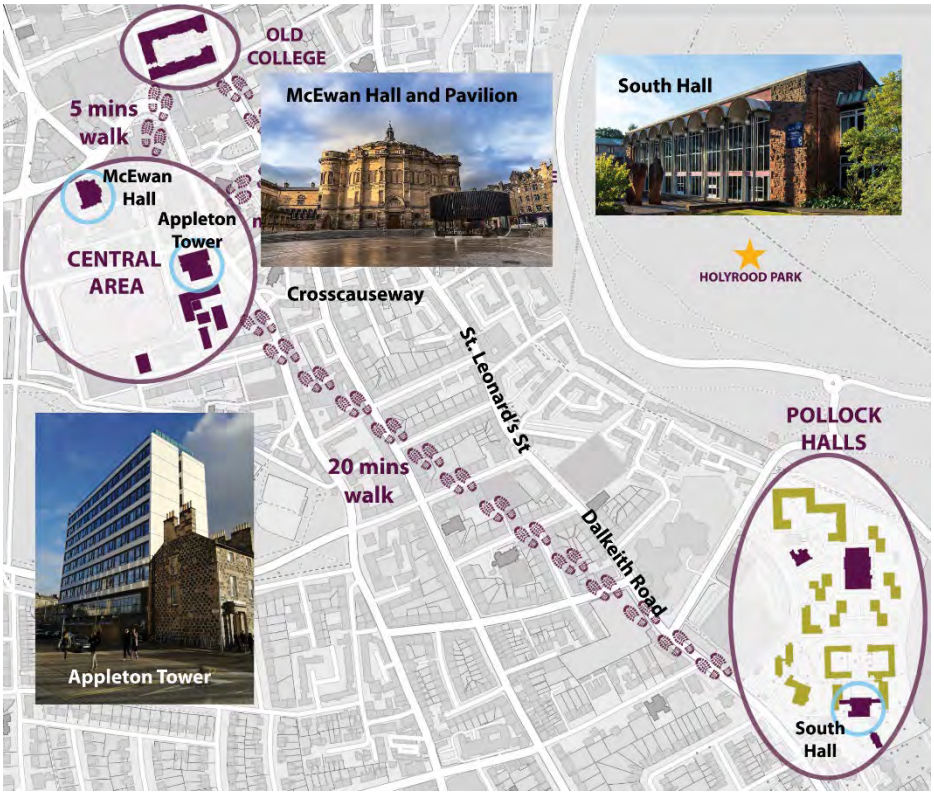
McEwan Hall, location of plenary sessions, stream one (Protists) and lunches, teas and coffees, along with the exhibitors and posters.

Appleton Tower, location of streams two and three. It is a short walk between the two and should talk just a couple of minutes.

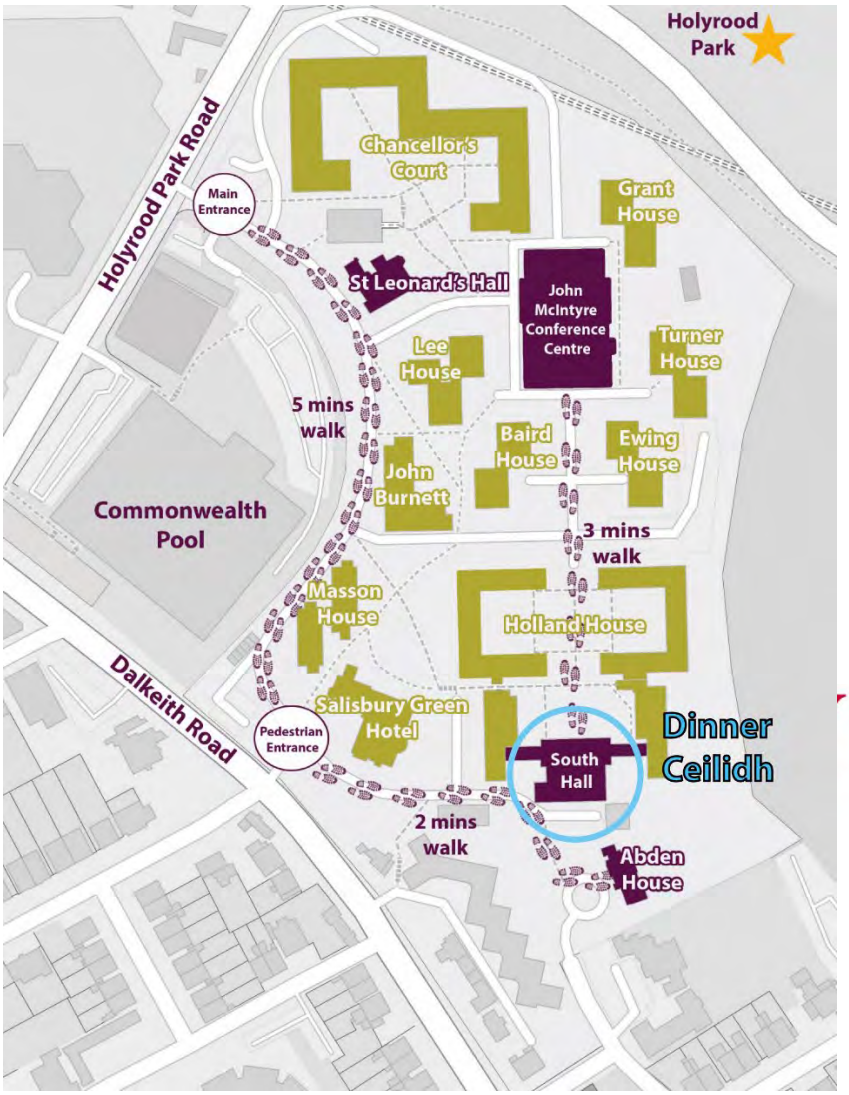


Map 1 – Central Campus

The **Conference dinner and ceilidh** are held at **South Hall** in **Pollock Halls**, which is about 20 mins walk from the Central Campus area (Map 2). Follow West and East Cross causeway, turn south on St. Leonards St, and continue on Dalkeith Road to the Pedestrian Entrance to the Pollock Halls area (Map 3).



Map 2 – Overview of venues and directions to South Hall / Pollock Halls



Map 3 – Pollock Halls and South Hall

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