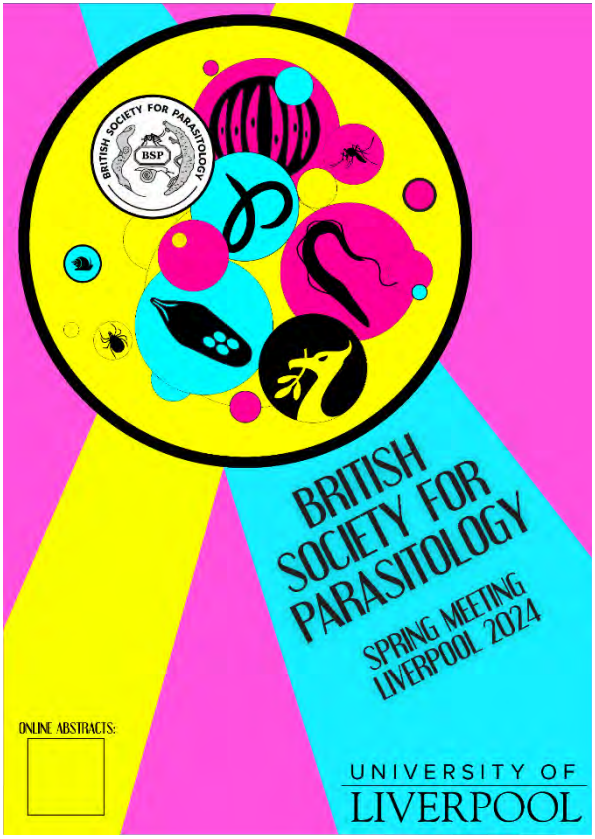


Go to Contents

Go to the Index

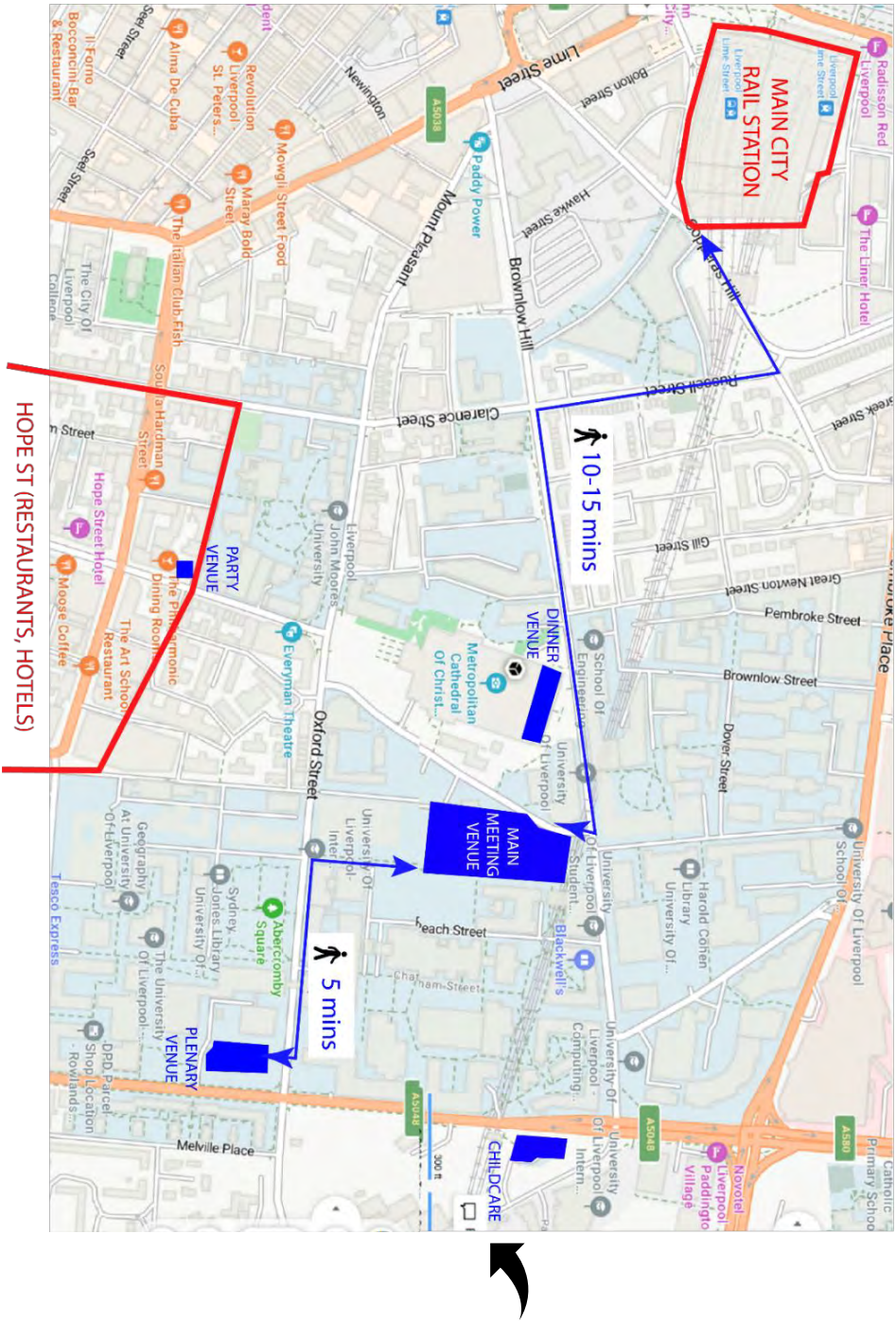
Go to the timetable

Go to the venue map

Please note there are significant changes between the printed version and this version of the abstract book. The programme was changed due to a national rail strike and sessions were moved to allow the meeting to close a day earlier.

This is the definitive version of the BSP Spring Meeting, Liverpool April 2-4 2024, abstract book.

Map of the Local area



A family-run business since 1967

APPLETON WOODS LTD

We're more than just a supplier!

- Family-run, independent business since 1967
- Superior technical support from a team of scientists
- Own van door to door delivery service
- ISO 14001 and ISO 9001 registered
- Look out for this logo for our sustainable products
- Dedicated customer service representative for each account
- Comprehensive range of quality equipment, consumables and life science reagents
- Own-branded 'appleton' equipment, consumables and reagents
- Competitive pricing available on various e-procurement catalogues
- Appleton promise, 2-year warranty on all benchtop equipment

Proud to be a supplier on the following Frameworks...

SUPC Southern Universities Purchasing Consortium

Inter-Regional Laboratory Agreement (IRLA/SUPC)

LONDON UNIVERSITIES PURCHASING CONSORTIUM

UKSBS Shared Business Services

UK Shared Business Services (UKSBS) for Equipment and Consumables

APUC

Advanced Procurement for Universities & Colleges

www.appletonwoods.co.uk info@appletonwoods.co.uk 0121 458 7740

BIO-RAD

BSP Spring Meeting – 2nd-5th April 2024 - University of Liverpool

COME AND TALK TO US ABOUT ddPCR AND MORE

DROPLET DIGITAL PCR™ (ddPCR)

Droplet Digital PCR (ddPCR) is an innovative technology that provides ultrasensitive nucleic acid detection and absolute quantification. It is effective in providing high-precision nucleic acid quantification in areas such as:

- Environmental Monitoring
- Rare Mutation Detection
- Low Level Gene Expression
- Gene Editing and QC in C&GT

SOME OF OUR TECHNOLOGIES:

ddPCR Systems & Assays

Flow Cytometers & Antibodies

Western Blotting Systems & Reagents

PCR/qPCR Systems & Reagents

NEW ENGLAND Biolabs®

Lighting the way.™

Luna® Universal qPCR & RT-qPCR Products

Find the right Luna product for your application

1 Select your target	2 Select your detection method: Dye-based	Probe-based
Genomic DNA or cDNA	Luna® Universal qPCR Master Mix (NEB #M3003)	Luna Universal Probe qPCR Master Mix (NEB #M3004)*
Purified RNA — One-Step RT-qPCR	Luna Universal One-Step RT-qPCR Kit (NEB #E3005)	Luna Universal Probe One-Step RT-qPCR: • Kit (NEB #E3006)* • 4X Mix with UDG (NEB #M3019)* • LyoPrime Luna™ Probe One-Step RT-qPCR Mix with UDG (NEB #L4001)
Purified RNA — Two-Step RT-qPCR	LunaScript® RT SuperMix (NEB #E3010/M3010) + Luna Universal qPCR Master Mix (NEB #M3003)	LunaScript RT SuperMix (NEB #E3010/M3010) + Luna Universal Probe qPCR Master Mix (NEB #M3004)
RNA from cell lysate	Luna Cell Ready One-Step RT-qPCR Kit (NEB #E3030)	Luna Cell Ready Probe One-Step RT-qPCR Kit (NEB #E3031)

*No ROX version available (OEM)
For bulk or custom options, contact us at www.neb.com/CustomContactForm

Visit **LUNAqPCR.com** to request your sample today.

Micro Clarity

The Microscope Experts

We are a leading independent supplier of microscopes , cameras, software and training in the UK. Not only do we supply the latest technology but we also offer service and support to new, recent and 'legacy' microscopes. With over 45 years of experience we hesitate to call ourselves experts but feel we have a depth of knowledge and experience that enables us to provide a high level of support to our customers.

Whether it is the supply of new microscopes, instrument service/repair and training or just advice we are always happy to help.

We supply and support a wide range of microscopes covering all applications from simple teaching or field microscopes through to research grade instruments of all types (stereo or compound). Alongside this we offer a complete range of accessories such as microscopy cameras, LED illuminators, hot stages, Image capture and analysis software to name a few.

If you require on site servicing or repair we have field service engineers who can visit and carry out routine servicing or repairs. We have links with all of the major manufacturers so have access to spare parts if they are available.

A popular addition to our offering is basic maintenance and set up training, one or two day courses tailored to meet your requirements.

For more information please visit our website or contact us directly via the email listed below.

www.microclarity.co.uk

e: info@microclarity.co.uk

Our sponsors: -

New England BioLabs : https://www.neb.com/en-gb

Promega: https://www.promega.co.uk/

Zeiss and Appleton Woods: https://www.appletonwoods.co.uk/

Elsevier: https://www.elsevier.com/

MDPI: https://www.mdpi.com/

Biomolecular Systems: https://biomolecularsystems.com/

Scientific Laboratory Supplies: https://www.scientificlabs.co.uk/

Drugs for Neglected Diseases initiative: dndi@dndi.org

Bio-Rad Laboratories Ltd: https://www.bio-rad.com/en-uk/

Brand: https://www.brand.de/en/

Springer Nature (Parasites and Vectors): https://parasitesandvectors.biomedcentral.com/

Quadratech Diagnostics: https://www.quadratech.co.uk/

PCR Biosystems Ltd: https://pcrbio.com/

IVES University of Liverpool: https://www.liverpool.ac.uk/infection-veterinary-and-ecological-sciences/

Royal Society: https://royalsociety.org/journals/

Starlab: https://www.starlab.co.uk

Sarstedt: http://www.sarstedt.com/

LSTM: https://www.lstmed.ac.uk/

Micro Clarity: https://www.microclarity.co.uk/

Calibre: https://calibrescientific.com/en

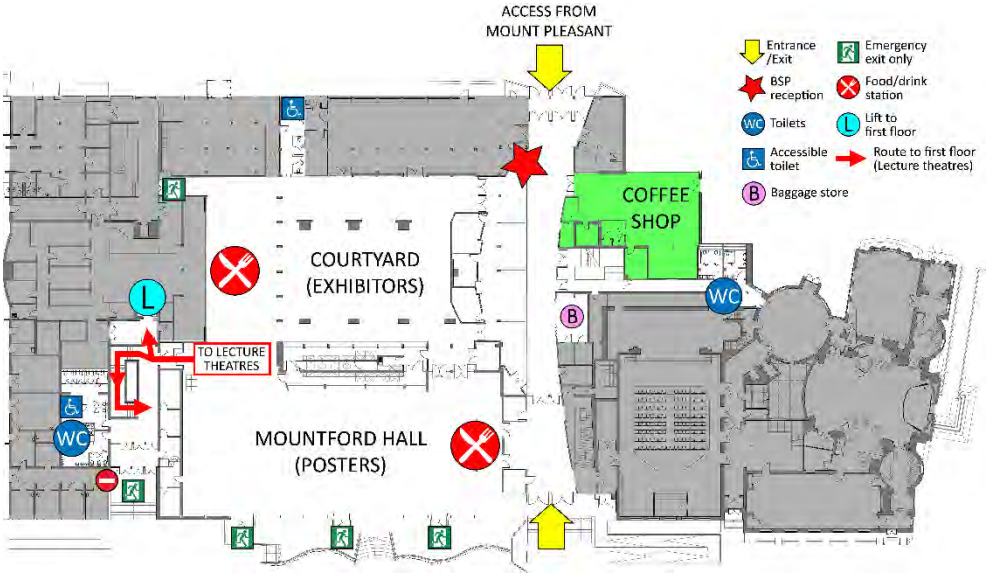
Sciontec: https://sciontec.co.uk/locations/liverpool-science-park/

PeerJ: https://peerj.com/

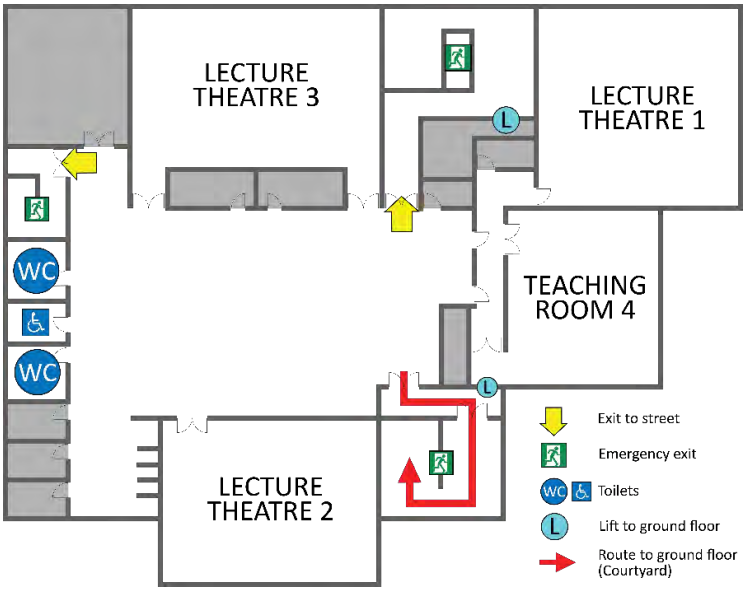
Life Tech and Fisher Scientfic: https://www.fishersci.co.uk/gb/en/home.html and www.thermofisher.com

The Venue

Ground floor, reception, food court and poster hall.



First floor Lecture theatres and break out area.



Welcome to the 2024 British Society for Parasitology Spring Meeting

Welcome to the City and University of Liverpool. We are delighted that you have joined us from across the UK and beyond for the annual festival of parasitology. Liverpool is a natural home for the Spring Meeting as the City, with its long history of global seafaring, has always been an international focus for parasitology. At the beginning of the twentieth century, pioneers like Sir Ronald Ross (1857-1932), who discovered that the mosquito is the malaria vector, Everett Dutton (1874-1905), who established trypanosomes as the cause of sleeping sickness, and Donald Blacklock (1879-1955) who discovered the vector for onchocerciasis, laid the basis for our modern science.

In recent times, parasitologists at both the University of Liverpool and Liverpool School of Tropical Medicine have continued to resolve parasite biology, for example, the role of neosporosis in bovine abortion, while developing novel therapeutic approaches, such as novel anthelmintics for lymphatic filariasis at LSTM and pen-side diagnostic tests for fasciolosis at the University of Liverpool.

The BSP Spring Meeting is always a special occasion, providing an essential forum for our community to share ideas and the latest discoveries. We all appreciate how important discussion is to our science, and so we are delighted to host the meeting, and indeed, to support the Society's missions to nurture young scientists, to grow parasitology internationally in those places that are the focus of parasitic diseases, and to foster conversations around the place of parasitology in society, and society in parasitology. To this end, our participants are drawn from 44 different countries, and over 40% of our speakers are early-career researchers.

Our participants can expect an engaging programme of speakers, presenting the most exciting and innovative parasitology across 24 sessions and three research streams, from fundamental work on 'Form, Function and Evolution', through 'Disease Complexity' to applied research in 'Control and Elimination'. The programme focuses on topics that cut across taxonomic boundaries, such as 'Organoid models' and 'Cellular Heterogeneity', so that experience in one part of our community can influence the broader field.

Besides talks, we also have training workshops in parasite database resources on Day 1, and poster exhibitions on Days 2 and 3. We are particularly pleased to welcome special guest speakers to our two lunchtime 'conversations' on equality, diversity and inclusion in parasitology and on development for early-career parasitologists. In the evenings, there is an entertaining social programme of drinks receptions (Days 1 and 2), the Young Parasitologists' Party (Day 2) and the Conference Dinner (Day 3) in the crypt of Liverpool Metropolitan Cathedral. Plus, we are situated in the heart of the University Campus, close to both the City Centre and the vibrant Hope Street quarter, where participants will find a lively mix of bars and restaurants.

We really hope that you enjoy your stay in Liverpool and wish you a fun and stimulating meeting.

The BSP 2024 Organising Committee

Content

Business information and Local Information.

Local Travel

Taxi

Ask to be collected on Mount Pleasant, Mountford Hall, L3 5TR

ComCab

0151 298 2222

https://comcab.co.uk/

One Call Taxis

0151 928 3535

https://onecalltaxis.com/

Alpha Taxis

0151 722 8888

https://alphataxis.co.uk/

Britannia Taxis

0151 708 7080

https://www.britanniataxis.co.uk/

A1 Taxis

0151 480 7777
https://a1-taxi.co.uk/

Bus

The meeting venue may be reached bus, see Merseytravel for details:
https://www.merseytravel.gov.uk/

Bus routes 7 and 79 travel from the City Centre to the University campus via Brownlow Hill and you should alight opposite the Guild of Student

Organising Committee and Session Organisers

Andrew Jackson, University of Liverpool
Ben Makepeace, University of Liverpool
Mark Viney, University of Liverpool
Jane Hodgkinson, University of Liverpool
Andy Fenton, University of Liverpool
Krystyna Cwiklinski, University of Liverpool
Al Darby, University of Liverpool
Catherine Hartley, University of Liverpool
James LaCourse, Liverpool School of Tropical Medicine
Russell Stothard, Liverpool School of Tropical Medicine
Alexandra Juhasz, Liverpool School of Tropical Medicine
Helen Price, Keele University

Volunteers

Sophie Park, University of Liverpool
Elly Lester, University of Liverpool
Jude Ogunmola, University of Liverpool
Isabelle Endacott, University of Liverpool
Emily Herschel-Kelly, University of Liverpool
Yuchen Liu, University of Liverpool
Nancy Shepherd, Liverpool School of Tropical Medicine
Shea Murray, Liverpool School of Tropical Medicine
Fatima Ahmed, Liverpool School of Tropical Medicine

Guilleary Deles, Liverpool School of Tropical Medicine
Lois Bent, Liverpool School of Tropical Medicine
Justin Jelason, Liverpool School of Tropical Medicine
Kimberley Muneno, York University
Ella Foreman, Liverpool School of Tropical Medicine
Laura Hill, Liverpool School of Tropical Medicin

Admin Support
Julian Fuller, Hazel Fuller & Cathy Fuller (Academic Events) info@academic-events.com
Abstract and Booking System – (Eventflo). Info@eventflo.com

Sponsorship team:
Dr Paul McCusker, Queen's University Belfast
John Archer, Liverpool School of Tropical Medicine and The Natural History Museum

The BSP Council
Prof J V Hamilton (President)
Prof Helen Price (Vice President)
Prof M Llewellyn (Hon Gen Sec)
Prof P Lamberton (Honorary Treasurer) Maternity leave
Dr J Pachebat (Temp Honorary Treasurer) Acting in role for maternity cover
Dr J LaCourse (Meeting Secretary)
Prof P Walrad (Communications Secretary)
Dr B J Power (Ordinary Member) Social media brief
Dr P McCusker (Ordinary Member) Sponsorship brief
Dr D Xia (Ordinary Member) Website brief
Dr C Tiengwe (Ordinary Member)
Dr Nicolas Pionnier (Ordinary Member)
Dr Emma Briggs (Ordinary Member) Membership brief
Dr Slava Yurchenko (Ordinary Member)
J Archer (Student Member)
Nada Abdalrahman (Student Member)
Isobel Gabain (Student Member)
Sophia DonVito (Student Member)

Becoming an active member of the BSP
To Join the BSP go to https://bsp.uk.net/Membership

Presentations

Information for Oral Presentations

Oral presentations need to be loaded to the rostrum PC at the break before your talk. 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

10 mins – 7-8 mins oral with 3-2 mins for questions.

Presentation Guidelines

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.

Information for Poster Presentations

Posters will be displayed in the Mountford 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). Velcro coins will be available on your board which will be numbered with your poster number.

The Full Programme

Day 1 - 2-April-2024

Workshops

Wormbase Parasite workshop part 1 - (PC Suite C, Hub502)
13:00 to 15:30

VEuPathDB workshop part 1- (PC Suite B, Hub502)
13:00 to 15:30

Break

Wormbase Parasite workshop part 2- (PC Suite C, Hub502)
16:00 to 18:00

VEuPathDB workshop part 2- (PC Suite B, Hub502)
16:00 to 18:00

Reception

Drinks (LGS Courtyard)
18:00 to 20:00

Day 2 – 3-April-2024

Plenary and Sessions

Plenary session 1 - (Brett Lecture Theatre) at 09:00 to 10:30
09:00 (35 mins) - Malaria Vaccines – at last! (Adrian Hill)
09:35 (35 mins) - Human African Trypanosomiasis - can we break the endemic, outbreak, epidemic cycle of infection? (Sue Welburn)

Drinks Break - (LGS courtyard)

(1) Drug development 1 - Sponsored by DnDi - (Lecture theatre 1) at 11:10 to 12:40
11:10 (25 mins) - Development of alternative treatments for filarial diseases (Sabine Specht)
11:30 (15 mins) - Pharmacological targeting of bioactive lipid production improves experimental lymphatic filariasis pathology (Shrilakshmi Hegde)
11:50 (15 mins) - Stem cell screening to identify novel drug targets in the human parasite *Schistosoma mansoni* (Sewwandi Perera)
12:05 (15 mins) - Development of azaquinazoline anti-*Wolbachia* drugs for veterinary zoonotic filariasis (Jessica Dagley)

12:20 (20 mins) Developing the natural product Corallopyronin A to treat filariasis (Kenneth Pfarr)

(2) Population genomics 1 - Sponsored by BioRad - (Lecture theatre 2) at 11:10 to 12:40

11:10 (25 mins) - Metabarcoding and targeted deep sequencing of parasitic nematode communities: applications and future directions. (John Gilleard)
11:35 (25 mins) - Finding a needle in a haystack: Genome-wide analyses of anthelmintic resistance in helminths of livestock (Roz Laing)
12:00 (15 mins) - *Strongyloides stercoralis* complex in humans and dogs: insights from population genomics in Asia (Yuchen Liu)
12:15 (25 mins) - Does hybridization amongst *Schistosoma* spp. matter? (Joanne Webster)

(3) Antigenic variation 1 - (Lecture theatre 3) at 11:10 to 12:40

11:10 (25 mins) - Antigenic variation at the macro and micro scale: how the African trypanosome coat structure helps it evade a highly diverse immune response (Nina Papavasiliou)
11:35 (25 mins) - A sub-nuclear *super-factory* for singular antigen expression: novel stage-specific regulators finetune expression at the active *VSG* expression-site. (Joana Correia Faria)
12:00 (15 mins) - RNA polymerase III is involved in regulating *Plasmodium falciparum* virulence (Gretchen Diffendall)
12:15 (25 mins) - Antigenic variation in *Babesia*: do we even know what we don't know? (David Allred)

(20) Parasite wildlife ecology – (Teaching room 4) at 11:10 to 12:40

11:10 (25 mins) - Nutritional supplementation has diverse impacts on the parasite community of wild mice (Amy Pedersen)
11:35 (20 mins) - Parasites in Plastic Environments (Jo Cable)
11:55 (15 mins) - Do woodland patch size and connectivity influence tick density, nymph infection prevalence and Lyme Disease hazard through impacts on key tick hosts? (Saudamini Venkatesan)
12:10 (15 mins) - Nematode co-infection dynamics: exploring variation in wild sheep (Mike Evans)
12:25 (15 mins) - Diversity of parasite communities in co-existing wild and domestic ungulates in Kenya (Kim van de Wiel)

Lunch - (LGS courtyard)

Equality, Diversity and Inclusion workshop - (PC Suite C, Hub502) at 13:00 to 14:00

13:00 (15 mins) - Wellcome's recent work on Equity, Diversity, and Inclusion (Diego Baptista)
13:15 (15 mins) - Equality, Diversity, and Inclusion at the University of Liverpool (Lesley Iwanejko)

(4) Drug development 2 – Sponsored by DnDi – (Lecture theatre 1) at 14:00 to 15:30

14:00 (25 mins) – Applying AI to anti-parasitic drug discovery (Manu De Rycker)
14:25 (20 mins) - Structural insight into the apicomplexan drug target cytochrome bc1 (Andrew Maclean)
14:45 (15 mins) – Non-natural myristate analogues: Synthesis and biochemical characterization of their activity in protozoan parasites (Rachel Humann)

15:00 (15 mins) – Histone modifying enzyme (HME) inhibitors demonstrate anthelmintic activity against *Fasciola hepatica*. (Sarah Davey)
15:15 (15 mins) – PfGCN5 bromodomain, a novel drug target (Mukul Rawat)

(5) Population genomics 2 - (Lecture theatre 2) at 14:00 to 15:30

14:00 (25 mins) - Evolutive Dynamics and Biological Interplay of *Leishmania (Viannia)* Species with Their Endosymbiotic Partner, Leishmania RNA Virus 1 (Elisa Cupolillo)
14:25 (15 mins) - Whole genome sequencing of *Leishmania* species causing Cutaneous Leishmaniasis in South America (Cián Lynch)
14:40 (15 mins) - Whole genome sequencing of *Leishmania braziliensis* in clinical samples demonstrates long-term recurrent recombination in a population of parasites from Southern Peru (Jean-Claude Dujardin)
14:55 (15 mins) - The dispersal of visceral leishmaniasis during the peak of the Roman Empire. (Daniel Jeffares)
15:10 (20 mins) - Genomic epidemiology of *Trypanosoma cruzi* and its vectors (Martin Llewellyn)

(6) Antigenic variation 2 - (Lecture theatre 3) at 14:00 to 15:30

14:00 (20 mins) - Mechanisms of *Pneumocystis jirovecii* surface antigenic variation (Philippe Hauser)
14:20 (25 mins) - High-resolution scRNA-seq reveals genomic determinants of antigen expression hierarchy in African Trypanosomes (Nicolai Siegel)
14:45 (20 mins) - A *Plasmodium*-specific AP2-P regulates multiple pathogenicity factors during the IDC (Arnab Pain)
15:05 (25 mins) - Challenging the Paradigm of Mutually Exclusive *var* Gene Expression for Antigenic Variation by *Plasmodium falciparum* (Kirk Deitsch)

(22) Innovations in vector control - (Teaching room 4) at 14:00 to 15:30

14:00 (25 mins) - Tiny Targets accelerate progress towards the elimination of sleeping sickness. (Andrew Hope)
14:25 (25 mins) - Vector control in response to the changing malaria landscape (Eric Ochomo)
14:50 (15 mins) - Monitoring biological age in mosquitoes using infrared spectroscopy (Mauro Pazmino)
15:05 (25 mins) - HumBug – developing an acoustic sensor to detect and identify mosquito vectors of disease. (Marianne Sinka)

Drinks Break – (LGS courtyard)

(7) Disease elimination – Sponsored by New England Biolabs - (Lecture theatre 1) at 16:00 to 18:00

16:00 (25 mins) – Elimination of Visceral Leishmaniasis from India (Shyam Sundar)
16:25 (20 mins) – ECLIPSE: Improving access to healthcare for cutaneous leishmaniasis in Brazil, Ethiopia and Sri Lanka (Helen Price)
16:45 (20 mins) - The elimination of lymphatic filariasis in Ghana: reality or pipeline dream (Dziedzom Komi de Souza)
17:05 (20 mins) - Schistosomes and how to find them – testing novel methods of *Schistosoma mansoni* molecular environmental monitoring in Lake Albert (Dr Zikmund Bartonicek)

17:25 (20 mins)– Community and Individual Preferences for a New Water Infrastructure for Non-Drinking Water in a Schistosomiasis Endemic Area (Raheema Chunara)
17:45 (15 mins)– Caught in a trap: DNA contamination in tsetse xenomonitoring can lead to over-estimates of *Trypanosoma brucei* infection (Isabel Saldanha)

(8) Organoid models - Sponsored by Promega - (Lecture theatre 2) at 16:00 to 18:00

16:00 (25 mins) - Small but mighty! – Using primary organoids to model host responses to pathogens in the lab (David Smith)
16:25 (25 mins) - Caecaloids, imaging and transcriptomics to unravel the whipworm niche at the host intestinal epithelia (Maria Duque-Correa)
16:50 (25 mins) - Intestinal organoid models for studying *Cryptosporidium* (Mattie Pawlowic)
17:15 (15 mins) - Utilizing equine enteroid-derived monolayers for studying parasitic intestinal nematode infection (Eva Tydén)
17:30 (15 mins) - Developmental biology of *Fasciola hepatica*: 3D co-culture using HepG2 spheroids to create mini-livers allows investigation of host-pathogen interactions (Aiste Vitkauskaite)
17:45 (15 mins) - A microRNA in the excretory-secretory products of helminth parasites induces dedifferentiation in epithelial cells within gastrointestinal organoids. (Matias Perez)

(9) Origins of parasitism - (Lecture theatre 3) At 16:00 to 18:00

16:00 (25 mins) - Gregarine apicomplexans as model systems to better understand the evolution of parasitism in the phylum Apicomplexa (Sonja Rueckert)
16:25 (20 mins) - Not so picky Colpodellids: Novel diversity of free-living bacterivorous colpodellids, relatives of Apicomplexa (Martin Kolisko)
16:45 (20 mins) - Using genome data to understand the diversification in microsporidia in invertebrates (Bryony Williams)
17:05 (15 mins) - Comparative analysis of trypanosomatids from the genera *Blastocrithidia* and *Obscuromonas* with non-canonical and standard genetic codes (Julius Lukes)
17:20 (15 mins) - Ancestral aneuploidy and stable chromosomal duplication resulting in differential genome structure and gene expression control. The case of Trypanosomatid parasites (Joao Cunha)
17:35 (25 mins) - Next-generation sequencing to decipher genome evolution of microscopic parasites: insights from Myxozoa (Cnidaria) (Dorothee Huchon)

(21) Molecular and cellular biology 1 - (Teaching room 4) at 16:00 to 18:00

16:00 (15 mins) - The *Toxoplasma gondii* mitoribosome reveals novel features of ribosome evolution and exciting differences from human mitoribosomes (Lilach Sheiner)
16:15 (15 mins) - Structural and functional insights into ESAG3 and GRESAG3 proteins in African trypanosomes (Calvin Tiengwe)
16:30 (15 mins) - Revisiting trypanosome transferrin receptor: unveiling novel insights in localization and ligand uptake (Sourav Banerjee)
16:45 (15 mins) - Diverse functions of SHIPPO-domain proteins in flagellar assembly (Samuel Dean)
17:00 (15 mins) - The Lectin Pathway of Complement Regulation by the infectious *Fasciola hepatica* newly excysted juvenile (NEJs) (Carolina De Marco Verissimo)
17:15 (15 mins) - Nanopore sequencing-based deep learning reveals the complete DNA replication landscape in *Leishmania* and its connection with genome variability. (Jeziel Dener Damasceno)

Posters – (LGS Mountford hall) at 18:00 to 19:30

Young Parasitologists Party (Frederiks Bar) at 19:30

Day 3 – 4-April-2024

Sessions and Plenary

(10) Helminth epidemiology 1 - Sponsored by Quadratech Diagnostic - (Lecture theatre 1) at 09:00 to 10:30

09:00 (25 mins) - The Lawa model: An integrated liver fluke control program using One Health approach (Banchob Sripa)
09:25 (20 mins) – Characterization of morbidity profiles in a context of hybridization and co-infection of human urogenital *Schistosoma haematobium* with livestock and human intestinal *Schistosoma* species in Senegal (Cheikh Fall)
09:45 (15 mins) – Can many biomarkers make light work of ovine fasciolosis diagnostics? (Christy Wray)
10:00 (15 mins) - The ABCs of liver fluke: Predicting the efficacy of Augmented Biological Control against *Fasciola hepatica* using an agent-based model (Daniel McDowell)
10:15 (15 mins) - Malaria and schistosomiasis surveillance prior to the implementation of a large-scale irrigation scheme reveals potential for future transmission (Rex Mbewe)

(11) Parasite-immune interactions 1 - (Lecture theatre 2) at 09:00 to 10:30

09:00 (25 mins) - Immunopathology of leishmaniasis: a spatial perspective on the regulation of immune checkpoint molecules. (Paul Kaye)
09:25 (15 mins) - IL-17 producing T cells in the control of skin inflammation and subcutaneous adipose wasting during chronic *Trypanosoma brucei* infection (Matthew Sinton)
09:40 (10 mins) - Understanding Trypanosome Lytic Factor biogenesis through human serum, tissue culture, and murine models (Sara Fresard)
09:50 (20 mins) The interplay between salivarian trypanosomes, host B cells, and neutrophils demonstrates the capacity of the parasite to evade immune defences (Magdalena Radwanska)
10:10 (20 mins) - Trypanosomatid virulence factors and new perspectives in vaccine development for leishmaniasis and Chagas disease (Santuza Maria Teixeira)

(12) Cellular heterogeneity - (Lecture theatre 3) at 09:00 to 10:30

09:00 (25 mins) - Drugs, sex, and schistosomes: control of female schistosome sexual development by a male-derived non-ribosomal peptide pheromone (Jim Collins)
09:25 (20 mins) - From whole worm to single cells: using transcriptomics to understand how a gastrointestinal nematode thrives in its host. (James Wasmuth)
09:45 (15 mins) - Capturing motile cells poses challenges to microfluidic encapsulation in scRNAseq (Lara Lopez Escobar)
10:00 (15 mins) - Schistosomes – old questions, new technologies (Gabriel Rinaldi)
10:15 (15 mins) - Within-host population dynamics of *Trypanosoma brucei* infections (Fatima Taha)

(24) Molecular and cellular biology 2 – (Teaching room 4) at 09:00 to 10:30

09:00 (15 mins) - Integrating cell signalling and host-parasite interactions to determine important drivers for schistosome growth, development, and survival in the human host (Anthony Walker)

09:15 (15 mins) - *Plasmodium* sporozoite excystation involves local breakdown of the oocyst capsule (Sadia Saeed)
09:30 (15 mins) - *Cryptosporidium* Remodels Host Microvilli Through an Exported Virulence Factor (Elena Rodrigues)
09:45 (15 mins) - Biochemical characterisation and essentiality of proteins involved in myo-inositol metabolism from the parasite *Trypanosoma cruzi* (Veronica Harris)
10:00 (15 mins) - *Blastocrithidia nonstop* mitochondrial genome and its expression are remarkably insulated from nuclear codon reassignment (Vyacheslav Yurchenko)
10:15 (15 mins) - Key *Leishmania* trans-regulators are essential for parasite surveillance and infectivity (Pegine Walrad)

Drinks break – LGS (Courtyard)

(13) Helminth epidemiology 2 - (Lecture theatre 1) at 11:00 to 12:30

11:00 (25 mins) - A Tale of Schistosomiasis in Malawi: From Burden to Prevention (Janelisa Musaya)
11:25 (20 mins) - Bovine schistosomiasis in Malawi emerging public health problem: revealing zoonotic haematobium hybrid (Alexandra Juhasz)
11:45 (15 mins) - Evaluation of surveillance-response interventions for *Schistosoma haematobium* elimination on Pemba Island, Tanzania: A 4-year intervention study with repeated cross-sectional surveys (Lydia Trippler)
12:00 (15 mins) - The short-term impact of *Schistosoma mansoni* infection on liver morbidity and health-related quality of life: implications for current elimination policies (Poppy Lamberton)
12:15 (15 mins) - Opening a can of worms: Detecting zoonotic *Strongyloides* species within strongyloidiasis. (Lucas Cunningham)

(14) Parasite-immune interactions 2 - Sponsored by PCR Biosystems - (Lecture theatre 2) at 11:00 to 12:30

11:00 (25 mins) - Life stage-specific glycosylation of schistosome-derived extracellular vesicles (EV) directs functional interactions of EV with host immune cells (Cornelis Hokke)
11:25 (25 mins) - Helminth immunomodulatory protein activity controlled by location and timing. (Henry McSorley)
11:50 (10 mins) - Identification of species-specific glycan antigens of *Schistosoma haematobium* (Laudine Petralia)
12:00 (10 mins) - Human immune responses to *Schistosoma mansoni*, lessons from controlled human infection models and natural endemic infection. (Emma Houlder)
12:10 (20 mins) - Disease susceptibility and gut health in the wild: Determining interactions between diet, gut microbiome, and immunity (Kathryn Else)

(15) Subcellular structure - Sponsored by ZEISS/Appleton Woods - (Lecture theatre 3) at 11:00 to 12:30

11:00 (25 mins) - Resolving the spatial proteome of *Plasmodium falciparum* asexual stages and their interaction with the erythrocyte. (Ross Waller)
11:25 (15 mins) - A novel approach to understanding parasite nutrient uptake and metabolism at a subcellular scale using Nanoscale Secondary Ion Mass Spectrometry (Macaulay Turner)
11:40 (10 mins) - Advanced imaging methods for investigating parasite structure and function (Matt Haley)

11:50 (15 mins) - Characterization of novel and essential kinetoplast components in *Trypanosoma brucei*. (Michael Hammond)
12:05 (25 mins) - Deciphering the conoid of *Toxoplasma gondii*: Insights into ultrastructural components and their functions (Dominique Soldati)

(23) Complex ecology data analysis - (Teaching room 4) at 11:00 to 12:30

11:00 (25 mins) - Modelling helminth transmission at the wildlife-livestock interface: a difficult relationship with data? (Eric Morgan)
11:25 (20 mins) - Quantifying complex outcomes of disease control interventions (Mafalda Viana)
11:45 (15 mins) - Current *Schistosoma mansoni* exposure and infection have distinct determinants: a data-driven population-based study in Uganda (Fabian Reitzug)
12:00 (15 mins) - Understanding the influence of environmental factors on disease dynamics: Insights from Desert Bighorn Sheep Populations (Diana Meza)
12:15 (15 mins) - Quantifying demographic contributions to helminth transmission dynamics in wild sheep (Andrew Dean)

Lunch – (LGS Courtyard)

Early-Career Researcher workshop - (PC Suite C, Hub502) at 13:00 to 14:00

13:00 (15 mins) - The Science Media Centre (Ed Day)
13:15 (15 mins) - Career progression and the PROSPER programme at the University of Liverpool (Fiona McBride)

(16) Control and elimination open session - (Lecture theatre 1) at 14:00 to 15:30

14:00 (10 mins) - Does the removal of macroparasites have an effect on the microparasite community in Bighorn sheep? (Alex Morris)
14:10 (10 mins) - How does the composition of mixed wildlife-livestock communities' impact ungulate parasite burden and diversity in Botswana? (Isabella Endacott)
14:20 (10 mins) - Mental health and skin Neglected Tropical Diseases (NTDs) - A participatory mixed method evaluation of integrated mental health and NTDs in Liberia (Carrie Barrett)
14:30 (10 mins) - Molecular detection of host blood meal and pathogen diversity in bat-associated ticks in Europe (Tamara Szentivanyi)
14:40 (10 mins) - Reproducibility matters: intra- and inter-sample variation of the point-of-care circulating cathodic antigen test in two *Schistosoma mansoni* endemic areas in Uganda (Elías Kabbas Piñango)
14:50 (10 mins) - Development of a whipworm vaccine using virus-like particles (Jacob Thompson)
15:00 (10 mins) - A vaccine dose and a worm's host: Malaria vaccination in a schistosome-endemic region of Malawi (Sarah Rollason)
15:10 (10 mins) - Comparative analysis of the immune responses elicited by native versus recombinant *Fasciola hepatica* vaccines (Krystyna Cwiklinski)

(17) Parasite-microbiome interactions - Sponsored by Royal Society Publishing, MDPI and MicroMolecular Systems - (Lecture theatre 2) at 14:00 to 15:30

14:00 (25 mins) - Structural and functional analyses of antimicrobial peptides in worm excretory/secretory products (Cinzia Cantacessi)

14:25 (20 mins) - Not only *Orientia* and scrub typhus? New frontiers in microbiome research for chiggers, the world's tiniest vectors (Benjamin Makepeace)
14:45 (20 mins) - *Trichomonas* – Bacteria interactions: A Laterally Acquired Molecular Toolkit to Target the Microbiota and Potentially Enable Zoonotic Events. (Adam Hart)
15:05 (25 mins) - Towards the Use of Novel High Density *Anopheles*-Specific *Wolbachia* Strains for *Anopheles* Vector Control. (Grant Hughes)

(18) Life-cycle interfaces - (Lecture theatre 3) at 14:00 to 15:30

14:00 (25 mins) - From Receptors to Lipolysis: Tracing *T. brucei*'s Route in Host Fat Tissue (Luisa Figueiredo)
14:25 (15 mins) - Mechanisms of life cycle simplification in field-derived and laboratory-selected African trypanosomes (Guy Oldrieve)
14:40 (15 mins) - Cytoadhesion of *Trypanosoma congolense* to bioengineered 3D bovine microvessels (Sara Silva Pereira)
14:55 (15 mins) - Bottling it all up: Using parasite population biology to identify susceptibility pathways in leishmaniasis (Ciara Loughrey)
15:10 (20 mins) - Stuck in the throat: Dissection of *Leishmania* parasite adhesion in the sand fly vector (Jack Sunter)

(19) Veterinary vaccines - Sponsored by Elsevier - (Teaching room 4) at 14:00 to 15:30

14:00 (25 mins) - Developing subunit vaccines for Animal African Trypanosomiasis (Gavin Wright)
14:25 (25 mins) - Controlling parasitic nematodes of sheep with sub-unit vaccines – the state of play (Alasdair Nisbet)
14:50 (15 mins) - Combined mucosal and systemic immunisation strategy against bovine neosporosis (Alexandra Correia)
15:05 (25 mins) - Recombinant anticoccidial vaccines for chickens – how good is good enough? (Damer Blake)

Drinks break - (LGS courtyard)

President's Medal - (Brett Lecture Theatre) at 16:00 to 16:30

16:00 (30 mins) - Using trypanosomes to understand the inner workings of the brain (Juan Quintana)

Wright Medal - (Brett Lecture Theatre) at 16:30 to 17:30

16:30 (60 mins) - Seeking simplicity in the complexity: the community epidemiology of multihost/multiparasite systems (Andy Fenton)

Closing remarks and poster prizes - (Brett Lecture Theatre)

BSP Annual general meeting - (Brett Lecture Theatre) at 17:30 to 18:00

Gala Dinner – (Cathedral Crypt) at 19:30 to 23:30

Oral Abstracts

Day 2 - 3-April-2024

Plenary session 1 - (Brett Lecture Theatre)

Chair: Andrew Jackson

09:00 (45 mins)

Malaria Vaccines – at last!

Prof Sir Adrian Hill, *Director, Jenner Institute*

A Hill[1];

[1] Jenner Institute, University of Oxford, UK

Malaria remains a major cause of mortality in children in sub-Saharan Africa with a total of over 600,000 deaths and 200 million clinical cases globally each year. Technically it has been very difficult to design effective anti-parasitic vaccines, over 140 distinct malaria candidate vaccines have reached clinical trials, and last year none were in programmatic deployment. A new nanoparticle-in adjuvant vaccine, R21 in Matrix-M™ adjuvant, has been designed by the Jenner Institute at the University of Oxford and initial clinical testing showed efficacy in a controlled human infection model. From 2017, in partnership with the Serum Institute of India, an accelerated programme of vaccine development in African children included a phase IIb trial at Nanoro, Burkina Faso which showed unprecedented efficacy of >75%. In a recently reported phase III trial in 4800 children across four countries in sub-Saharan Africa (Lancet 10426: 533-544, 2024) good safety was documented and high-level efficacy was confirmed. Immunological analyses have identified likely immune correlates of protective efficacy. This vaccine has now received initial regulatory and policy approvals and plans for its deployment, scale up, wider use and potential impact will be discussed. In parallel, the RTS,S/AS01 vaccine from GSK is also set for roll out at a smaller scale this year and several blood-stage and transmission-blocking vaccines are showing promise in African trials.

09:45 (45 mins)

Human African Trypanosomiasis - can we break the endemic, outbreak, epidemic cycle of infection?

Prof Sue Welburn, *University of Edinburgh*

S Welburn[1];

[1] Edinburgh Medical School, College of Medicine and Veterinary Medicine, The University of Edinburgh, UK

Despite concerted efforts for control, Human African Trypanosomiasis (HAT) is now endemic across vast swathes of sub-Saharan Africa where tsetse distribution is widespread. In this new era of endemic HAT we look at how peculiarities in the epidemiologies of Gambiense (gHAT) and Rhodesian HAT (rHAT) impact on contemporary strategies for elimination of HAT "as a public health problem". Epidemics of both forms of sleeping sickness have emerged with remarkable periodicity within spatially stable foci in sub-Saharan Africa, but are able to migrate if conditions are right. While tsetse flies are the primary vector, gHAT can also be sustained without tsetse. Long-term silent infections, maternal and sexual transmission and the implication of human genetic factors in HAT epidemiology including the role of stress causing breakdown of heritable tolerance in silent disease carriers may all contribute to generation of new gHAT outbreaks. Similarly, long term infection of animal carriers of zoonotic rHAT only serve to complicate control strategies at the human animal interface. Here we look back on the

epidemiology and management of gHAT and rHAT epidemics of the past and speculate as to what the future holds for this still neglected disease.

(1) Drug development 1 - (Lecture theatre 1)

Sponsored by Drugs for Neglected Diseases (DnDi)

Chair: Mark Taylor

11:10 (25 mins)

Development of alternative treatments for filarial diseases

Dr Sabine Specht, *DnDi*
S Specht[1];
[1] Drugs for Neglected Diseases initiative (DNDi), Switzerland

Twenty diseases are recognized as neglected tropical diseases (NTDs) by World Health Assembly resolutions, including human filarial diseases. The end of neglected tropical diseases is now firmly embedded within the Sustainable Development Goals for 2030, under target 3.3. Filarial diseases still affect an estimated 200 million people worldwide and global efforts undertaken in recent decades have enabled elimination of filariasis as a public health problem. It is recognized that new drugs or drug regimens that kill or permanently sterilize adult filarial worms would significantly improve elimination timelines and accelerate achievement of the program goal of disease elimination. Drug development is, however, handicapped by high attrition rates, and many promising molecules fail in preclinical development or in subsequent toxicological, safety and efficacy testing; thus, research and development (R&D) costs are, in aggregate, very high. Drug discovery and development for NTDs is largely driven by unmet medical needs put forward by the global health community; the area is underfunded and since no high return on investment is possible, there is no dedicated drug development pipeline for human filariasis. Product development partnerships between the private sector, academic institutions and NGO´s are a vital part of enabling drug development in an otherwise underfunded area. In addition, repurposing existing drugs is one approach to filling the drug development pipeline for human filariasis. While the *de novo* development of anthelmintics is commercially not attractive for human use, development of new drugs for animal health is (relatively) financially rewarding and therefore much better supported and further advanced. Furthermore, drug repurposing typically has a higher chance of success with an already proven drug target in nematodes of veterinary importance. Some impressive examples demonstrate successful repurposing of veterinary drugs for human use, including benzimidazoles, IVM, praziquantel, moxidectin and triclabendazole. This approach has also been adopted by the DNDi, which is currently investigating emodepside (in collaboration with Bayer AG) and ABBV-4083 (a tylosin derivative, jointly developed in collaboration with the Anti-Wolbachia (AWOL) consortium and the pharma partner AbbVie). The third lead compound is the off-patent veterinary product oxfendazole for potential human use. Here we present a project update and discuss the considerations to enable patient`s access to new medicines.

11:35 (15 mins)

Pharmacological targeting of bioactive lipid production improves experimental lymphatic filariasis pathology

Dr Shrilakshmi Hegde, *Postdoctoral research associate, Liverpool School of Tropical Medicine*

S Hegde[1]; J Furlong Silva[1]; A Steven[1]; JL Dagley[1]; MJ Taylor[1]; JD Turner[1];
[1] Liverpool School of Tropical Medicine, UK

Lymphatic filariasis (LF) is a Neglected Tropical Disease prioritised for global elimination, one of the main aetiological agents of non-hereditary lymphoedema and a major cause of global disability. An estimated 36 million individuals with LF infections worldwide suffer stigmatising chronic morbidities (elephantiasis or hydrocoele) which can drive depressive illness. The current LF elimination strategy by mass drug administration of standard anti-filarial drugs has negligible impact at moderating pre-established lymphatic disease. However, long-courses of high dose doxycycline treatment have shown promising results in reversing early lymphoedema grade in phase II clinical trials, demonstrating proof-of-concept that filarial pathology can be pharmacologically targeted. Our recent research has shown that in LF laboratory models, the anti-morbidity mechanism of doxycycline is via targeting a type-2 adaptive immune-mediated inflammatory pathway responsible for triggering lymphangiogenesis, aberrant lymphatic vessel formation, dilatation and lymphatic insufficiency. In other secondary lymphoedemas, emerging evidence implicates eicosanoid lipid inflammatory mediators in disease progression. Here, using an established murine experimental hind-limb model of *Brugia malayi* infection, we evaluated the role of eicosanoids in LF lymphatic pathology. The cyclooxygenase-(COX)2 and lipoxygenase(LOX)5 terminal metabolites, prostaglandin(PG)E2 and leukotriene(LT)B4 were significantly upregulated in circulation of *B. malayi* infected mice and were modulated following effective anti-morbidity doxycycline treatment. Both eicosanoids directly stimulated lymphatic endothelial cell proliferation *in vitro.* We therefore investigated the effect of COX/LOX pharmacological inhibitor treatment on lymphatic structure and function in *B. malayi* infected mice using near-infrared (NIR) intravital indocyanine green (ICG) lymphography. COX/LOX inhibitor treatment significantly decreased lymphatic remodelling in dorsal, lateral, and ventral aspects of the infected limb compared to vehicle treated animals. These initial results uncover the novel potential of targeting bioactive lipid pathways with affordable and safe non-steroidal anti-inflammatory drugs for the treatment of filarial lymphatic disease.

11:50 (15 mins)

Stem cell screening to identify novel drug targets in the human parasite *Schistosoma mansoni*

Sewwandi Perera, *Postgraduate researcher, Kingston University*

S Perera[1]; AM Chioni[1]; AJ Walker[1];
[1] Kingston University, UK

Human schistosomiasis affects approximately 250 million people in 78 developing countries and 0.8 billion people are at risk of infection. There is no approved vaccine for this disease and treatment relies solely upon one drug, praziquantel, raising concern about potential drug resistance. Schistosomes have a complex life cycle, involving several life stages in molluscan intermediate and mammalian definitive hosts. During schistosome development in the mammalian host, rapid growth and physiological change

occurs, particularly during the early- to late-liver stage, where the schistosomula (somules) become packed full of proliferating somatic stem cells. These stem cells are vital for the survival and development of the parasite. This research aims to develop a better understanding of the pre-adult intra-mammalian life stages of *Schistosoma mansoni*, particularly the early and late liver stages, with a specific focus on their stem cell biology and to discover novel therapeutic targets. Early and late liver stage somules were chased using EdU in cell proliferation assays and somules co-stained with anti-phospho-kinase and anti-phospho-(kinase) substrate antibodies, revealing that multiple protein kinase pathways including protein kinase C, protein kinase A and AKT are activated and localise to the proliferating stem cells. Next a commercial stem cell library, containing 280 compounds aimed at human stem cell processes, was screened against the liver somules, using a developed pipeline employing confocal microscopy to assess the effects of each compound on stem cell proliferation in the parasite. Numerous 'hit' compounds have been identified within the library and their effects on somule viability, growth, and development are currently being explored. Ultimately, the 'hit' compounds might prove suitable as novel drugs against schistosomes or might help us identify other drugs that target specific mechanisms to interfere with schistosome growth and development.

12:05 (15 mins)

Development of azaquinazoline anti-*Wolbachia* drugs for veterinary zoonotic filariasis

Jessica Dagley, *Research assistant, Liverpool School of Tropical Medicine*

JL Dagley[1]; S Hegde[1]; AE Marriott[1]; A Steven[1]; C Fricks[4]; U DiCosty[4]; A Mansour[4]; EJ Campbell[3]; CM Wilson[3]; SA Ward[1]; D Hong[2]; P O'Neill[2]; A Moorhead[3]; S McCall[4]; JW McCall[4]; M Taylor[1]; JD Turner[1]; *[1] Liverpool School of Tropical Medicine, UK; [2] University of Liverpool, UK; [3] University of Georgia, United States; [4] TRS Laboratories Inc, United States*

Dirofilaria immitis is a mosquito-borne filarial nematode causing potentially lethal heartworm disease in cats and dogs. *Dirofilaria spp.* also cause zoonotic infections and pathologies in humans. Currently, control of veterinary dirofilariasis relies on chemoprophylaxis using macrocyclic lactone drugs, however, resistant isolates are increasing and resulting in preventative treatment failure.

Wolbachia is an essential endosymbiont necessary for development, reproduction, and survival of filarial nematodes. Targeting *Wolbachia* within filarial parasites using second generation tetracyclines is a relatively new curative treatment approach for both human and veterinary filariasis. Doxycycline, whilst effective at sterilising worms and leading to the gradual death of adult filariae, requires long treatment durations, can cause dysbiosis side-effects and raises antibiotic stewardship concerns, precluding the widespread use of tetracyclines in companion animals. We have developed a new azaquinazoline class of anti-*Wolbachia* drug with no broad-spectrum antibiotic activities. The first-in-class clinical candidate, AWZ1066S, mediates 5-day curative activity in human filariasis infection models. Here we report that AWZ1066S also mediates complete chemoprophylaxis (blocking adult infections) in a *Brugia malayi* mouse model when administered at day 1 and 29 after larval infection. We therefore selected four back-up azaquinazoline analogues of AWZ1066S for comparative *in vivo* efficacy against *D. immitis,* based on drug-like properties and *in vitro* anti-*Wolbachia* activity. We determined three analogues had equipotent or enhanced *Wolbachia* depletions in comparison to AWZ1006S using one-day oral exposures. One candidate, AWZ1023, was selected for further development in a proof-of-concept dog chemoprophylactic pilot study. Three dogs receiving twice-daily intramuscular dosing of AWZ1023 (day 1 and 29) had complete absence of adult heartworm at study termination (day 177), demonstrating the effectiveness of AWZ1023 at blocking juvenile nematode development.

In conclusion, this study provides an early proof-of-concept that azaquinazolines targeting nematode *Wolbachia* are a promising new class of drug for the treatment and prevention of veterinary zoonotic filarial infections, providing a potential alternative to the current reliance on macrocyclic lactone drugs.

12:20 (20 mins)

Developing the natural product Corallopyronin A to treat filariasis

Dr Kenneth Pfarr, *University Hospital Bonn*

K Pfarr[1];
[1] *Institute for Medical Microbiology, Immunology and Parasitology, University Hospital Bonn, Germany*

The bacterial DNA-dependent RNA polymerase inhibitor Corallopyronin A (CorA) binds to a different site than rifampicin, killing rifampicin-resistant *Staphylococcus aureus*. CorA also kills the Gram-negative *Wolbachia* endobacteria of filarial nematodes that cause onchocerciasis (river blindness) and lymphatic filariasis (elephantiasis). Depleting the essential endosymbionts causes worm sterility and slow adult worm killing. Using the *Litomosoides sigmodontis* infection model in Mongolian gerbils, we demonstrated that CorA depletes *Wolbachia* from microfilariae and adult filariae by more than 2-logs and is macrofilaricidal. The macrofilaricidal effect can be reached with a 2 weeks regimen and can be further reduced to ten days by administering CorA and albendazole, a significant advance over the current 4-week doxycycline regimen. We have also demonstrated macrofilaricidal activity using the *Onchocerca ochengi* mouse model. To develop CorA as a novel solution to filarial infection, we have conducted standard non-GLP ADMET studies. *In-vitro* toxicity tests (off-target, AMES, micronucleus, hERG, phototoxicity) demonstrated that it is non-toxic and pharmacologically safe; *in vivo* toxicity studies in rats and dogs measured a maximal tolerated dose (MTD) of 1000 mg/kg in both species. Seven-day repeated dose studies in rats and dogs demonstrated no prohibitive safety issues: predicted NOEL=150 mg/kg/d; predicted HED=4 mg/kg. CorA drug substance is heterologously produced in genetically modified *Myxococcus xanthus* and has been upscaled 15 m3 (kilograms) in 2022 at Bio Base Europe Pilot Plant (Belgium). The Helmholtz Centre for Infection Research purified this large amount of material, achieving 90-95% pure HQ-RGM material. With amorphous solid dispersion formulation principles, two solid oral formulations were developed that increased stability (>3 months at 30 °C, >6 months at 5 °C) and oral bioavailability (mouse >59%, dog>53%) compared to neat CorA. We are establishing drug product production at GMP facilities. GLP safety and toxicology studies will be conducted in 2024. After finalization of the pre-clinical work, we plan to enter the clinical phase I in 2025/2026

(2) Population genomics 1 - (Lecture theatre 2)
Sponsored by BioRad

Chair: Mark Viney

11:10 (25 mins)

Metabarcoding and targeted deep sequencing of parasitic nematode communities: applications and future directions

Prof John Gilleard, *Faculty of Veterinary Medicine , University of Calgary*

J Gilleard[1];
[1] Faculty of Veterinary Medicine , University of Calgary, Canada

Advances second and third-generation sequencing technologies over the last decade have revolutionised the analysis of microbial communities and are now being increasingly applied in helminthology research. There are many opportunities, as well as some specific challenges, when applying these approaches to helminths. Long-read and short-read shotgun sequencing are generating many more, and higher quality, reference helminth genomes which will be crucial to apply genome-wide approaches to helminth population genetic studies. However, metabarcoding and targeted deep sequencing have an important roles and are particularly powerful when dealing with large sample numbers, low amounts and/or low quality template DNA (for example in stool samples), and complex and/or poorly defined helminth communities. This talk will focus on recent progress, current resources and future directions in both short-read and long-read metabarcoding (nemabiome metabarcoding) and targeted deep sequencing. Examples will include the use of these methods in the study of anthelmintic drug resistance, molecular epidemiology and molecular diagnostics of parasitic nematodes in both domestic animals and humans.

11:35 (20 25 mins)

Finding a needle in a haystack: Genome-wide analyses of anthelmintic resistance in helminths of livestock

Dr Roz Laing, *University of Glasgow*

R Laing[1];
[1] University of Glasgow , UK

Control of parasitic infections in animals and humans currently relies on mass drug administration of a limited number of anthelmintics. However, this approach is not sustainable due to the emergence and spread of anthelmintic resistance. *Haemonchus contortus* is an economically important and highly pathogenic gastrointestinal nematode of small ruminants, which is becoming increasingly difficult to control due to multi-drug resistance. The mechanisms underlying anthelmintic resistance are generally poorly understood, with studies comparing resistant and sensitive parasites confounded by high levels of genetic diversity within and between and populations. To overcome this, we crossed a well-characterised multi-drug resistant isolate of *H. contortus* with a drug susceptible isolate to study resistance to three major anthelmintic classes, while controlling for background genetic variation. In this talk I will describe recent progress in understanding the genetic mechanisms underlying resistance to levamisole and describe ongoing work investigating the evolution of resistance to the macrocyclic lactones.

11:5512:00 (15 mins)

Strongyloides stercoralis complex in humans and dogs: insights from population genomics in Asia

Yuchen Liu, *University of Liverpool*

Y Liu[2]; R Sarker[4]; B Sripa[3]; V Khieu[5]; W Nevin[1]; M Viney[2];
[1] Liverpool School of Tropical Medicine, UK; [2] University of Liverpool, UK; [3] Khon Kaen University, Thailand; [4] University of Chittagong, Bangladesh; [5] National Center for Parasitology, Entomology and Malaria Control, Cambodia

Strongyloides stercoralis is a parasitic nematode that infects people and is widespread in tropical and subtropical regions. It has been assumed that *S. stercoralis* transmits only among people. However, accumulating evidence has suggested that *Strongyloides* from people and dogs are the same species, so that dogs can be infected with it and act as a source of human infection. To investigate the host range of *S. stercoralis*, the current study sampled sympatric populations of worms from people and dogs in Asia. Individual *Strongyloides* larvae from people and dogs were subjected to whole genome sequencing. DNA reads from each individual sample were then used to investigate the parasite's population genetics. The epidemiological data revealed a higher *Strongyloides* prevalence in people than in dogs in Thailand, though in communities in Bangladesh and Cambodia the prevalence in people and dogs was more similar. This indicates that the transmission of *Strongyloides* between people and dogs might vary across Asia. Population genomic analyses showed that people were infected with a range of closely related *S. stercoralis* genotypes that were widely dispersed across Asia. In contrast, parasites from dogs clustered into five genetically distinct groups, with four clusters differing from those found in humans. Interestingly, one genotype from a dog in Cambodia clustered with those found in humans. These data suggest that *Strongyloides* in people and dogs in Asia are different parasites, though dogs may be able to be infected with *Strongyloides* from people.

12:10 15 (25 mins)

Does hybridization amongst *Schistosoma* spp. matter?

Prof Joanne Webster, *Royal Veterinary College*

J Webster[1];
[1] *Royal Veterinary College, UK*

Since 2021 we have seen the launch of a new WHO Neglected Tropical Diseases (NTD) Roadmap, together with revised Disease Control and Elimination Guidelines and Targets. Across all there is now a clear emphasis on the need to incorporate a One Health approach, recognizing the critical links between human and animal health and the environment. Schistosomiasis is a NTD of global medical and veterinary importance, with over 220 million people currently infected as well as untold millions of livestock. Despite over two decades of mass administration of the anthelmintic praziquantel to, predominantly, school-aged children, the burden of schistosomiasis remains extremely high in certain regions. Whilst animal hosts have been long acknowledged as zoonotic reservoirs across Asia, within Africa or the Americas, in contrast, any zoonotic component of schistosomiasis transmission and its implications for disease control has, until now, been largely ignored. This is true of both *S. mansoni*, but also notably, *S. haematobium*, the latter of which was assumed to be an exclusively human infection – and thus amenable to elimination by targeting treatment of humans alone. However, an increasing body of work has revealed widespread viable hybridization between *S. haematobium* of humans with *Schistosoma* spp. (notably *S. bovis, S. curassoni* or *S. mattheii*) of livestock throughout Africa and beyond. The dynamics of which species/species-combination predominates varies focally. Moreover, whilst recent genomic evidence suggests the majority of such hybrids may be ancient, there is also evidence of rare ongoing contemporary hybridization. Here I will present some of our recent research focusing on the transmission dynamics of *Schistosoma* spp. – notably that of the potential risk raised by ongoing viable hybridization between *Schistosoma* species of both humans and animals.

(3) Antigenic variation 1 - (Lecture theatre 3)

Chair: David Horn

11:10 (25 mins)

Antigenic variation at the macro and micro scale: how the African trypanosome coat structure helps it evade a highly diverse immune response

Prof Nina Papavasiliou, *German Cancer Research Center*

N Papavasiliou[1];
[1] *German Cancer Research Center, Germany*

The African trypanosome survives the immune response of its mammalian host by antigenic variation of its major surface antigen (the Variant Surface Glycoprotein, or VSG). Whilst the genome encodes around 400 complete variants, the genomic archive contains several thousand variant gene fragments that can serve as donor sequences for gene conversion, making the potential number of possible variants very high. The host response to trypanosomes is chiefly antibody based, and antibodies are also capable of extreme diversification through recombination (Ig gene rearrangements). And yet, this very diverse host response is routinely evaded by trypanosomes. In recent work we have found that antibody repertoires against specific VSGs are highly restricted, and are directed predominantly to distinct epitopes on the surface of the VSGs. They are also highly discriminatory: minor alterations within these exposed epitopes confer antigenically-distinct properties to these VSGs and elicit different repertoires. We propose that the patterned and repetitive nature of the VSG coat focuses host immunity to a restricted set of immunodominant epitopes per VSG, eliciting a highly stereotyped response, minimizing cross reactivity between different VSGs and facilitating prolonged immune evasion. Therefore, trypanosomes evade the antibody response not only at the macro scale (through antigenic variation and replacement of entire VSG genes) but also at the micro scale (through the establishment of focused immunodominant epitopes that, when replaced, can fuel immune evasion).

11:35 (25 mins)

A sub-nuclear *super-factory* for singular antigen expression: novel stage-specific regulators finetune expression at the active *VSG* expression-site.

Dr Joana Correia Faria, *Principal Investigator / Lecturer, University of York*

J Correia Faria[1];
[1] *Biology Department & York Biomedical Research Institute, University of York, UK*

African trypanosomes are 'masters of disguise'. They rely on a vast genetic repertoire (>2,600 genes and pseudogenes) encoding for their variant surface glycoprotein (*VSG*) to undergo antigenic variation and successfully evade the host immune response. Their ability to express a single *VSG* at any given time, known as 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 a dedicated sub-nuclear compartment designated expression-site body (ESB). To date, only two proteins have been shown to specifically localise to the ESB: ESB1, a stage-specific transcriptional activator; and VSG-exclusion-2 (VEX2), a large and enigmatic RNA:DNA helicase that is critical for VSG monogenic expression. Further, VEX2 integrates transcription and splicing, whereby the active-*VSG* gene establishes a stable inter-chromosomal interaction with a locus involved in trans-splicing, therefore enabling high levels of *VSG* expression. We sought to define the spatial interactome within the ESB using TurboID-mediated proximity labelling combined with quantitative LC-MS/MS analysis. We found new components of the SL-array and NUFIP bodies, several ESB-enriched proteins, but most notably, three novel ESB-specific components. Moreover, these three novel factors are compartmentalised at the ESB in a VEX2-dependent manner, two are stage-specific and likely to be involved in splicing and

RNA decay. Transcriptomic analysis following the depletion of either of these three factors shows a specific role in the regulation of expression-site-associated-genes (*ESAGs*) expression. Notably, it has long been known that *ESAGs* upstream of *VSGs*, despite co-transcription in the same polycistron, yield far less abundant transcripts, and no factor responsible for this differential control has previously been identified.

~~12:00 (15 mins)~~ Not presented

~~RNA polymerase III is involved in regulating *Plasmodium falciparum* virulence~~

~~Dr Gretchen Diffendall, *Institut Pasteur*~~

~~G Diffendall[1]; A Claes[1]; A Barcons-Simon[4]; P Nyarko[3]; D Florent[2]; M Santos[5]; D Loew[2]; A Claessens[3]; A Scherf[1];~~

~~[1] *Institut Pasteur, Paris, France;* [2] *Institut Curie, Paris, France;* [3] *University of Montpellier, France;* [4] *Ludwig Maximilian University of Munich, Germany;* [5] *University of Lisbon, Portugal*~~

~~Malaria disease severity is correlated with the levels of infected red blood cells (iRBCs) adhering within blood vessels. Our research has revealed that an RNA Polymerase III (RNA Pol III) dependent process regulates pathogen growth and the expression of a key virulence factor in response to external factors. This discovery links the sequestration of *P. falciparum* iRBCs to a specific group of non-coding RNAs transcribed by Pol III. Moreover, we identified the *P. falciparum* Maf1 protein as a crucial regulator of transcription by Pol III, essential for both maintaining cellular balance and by responding to environmental stimuli. Previous studies have linked changes in iRBC adhesion capacity to seasonal asymptomatic malaria infections, although the reasons behind this remain unclear. Our findings show that in *P. falciparum*, RNA Pol III transcription is reduced in samples taken from asymptomatic individuals during the dry season from the Gambia. Additionally, we have discovered seasonal variations in plasma metabolites among individuals, with evidence suggesting these differences can influence Pol III activity. Our results introduce a novel perspective that contributes to our understanding of *P. falciparum* virulence. Furthermore, it establishes a connection between this regulatory process and the occurrence of seasonal asymptomatic malaria infections.~~

12:15 (25 mins)

Antigenic variation in *Babesia*: do we even know what we don't know?

Prof David Allred, *University of Florida*

D Allred[1];

[1] *University of Florida, United States*

Tick-borne intraerythrocytic parasites of the genus *Babesia* are notoriously effective at establishing infections of extreme duration. Cytoadhesion and rapid structural and antigenic variation of the cytoadhesion ligand, two known processes contributing to this success, are both embodied in the heterodimeric protein, VESA1. VESA1 subunits are encoded by separate branches of the *ves* multigene family and expressed in a monoparalogous manner. The *ves* family is present in a highly amplified and diversified form among all *Babesia sensu stricto* parasites observed to date, but is largely absent from species outside this group. Sudden wholesale changes in expressed *ves* sequences may occur through in situ transcriptional switching (isTS) among *ves* family members. Lacking specific binding domains like those found in the analogous PfEMP1 proteins of *Plasmodium falciparum*, novel adhesive VESA1 isoforms must be generated *de novo*. This appears to occur through the random recombination events of segmental gene conversion (SGC), with simultaneous generation of hugely diverse antigenic variants. The enzymatic mediators of this process have not yet been identified. While resembling

homologous recombination (HR), SGC is undeterred by knockout of the lone *rad51* gene, rendering HR unlikely as a major pathway. Functional knockout of the translesion Rev1/Polζ repair pathway renders parasites highly sensitive to DNA base damage but does not appear to significantly impact SGC (analysis ongoing), suggesting that frequent template-switching also is not responsible. Triggers of the DNA breaks leading to SGC are not known, but occur spontaneously and must target the *ves* family specifically. We hypothesize this may be related to the extreme enrichment of the *ves* multigene family with oxidation-sensitive G-quadruplex sequences. These disparate points will be contrasted between *Babesia* and other parasite species. Supported by NIAID, American Heart Association, USDA, USAID, University of Florida.

(20) Parasite wildlife ecology - (Teaching room 4)

Chair: Hannah Vineer

11:10 (25 mins)

Nutritional supplementation has diverse impacts on the parasite community of wild mice

Dr Amy Pedersen, *University of Edinburgh*

A Pedersen[1];
[1] *University of Edinburgh, UK*

Understanding the relationship between diet availability and parasitic infection in humans and animals is of increasing importance, given rising rates of habitat modification, land-use change and other anthropogenic effects. These changes can alter the distribution, quality and availability of resources, either accidentally (e.g. through consumption of urban waste or agricultural products, the reduction of naturally occurring food sources) or deliberately (e.g. garden bird feeders, tourism, supplemental feeding stations). However, understanding how different diet availability scenarios, either natural or anthropogenic, affect infection levels in wildlife is a major conservation challenge, due to the myriad of ways in which resources affect hosts, both at the individual and population scales. Different resource scenarios can alter a range of biological traits, all of which influence different processes (contact with infective stages, susceptibility, resistance or tolerance to infection, infectivity to other hosts, etc.) that determine parasite infection risk and outcome in different ways.

From a series of field and controlled laboratory infection/coinfection experiments, I will demonstrate the effects of nutritional supplementation on both the host (health, behaviour and contacts) and the parasite community (infection, intensity, etc) using a wood mouse system. I will show evidence that nutritional supplementation significantly impacts the gastrointestinal worm *Heligomosomoides polygyrus* infection intensity and immunity in the wild and lab. In addition, I will present recent results that demonstrate that the composition of the gut microbiome, which is changed by diet, may impact *H. polygyrus* establishment and resistance. Importantly, I will provide evidence that providing wild mice with a high-quality diet does not always lead to a reduction in parasite infection and intensity, and in contrast sometimes increases the likelihood of infection and even reduces immuno-responsiveness to immunisation. These results highlight how pairing both the lab and natural setting provides a unique and powerful opportunity to understand how nutritional supplementation can impact host-parasite dynamics with implications for better understanding infection, immunity and disease control.

11:35 (20 mins)

Parasites in Plastic Environments

Dr Jo Cable, *Cardiff University*

J Cable[1];
[1] Cardiff University, UK

'Human-built, modified, or engineered niches of the Anthropocene' cover most of the Earth's landmasses. These novel ecosystems are constantly changing with increased urbanisation, production, climate shifts and pollution. Parasites, like other animals, are adapting to these changing conditions. To ameliorate the impact of future epidemics, we need to understand how multiple stressors affect our ability to combat infections. Among the most prevalent pollutants are plastics, indispensable in modern life with significant medical uses. However, their durability and resistance to degradation also make them recalcitrant waste sources. Here, we explore the impact of both petroleum-based and bio-based plastics on a model freshwater-ectoparasite system, focussing particularly on the additives that enhance polymer functionality. Without comprehensive risk assessments of all manufactured products, we risk 'green-washing', substituting one pollutant with another, and inadvertently fostering future disease outbreaks.

11:55 (15 mins)

Do woodland patch size and connectivity influence tick density, nymph infection prevalence and Lyme Disease hazard through impacts on key tick hosts?

Dr Saudamini Venkatesan, *University of Liverpool*

S Venkatesan[2]; K Hansford[3]; S Gandy[3]; M Greener[1]; R Hassall[4]; B Purse[4]; R Biek[1]; T Morrison[1]; L Gilbert[1]; J Medlock[3]; C Millins[2];
[1] University of Glasgow , UK; [2] University of Liverpool, UK; [3] UK Health Security Agency, UK; [4] UK Centre for Ecology & Hydrology, UK

Zoonotic tick-borne diseases are a rising concern in the UK, with Lyme disease incidence increasing over the last two decades. Changing land use across UK through government policies to expand woodlands and green spaces may affect human risk of exposure to tick-borne diseases by creating more suitable habitats for *Ixodes ricinus* as well as by impacting abundance and movement of key tick hosts such as rodents and deer. We used a systems approach to investigate how changing landscape structure, specifically woodland size and patch connectivity will impact *I. ricinus* densities, pathogen infection prevalence as well as Lyme disease hazard in two contrasting landscapes endemic for Lyme disease pathogens (*Borrelia burgdorferi sensu lato*). We expected larger and more connected woodland patches to have higher tick densities and *B. burgdorferi s.l.* prevalence due to higher host habitat use by tick and pathogen transmission hosts. We also predicted that woodlands would have higher densities of infected ticks than adjacent open habitat due to more suitable habitat and abiotic conditions.

To test this, we sampled *I. ricinus* nymphs from 60 woodlands and 30 adjacent open habitats, across a gradient of woodland patch size and connectivity in Aberdeenshire, Scotland and Dorset, Wiltshire and Hampshire (collectively referred to as Wessex), England and tested the nymphs for infection with *B. burgdorferi s.l.* In addition, we deployed 180 trail cameras in the woodland and open habitats, to measure habitat use of important tick hosts specifically roe, fallow, red and muntjac deer as well as livestock. We used a set of general linear mixed models, to address how woodland size and connectivity affects nymph densities, *Borrelia* infection prevalence and the density of infected nymphs (Lyme disease hazard), while accounting for other variables such as climate, vegetation and host habitat use.

Consistent with our predictions, we found significantly higher densities of infected ticks in woodland patches compared to adjacent open habitats. In contrast to our predictions, we found that woodland size did not have an impact on nymph densities, infection prevalence or Lyme Disease hazard. Patch connectivity on the other hand had opposite effects on nymph densities in the two landscapes. More

connected patches had significantly higher nymph densities in Aberdeenshire, whereas the more connected patches in Wessex had significantly lower nymph densities. These opposing effects could be driven by the differing landscape features and host habitat use within the two landscapes. Specifically, the matrix between woodland patches in Aberdeenshire is dominated by fenced pastureland and arable crops while the Wessex landscape matrix comprises large, open grazing commons with livestock freely roaming between and within woodland patches. Our study illustrates the possible impacts of large-scale woodland management policies, specifically those resulting in increased woodland area and more connected woodlands, on the distribution and density of *I. ricinus* as well as the Lyme disease pathogens. Importantly, we show that these impacts can be context-dependent, driven by the specific features of the landscape.

12:10 (15 mins)

Nematode co-infection dynamics: exploring variation in wild sheep

Mike Evans, *University of Edinburgh*

MJ Evans[3]; X Bal[3]; Y Corripio-Miyar[1]; A Dean[2]; A Fenton[2]; A Hayward[1]; F Kenyon[1]; H Lemon[3]; AB Pedersen[3]; JM Pemberton[3]; J Pilkington[3]; A Sweeny[4]; T McNeilly[1]; DH Nussey[3];
[1] Moredun Research Institute, UK; [2] University of Liverpool, UK; [3] University of Edinburgh, UK; [4] University of Sheffield, UK

Co-infections with multiple species of gastrointestinal nematodes (GIN) cause significant impacts on human health and development, wild animal survival and fitness, and livestock welfare and productivity. The prevalence and intensity of these co-infections are subject to complex dynamics, and the management of them is further complicated by anthelmintic resistance and climate-linked temporo-spatial changes in the different species' epidemiologies. Wild animal systems are well suited to studying these dynamics in the absence of human interventions and, in addition to advancing our fundamental understanding of disease ecology, may inform alternative management strategies with reduced reliance on anthelmintics. The wild Soay sheep of St Kilda provide an ideal such system, given their unmanaged nature and transferable tools developed in domestic sheep. Here we apply ITS-2 sequence-based nematode speciation ('Nemabiome' sequencing) to approximately 2000 faecal samples collected non-invasively up to four times annually from over 500 Soay sheep over a four-year period. These species proportions were then corrected for faecal egg count and analysed using modern mixed-modelling approaches, incorporating the wealth of host phenotypic data for which this study system is renowned. Our analyses of these data reveal seasonal epidemiological patterns in *Nematodirus battus* and *Teladorsagia circumcincta* that are consistent with those observed in managed domestic sheep and are well explained by temporal variation in egg shedding and the free-living ecologies of these species. However, contrary to expectations derived from experience in domestic sheep, *Trichostrongylus vitrinus* is found at consistent levels throughout lambs' first year of life, whilst levels of *Trichostrongylus axei* rise into the early spring of the second year of the sheep's life. These differences are similar to early reports of seasonal infections in domestic sheep prior to modern anthelmintics, but are not easily explained by the ecologies of the free-living larval stages alone, raising important questions about within-host dynamics. Our work also sheds important insight into the epidemiology of *Bunostomum trigonocephalum* a poorly studied nematode parasite of sheep that was considered a significant parasite in the UK prior to widespread anthelmintic use and continues to cause impacts in other global settings. Our results then show further variation in nematode co-infections between males and females of different ages, leading on to planned investigations into whether co-infection patterns are repeatable or heritable under natural conditions.

12:25 (15 mins)

Diversity of parasite communities in co-existing wild and domestic ungulates in Kenya
Kim van de Wiel, *University of Liverpool*

K van de Wiel[2]; B Karani[4]; A Sweeny[3]; Y Corripio-Miyar[1]; P Toye[4]; F Kenyon[1]; A Fenton[2]; J Bro-Jorgensen[2];
[1] Moredun Research Institute, UK; [2] Institute of Infection, Veterinary and Ecological Sciences, University of Liverpool, UK; [3] University of Sheffield, UK; [4] Centre for Tropical Livestock Genetics and Health, International Livestock Research Institute, Kenya

Co-existence of wildlife and livestock is a common occurrence, yet there remains a limited understanding of parasite sharing between these species across the wildlife-livestock interface. Gastrointestinal nematodes pose significant concerns due to their association with production losses in livestock and potential detrimental impacts on species abundance in wildlife. This concern is particularly pronounced in regions experiencing increased interactions between livestock and wildlife, such as the Maasai Mara ecosystem in Kenya. In our study, we aimed to evaluate variations in gastrointestinal parasite communities in co-grazing animal species across different interface areas. We collected nematode infective larvae from ~1000 fresh faecal samples obtained from 14 sympatric wild and domestic herbivore species (>10 kg) across mixed livestock-wildlife and single-occupancy pastures. Larval DNA was analysed through metabarcoding of the variable ITS-2 region, and sequences were grouped into Amplicon Sequence Variants (ASVs) and cross-referenced with a database (nemabiome.ca) to identify nematode species. We will present the parasite diversity observed among the 14 co-existing host species and highlight the impact of host traits and phylogenetic relationships on the composition of parasites within the sampled animal hosts. Our findings shed a light on the parasite communities inhabiting wild and domestic animals in the Maasai Mara ecosystem and underscore the implications of parasite transmission dynamics in wildlife-livestock interface areas.

(4) Drug Development 2 – (Lecture theatre 1)

Sponsored by Drugs for Neglected Diseases (DnDi)

Chair: Mark Taylor

at 14:00 (25 mins)

Applying AI to anti-parasitic drug discovery
Dr Manu De Rycker, *University of Dundee*

M De Rycker[1];
[1] University of Dundee, UK

The development of new treatments for parasitic diseases is a complex multifactorial process, with high rates of attrition. Computational approaches are increasingly applied to expedite the development of new treatments. Methods to interpret large datasets, build predictive models and design new compounds all contribute to better decision making. Here I will outline a series of computational approaches that we are using or developing for our kinetoplastid and apicomplexan drug discovery programmes. We are applying data-driven deep learning models to predict activity against key undesirable targets for *Trypanosoma cruzi*, allowing us to screen bespoke library collections depleted of likely non-progressable compounds. Using high-content imaging and image-based profiling we are developing methods to cluster compounds by their resulting phenotype and the underlying mechanism of action. Finally, I will introduce quantum mechanics approaches to understand ligand–protein

interactions driving the molecular recognition process and to build predictive affinity models to guide compound design.

14:25 (20 mins)

Structural insight into the apicomplexan drug target cytochrome *bc*1

Dr Andrew MacLean, *Research Fellow, University of Glasgow*

A MacLean[1]; L Sheiner[1]; A Mühleip[2];
[1] University of Glasgow , UK; [2] University of Helsinki, Finland

The mitochondrial electron transport chain (mETC) and F_1F_o-ATP synthase are of central importance for energy and metabolism in eukaryotic cells. The Apicomplexa, important pathogens of humans causing diseases such as toxoplasmosis and malaria, depend on their mETC in every known stage of their complicated life cycles. Complex III, also known as the cytochrome bc_1 complex, is the target of clinically used drugs such as atovaquone. The apicomplexan mETC is highly divergent from the mammalian system. Our previous proteomic work uncovered the composition of the *Toxoplasma* mETC complexes and F_1F_o-ATP synthase identifying 70 proteins, including 20 newly discovered protein subunits, highlighting their divergence from mammals.
To understand how these divergent complexes work and elucidate the mechanism of action of drug binding we used Cryo-EM to determine the structure of complex III and IV. Using native purification approaches we solved the structure of the respiratory supercomplex of complex III-IV from *Toxoplasma*, identifying new subunits and parasite-specific domains, as well as unique supercomplex architecture. Using a combination of native and immunoprecipitation approaches we were able to determine high resolution structures of the drug target *Toxoplasma* complex III in *Toxoplasma* with the inhibitors atovaquone or ELQ-300 bound. This gave us a detailed understanding of the mechanism of inhibitor binding and species specificity of drug action. This includes insight into why atovaquone displays much higher potency against apicomplexan complex III, compared to host, as well as showing that ELQ-300 has a different binding mode in apicomplexans compared to mammals. Insights from structural work opens the way for future drug design in both *Toxoplasma* and *Plasmodium*.

14:45 (15 mins)

Non-natural myristate analogues: Synthesis and biochemical characterization of their activity in protozoan parasites

Rachel Humann, *Postgraduate researcher, St Andrews University*

R Humann[1];
[1] St Andrews University, UK

Inadequate and antiquated drugs for treating a wide range of neglected tropical diseases are limiting their eradication. Despite there being some research into the potential of analogues of myristate as anti-trypanosomal agents, the biochemical characterization of their mode-of-action is largely unreported, which limits their use as potential therapeutics. This research is focused on characterising the phenotypes of known/novel myristate analogue effects in *Trypanosoma brucei*. The use of complimentary small molecule probes based upon myristate to identify compound protein targets. These myristate analogues showed EC_{50} values of <10 μM in the presence of 10 % foetal bovine serum (FBS) against *Trypanosoma brucei*, but significantly lower EC_{50} values (nanomolar) in more physiologically relevant (5%) FBS conditions. Through a series of gas chromatography mass spectrometry (GC-MS) based biochemical characterizations and metabolomic analysis, these myristate analogues were shown to sequester/ accumulate as probable acyl-CoA species within *T. brucei*. A metabolomics approach

confirmed the elongation of one fatty acid analogue, 10-(propoxy)decanoic acid, O11, which is a novel finding for this known myristate analogue. Herein there is also evidence for the likely interaction of myristate analogue acyl-CoA species with the *N*-myristoyltransferase enzyme. Using bi/mono-functional molecular it was found that these myristate analogues are used for the lipidation of a number of proteins, with the likely targets being the inositolphosphoceramide synthase (Tb927.9.9380) and the flagellar Ca^{2+} binding protein (Tb927.8.5460), the knock down of the latter giving the same unusual detached flagellar as treatment with these myristate analogues.

15:00 (15 mins)

Histone modifying enzyme (HME) inhibitors demonstrate anthelmintic activity against *Fasciola hepatica*

Sarah Davey, *Aberystwyth University*

S Davey[1]; K Hoffmann[1]; IW Chalmers[1]; J Forde-Thomas[1]; G Padalino[2];
[1] Aberystwyth University, UK; [2] Swansea University, UK

Fascioliasis remains a foodborne disease of considerable global impact. In addition to primary production and economic losses in the livestock sector, the World Health Organisation estimates over 2.39 million people are also affected globally, which justifies its inclusion on the list of communicable diseases prioritised under the Elimination Initiative of 2030. A key goal of research into the sustainable control of fascioliasis is the identification of novel anthelmintics as treatment in both humans and animals is primarily reliant on a single chemotherapeutic: triclabendazole (TCBZ). Here, we present evidence that compounds affecting histone modifying enzymes (HMEs) are flukicidal as identified during *ex vivo* screening of the Structural Genomics Consortium's epigenetic compound library. In brief, a 61-compound collection containing methyltransferase (n = 20), demethylase (n = 3), acetyltransferase (n = 6), bromodomain (n = 20) and other histone modification (n = 12) inhibitors was screened against newly excysted juvenile *F. hepatica* at an initial concentration of 10 μM (primary screen). Treated parasites were categorically scored for phenotype and motility at 24, 48 and 72 hours, with hit compounds identified as those inducing a significant degradation of parasite viability in comparison to DMSO treated controls. Hit compounds were then titrated from 10 to 0.3125 μM in a six-point serial dilution to calculate EC_{50} values (secondary screen). Putative targets of each hit compound were also identified via bioinformatic characterisation of their respective protein families. Of the 61 compounds screened, three (GSK-J4, LLY507 and NVS-CECR2-1) were found to induce significant negative phenotypes at all timepoints during primary screens. Each compound performed comparatively to TCBZ, with earliest observable affects at 1 hour post compound treatment. Titrations of each of the compounds revealed EC_{50} values of 4.17, 2.32 and 2.88 μM for GSK-J4, LLY507 and NVS-CECR2-1, respectively (72 hours post treatment). Orthologues of the human protein targets were also identified for GSK-J4 (lysine demethylase 6A, *Fh*KDM6A) and LLY507 (SET and MYND domain containing protein 2/3-like, *Fh*SMYD2/3), but only distant homologues were identified for the target of NVS-CECR2-1. Each proposed *F. hepatica* target protein was found to possess the expected domains and motifs upon analysis with InterPro and direct alignment to *Homo sapiens*- and *Schistosoma mansoni*- sequences. Complementary characterisation of *F. hepatica* HMEs involved in acetylation and methylation also uncovered 63 previously uncharacterised family members, including an expansion of the sirtuin deacetylases in the liver flukes (*Fasciola gigantica, Fasciolopsis buski, Opisthorchis felineus* and *Clonorchis sinensis*) and homologous drug targets prioritised in the blood flukes (e.g., *S. mansoni* CREB-binding protein (CBP) and bromodomain-containing protein-3 (BRD3) orthologues). We believe that the compounds identified by *ex vivo* screening, in addition to the characterisation of a large

complement of potential future epigenetic targets, represents an exciting opportunity for the future of *F. hepatica* drug discovery.

15:15 (15 mins)

PfGCN5 bromodomain, a novel drug target

Mukul Rawat, *Postgraduate researcher, University of Dundee*

M Rawat[1]; S Adjalley[2]; C Cao[2]; J Hoshizaki[2]; T Qahash[3]; R Shaik[2]; C Smidt[2]; M Luth[4]; E Winzeler[4]; M Llinás[3]; M Lee[1];
[1] University of Dundee, UK; [2] Wellcome Sanger Institute, UK; [3] Pennsylvania State University, United States; [4] School of Medicine, University of California, San Diego, United States

The emergence of resistance to existing drugs has highlighted the need for new antimalarials. Bromodomain-containing proteins (BDPs) bind to acetylated lysine residues in histones and regulate transcription involved in the pathogenesis of a variety of diseases. BDPs have been exploited as drug targets in various diseases for new therapeutic development. *Plasmodium falciparum* General Control Non-repressed 5 protein (GCN5) has been shown to play a role in invasion and virulence. Here, we show that conditional knockout of the PfGCN5 bromodomain is essential for parasite survival in the blood stage. Next, we investigated the activity of small molecule inhibitor L45 and the possibility of exploiting PfGCN5 bromodomain as a potential drug target. The protein structure of the PfGCN5 bromodomain in complex with the inhibitor has been previously resolved. L45 is active against the blood and liver stage of *P. falciparum* and *P. berghei*, respectively. *In vitro* selection of drug-resistant parasites identified point mutations in the mitochondrial carrier protein (PfMCP1). Interestingly, L45-resistant parasites were hypersensitive to other mitochondrial drugs including atovaquone, DSM1, and myxothiazol. Furthermore, metabolomics studies showed the upregulation dUMP and peptides on L45 treatment. This suggests a link between PfGCN5 bromodomain inhibition and mitochondrial function. Our data indicate that L45 has a novel mode of inhibiting *Plasmodium* and that PfGCN5 bromodomain inhibition may be a promising starting point for rational drug design.

(5) Population genomics 2 - (Lecture theatre 2)

Sponsored by Calibre Scientific

Chair: Steve Paterson

14:00 (25 mins)

Evolutive Dynamics and Biological Interplay of *Leishmania (Viannia)* Species with Their Endosymbiotic Partner, Leishmania RNA Virus 1

Dr Elisa Cupolillo, *Instituto Oswaldo Cruz*

E Cupolillo[1]; L Motta Cantanhêde[1]; K Chourabi[1]; M Côrtes Boité[1]; C Dujardin[2]; S Heeren[2]; F Van den Broeck[2];
[1] Leishmaniasis Research Laboratory, Instituto Oswaldo Cruz, Brazil; [2] Department of Biomedical Sciences, Institute of Tropical Medicine, Belgium

The parasitic flagellates of the *Leishmania* genus (Family Trypanosomatidae) cause leishmaniasis, a vector-borne disease with diverse clinical manifestations. Dixenous *Leishmania* parasites likely evolved from monoxenic Trypanosomatidae, with phylogenetic studies placing them in the Leishmaniinae subfamily. Their origin dates back to the Mesozoic era, dispersing globally before the Gondwana supercontinent breakup. These protists host negative-sense single-stranded RNA viruses (Family

Leishbunyaviridae) and double-stranded RNA viruses (Family Totiviridae). *Leishmania martiniquensis* leishbunyavirus 1 (LmarLBV1) enhance infectivity *in vitro* and is the only known bunyavirus in *Leishmania*, found in the Mundinia subgenus, represented by worldwide dispersed Leishmania species. Totiviridae, commonly known as myco-viruses, infect also several protists. Leishmania RNA virus (LRV) includes LRV1 and LRV2, infecting *Viannia* and *Leishmania* subgenera, respectively. LRV1, influencing infection pathology, exhibits regional specificity, with Northern South American strains predominantly infected. Population genetic studies reveal LRV1 distribution aligning with Brazilian Amazon strains, emphasizing the importance of LRV surveys due to their impact on infection outcomes. LRVs provide *Leishmania* a survival advantage by suppressing anti-leishmanial immunity in vertebrate hosts. LRV1's interaction with the TLR3 endosomal receptor promotes chronic inflammation and facilitates *Leishmania* spread, causing persistent "metastatic" infection. The presence of viruses in certain *Leishmania* spp. may result from recent horizontal transfers, supported by experimental evidence of LRV transmission via exosomes. Understanding the co-evolution of LRV1 and *Leishmania* (*Viannia*) parasites, their species-specific relationships, and the endosymbiont's impact on parasite biology is crucial for comprehending *Leishmania* infection dynamics in hosts.

14:25 (15 mins)

Whole genome sequencing of *Leishmania* species causing Cutaneous Leishmaniasis in South America

Cián Lynch, *Postgraduate researcher, University of York*

C Lynch[2]; JL Reis-Cunha[3]; S James[3]; SJ Smit[2]; DC Jeffares[1]; E Cupolillo[4];
[1] University of York, UK; [2] Department of Biology, University of York, UK; [3] York Biomedical Research Institute, Department of Biology, University of York, UK; [4] Laboratório de Pesquisa em Leishmaniose, Instituto Oswaldo Cruz, Fiocruz, Rio de Janeiro-RJ, Brasil, UK

Leishmania protozoans cause significant disease burden in many regions of the world. Many Leishmania species endemic to South America cause Cutaneous Leishmaniasis (CL), with limited disease treatment options and efficacy. There are differences in the CL clinical outcomes, varying from a single to diffuse lesions and mucocutaneous manifestations, which might be a consequence of differences in the host and/or the parasite genome.

The delimitation of *Leishmania* species that causes CL species in Brazil is not completely understood, due to a lack of chromosomal level genome assemblies to represent the full gene repertoire of some species; and/or lack of substantial data from field isolates to estimate its intra-population variation. These parasites often have similar genomes, and natural hybridization was already detected in multiple populations, such as between *L. braziliensis* and *L. peruviana*, which hampers discerning between species limits and geographic variation. The occurrence of hybridization allows beneficial mutations to spread throughout populations, which is of great importance to study virulence factors and drug sensitivity .

Here we have generated chromosome-level assemblies of 12 strains/species of *Leishmania: L. braziliensis* (M2903), *L. equatorensis*, two strains of *L. lainsoni, L. naiffi, L. panamensis, L. shawi, L. adleri, L. hertigi, L. pifanoi, L. colombiensis*, and an new *L. Viannia* hybrid isolate. Using a combination of Oxford Nanopore long reads and Illumina short reads, we have built a genome assembly pipeline using the Necat assembler, and extensive polishing steps before annotation. This resulted in a significant improvement in the quality and continuity of the genome assemblies. In the case of four of these species (*L. lainsoni, L. panamensis, L. naiffi,* and *L. guyanensis*) we have significantly improved the quality of genome assembly over the currently available data, while for the remaining species, we generated the first chromosomal level genome assembly reference.

14:40 (15 mins)

Whole genome sequencing of *Leishmania braziliensis* in clinical samples demonstrates long-term recurrent recombination in a population of parasites from Southern Peru

Prof Jean-Claude Dujardin, *Head of Molecular Parasitology, Institute of Tropical Medicine, Belgium*

P Monsieurs[1]; ME Cruz[3]; A Aroni[1]; M Sernaque[2]; M Pinedo[1]; J Arevalo[2]; F Van den Broeck[1]; S Heeren[1]; MA Domagalska[1]; JC Dujardin[1];
[1] Institute of Tropical Medicine, Antwerp, Belgium; [2] Universidad Peruana Cayetano Heredia, Peru; [3] Consultorio de Enfermedades Infecciosas y Tropicales, Cusco, Peru

Peru is one of the countries with the highest burden of tegumentary leishmaniasis (TL) in the world. In the Amazonian Forest, *L. braziliensis* is the most prevalent species of *Leishmania*, which is known to be genetically very heterogeneous. Using whole genome sequencing (WGS) of cultivated parasites isolated from 1994 to 2002, our group discovered ancestral populations isolated in patches of tropical rainforest [Heeren et al., 2023]. In addition, a large number of parasites from Southern Peru showed mixed ancestry, resulting from multiple intra-species hybridization events, probably favoured by environmental destruction in that zone, together with migration of human and hemerophile reservoir like dogs and rats. Interestingly, these hybrids were often associated with treatment failure and 80% of them were shown to harbour the endosymbiotic LRV1 virus. The presence of these hybrids in that region could be of relevance for public health, and their fate since the early sampling was studied in the present follow-up study.

More specifically, we aimed to assess if these hybrid parasites are still dominant today in southern Peru and if they continue to recombine or predominantly reproduce clonally. We sampled patients from Southern Peru in 2019-2020 and used a culture-independent WGS protocol to avoid selection biases associated with *in vitro* maintenance. Our study consists of two parts. 1) the validation of a robust direct sequencing protocol on clinical samples with extremely low parasitaemia levels (median 0.01% of parasite DNA): we compared the performance of two direct sequencing methods (Selective Whole Genome Amplification [SWGA] and SureSelect-based target genome capture [SuSL]) on 17 samples collected from TL patients in the departments of Cusco and Madre de Dios. SuSL outperformed SWGA by providing more even and higher genomic coverage which allowed reliable analysis of population genomic structure, somy levels, and maxicircle sequence, identification of major copy number variations, and better coverage of genes associated with drug resistance and virulence. 2) population genomic analyses of these clinical samples was integrated with our previously obtained genomic data of cultured isolates collected in this region. SuSL allowed identification and ancestry analysis of hybrid parasites: 13 out of the 17 clinical samples from 2019-2020 exhibited admixed ancestry patterns as the cultured/cryopreserved isolates from 1991-2003: close inspection of local ancestry assignment in individual chromosomes revealed that all hybrid parasites had unique mosaic ancestry patterns between their ancestral components, suggesting that *L. braziliensis* has recurrently recombined in this region for several decades. Besides the technological innovation of SuSL, our study confirms the importance of sexual recombination in *L. braziliensis*, the dominance of hybrids in Southern Peru and the need for genomic surveillance.

Heeren et al. Nat Commun. 2023 Dec 15;14(1):8343

14:55 (15 mins)

The dispersal of visceral leishmaniasis during the peak of the Roman Empire

Dr Daniel Jeffares, *Lecturer, University of York*

DC Jeffares[1]; C Grace[3]; JL Reis-Cunha[3]; S Ahmed[2]; S Harnqvist[4]; M Côrtes Boité[5]; G Barcellos[5]; L Lachaud[6]; P Bastien[6]; H Munt[7]; J Mottram[1]; E Cupolillo[5];
[1] University of York, UK; [2] Department of Biology, University of York, UK; [3] York Biomedical Research Institute, Department of Biology, University of York, UK; [4] University of Edinburgh, Institute for Ecology and Evolution, UK; [5] Laboratório de Pesquisa em Leishmaniose, Instituto Oswaldo Cruz, Fiocruz, Rio de Janeiro-RJ, Brasil, UK; [6] Laboratoire de Parasitologie-Mycologie, Centre Hospitalier Universitaire de Montpellier, Centre National de Référence des Leishmanioses, Montpellier, France, UK; [7] Department of History, University of York, UK

The *Leishmania donovani* species complex (LDSC, *L. donovani* and *L. infantum*) causes visceral leishmaniasis. These species are currently widely distributed throughout north and east Africa, the Arabian Peninsula, central Asia, and the Indian subcontinent. Previous reports have suggested that *L. donovani* and *L. infantum* diverged between one and ten million years ago. Here, we use population genomic analysis of over 800 genomes to show that the divergence of this species was far more recent – most likely within the last 2000 years of human history.

Details: Ancient DNA and population genomic data indicate that the visceral leishmaniasis originated in East Africa - but the timing and route of global dispersal of this disease are not well understood. We used genome data to estimate regional population split dates, showing that the LDSC began to migrate of Africa in approximately 200 CE, consistent with dispersal during the height of the Roman Empire, when the Axumite Empire in modern day Ethiopia was a major international trade route. From the Axumite Empire, passage on the Nile River would lead to the populous Roman city of Alexandria.

We show that the complex likely arrived in Turkey and the Arabian Peninsula by 700 CE, India by 750 CE. The *L. infantum* clade is not well-supported as a species, but rather a sub-population of *L. donovani*, which arose in approximately 722 CE. We estimate that *L. infantum* was introduced to South America from the Iberian Peninsula population almost a millennium later (~1650 CE), clearly implicating the Portuguese colonisers.

These results revise our understanding of the history of visceral leishmaniasis. Importantly, the people of East Africa appear to have been exposed to *L. donovani* for at least one thousand years before European colonisers introduced *L. infantum* to native American people.

15:10 (20 mins)

Genomic epidemiology of *Trypanosoma cruzi* and its vectors

Dr Martin Llewellyn, *Reader, University of Glasgow*

M Llewellyn[1];
[1] University of Glasgow , UK

Trypanosoma cruzi causes Chagas disease, a neglected disease even amongst the neglected tropical diseases. Chagas disease is spread by triatomine bugs, a group of reduviids that have made the switch to haematophagy. Chagas disease kills > 10,000 annually, with 6-7 million people infected, principally in South and Central America. Little is understood about the genomic epidemiology of Chagas disease and its vectors. The parasite is highly genetically diverse, with six discrete typing units (DTUs) reported and substantial genetic diversity also reported with these DTUs. Chagas disease prognosis is similarly variable, but, as yet, there is no clear link between parasite genotype and disease outcome. Over 100 species of triatomine vectors species are thought to transmit Chagas disease, but a smaller number drive most human transmission. Since the eradication of major domestic species in the late 20th century, several 'secondary' vectors species are in the process of re-colonising. In this talk, I'll present our work characterising the population genomics of *T. cruzi* and it vectors, exploring parasite mating

systems, invasion of the domestic setting and highlighting the need for new resources and perspectives to improve our understanding of the parasite and to achieve its sustainable control.

(6) Antigenic variation 2 - (Lecture theatre 3)

Chair: David Horn

14:00 (20 mins)

Mechanisms of *Pneumocystis jirovecii* surface antigenic variation

Dr Philippe Hauser, *Centre Hospitalier Universitaire Vaudois*

P Hauser[1];
[1] Centre Hospitalier Universitaire Vaudois, Switzerland

Surface antigenic variation is crucial for major pathogens that infect humans. To escape the immune system, they exploit various mechanisms to modify or exchange the protein that is exposed on the cell surface, at the genetic, expressional, and/or epigenetic level. We studied those used by the fungus *Pneumocystis jirovecii* that causes life-threatening pneumonia in immunocompromised individuals. Though this fungus is currently not cultivable, our detailed analysis of the subtelomeric sequence motifs and genes encoding six families of major surface glycoproteins suggests that the system involves homologous recombination. Translocations of entire genes lead to the reassortment of the repertoire of ca. 80 non-expressed alleles of family I present in each strain, from which single genes are retrieved over time for mutually exclusive expression within subpopulations of cells. The recombination also leads to allele mosaicism and rearrangement of the subtelomeres. In addition, imperfect mirror sequences forming DNA triplexes are likely to play a role in the system

14:20 (25 mins)

High-resolution scRNA-seq reveals genomic determinants of antigen expression hierarchy in African Trypanosomes

Prof Nicolai Siegel, *Ludwig-Maximilians University*

N Siegel[1];
[1] Ludwig-Maximilians University, Germany

Antigenic variation is an immune evasion strategy used by many different pathogens. It involves the periodic, non-random switch in the expression of different antigens throughout an infection. How the observed hierarchy in antigen expression is achieved has remained a mystery. A key challenge in uncovering this process has been the inability to track transcriptome changes and potential genomic rearrangements in individual cells during a switch event. Here, we report the establishment of a highly sensitive single-cell RNA-seq (scRNA-seq) approach for the model protozoan parasite *Trypanosoma brucei*. This approach has revealed genomic rearrangements that occur in individual cells during a switch event. Our data show that following a double-strand break (DSB) in the transcribed antigen-coding gene – an important trigger for antigen switching – the type of repair mechanism and the resultant antigen expression depend on the availability of a homologous repair template in the genome. When such a template was available, repair proceeded through segmental gene conversion, creating new, mosaic antigen-coding genes. Conversely, in the absence of a suitable template, a telomere-adjacent antigen-coding gene from a different part of the genome was activated by break-induced replication. Our results reveal the critical role of available repair sequence in the antigen selection mechanism. Additionally, our study demonstrates the power of highly sensitive scRNA-seq methods in detecting genomic rearrangements that drive transcriptional changes at the single-cell level.

14:45 (20 mins)

A *Plasmodium*-specific AP2-P regulates multiple pathogenicity factors during the IDC

Prof Arnab Pain, *KAUST*

A Pain[1];

[1] KAUST, Saudi Arabia

Apicomplexans are a diverse group of obligate parasites of humans and animals, with a high impact on human health, food security and economics. Several studies on apicomplexan evolution have revealed that before the switch to parasitic life, the ancestor contained a broad repertoire of genes many of which were subsequently repurposed towards parasitism, such as extracellular proteins, components of a motility apparatus, and DNA- and RNA-binding protein families. Proteins with DNA-binding domains (DBD) or RNA-binding domains (RBD) regulate various molecular processes of apicomplexan parasites. Indeed, during apicomplexan evolution, we have seen major lineage-specific expansions of proteins with AP2 (apiAP2) DBD – many of which have been shown to act as genetic control switches for diverse apicomplexan processes including pathogenesis. Several studies that focused on understanding gene regulation suggest that malaria parasites have evolved multilayered gene regulatory mechanisms to fine-tune the expression of genes that include genes encoding pathogenicity factors crucial for its growth and development inside the host. Recently, our group, in collaboration with several international partners, have characterized an essential *Plasmodium*-specific Apicomplexan AP2 (ApiAP2) transcription factor in *Plasmodium falciparum* (PfAP2-P; Pathogenesis) during the intraerythrocytic developmental cycle (IDC). An inducible gene knockout approach showed that PfAP2-P is essential for development during the trophozoite stage, and critical for *var* gene regulation, merozoite development and parasite egress. ChIP-seq experiments were performed at 16 hours post-invasion (h.p.i.) and 40 h.p.i. matching the two peaks of PfAP2-P expression, demonstrating the binding of PfAP2-P to the promoters of genes controlling antigenic variation, host cell remodelling and trophozoite development at 16 h.p.i. and antigenic variation and pathogenicity at 40 h.p.i. Using single-cell RNA-seq and fluorescence-activated cell sorting, we show the de-repression of *var* gene expression in *Δpfap2-p* parasites that express multiple PfEMP1 proteins on the surface of infected RBCs. In addition, the *Δpfap2-p* parasites overexpress several early gametocyte marker genes at both 16 and 40 h.p.i., indicating a putative regulatory role in the sexual stage conversion. Using the Chromosome Conformation Capture (Hi-C) experiment, we demonstrate that deletion of PfAP2-P results in a significant reduction of both intra-chromosomal and inter-chromosomal interactions in heterochromatin clusters. We conclude that PfAP2-P is a vital upstream transcriptional regulator controlling essential pathogenic processes in two distinct developmental stages during the IDC that include parasite growth, egress and invasion, chromatin structure and *var* gene expression.

15:05 (25 mins)

Challenging the Paradigm of Mutually Exclusive *var* Gene Expression for Antigenic Variation by *Plasmodium falciparum*

Prof Kirk Deitsch, *Cornell University*

Malaria parasites have evolved mechanisms to enable them to avoid their host's immune response and maintain an infection that is sufficiently persistent to allow efficient transmission to their mosquito vector. This is largely accomplished through antigenic variation, the systematic alteration of surface proteins exposed to the immune system. The parasite's genome contains large, multicopy gene families, with each individual gene encoding an antigenically distinct form of the surface protein. By systematically activating and silencing individual genes, the parasites can alter their antigenic signature and thus avoid antibodies produced by the host directed toward these surface antigens. This process is remarkably

effective, with infections typically able to persist for over a year. For *P. falciparum*, the gene family encoding the primary antigenic determinant is called *var* and consists of 40-90 genes per haploid genome. Current models indicate that only a single gene is expressed at any given time, with the remainder of the family maintained in a transcriptionally silent state via repressive epigenetic chromatin modifications. However, using single cell transcriptomic analysis, we have recently discovered that *var* gene expression is much more flexible than previously appreciated. We found that while parasites typically express a single *var* gene, individual parasites can also express multiple *var* genes simultaneously or enter a state in which little or no *var* gene expression is detectable. Further, by manipulating the availability of the methyl donor S-adenosyl methionine, we can completely disrupt mutually exclusive expression, leading to stable, high-level expression of several *var* genes in each cell. These data challenge the dogma that *var* gene expression is limited to a single gene at a time and shed light on the mechanisms underlying mutually exclusive expression, transcriptional switching and *var* gene choice.

(22) Innovations in vector control - (Teaching room 4)

Chair: Al Darby

14:00 (25 mins)

Tiny Targets accelerate progress towards the elimination of sleeping sickness

Dr Andrew Hope, *Liverpool School of Tropical Medicine*

A Hope[1];
[1] *Liverpool School of Tropical Medicine, UK*

Sleeping sickness (human African trypanosomiasis, HAT) is a neglected tropical disease which is fatal if untreated. The most common (>90% of cases) form of the disease occurs in west and central Africa and is caused by *T. b. gambiense* (gHAT) transmitted by riverine species of tsetse. A programme of mass screening and treatment of cases, co-ordinated by the WHO, reduced the number of cases reported globally from an average of ~23,000 (range=10,987-37,385) cases in the 1990s to ~7,000 cases/year by 2010. Control of the tsetse vectors did not play a major role during this period due to the costs and logistical constraints associated with the available technologies. To address this, an international collaboration of researchers and industry undertook field research on the host-finding behaviour of riverine tsetse. This led to the development of Tiny Targets, insecticide-treated baits which attract and kill riverine tsetse. Initial field trials in Uganda, Chad and Guinea demonstrated that Tiny Targets could reduce tsetse densities by >80% at a reduced cost (<20%) compared to standard methods of tsetse control. Following these successful trials, Tiny Targets were introduced in Côte d'Ivoire and Democratic Republic of Congo (DRC) and implemented alongside screening and treatment. These five countries historically accounted for >80% of gHAT cases. Modelling has demonstrated the impact of Tiny Targets on disease incidence. In Chad for instance, >70% of a decline in the annual incidence of gHAT in the Mandoul focus was due to the deployment of Tiny Targets. Tiny Targets have contributed to achieving the WHO 2020 goal of reducing the number of new cases to fewer than 2,000 per year, achieved consistently since 2017. In 2021, WHO declared that Côte d'Ivoire had achieved elimination of gHAT as a public health problem, and in 2022 Uganda also achieved this milestone; the role of Tiny Targets in this success was recognised in both countries. More recently, Tiny Targets have been introduced in South Sudan and Angola and continue to be scaled-up in DRC, the country with the largest burden of disease. Introduction of Tiny Targets to other countries where gHAT persists is required to meet WHO's 2030 goal of eliminating transmission of gHAT.

14:25 (25 mins)

Vector control in response to the changing malaria landscape
Dr Eric Ochomo, *Kenya Medical Research Institute*

E Ochomo[1];
[1] Kenya Medical Research Institute, Kenya

A quick scan of the world malaria reports in the past decade will tell you a story of despair. Malaria has stagnated and even increased in some places. This despite the recent celebrations of >two billion insecticide-treated nets (ITNs) distributed globally and the billions of dollars invested this far. Multiple reasons are suggested as being responsible for this stagnation; insecticide resistance, changing vector behaviour, changing land use and climate change, invasive vectors, human displacement, urbanization and human behaviour. Appreciating that vector control has been central in all the areas where malaria has been eliminated and was observed to contribute to bulk of the decline between 2000 and 2015, our research focuses on strengthening entomology surveillance and evaluating novel vector control tools. We look at traditional and current approaches such as house modification, larval control, indoor residual spraying (IRS) and ITNs and evaluate novel tools such as spatial repellents, attractive targeted sugar baits, endectocides, next generation ITN and IRS products and genetically modified mosquitoes. These evaluations inform the recommendation of novel vector control tools at World Health Organization (WHO) in addition to contributing to local vector control policy. Additionally, our research focuses on the gaps that vector control needs to fill to ensure a trend in the right direction in the fight against malaria.

14:50 (15 mins)
Monitoring biological age in mosquitoes using infrared spectroscopy
Dr Mauro Pazmino, *University of Glasgow*

J Mgaya[3]; M Pazmiño Betancourth[1]; DJ Siria[3]; F Baldini[1]; FO Okumu[3]; M Gonzalez-Jimenez[1]; M Sikulu[2];
[1] University of Glasgow , UK; [2] The University of Queensland, Australia; [3] Ifakara Health Institute, Tanzania

Background: The age of vector populations is the most important factor that determines vectorial capacity, hence disease transmission. However, there are no effective methods to measure age structures in mosquitoes. Infrared spectroscopy (IRS) with machine learning (ML) models have shown promising results as all-in-one solution to determine the age of malaria vectors. However, models trained with laboratory reared mosquitoes require re-calibration to generalize and predict the age of wild samples. This extra step is caused by the different ageing rates between laboratory reared and field collected, which are exposed to different environmental conditions. Here, we characterized how temperature affects ageing rates in mosquitoes and how these changes are reflected in IRS spectra to produce ML models that can predict the biological age of mosquitoes and generalize across different ecological settings.

Methods: The effect that temperature has on biological age was tested using two different average temperatures: 24 and 27°C and with two different ranges: ±3°C and ±6°C using environmental chambers. The survival of two major malaria vectors (*Anopheles coluzzii* and *An. gambiae)* was monitored for approximately 30 days across different replicates, in the presence and absence of a sublethal exposure of deltamethrin insecticide. Mid infrared spectra of mosquitoes at different time points were obtained and their life expectancy was analysed to determine their biological age.

Results: Mosquito survival varied depending on temperature, insecticide exposure and species. Accuracy for predicting biological age was higher compared to chronological age, suggesting that IRS

signal is directly associated with mosquito ageing rates. Ongoing analysis will validate these models in mosquitoes reared in semi field settings and collected from the field.

15:05 (25 mins)

HumBug – developing an acoustic sensor to detect and identify mosquito vectors of disease

Dr Marianne Sinka , *University of Oxford*

M Sinka [1];
[1] University of Oxford, UK

Of the 3500 species of mosquito in the world, only around 100 are able to transmit human diseases with sufficient efficiency to be dangerous. What tends to distinguish these species is their affinity with humans; making use of the altered environments we create. Their species-specific behavioural characteristics (e.g. a preference for human blood or the drive to search indoors for a blood meal) influence their capacity to spread disease as well as how effective our current arsenal of (most indoor and insecticide-based) interventions will be at controlling them. Thus correctly identifying which species of vector are found at a location is fundamental to successful vector control. Methods: Here I will describe HumBug – an acoustic mosquito sensor that uses a budget smartphone to detect and identify mosquito vectors as they attempt to bite people indoors during the night. I will highlight the challenges (mosquitoes are very small and not very loud) and the benefits (cheap, standardised vector surveillance over spatial and temporal ranges that are impossible to achieve using traditional surveillance methodologies) and present field results from our trials conducted in Tanzania and the Democratic Republic of Congo. Results: The HumBug tool successfully captured mosquito flight tones from every house where it was deployed. Its performance was compared to CDC-LTs and human baited nets and accurately represented the seasonal fluctuation in abundance. Nocturnal peak biting activity was also clearly seen via the acoustic data. Community acceptance of the tool was high and few issues were reported Conclusion: The HumBug tool could be of significant value in long term monitoring over spatial scales not possible to achieve via traditional sampling methodologies. Moreover, it could provide a solution for long term passive monitoring for invasive vector species such as *An. stephensi* in Africa.

(7) Disease elimination -– (Lecture theatre 1)

Sponsored by New England Biolabs

Chair: Poppy Lamberton

16:00 (25 mins)

Elimination of Visceral Leishmaniasis from India

Prof Shyam Sundar, *Banaras Hindu University*

S Sundar[1];
[1] Banaras Hindu University, India

Visceral leishmaniasis (VL), also known as Kala-azar, is the most severe form of leishmaniasis. VL epidemics have been known for several centuries in India. However, the current epidemic began in the early 1970s, with estimated annual incidences of several hundred thousand. Unfortunately, drug resistance to pentavalent antimonials in Bihar has limited the effectiveness of treatment options. In the year 2000, amphotericin B deoxycholate was recommended as a substitute; however, the lack of infrastructure in public health facilities made it difficult to implement. In 2002, oral miltefosine was

licensed for the treatment of VL in India after a high cure rate in the pivotal phase 3 trial. In 2005, the Kala-azar Elimination Program (KAEP) was launched jointly in India, Nepal, and Bangladesh with early diagnosis using the rK39 strip test and treatment with miltefosine for four weeks, residual insecticide spray, and public awareness. The selection of miltefosine was based on its high cure rate and the ease of using an oral drug in the field. However, after nearly seven years of use, poor compliance was common due to the 28-day long treatment and reports of declining efficacy. Finally, the Regional Technical Advisory Group recommended a change in treatment. In 2010, a single dose of Liposomal Amphotericin B (10mg/Kg) demonstrated very high efficacy, leading to its approval by the WHO in the same year. In 2013, treatment was changed, leading to a significant impact on the incidence of VL. After ten years of its use, the elimination target has been achieved in India and Bangladesh. In October 2023, Bangladesh was declared by the WHO to have eliminated the disease. In India, all 633 blocks have been declared to have reached the elimination target. However, the status has to be maintained for three consecutive years for WHO certification.

16:25 (20 mins)

ECLIPSE: Improving access to healthcare for cutaneous leishmaniasis in Brazil, Ethiopia and Sri Lanka

Prof Helen Price, *Keele University*

HP Price[1]; S Agampodi[5]; TC Agampodi[5]; L Dikomitis[2]; PR Machado[3]; A Mulugeta[4]; L Trad[3];
[1] Keele University, UK; [2] University of Kent , UK; [3] Federal University of Bahia, Brazil; [4] Mekelle University, Ethiopia; [5] Rajarata University of Sri Lanka, Sri Lanka

Cutaneous leishmaniasis (CL) is a stigmatizing neglected tropical disease (NTD) which affects highly marginalized and underserved communities across the globe. While there are new WHO initiatives to address skin-related NTDs, effective control will require a clearer understanding of the barriers to accessing healthcare for CL and the wider challenges and effects of the disease on individuals and their communities. We present here findings from the five-year interdisciplinary ECLIPSE programme which aims to improve the CL patient journey and reduce stigma in the most underserved communities in Brazil, Ethiopia and Sri Lanka. We have used qualitative, quantitative and creative approaches to investigate the impacts of CL on individuals and communities, levels of disease awareness and the barriers and facilitators for accessing diagnosis and treatment. Through dedicated Community Advisory Groups and Communities of Practice we ensured that our approach, research tools and interventions are appropriate for each context and that all activities are underpinned by stakeholder involvement. Findings from over 200 interviews and 2,500 surveys across three countries showed that understanding of the causes and transmission of CL was low and that misinformation contributed to fear and stigmatization of affected individuals and, in some cases, of whole communities. Barriers to accessing healthcare included distance of travel to clinics, lack of childcare, fear of drug side-effects, inability to work, and low disease awareness in communities and healthcare professionals. We found the burden of CL to be particularly high in Tigray, Ethiopia, where the ECLIPSE team evidenced the devastating impact of the recent conflict on healthcare systems, together with a sharp increase in CL cases due to population displacement to caves and greater contact with rock hyrax, a reservoir host for *Leishmania aethiopica*. Understanding and awareness of CL was very low in affected communities. There was a view at primary healthcare level that the disease was untreatable, which impacted on referral for diagnosis and treatment. Conversely, use of traditional remedies was very high. There was evidence of severe stigma of individuals, which was linked to local beliefs about CL. The ECLIPSE team are now addressing identified challenges and barriers through the co-production and implementation of bespoke

community-facing interventions, with shared learning across country teams. Interventions include awareness campaigns, community-based films and books, a traditional masked folk theatre, video animation, television and radio programmes, podcasts, and training courses for healthcare professionals. In parallel, we are engaging with policymakers across multiple sectors and influencing policy change around decentralisation and increasing capacity and access for CL diagnosis and treatment. Work with the Tigray Regional Health Bureau and Ayder Hospital has resulted in the establishment of a new inpatient centre and five new outpatient treatment centres for CL in Tigray.

16:45 (20 mins)

The elimination of lymphatic filariasis in Ghana: reality or pipeline dream

Prof Dziedzom Komi de Souza, *University of Ghana*

DK de Souza[1];
[1] University of Ghana, Ghana

The establishment of Global Programme to Eliminate Lymphatic Filariasis (GPELF) in the year 2000 was made possible by the availability of an easy-to-use rapid diagnostic test and the drugs ivermectin, albendazole and diethylcarbamazine. At the onset of the programme, and based on modelling studies, it was assumed that 5 to 6 years of treatment with 80% coverage of the entire population should be sufficient to break the cycle of transmission of *W. bancrofti* and *Brugia spp.* and eliminate LF as a disease of public health concern in endemic communities. Since the launch of the GPELF there has been significant progress with many implementation units stopping mass drug administration (MDA). However, the infection persists in many endemic country settings despite more than two decades of control activities. Using Ghana as an example, we will delve into the LF situation in one of the first countries to implement MDA using ivermectin and albendazole, based on the WHO guidelines for countries that are co-endemic for LF and onchocerciasis and where the antigen prevalence is above 2%. Monitoring and impact assessments of the Ghana LF programme followed WHO guidelines and protocols. However, in the implementation of these guidelines various challenges were encountered. This presentation is an overview of the evolution of the LF situation in Ghana, the impact of MDA on the transmission of LF, the challenges encountered, the role of vectors in the persistent transmission, and current studies to address the endgame challenges.

17:05 (20 mins)

Schistosomes and how to find them – testing novel methods of *Schistosoma mansoni* molecular environmental monitoring in Lake Albert

Dr Zikmund Bartonicek *Natural History Museum London & Institute of Parasitology, Biology Centre CAS*

Z Bartonicek[1]; J Dvorak[4]; F Allan[1]; AM Emery[1]; P Isingoma[3]; JJ Day[2]; BL Webster[1];
[1] Natural History Museum, UK; [2] University College London, UK; [3] Vector Control Division, Ministry of Health, Uganda; [4] Center of Infectious Animal Diseases, Faculty of Agrobiology, Food and Natural Resources, Czech University of Life Sciences, Czechia

Introduction: Sensitive and specific surveillance methods are needed to detect and monitor schistosomiasis transmission, particularly as interventions decrease disease prevalence. *Schistosoma mansoni* causes intestinal schistosomiasis and is endemic in sub-Saharan Africa, parts of South America and the Caribbean. Its lifecycle depends on zooplanktonic larvae (cercariae and miracidia) and intermediate freshwater snail hosts, all of which are a part of freshwater food webs.

Traditionally, environmental transmission monitoring is achieved via malacological surveys where snails are collected and screened for *Schistosoma* cercariae or DNA. Although informative, these can be laborious and insensitive, and new, more sensitive monitoring methods are needed.

This study field-tested a new molecular approach – Fish Faecal Xenomonitoring (FFX), detecting *S. mansoni* DNA in the faeces of juvenile *Oreochromis niloticus*, a natural fish predator of cercariae and other zooplankton, and compared it to traditional and environmental DNA (eDNA) methods.

Methods: After laboratory tests of the FFX method showed promising results confirming that *S. mansoni* DNA can reliably be detected in faeces of *O. niloticus* that consumed cercariae, the method was tested in *S. mansoni* - endemic north-eastern Lake Albert (Uganda).

Across ten sampling sites, juvenile (1 - 4 cm standard length) *O. niloticus* were caught and pooled in groups of five. Fish were fed and kept in aquaria for 18 hours, after which they were released back into the lake. All fish faeces were collected from aquaria, stored in ethanol, and subsequently molecularly analysed for the presence of *Schistosoma* DNA by qPCR. The FFX results were compared to the detection of transmission by traditional xenomonitoring, where *Biomphalaria* and *Bulinus* snails were collected and checked for emergent schistosome cercariae. Alongside, we also tested *Schistosoma* detection using eDNA methods, where the water from tested sites was filtered through 0.45 µm Sterivex PVDF filters and subsequently tested by qPCR.

For FFX and eDNA, samples were analysed using a newly developed multiplex qPCR assay targeting the 16S region of African *Schistosoma* species and also *O. niloticus* as an internal control.

Results: All tested methods (FFX, eDNA and shedding) were in agreement at 4/10 sites tested. The FFX approach was most sensitive, indicating *Schistosoma* presence at 9/10 sites, followed by eDNA (5/10 sites) and shedding analysis, which identified 4/10 inspected sites with patently infected snails.

These results clearly demonstrate that *Schistosoma mansoni* transmission is ongoing in Lake Albert and that molecular methods are more sensitive than traditional shedding analysis.

Conclusions: Our findings show that juvenile *O. niloticus* readily consume *Schistosoma* cercariae with DNA detectable in their faeces post-consumption. We also demonstrate that both the FFX end eDNA methods can detect schistosomiasis transmission in water bodies such as Lake Albert, perhaps with higher sensitivity than traditional methods, enhancing schistosomiasis transmission monitoring.

17:25 (20 mins)

Community and Individual Preferences for a New Water Infrastructure for Non-Drinking Water in a Schistosomiasis Endemic Area

Raheema Chunara, *University of Glasgow*

R Chunara[1]; L Mujumbusi[3]; E Nalwadda[3]; M Arinaitwe[2]; L Pickering[1]; MR Templeton[4]; P Lamberton[1];
[1] University of Glasgow , UK; [2] Ministry of Health, Uganda; [3] Medical Research Council/Uganda Virus Research Institute & London School of Hygiene and Tropical Medicine Uganda Research Unit, Uganda; [4] Department of Civil and Environmental Engineering, Imperial College London, UK

Background: Schistosomiasis is a water-borne parasitic disease affecting 240 million people. Schistosomes reproduce sexually in humans, releasing eggs in urine and faeces which hatch in freshwater and infect snails, where they reproduce asexually releasing hundreds of cercariae/day. These cercariae burrow directly into humans on contact with contaminated water, continuing the cycle. Mass drug administration has been the WHO recommended strategy for nearly 20 years, and whilst

successful in some areas, there are hotspots across sub-Saharan Africa. Additional nonpharmaceutical interventions are needed to meet the WHO goal of eliminating schistosomiasis as a public health problem by 2030. The WHO roadmap states that improved access to safe water, sanitation and hygiene (WaSH) is needed. In low-income countries, non-governmental organizations are essential actors that implement WaSH infrastructure however 30-50% of WaSH projects implemented in communities cease to be used after 2 – 5 years. To increase access to safe water, both uptake and sustainability of the WaSH infrastructure needs to be considered. Qualitative research in the community can deepen our understanding of the needs of the community and provide an evidence base to help co-design a solution that best meets those needs.
Methods: Data were collected in February 2023 with community members from Bugoto, a high endemicity community located in eastern Uganda on the shores of Lake Victoria through in-depth interviews (IDIs) (n=21) and focus group discussions (FGDs) (n=4). The IDIs were conducted with adult women and the FGDs were conducted with both women and men. The IDIs and FGDs with community members were conducted in Lusoga, the language spoke in Bugoto. Prior to the IDIs and FGDs, the study objectives and overview were read in Lusoga to the participant followed by obtaining consent through a signature or thumbprint. Thematic analyses were used to analyse the data. Data were coded into themes using the software NVIVO14. Iterative characterization was then utilized to analyse chosen themes using the process of descriptive, followed by Interpretive, analysis.

Findings/Discussion: Insights were obtained regarding non-drinking water usage patterns, including facilitators and barriers to accessing various water sources. This provided a contextual understanding of the community's water needs. Subsequently, analyses were conducted to determine preferences for future water infrastructure for non-drinking purposes, resulting in the identification of five major themes. These themes, coupled with observational data collected during the researcher's time in Bugoto will be presented and will inform the design of future interventions tailored to the community's preferences and requirements.

17:45 (15 mins)

Caught in a trap: DNA contamination in tsetse xenomonitoring can lead to over-estimates of *Trypanosoma brucei* infection

Isabel Saldanha, *Liverpool School of Tropical Medicine*

I Saldanha[1]; R Lea[1]; O Manangwa[6]; G Garrod[1]; LR Haines[5]; A Acosta-Serrano[5]; H Auty[2]; M Betson[3]; JS Lord[1]; LJ Morrison[4]; F Mramba[6]; SJ Torr[1]; LJ Cunningham[1];
[1] Liverpool School of Tropical Medicine, UK; [2] University of Glasgow , UK; [3] University of Surrey, UK; [4] The Roslin Institute, University of Edinburgh, UK; [5] Department of Biological Sciences, University of Notre Dame, United States; [6] Vector and Vector-borne Diseases Research Institute, Tanzania

Tsetse flies (*Glossina sp*.) are vectors of *Trypanosoma brucei* subspecies that cause human African trypanosomiasis (HAT). Capturing and screening tsetse is critical for HAT surveillance. Classically, tsetse are microscopically analysed to identify trypanosomes but this is increasingly replaced with molecular xenomonitoring. Nonetheless, sensitive *T. brucei*-detection assays, such as TBR-PCR, are vulnerable to DNA cross-contamination. This may occur at capture, when live tsetse are retained temporarily in the cage of a trap. This study set out to determine whether infected tsetse can contaminate naïve tsetse with *T. brucei* DNA via faeces when co-housed.

Insectary-reared teneral *G. morsitans morsitans* were fed an infectious *T. b. brucei*-spiked bloodmeal. At 19 days post-infection, infected flies and naïve flies were caged together in the following ratios: (T1) 9:3, (T2) 6:6 (T3) 1:11 and a control (C0) 0:12 in triplicate. Following 24-hour incubation, DNA was extracted from each fly and screened for parasite DNA presence using PCR and qPCR.

All insectary-reared infected flies were positive for *T. brucei* DNA using TBR-qPCR, however naïve flies also tested positive. Even at a ratio of 1 infected to 11 naïve flies, 91% of naïve flies had positive TBR-qPCR results. Furthermore, the quantity of *T. brucei* DNA detected in naïve flies was significantly correlated with cage infection ratio. With evidence of cross-contamination, field-caught flies from Tanzania were then assessed using the same screening protocol. End-point TBR-PCR assays predicted an infection rate of 24.77%. By employing qPCR and Cq cut-offs optimised on insectary-reared flies, a more realistic parasite prevalence for field-trapped flies was estimated at 0.47% (95% confidence intervals [0.36, 0.73]).

Our results show that infected tsetse can contaminate naïve flies with *T. brucei* DNA when co-caged, and that the level of contamination can be extensive. Whilst simple PCR may overestimate infection rates, quantitative PCR offers a means of eliminating false positives.

(8) Organoid models - (Lecture theatre 2)
Sponsored by Promega

Chair: Krystyna Cwiklinski

16:00 (25 mins)

Small but mighty! – Using primary organoids to model host responses to pathogens in the lab

Dr David Smith, *Moredun Research Institute*

D Smith[1];
[1] *Moredun Research Institute, UK*

Organoids are stem cell-derived organized multicellular structures that mimic a specific tissue type. In recent years, we have shown that primary organoids derived from gastrointestinal tissue in ruminants are tissue-, species- and even individual animal-specific. In a recent study, this latter point inspired us to determine whether organoids derived from hyper-immune animals infected with a gastrointestinal nematode retained an epigenetic "blueprint" of infection/immune status. Here, we report that duodenal organoids derived from longitudinally *Trichostrongylus colubriformis* trickle-challenged sheep have a distinctly different gene expression profile compared to organoids derived from healthy animals, after multiple organoid passages in a parasite-free environment. Our results indicate that *T. colubriformis* infection has a reprogramming effect on the host that diminishes intestinal cell differentiation and gut motility and alters immune signalling. Moreover, gene expression of known markers of resistance are also increased in organoids derived from hyper-immune animals, despite multiple rounds of passaging in a worm-free environment. We have now established organoid cultures from diverse tissue types and across various large animal species and we are now applying these tissue culture systems to model a range of host:pathogens interactions and vaccine delivery systems in the lab.

16:25 (25 mins)

Caecaloids, imaging and transcriptomics to unravel the whipworm niche at the host intestinal epithelia

Dr Maria Duque-Correa, *University of Cambridge*

M Duque-Correa[1];
[1] *University of Cambridge, UK*

Whipworms (*Trichuris spp*) are large metazoan parasites that inhabit multi-intracellular epithelial tunnels in the caecum and proximal colon of their hosts, causing chronic disease in humans and other mammals. Whipworms manipulate mucosal physiology and inflammation through interactions with the intestinal epithelial cells and stem cell niche. These interactions enable chronic infections where whipworms are tolerated for years; but at a mechanistic level, how they operate is not understood. Our research aims to define these interactions and bring a mechanistic understanding to how they underpin whipworm invasion, colonisation, and persistence in their mucosal niche. To address this aim, we have established a model that for the first time effectively reproduces whipworm (*Trichuris muris*) infection *in vitro* using caecal organoids (caecaloids) and combined it with *in vivo* infections and imaging and transcriptomic analysis. Utilising these models, we have shown that *T. muris* first-stage (L1) larvae degrade mucus layers to access epithelial cells and have visualised "live" the invasion of IECs and the formation of syncytial tunnels. In early syncytial tunnels, larvae are completely intracellular, woven through multiple live dividing cells. Moreover, using single-cell RNA sequencing, we revealed that progression of infection results in cell damage and an expansion of enterocytes expressing of *Isg15*, potentially instigating the host immune response to the whipworm and tissue repair. Excitingly, we have recently observed larval development from early to late stages inside syncytial tunnels in caecaloids akin to those seen *in vivo*. Further, we are currently working towards translating this system to human organoids and the human whipworm (*T. trichiura*). Caecaloids have unlocked new opportunities to study whipworm developmental and biology, while reducing the number of animals required for these studies. Collectively, our research is unravelling intestinal epithelium invasion by whipworms and revealing specific host-parasite interactions that allow the whipworm to establish and persist in its multi-intracellular niche.

16:50 (25 mins)

Intestinal organoid models for studying *Cryptosporidium*

Dr Mattie Pawlowic, *University of Dundee*

M Pawlowic[1];
[1] *University of Dundee, UK*

Cryptosporidium is an apicomplexan parasite that causes diarrheal disease, and is especially dangerous in young, malnourished children and immunocompromised adults. Unfortunately, there is no vaccine and no effective treatments for these patient populations. One of the major hurdles in advancing our understanding of the biology of this parasite, as well as developing new therapeutics, is lack of a simple, continuous *in vitro* culture system. Current co-culture of *Cryptosporidium* with a transformed intestinal cell line supports limited parasite growth with a parasite life cycle arrest at fertilisation. Over the past five years, many groups have explored the use of organoid-based culture systems, and report success in observation of the full parasite life cycle. We will discuss these new models, both the possibilities and limitations. In addition to some of the published organoid culture models, the Pawlowic lab is implementing the use of human intestinal organoid model in collaboration with the Thorne Lab (University of Arizona) for co-culture with *Cryptosporidium*. The organoid monolayer organises into zones of proliferation and differentiation, containing all main intestinal cell types. We will report the results of establishing this model and our insights about challenges encountered along the way. This model permits the use of live imaging. Coupled with sophisticated image analysis tools, we believe this

model will allow for expanded insight into parasite biology and advance the development of new anti-cryptosporidial therapeutics.

17:15 (15 mins)

Utilizing equine enteroid-derived monolayers for studying parasitic intestinal nematode infection

Dr Eva Tydén, *Swedish University of Agricultural Sciences*

E Tydén[1]; M Sellin[2]; F Martin[1]; C Fossum[1]; S Hellman[1];
[1] Swedish University of Agricultural Sciences, Sweden; [2] Uppsala University, Sweden

Organoid cultures derived from stem cells have become increasingly popular as experimental models for studying infections caused by various gastrointestinal pathogens in different host species. However, the size of infectious nematode larvae and the closed structure of 3-dimensional organoids often pose challenges when studying the natural route of infection. In order to address this issue, the present study utilized enteroids, organoids derived from the equine small intestine, to establish monolayer cultures on the apical surface of the epithelium, allowing for easier administration of infectious agents. To evaluate the functionality of these monolayers, they were stimulated with IL-4 and IL-13, and/or exposed to infectious stage larvae of equine nematodes such as *Parascaris univalens*, cyathostominae, and *Strongylus vulgaris*. The effects of these stimuli were assessed through qPCR analysis, histochemistry, immunofluorescence, live-cell imaging, and scanning electron microscopy. These analyses revealed that the monolayers were heterogeneous, consisting of both immature and differentiated cells including tuft cells and mucus-producing goblet cells. Stimulation with IL-4/IL-13 led to an increase in the differentiation of tuft and goblet cells, as evidenced by the expression of DCLK1 and MUC2. Co-culture with *P. univalens* further enhanced the expression of MUC2 in these cytokine-primed monolayers. Additionally, live-cell imaging showed morphological changes in the epithelial cells upon exposure to larvae, even in the absence of cytokine stimulation. Overall, this study presents the design, characterization, and usability of an experimental model representing the equine nematode-infected small intestinal epithelium. The presence of tuft cells and goblet cells, whose mucus production is influenced by Th2 cytokines and/or the presence of larvae, provides an opportunity for mechanistic studies on the physical interactions between nematodes and the equine intestinal mucosa.

17:30 (15 mins)

Developmental biology of *Fasciola hepatica*: 3D co-culture using HepG2 spheroids to create mini-livers allows investigation of host-pathogen interactions

Aiste Vitkauskaite, *University of Galway*

A Vitkauskaite[1]; K Cwiklinski[2]; E McDermott[1]; R Lalor[1]; C De Marco Verissimo[1]; M Hussein Dehkordi[1]; K Thompson[1]; P Owens[1]; HO Fearnhead[1]; NE Calvani[1]; JP Dalton[1];
[1] The University of Galway, Ireland [2] The University of Liverpool, UK

The helminth parasite *Fasciola hepatica* is a significant cause of animal and human morbidity worldwide. Investigations of the parasite's developmental biology are hampered by our inability to culture and propagate juvenile worms *in vitro*. HepG2 is a human non-tumorigenic liver cell line with high proliferation rates and epithelial-like morphology. We have shown that co-culture with three-dimensional HepG2 cell aggregates (3D spheroids) promotes the survival, growth and development of the infective stage of the parasite, the newly excysted juvenile (NEJ) *in vitro*. Parasites grown in the presence of

HepG2 spheroids, mini-livers, were observed regularly interacting with the spheroids, invading the tissue, indicating the importance of tactile stimuli. Parasites actively feed on and ingest the peripheral cells of the spheroids. We investigated parasite development using immunohistochemistry and scanning electron microscopy (SEM). The parasites exhibited not only a rapid increase in size and temporal expression of developmental genes, but also extensive development of the gut caecum, musculature, and surface sensory system. Parasites grown with 3D mini-livers mimic *in vivo* parasite-host liver interactions, greatly improving our ability to investigate and understand *F. hepatica*-host biology. This co-culture system has the potential to facilitate the development of new parasite control methods. Therefore, we are continuing to improve culture conditions to favour parasite growth and development.

17:45 (15 mins)

A microRNA in the excretory-secretory products of helminth parasites induces dedifferentiation in epithelial cells within gastrointestinal organoids

Dr Matias Perez, *University of Glasgow*

M Perez[3]; R Laing[3]; K Hildersley[1]; T McNeilly[1]; R Meizels[2]; E Devaney[3]; C Britton[3];
[1] Moredun Research Institute, UK; [2] School of Infection & Immunity, University of Glasgow, UK; [3] School of Biodiversity, One Health and Veterinary Medicine University of Glasgow, UK

Infection with helminth parasites is a major health and economic problem in humans and livestock. Helminths can modulate host immune responses, mainly via release of excretory-secretory (ES) products, including proteins and microRNAs (miRNAs). The impact of parasite miRNAs on host gene expression and immune outcome remains unclear. Nematodes infecting the gastrointestinal (GI) tract are closely associated with host epithelial cells that initiate a type 2 response and are responsible for the 'weep and sweep' response to expel GI nematodes. In this study, we detail the effect of a pan GI-nematode-secreted miRNA on host epithelial cells using GI organoids. We show that this secreted parasite miRNA can suppress the effects of the type 2 cytokine IL-13, resulting in reduced expansion of tuft and mucous-secreting cells. Bioinformatic and organoid studies indicate that the parasite miRNA regulates host transcription factors, stimulating GI cell proliferation and suppressing differentiation and, at the same time, promoting tissue regeneration following IL-13 stimulation. Our data advance understanding of the intricate crosstalk between host and parasite and identify novel functions of a parasite miRNA with potential therapeutic benefits in the repair of GI tissue caused by infection or immune-mediated damage.

(9) Origins of Parasitism - (Lecture theatre 3)

Chair: Andrew Jackson

16:00 (25 mins)

Gregarine apicomplexans as model systems to better understand the evolution of parasitism in the phylum Apicomplexa

Dr Sonja Rueckert, *University of Duisburg-Essen*

S Rueckert[1];
[1] Department of Eukaryotic Microbiology, Faculty of Biology, University of Duisburg-Essen, Germany,

The roughly 6000+ species of the phylum Apicomplexa are said to all be obligate parasitic. Some species are very well known in public as they cause notorious diseases such as Malaria, Toxoplasmaosis, Babesiosis, etc. in humans and livestock. In addition to these (un)popular species there is a large, diverse group of apicomplexans that only infects invertebrates, the gregarines. The

impact gregarines pose on their hosts vary and are spread across the spectrum of symbiosis from positive to negative. Available molecular data show that they occupy a basal phylogenetic position within the Apicomplexa, and recent transcriptomic data have shown that some previously as gregarine described species are actually close relatives to the Apicomplexa. With a vast range of techniques now available the understanding of the evolution of the Apicomplexa and close relatives with similar lifestyles has begun to unfold. To fully understand the evolution from free-living, photosynthetic algae to intracellular parasites, it is important to study the adaptations gregarine's have undergone on the spectrum of symbiosis. Unfortunately, there is still one aspect that slows this area of research down, which is the inability to culture gregarines. We are working on and have already made progress towards the development of a gregarine culture system.

16:25 (20 mins)

Not so picky Colpodellids: Novel diversity of free-living bacterivorous colpodellids, relatives of Apicomplexa

Dr Martin Kolisko, *Institute of Parasitology, Biology Centre, Czech Academy of Sciences*

M Kolisko[1];
[1] Institute of Parasitology, Biology Centre, CAS, Czechia

Colpodellids are free-living marine, freshwater, and soil predatory microbial eukaryotes. They consume their prey through a unique process called myzocytosis, in which the predator attaches to and penetrates the prey's surface and cell membrane and 'sucks up' the cell contents. These predators are closely related to Apicomplexan parasites and to two free-living algae, *Chromera* and *Vitrella*. All currently described colpodellids live aerobically and feed specifically on other microbial eukaryotes. Most apicomplexans are obligatory intracellular parasites that invade their host cells using a characteristic apical complex. Colpodellids also possess apical complex-like structures, despite the dramatic difference in life history compared to Apicomplexa. The apical complex of Colpodellids facilitates predation by myzocytosis, where attachment to the prey is followed by formation of a microtubule ring derived from the pseudoconoid that delimits the connection between the predator and prey. Overall, colpodellids together with *Chromera, Vitrella* and Apicomplexa represent an incredibly diverse group of organisms whose lifestyles range from primary production, through predation to parasitism. Moreover, they all share the apical complex that has been repurposed for different activities. We have recently discovered a colpodellid lineage containing isolates that break the paradigm inferred from all other known colpodellids, as they are bacterivorous and clearly capable of living both aerobically and anaerobically. Here, I will present morphological and molecular characterizations for several isolates of these novel Colpodellids, including light and electron microscopy observations demonstrating feeding behaviour and apical-complex structures. I will also show the results of phylogenomic analysis that pinpoints their precise phylogenetic position. Since these colpodellids are anaerobes, I will also show the results of *in-silico* predictions of mitochondrial and putative plastid metabolism. These results add to the existing diversity of life histories within colpodellids, apicomplexans, and chromerids.

16:45 (15 20 mins)

Using genome data to understand the diversification in microsporidia in invertebrates

Dr Bryony Williams, *Exeter University*

B Williams[1];
[1] Exeter University, UK

Microsporidia possess eukaryotic genomes in arguably their most streamlined state. Starting with the sequencing of the genome of *Encephalitozoon cuniculi* in 2002, an increasing number of species have had their genomes sequenced year on year and draft genome sequences are now starting to accompany new species descriptions. This growing genomic resource is allowing us to better understand the processes that have shaped miniaturised microsporidian genomes as well giving the data to infer relationships between major groups within this phylum. One animal type that are particularly intensively affected by microsporidia are the invertebrates, in which most species have been described. However, their full diversity with invertebrates is not yet known, in spite of the fact that microsporidia are major pathogens intensively reared crustacea and insects. With a view to better understanding how microsporidia have diversified within these animals, we use available genomic data from Chelicerata, Crustacea and Hexapoda to determine which microsporidian lineages infect which invertebrate groups, infer co-evolutionary relationships and relate parasite groups to host taxonomic and ecological traits.

17:00 05 (20 15 mins)

Comparative analysis of trypanosomatids from the genera *Blastocrithidia* and *Obscuromonas* with non-canonical and standard genetic codes

Prof Julius Lukes, *Biology Centre, Czech Academy of Sciences*

K Záhonová[1]; Z Fussy[2]; A Albanaz[3]; A Butenko[1]; J Votýpka[4]; A Kostygov[3]; A Kachale[1]; F Fakih[1]; Z Paris[1]; V Yurchenko[3]; J Lukes[1];
[1] Institute of Parasitology, Biology Centre, Czech Academy of Sciences, Czechia; [2] Scripps Institution of Oceanography, University of California, United States; [3] Life Science Research Centre, University of Ostrava, Czechia; [4] Faculty of Sciences, Charles University,, Czechia

Blastocrithidia nonstop, a trypanosomatid closely related to the parasitic genera *Trypanosoma* and *Leishmania*, has reassigned all three stop codons into sense codons, yet it also uses UAA as a universal stop codon. We have sequenced, assembled and analyzed the genomes of four members of the genus *Blastocrithidia* and four members of the closely related genus *Obscuromonas*, which has a canonical genetic code. The genome-wide comparative analysis has shown that all *Blastocrithidia* species share the same codon reassignment and are exceptionally AT-rich, but other genomic features are remarkably similar to *Obscuromonas* and other trypanosomatids. In *B. nonstop* the in-frame UAA and UAG are decoded by tRNAs with a matched anticodon, yet UGA is decoded by a tRNA with uniquely shortened anticodon stem recognizing non-canonically both UGG and UGA codons. Corresponding tRNAs seem to be absent in *Obscuromonas* species. We aim to turn *B. nonstop* into a genetically tractable organism, which would allow addressing questions such as: Why did the massive reassignment occur in this lineage? How do in-frame stop codons influence translation? What is the role of tRNAs with short anticodon stems? Why are the in-frame stop codons more frequent in certain genes but absent in others? How does the ribosome distinguish between the in-frame and genuine UAA termination codons?

17:20 (20 15 mins)

Ancestral aneuploidy and stable chromosomal duplication resulting in differential genome structure and gene expression control. The case of Trypanosomatid parasites

Joao Cunha*, University of York*

JL Reis-Cunha[7]; SA Pimenta-Carvalho[6]; LV Almeida[1]; A Coqueiro-dos-Santos[6]; C Marques[5]; J Black[4]; J Damasceno[5]; R McCulloch[2]; D Bartholomeu[1]; DC Jeffares[3];

[1] *Universidade Federal de Minas Gerais, Brazil;* [2] *University of Glasgow , UK;* [3] *University of York, UK;* [4] *University of São Paulo Medical School, Brazil;* [5] *Wellcome Centre for Integrative Parasitology, Institute of Infection, Immunity and Inflammation, University of Glasgow, UK;* [6] *Federal University of Minas Gerais - UFMG, Brazil;* [7] *York Biomedical Research Institute, Department of Biology, University of York, UK*

Aneuploidy is widely observed in both unicellular and multicellular eukaryotes, usually associated with adaptation to stress conditions. Chromosomal duplication stability is a trade-off between the fitness cost of having unbalanced gene copies and the potential fitness gained from increased dosage of specific advantageous genes. Trypanosomatids, a family of protozoans which include species that cause neglected tropical diseases, are a relevant group to study aneuploidies, as their life cycle has several stressors that could select for different patterns of chromosomal duplications and/or losses. Moreover, trypanosomatid biology is unusual, and one reason for aneuploidy-driven gene expression control could be linked to their near universal use of polycistronic transcription, limiting their capacity to alter the transcription of individual genes via promoters, and instead increasing their reliance on mechanisms of gene expansion and contraction, and post-transcriptional control mechanisms. However, it is still unclear when the capacity for aneuploidy arose during trypanosomatid evolution, and its relevance for the parasite long-term evolution. By evaluating the whole genome sequencing data from 866 isolates covering 7 trypanosomatid genera
(*Crithidia*, *Endotrypanum*, *Leishmania*, *Leptomonas*, *Paratrypanosoma*, *Porcisia*, *Trypanosoma*), we have revealed three features of aneuploidy in these parasites. First, aneuploidy tolerance is an ancestral characteristic of trypanosomatids, suggesting it is central to their genome functionality. Second, *T. brucei* and related African trypanosomes have more recently evolved to largely dispense with aneuploidy, perhaps reflecting genome reorganisation. Third, we have identified the presence of an ancestral chromosomal duplication, named collectively
as *Trypanosomatid Ancestral Supernumerary Chromosome* "TASC", which has been maintained throughout Trypanosomatid evolution either as a greater than diploid chromosome or a syntenic duplication in two chromosomes in African trypanosomes. The number of chromosomes with extra copies in a given isolate is usually low, and only TASC was kept for long enough to greatly impact its nucleotide diversity, gene structure, expression control and evolution. TASC has most genes in the same coding strand, is expressed as a disomic chromosome even having four copies and have increased potential for functional variation, but purge highly deleterious mutations more efficiently than other chromosomes. The evidence of stringent control over gene expression in this chromosome suggests that these parasites have adapted to mitigate the fitness cost associated with this ancient chromosomal duplication. What processes govern aneuploidy in these protozoans and the underlying molecular mechanisms that regulate TASC expression remains unknown. New studies investigating these modifications will be important to address these deficits in our understanding.

17:40 35 (20 25 mins)

Next-generation sequencing to decipher genome evolution of microscopic parasites: insights from Myxozoa (Cnidaria)

Prof Dorothee Huchon, *Tel Aviv University*

D Huchon[1];
[1] *Tel Aviv University, Israel*

Myxozoa are a diverse and enigmatic group of microscopic parasitic animals closely related to jellyfish and hydroids. Evolutionary research on Myxozoa has long been hampered by their small size and lack of informative morphological characteristics. The challenges extended to DNA sequencing, where

issues such as obtaining substantial DNA quantities, fast evolutionary rates, and host contaminations presented hurdles. This lecture explores how high-throughput sequencing methodologies provided novel insights into myxozoan evolution and genomics. Leveraging genomic data, we could conclusively confirmed the phylogenetic positioning of myxozoans as the sister clade of *Polypodium hydriforme*. We revealed that myxozoans have a small nuclear genome, characterized by an exceptionally fast evolutionary rate. Intriguingly, all myxozoans have lost crucial animal genomic and developmental pathways, including cytosine methylation and all Hox genes. Moreover, our investigations have brought to light the fascinating case of *Henneguya salminicola*, a myxozoan species that has lost aerobic respiration, marking the first molecular characterization of an anaerobic animal. In contrast to the compact nuclear genome, myxozoans feature colossal mitochondrial genomes, which contain many non-coding regions comprised of repetitive elements. Few myxozoan species harbour a partitioned mitochondrial genome organized into several mega-chromosomes. Most canonical genes commonly found in all metazoan mitochondrial genomes are absent in myxozoans, likely due to their accelerated evolutionary rate. Conversely, numerous open reading frames with unknown functions have been identified. While comparative genomics and transcriptomics of myxozoan species remain challenging due to their rapid evolutionary rate and the subsequent complexities in reliable genomic annotation, future genomic data are expected to revolutionize our understanding of myxozoan host relationships, evolution, and ecology.

(21) Molecular and cellular biology 1 – (Teaching room 4)

Chair: Pegine Walrad

16:00 (15 mins)

The *Toxoplasma gondii* mitoribosome reveals novel features of ribosome evolution and exciting differences from human mitoribosomes

Dr Lilach Sheiner, *University of Glasgow*

L Sheiner[1];
[1] Wellcome Trust Centre for Molecular Parasitology, University of Glasgow, UK

Mitochondrial ribosomes (mitoribosomes) are fundamental, and their function of synthesising mitochondrial proteins is universal, including in parasites. The apicomplexan mitochondrion is essential for parasite survival, virulence, and dissemination, and the same is thus expected for its mitoribosome. In agreement with this prediction, evidence of the essentiality of conserved mitoribosomal proteins in apicomplexan, accumulates, including from our own work. In addition to being essential, indirect observations further suggest that this mitoribosome is highly divergent from its human equivalent. Divergence is expected from the prediction of rRNA fragmentation, based on different apicomplexan mitochondrial genome sequences, as well as on the sensitivity profile of some apicomplexan parasites to mitoribosome inhibitors.

Despite its essentiality and divergence, the biology of the apicomplexan mitoribosome is poorly studied. Here, using *Toxoplasma gondii* as a model organism, we employed a combination of complementary approaches to expand our understanding of the apicomplexan mitoribosome function and assembly. We discovered an rRNA fragmentation that is much more extensive than predicted according to the parasite mitochondrial genome, and revealed several novel features that enable this highly divergent ribosome to still perform its critical function.

One examples of an apicomplexan, and likely myzozoan, mitoribosome signature feature we discovered is the repurposing of several transcription factors as new mitoribosomal proteins, which we believe compensate for rRNA remodelling; and we postulate that they effectively replace conserved and critical ribosomal domains.

On top of addressing the fundamental question of how divergent ribosomes function, our work has further potential to inform apicomplexan drug discovery, which we demonstrate by revisiting a previously proposed resistance mechanism of apicomplexan to a known mitoribosome inhibitor.

16:15 (15 mins)

Structural and functional insights into ESAG3 and GRESAG3 proteins in African trypanosomes

Dr Calvin Tiengwe, *Imperial College London*

Q Zhong[3]; J Barritt[3]; C Gilabert Carbajo[3]; E Nji[4]; S Dean[2]; K Gull[3]; M Tinti[1]; MA Ferguson[1]; E Hohenester[3]; S Rouse[3]; C Tiengwe[3];
[1] University of Dundee, UK; [2] University of Warwick, UK; [3] Imperial College London, UK; [4] Strathmore University, UK

African trypanosomes are highly effective extracellular parasites that evade host immune responses through antigenic variation of variant surface glycoproteins (VSGs). VSGs are co-transcribed in blood-stage parasites alongside expression site-associated genes (ESAGs) that play key roles in host adaptation, including nutrient acquisition and resistance to human serum. Many ESAGs have

homologues outside telomeric VSG expression sites, termed Genes Related to ESAGs (GRESAGs), which are expressed across both mammalian and insect lifecycle stages and are thought to have redundant functions with ESAGs. However, the extent to which GRESAGs can compensate for ESAG functions within their gene family is not well understood, and the specific functions of many ESAGs remain unknown.

Previous studies indicate that (GR)ESAG3 belong to the Fam53 family, is exclusively expressed in blood-stage trypanosomes, and is upregulated during chronic human infections, but the exact function of (GR)ESAG3 is unknown. Here, we show that although ESAG3 and GRESAG3 are phylogenetically distantly related gene families, both exhibits conserved key residues and a tertiary structure characteristic of class-A glycosyltransferases (GT). RNAi-mediated depletion of ESAG3 inhibits parasite growth *in vitro* without altering GRESAG3 expression levels. Biochemical fractionation and immunofluorescence analyses reveal that ESAG3 is localised to the ER, whereas GRESAG3 is localised to the Golgi apparatus, suggesting distinct functions despite their shared structural features.

Moreover, we demonstrate that purified recombinant ESAG3 exhibits GT activity. Size exclusion chromatography and native gel analysis of trypanosome extracts reveal that ESAG3 forms higher-order oligomers of ~700 kDa. Single-particle cryo-electron microscopy analysis at a resolution of 3.2 Angstroms reveals the ESAG3 complex as a ring-shaped structure formed by eighteen monomers organised into three hexamers. The structure reveals that the fundamental building block is a dimer with the GT active sites facing each other. These findings provide the first structural insights into the domain organisation of a GT from Kinetoplastids, generating testable hypotheses to uncover the precise role of potentially novel GT functionalities in Kinetoplastids in general or *T. brucei* in particular. It is tempting to speculate that (GR)ESAG3 is the GT responsible for *O*-glycosylating trimeric VSGs, which also assemble into an 18-mer structure, for which the GT remains elusive.

16:30 (15 mins)

Revisiting trypanosome transferrin receptor: unveiling novel insights in localization and ligand uptake

Dr Sourav Banerjee, *University of Cambridge*

S Banerjee[1]; N Minshall[1]; H Webb[1]; M Carrington[1];
[1] *University of Cambridge, Department of Biochemistry, UK*

Trypanosoma brucei acquires Fe^{3+} from mammalian hosts through receptor mediated endocytosis of transferrin (Tf). The trypanosome transferrin receptor (TfR) is a heterodimer of ESAG6 and ESAG7 and is attached to the external face of the plasma membrane by a single glycophosphatidylinositol (GPI) anchor at the C-terminus of ESAG6. The TfR is clearly accessible to Tf so how does the trypanosome protect itself against host TfR antibodies? One model has been seclusion of the TfR in the flagellar pocket, effective at protecting it from the cellular arm of the immune system. Based on previous reports on the localisation of TfR all agree on the presence of it in endosomal compartments with some placing it in the flagellar pocket (lumen) and others on the cell surface in presence of canine transferrin which did not bind the expressed TfR.
To characterise the cellular evasion strategies evolved by *T. brucei* to avoid the host adaptive immune system, we have re-visited the expression, localisation and functioning of the TfR. We show that the expression level of TfR varies several folds in cells expressing ESAG6 and ESAG7 from one bloodstream form expression site (BES) to another. We find that the determination of the localisation is affected by the expression level but this is likely due to detection limits and fixation protocol rather than representing a real difference. Next, we took advantage of the endogenous BES7 TfR with GPI-anchors on both ESAG6 and ESAG7 to compare the properties of single and double anchored TfR. We found

that the double anchored TfR was expressed at slightly higher (1.4-fold) levels but otherwise had similar properties. Assays of Tf uptake showed that Tf was internalised within a couple of minutes for both single and double anchored receptors.

Together these observations have allowed us to develop a model in which the TfR is distributed all over the cell surface in a similar manner to other receptors. We have previously shown that Tf binding along with the presence of N-linked glycans effectively mask exposed parts of the receptor. Here we add cellular mechanisms of the likely rapid endocytosis of any TfR- antibody complex along with a low copy number effectively reducing the residence time of TfR immunoglobulins on the cell surface.

We would like to thank Professor Piet Borst for the gift of TfR antiserum.

16:45 (15 mins)

Diverse functions of SHIPPO-domain proteins in flagellar assembly

Dr Samuel Dean, *University of Warwick*

J Sáez Conde[2]; A Paterou[2]; F Moreira-Leite[1]; S Dean[2];
[1] Oxford Brookes University, UK; [2] Division of Biomedical Sciences, Warwick Medical School, UK

The flagellum of the African trypanosome is essential for their ability for infect new hosts and their transmission via tsetse. The flagellum has 3 major structural domains – the basal body (BB) that templates new flagellar growth, the axoneme that "beats" for cellular propulsion, and the transition zone (TZ) that separates the two. The TZ is the structural intermediate between the BB and the axoneme and is fundamental for flagellar assembly and function. For example, the TZ harbours the "basal plate", an electron dense structure at the distal TZ-axoneme boundary that nucleates axonemal microtubules essential for flagellar beating. Despite its importance, assembly of the TZ is not well understood.

Our previous work revealed the TZ to be extraordinarily complex, comprised of >70 proteins. We showed that one of these, TZP250 (transition Zone Protein 250kD) has an RNAi phenotype that includes severe axoneme defects, suggesting an important role in flagellar assembly. Here, we show that the primary defect in TZP250 mutants is TZ length dysregulation and basal plate mispositioning, revealing a key role for TZP250 in TZ morphogenesis. To investigate TZP250 function further, we used ultra-expansion microscopy (UexM) on bi-terminally tagged TZP250 to show that while the N terminus of TZP250 encircles the TZ at the (proximal) BB-TZ boundary, the C terminus lies at the (distal) TZ-axoneme boundary. Hence, TZP250 molecules lie along the entire ~350nm length of the TZ. Combining structural predictions, sophisticated phenotypic assays and super-resolved domain localisation, we propose that TZP250 acts as a molecular ruler that defines the length of the TZ.

Protein domain analysis shows that the central part of TZP250 is dominated by repeated 'SHIPPO' domains, a Pro-Gly-Pro-Gly-X-Tyr motif that was recently shown to mediate binding to the outer wedge of the axonemal doublet microtubules and are likely to represent key biochemistry for TZP250 function. Interestingly, we reveal that, despite representing a complex, multi-copy gene family, SHIPPO proteins are found only in ciliated organisms, and diversified in metazoa, such as humans and zebrafish. Using only the presence of this domain, we identify a second SHIPPO-containing protein in trypanosomes, which localises to the axoneme and is essential for its complete assembly, suggesting a role in maintaining axoneme stability.

In summary, we show the SHIPPO domain to represent a highly conserved but diverse protein family with different functions within a ciliary context.

17:00 (15 mins)

The Lectin Pathway of Complement Regulation by the infectious *Fasciola hepatica* newly excysted juvenile (NEJs)

Dr Carolina De Marco Verissimo, *National University of Ireland Galway*

T Kilbane[3]; J Dobó[2]; P Gál[2]; K Cwiklinski[1]; JP Dalton[3]; C De Marco Verissimo[3];
[1] *Institute of Infection, Veterinary and Ecological Sciences, University of Liverpool, UK;* [2] *Institute of Enzymology, Research Centre for Natural Sciences, Hungary;* [3] *Centre for One Health and Ryan Institute. University of Galway, Ireland*

The complement response is the first-line innate host defence against invading organisms and is activated via Classical, Lectin, and Alternative pathways. The Lectin pathway (LP) is initiated by the binding or recognition molecules to sugar arrays, e.g. mannose, on the pathogen's surfaces, which leads to the formation of C3-convertase, essential for propagation of the cascade. Recently, we showed that the invasive stage of *Fasciola hepatica*, newly excysted juveniles (NEJs), survives in normal human serum by inactivating the complement LP, despite being covered by glycans. Considering the rich glycan-coated surface displayed by *F. hepatica* NEJs, which includes both highly mannosylated and acetylated structures, it was surprising to observe that neither Mannose-binding lectin (MBL) nor Ficolin-2 (Fic-2) bind to live NEJs. To unveil how the parasite prevents the binding of these LP recognition molecules, we investigated the effect of NEJs' excreted-secreted (ES) molecules on recombinant human MBL and Fic-2. We discovered that 1 hour of incubation at 37°C of rhMBL or rhFic-2 with either live NEJs or ES alone results in their specific cleavage. Considering the presence of collagen-like domains in both MBL and Fic-2 and the well-known role of cathepsin L3 protease (FhCL3), highly secreted by the NEJs stage, we next assessed the ability of recombinant FhCL3 to digest rhMBL and rhFic-2. Co-incubation of the molecules at 37°C showed that rFhCL3 efficiently cleaves rhMBL and rhFic-2. Fascinatingly, our studies show that *F. hepatica* NEJs possess multiple and overlapping strategies to prevent LP activation, which also include the expression and secretion of serine protease inhibitors (Serpins; namely FhSrp1 and FhSrp2). We have shown that rFhSrp1 and rFhSrp2 inhibit the MBL-associated serine proteases (MASP-1 and MASP-2), the key initiators of the LP. Furthermore, rFhSrp1 and rFhSrp2 form complexes and inhibit rMASP-1/2, as shown by ELISAs, pull-down, SDS-Page, biochemical assays, and Mass spectrometry (MS). Similar to that demonstrated with live *F. hepatica* NEJs, incubation of either rFhSrp1 or rFhSrp2 with normal human serum leads to selective LP inhibition (>90%). Nevertheless, a time-course co-incubation of these serpins with rMASPs showed high efficiency of rFhSrp1 in binding and cleaving MASPs, and an interesting suicidal mechanism of inhibition between rFhSrp1 and MASP-1 and -2. The downstream effect of MASPs inhibition was verified by a proportional reduction in their ability to cleave complement C4, essential for forming C3-convertase. Here, we uncovered an array of novel mechanisms by which the invading *F. hepatica* NEJs circumvent the binding of MBL and Fic-2, and the activation of MASPs to become refractory to killing via the LP. The importance of such complement regulation during *F. hepatica* infection is stressed by the various strategies used to avoid an LP response.

17:15 (15 mins)

Nanopore sequencing-based deep learning reveals the complete DNA replication landscape in *Leishmania* and its connection with genome variability

Dr Jeziel Dener Damasceno, *University of Glasgow*

J Damasceno[1]; GL da Silva[1]; C Marques[1]; C Lapsley[1]; D Beraldi[1]; R McCulloch[1];
[1] *School of Infection and Immunity – University of Glasgow, UK*

Genomic plasticity through gene and chromosome copy number variation is a crucial adaptive mechanism employed by *Leishmania*, including during the evolution of drug resistance. How such genomic flexibility arises remains unclear. We have previously shown that genome duplication in *Leishmania* is temporally compartmentalized both intra- and inter-chromosomally. Duplication predominantly initiates at a single locus in each chromosome core in early S-phase and progresses toward sub-telomeres, which replicate during and after late S-phase. In addition, smaller chromosomes are duplicated earlier in S-phase than their larger counterparts. It seems unlikely these data present a complete picture of the *Leishmania* DNA replication programme, however. Here, we have used D-Nascent, a deep learning assay based on Oxford Nanopore sequencing, to detect DNA replication forks, origins, and termination sites across the *Leishmania* genome. Our findings confirm the pre-eminence of a single major locus of DNA replication initiation in each chromosome, but additionally reveal thousands of previously undetected replication initiation events. D-Nascent indicates that larger chromosomes display a denser concentration of DNA replication origins than smaller chromosomes, suggesting an evolutionary adaptation to counteract their delayed replication timing. Analysis of DNA replication forks provided a genome-wide assessment of *Origin Efficiency Metrics*, which delineated DNA replication initiation and termination zones within *Leishmania*'s genome. Initiation zones are marked by high AT content, increased G-quadruplex (G4) levels and lower chromatin occupancy. Indeed, DNA replication initiation efficiency shows a direct correlation with the presence of G4s and AT-rich regions, and an inverse correlation with chromatin density and GC content. Furthermore, we find markedly diminished transcription initiation at sites of DNA replication initiation zones, in contrast to heightened transcription initiation activity observed at termination zones. Finally, we show that zones with higher DNA replication initiation efficiency are linked to increased mutagenesis, as evidenced by increased accumulation of single nucleotide polymorphisms (SNPs). Moreover, our data uncover a correlation between copy number variation level, chromosome length, and DNA replication timing, with a higher prevalence of copy number variation in smaller chromosomes compared to larger ones. In total, D-Nascent provides a more complete picture of the DNA replication landscape in *Leishmania*, revealing that genome duplication is executed by a single putatively constitutive origin in each chromosome supported by more widespread, potentially stochastic replication events whose distribution reflects chromosome size and dictate replication timing and genomic variability. These insights offer a deeper understanding of *Leishmania* genome malleability and adaptability.

Day 3 - 4-April-2024

(10) Helminth epidemiology 1 - (Lecture theatre 1)

Sponsored by Quadratech Diagnostic

Chair: Alexandra Juhasz

09:00 (25 mins)

The Lawa model: An integrated liver fluke control program using One Health Approach

Prof Banchob Sripa, *Khon Kaen University*

B Sripa[1];

[1] WHO Collaborating Centre for Research and Control of Opisthorchiasis & Department of Tropical Medicine, Faculty of Medicine, Khon Kaen University, Thailand

Human liver fluke infections caused by *Opisthorchis viverrini* and *Clonorchis sinensis* are major neglected foodborne trematodiases in Eastern and Southeast Asia, affecting over 30 million people. These infections are linked to various hepatobiliary diseases, including cholangiocarcinoma (CCA), a fatal bile duct cancer. Northeast Thailand, where *O. viverrini* is endemic, reports the highest incidence of CCA globally. Despite decades of liver fluke control programs in Thailand, the current infection status of *O. viverrini* remains high in certain endemic areas due to its complex life cycle involving multiple hosts/environments, posing challenges for conventional control methods. Consequently, a new control strategy for liver fluke infection using the One Health approach was introduced in the Lawa Lake area of Khon Kaen province, where the liver fluke has been highly endemic for over 15 years. As a result, the infection rate in over 20 surrounding villages has decreased to less than 5% from an estimated baseline of 60%, indicating significant progress. The local population has gained enhanced knowledge of liver fluke transmission and control. Notably, the prevalence of Cyprinid fish species, the intermediate hosts, has reduced to less than 0.1% from a maximum of 70% during the baseline survey, and liver fluke parasite cercariae have not been detected recently. This liver fluke control program, now named the "Lawa model", has gained national recognition and its principle is incorporated into the national agenda against liver fluke and CCA since 2016, with expansion to other regions of Thailand. Internationally, the "Lawa model" is celebrated as one of the two showcases with successful control programs for helminths, recognized by the WHO/NZD4 meeting in 2014. Furthermore, the One Health approach has been endorsed in the report of the Expert Consultation to Accelerate Control of Foodborne Trematode Infections, Taeniasis, and Cysticercosis in Seoul, Korea, by the WHO Western Pacific Region in 2017.

09:25 (20 mins)

Characterization of morbidity profiles in a context of hybridization and co-infection of human urogenital *Schistosoma haematobium* with livestock and human intestinal *Schistosoma* species in Senegal

Dr Cheikh Fall, *Cheikh Anta Diop University, Senegal*

C Fall[2]; S Lambert[3]; E Leger[3]; L Yasenev[4]; A Garba[1]; SD Diop[5]; ND Diouf[7]; SA Sarr[6]; B Faye[6]; M Walker[3]; M Sene[7]; JP Webster[3];

[1] RISEAL (Reseau International Schistosomoses Environnements Amenagements et Lutte), Niger; [2] Cheikh Anta Diop University, Senegal; [3] Centre for Emerging, Endemic and Exotic Diseases, Department of Pathobiology and Population Sciences, Royal Veterinary College, UK; [4] London Centre for Neglected Tropical Disease Research (LCNTDR), Faculty of Medicine, Imperial College, UK; [5] Institut Supérieur de Formation Agricole et Rurale, Université de Thiès, Senegal; [6] Service de Parasitologie—Mycologie, Faculté de Médecine, Pharmacie et Odontologie, Université Cheikh Anta Diop, Senegal; [7] Unité de Formation et de Recherche des Sciences Agronomiques, d'Aquaculture et de Technologies Alimentaires, Université Gaston Berger, Senegal

Background: Schistosomiasis is a major neglected tropical disease. Across West Africa, co-infections between human urogenital *Schistosoma haematobium* and human intestinal *Schistosoma mansoni*, as well as infections with viable hybrids between human urogenital *S. haematobium* and intestinal livestock *Schistosoma bovis* are prevalent. Morbidity profiles are known to be influenced by mixed *S. haematobium* with *S. mansoni* coinfections, but the impact of *S. haematobium-S. bovis hybrid* infection on morbidity remains unknown.

Methods: Two surveys were conducted from May to August 2016 and from October 2017 to January 2018 Across three regions in Senegal (Richard Toll/ Barkedji/Dakar) in children (n=1326) and adults (n=304). Morbidity indicators of intestinal and urogenital schistosomiasis, measured using ultrasound of the liver and urogenital tract, anaemia and haematuria profiles, were assessed for each individual

according to the infecting species combination and intensity. Doppler echocardiography was used to measure the heart chambers. Pulmonary arterial systolic pressure was assessed by tricuspid regurgitation flow (n=250).
Findings: Hepatomegaly was significantly higher, whilst the number of ureter lesions significantly lower in *S. haematobium-S. bovis* hybrid infections compared to single species *S. haematobium* infections (controlling for *S. mansoni* co-infections). Haematuria and lesions of the urogenital tract were positively associated with presence and intensity of *S. haematobium* or *S. haematobium-S. bovis* urogenital schistosomiasis. The heart of the school-aged child in endemic area is characterized by larger cavitary dimensions. The average pulmonary systolic arterial pressure was greater in Richard-Toll: 26.38 ± 3.16 mmHg versus 21.82 ± 1.73 mmHg in Dakar ($p < 0.001$). There was a correlation between pulmonary systolic arterial pressure and presence of haematuria, anaemia and parasitological positivity.
Conclusion: The results of our study show the impact of parasite hybridization on bilharzian morbidity, with lesions that can progress to cancer and chronic heart failure. Further studies with larger numbers will be needed to confirm our results.

09:45 (15 mins)

Nematode-virus co-infection has a variable impact on the resistance and tolerance to *H.polygyrus* in genetically diverse mice

Insani Hubi Zulfa, *SRUCScotland's Rural College*

IH Zulfa[3]; M Chase-Topping[2]; I Dry[1]; A Doeschl-Wilson[1]; J Houdijk[3]; S Athanasiadou[3];
[1] The Roslin Institute, University of Edinburgh, UK; [2] Royal (Dick) School of Veterinary Studies and The Roslin Institute, University of Edinburgh, UK; [3] SRUC, UK

To combat infection, hosts develop two defence strategies: resistance, which is their ability to clear out the pathogen and tolerance, their ability to reduce the impact of the pathogen on their fitness. The aim of the study was to characterise the phenotypic variation of host resistance and tolerance to *Heligmosomoides polygyrus* in genetically diverse mice during co-infection (*H. polygyrus* and Theiler's murine encephalomyelitis virus). Three strains of mice were used: SJL mice (resistant to *H. polygyrus* but susceptible to TMEV), BALB/c mice (intermediate susceptible to both *pathogens)*, and C57BL/6 (susceptible to *H. polygyrus* but resistant to TMEV). Mice of each strain were infected with either a single (*H. polygyrus*) or two pathogens (*H. polygyrus* and TMEV) or were sham infected (n=15). Both pathogens were administered at the subclinical level (200 L_3 *H. polygyrus* in 0.2ml water and an avirulent TMEV at 10^6 pfu in 0.2 ml DMEM). Mice were euthanised at 14 dpi and 42 dpi to represent nematodes establishment period and nematodes clearance period. In the susceptible to *H. polygyrus* C57BL/6 mice, average daily gain (ADG 0.08 g/day) and FI (0.07g/day) was higher in Co-inf compared to Par, Vir, and Sham at 14 dpi (P<0.001). However, in the BALB/c mice, co-infection treatment resulted in 20% lower ADG compared to mice receiving *H. polygyrus*-only (P=0.034); 10% of ADG loss was observed in the resistant to *H. polygyrus* SJL mice (P<0.001). When compared to *H. polygyrus*-only infected C57BL/6 mice, co-inf resulted in 15% lower EIC (P<0.001), and 20% worm counts (P<0.05), whereas Co-inf BALB/c mice showed 5% elevated EIC (P<0.001) and 10% worm counts (P<0.05) compared to *H. polygyrus*-only mice. Co-inf did not have any impact on resistance traits in SJL mice. The trend was the same for all parasitological measurements at 42 dpi. Compared to *H. polygyrus* only SJL mice, Co-inf mice were 5% more tolerant (P<0.05). Co-inf C57BL/6 mice tended to be more tolerant (P=0.058) whereas Co-inf BALB/c mice tended to be less tolerant than their *H. polygyrus* only counterparts (P=0.051). Our data showed that the impact of co-infection with two intestinal pathogens resulted in significant variation on host resistance and tolerance to *H. polygyrus* in three inbred mouse strains. Contrary to expectation, mice susceptible to *H. polygyrus* benefited most

from co-infection, as their resistance was improved compared to *H. polygyrus*-only counterparts. On the other hand, mice already resistant to *H. polygyrus* improved their tolerance following co-inf, compared to *H. polygyrus* only mice. The underlying mechanisms of these co-infection phenotypes are currently under investigation.

10:00 (15 mins)

The ABCs of liver fluke: Predicting the efficacy of Augmented Biological Control against *Fasciola hepatica* using an agent-based model

Daniel McDowell, *Cardiff University*

D McDowell[1]; S Perkins[1]; FJ Van Veen[2]; J Lello[1];
[1] Cardiff School of Biosciences, Cardiff University, UK; [2] Biosciences, University of Exeter, UK

Trematode infections are a persistent problem for public and animal health, leading to serious economic and welfare issues. The liver fluke, *Fasciola hepatica*, is a trematode of global significance, due to its important economic and welfare impacts on livestock, and as an infection risk to 180 million people. Liver fluke infections cost the livestock trade an estimated two billion dollars per year, due to reduced meat and dairy yields, animal loss and treatment costs. The main treatments against *F. hepatica* are chemotherapeutics used to target the adult and juvenile fluke within the definitive host. In the past, these anthelmintics have proven highly efficacious, however, due to the over reliance on anthelmintics globally, the liver fluke has developed resistance, rendering the effective removal of this parasite increasingly difficult. Additional or alternative control is, therefore, needed for continued effective fluke control. Augmented biocontrol, alone or as an addition to chemotherapeutic treatment is one such alternative. Microinvertebrates are abundant in habitats where the parasites' intermediate host snail (*Galba truncatula*) resides, and our empirical work has shown these microinvertebrates consume the miracidia of *F. hepatica*. Here, we use an agent-based modelling approach to predict the efficacy of applying additional microinvertebrates within the habitat of the intermediate host, as a biological control, with and without the addition of chemotherapeutics control in the definitive host. We explore the efficacy of these different strategies, in reducing the number of liver fluke infections alongside various anthelmintic resistance scenarios. With the political goal of improving environmental practices and biodiversity within agricultural settings, providing a natural control method may create a win-win scenario that benefits the environment and reduces the burden of liver fluke.

10:15 (15 mins)

Malaria and schistosomiasis surveillance prior to the implementation of a large-scale irrigation scheme reveals potential for future transmission

Dr Rex Mbewe, *Malawi Liverpool Wellcome Programme*

R Mbewe[4]; S Gowelo[2]; J Chirombo[3]; B Chiepa[3]; B Kapumba[3]; C Nkolokosa[3]; T Mzilahowa[5]; S Coleman[1]; O Wetherill[1]; R Stothard[1]; C Jones[1];
[1] Liverpool School of Tropical Medicine, UK; [2] University of California, United States; [3] Malawi Liverpool Wellcome Trust clinical research program, Malawi; ; [4] Malawi Liverpool Wellcome Programme, Malawi; [5] Malaria Alert Centre, Malawi

The Malawi government is undertaking large scale irrigation project sponsored by the World Bank which will transform the lower Shire Valley in the southern part of the country. The irrigation scheme will convert over 40,000 hectares into agricultural land for small holder farmers in an area with large scale inter-annual variations in rainfall and unpredictable harvests. Malaria and Schistosomiasis are endemic

in the area. Therefore, the current study aims to ascertain current levels of disease endemicity and transmission potential as a baseline to be compared with transmission levels during and after implementation.

Malaria vectors are collected with CDC light traps and prokopack aspirations (indoor and outdoor) in 90 households across three villages in the irrigation catchment area. Schistosomiasis snail hosts are collected through the inspection of water habitats, dams, pond, rivers and irrigation canals. Cercaria shedding of schistosomes in infected snails are observed using a microscope and schistosomes identified using a qPCR tool. Malaria and schistosomiasis disease testing for cases and incidences are being monitored through school surveys on a biannual basis targeting 1000 pupils. These tests are being done using RDTs for malaria and urine and blood tests for schistosomiasis.

The preliminary data show malaria prevalence rates of 9.7% [95% CI 8.80%-10.59%], and 34.8% [95% CI 33.40%-36.29%] for urogenital schistosomiasis and 1.8% [95% CI 1.43%-2.24%] intestinal schistosomiasis. Key schistosome snails including *Biomphalaria pfeifferi* (the intermediate host for intestinal schistosomiasis) have been identified in the region. Entomological surveillance shows that *An. gambiae* s.l. dominates specimens caught with the presence of potential zoophillic and alternative malaria vector species (e.g. *An. pretoriensis*).

The implementation of the large-scale irrigation project in the lower Shire has the potential to escalate the already existing disease burden. There is urgent need to intensify surveillance of these diseases and promote control and prevention strategies that will enhance the economic gains from the irrigation scheme while minimizing the public health impacts that compromise the attainment of Malawi's Government vision of food self-sufficiency.

(11) Parasite-Immune interactions 1 – (Lecture theatre 2)

Chair: Nicolas Pionnier

09:00 (25 mins)

Immunopathology of leishmaniasis: a spatial perspective on the regulation of immune checkpoint molecules

Prof Paul Kaye, *University of York*

PM Kaye[1];
[1] York Biomedical Research Institute, Hull York Medical School, University of York, UK

The aberrant expression of immune checkpoint (IC) molecules is now well established as a mechanism regulating local immunity in cancer, autoimmunity, and infectious diseases, including those caused by parasitic protozoans. Focusing on the analysis of human skin biopsies from patients with various forms of cutaneous leishmaniasis and from volunteers enrolled in a human challenge study, we have applied spatial transcriptomics combined with conventional immunohistology to explore the diversity in cellular expression of important IC molecules (e.g. PD-L1, IDO1) and the impact of intracellular parasitism on their expression. Furthermore, using techniques in spatial mapping such as Delaunay triangulation, we have characterised the neighbourhood surrounding IC-expressing myeloid cells as a means of identifying the cellular and molecular pathways leading to IC molecule expression. Recent data will be discussed that points to an important role for IL-32-expressing $CD8^+$ T cells as drivers of IC molecule expression during human cutaneous leishmaniasis and hence as contributors to the immune dysfunction that limits the action of immune-dependent anti-leishmanial drugs.

09:25 (15 mins)

IL-17 producing T cells in the control of skin inflammation and subcutaneous adipose wasting during chronic *Trypanosoma brucei* infection

Dr Matthew Sinton, *University of Manchester*

M Sinton[1]; P Chandrasegaran[1]; A Nabilla Lestari[1]; B Cheaib[1]; A Cooper[1]; J Ogunsola[1]; NR Kuispond Swar[5]; R Heslop[1]; P Capewell[1]; D Ngoyi[5]; M Camara[7]; B Bucheton[6]; S Kajimura[3]; C Benezech[2]; SB Coffelt[1]; NA Mabbott[4]; A MacLeod[1]; JF Quintana[1];

[1] University of Glasgow , UK; [2] University of Edinburgh, UK; [3] Harvard University, United States; [4] The Royal (Dick) School of Veterinary Studies and the Roslin Institute, The University of Edinburgh, UK; [5] Department of Parasitology, National Institute of Biomedical Research, Congo; [6] Institut de Recherche pour le Développement, France; [7] Programme National de Lutte contre la Trypanosomiase Humaine Africaine, Guinea

African trypanosomes colonise the skin in a process critical for disease transmission. However, the immunological barriers that these parasites must overcome to ensure transmission are far from being fully understood. Here, we addressed this gap in knowledge by applying a combination of spatially resolved single cell transcriptomics, and *in vivo* murine models of infection. We observed a significant expansion of $Vg6^+$ gd T cell and T_H17 T cells in the chronically infected mouse skin compared to healthy controls, both of which produce significant levels of the inflammatory cytokine IL-17. *In silico* cell-cell interaction analysis suggests that the activation of these IL-17-producing T cells is mediated *via Cd40, Il6, Il10,* and *Tnfsf18* signalling derived from subcutaneous adipocytes. *In vivo*, we first observed that the absence of $Vg6^+$ gd T cells results in an exacerbated dermal inflammation during infection, characterised by a heightened frequency of IFNg-producing cytotoxic $CD8^+$ T cells. Interestingly, we also found that global deletion of IL-17 prevents the characteristic weight loss associated with this disease. Unexpectedly, we found that abrogation of IL-17 signalling exclusively on adipocytes results in a limited adipocyte turnover over time, characterised by a significant accumulation of $Dpp4^+$ $Pi16^+$ interstitial immature preadipocytes and a higher burden of extravascular parasites in the subcutaneous adipose tissue, demonstrating that IL-17 signalling is crucial for controlling preadipocyte fate, subcutaneous adipose tissue replenishment, and local parasite burden. These studies highlight a previously unappreciated crosstalk between subcutaneous adipocytes and gd T cells during chronic *T. brucei* infection, orchestrated by IL-17. In the context of *T. brucei* infection, IL-17 signalling plays pleiotropic roles in the skin, preventing excessive $CD8^+$ T cell activation and modulating subcutaneous adipose tissue remodelling to prevent wasting. Altogether, these studies reveal mechanisms of gd T cells-mediated immunity in the skin in the context of African trypanosome infection, as well as a novel role for adipocytes as regulators of skin immunity during chronic infection.

09:40 (105 mins)

Understanding Trypanosome Lytic Factor biogenesis through human serum, tissue culture, and murine models

Sara Fresard, *City University of New York, Hunter College and The Graduate Center*

S Fresard[1]; K Leiss[2]; R Thomson[2]; J Raper[1];

[1] City University of New York, Hunter College and The Graduate Center, United States; [2] CUNY Hunter College, United States

African Trypanosomiasis is a disease caused by the African Trypanosoma family of bloodborne parasites. Cattle are susceptible to trypanosomiasis, while humans and some non-human primates are protected against most species of trypanosomes due to an immunity complex called Trypanosome Lytic Factor (TLF). TLF is a specialized High-Density Lipoprotein (HDL), that carries a lytic cation channel-

forming protein (Apolipoprotein-L1 (APOL1)), and a ligand (Haptoglobin-related protein (HPR)), which binds to parasite receptors increasing uptake into the parasite. There are two TLF HDL complexes in blood: TLF1 (~500 kDa) and TLF2 (~1,200 kDa). How these TLFs are assembled is unknown. We have used human serum, tissue cell culture, and transgenic murine models to better understand how and where TLFs are assembled and how to translate that information to resistant transgenic cattle model. Generation of transgenic cattle constitutively expressing genes that encode for TLF components would reduce animal disease, as well as the reservoir for human disease.
To fully characterize all possible TLF species, we used anti-APOL1 affinity chromatography and size exclusion chromatography by FPLC. Both TLF1 and TLF2 were isolated, as well as a third complex of 180 kDa. Immunoprecipitation confirmed APOL1 and APOA-I were on the same complex, and activity assays based on normalized APOL1 concentration showed this complex lyses trypanosomes equivalently to TLF1, indicating the presence of HPR. We propose that this TLF complex, TLF3, is a nascent HDL made in hepatocytes based on its size, density, and predicted liver gene expression. These data inform us that liver specific promoters could be used to drive the expression of *APOL1* and *HPR* in transgenic cattle. We have generated many germline transgenic murine models to test different promoters for the expression of *HPR* and *APOL1*. However, we find that the Ubiquitin promoter (not liver specific promoters) drives the highest expression of both *HPR* and *APOL1,* which is key for sustained and robust protection against trypanosome challenges.

To revisit the biogenesis of TLF3 we turned to *in vitro* studies of human hepatocyte cell lines, HepG2. We find co-assembly of APOL1 and APOA-I by size fraction, and plan to affinity purify APOL1 complexes secreted by HepG2 cells to evaluate their protein composition by mass spectrometry and the trypanosome lytic capacity of the complexes. We hypothesize that all three proteins are assembled together in/on the hepatocyte with minimal but sufficient lipids to generate a nascent HDL. Thereafter, the TLF3 complex is released into the blood and matures into TLF1 by accumulating lipids and potentially more APOL1 from peripheral tissues. By understanding TLF biogenesis, we can use the appropriate promoters in transgenic cattle models to generate cattle resistant to trypanosomiasis.

09:505 (20 mins)

The interplay between salivarian trypanosomes, host B cells, and Neutrophils demonstrates the capacity of the parasite to evade immune defences

Dr Magdalena Radwanska, *Ghent University*

M Radwanska[1]; V Bockstal[1]; J Cnops[1]; S Moon[1]; HT Nguyen[1]; I Janssens[1]; HT Pham[1]; B Choi[1]; V Deleeuw[1]; FE Obishakin[1]; C De Trez[1]; S Magez[1];
[1] Laboratory for Biomedical Research, Department of Environmental Technology, Food Technology and Molecular Biotechnology, Ghent University Global Campus, South Korea

Salivarian trypanosomes are extracellular protozoan parasites causing infections in a wide range of mammalian hosts. The success of these parasites is attributed to their capacity to disable the immune system, resulting in a long-lasting infection, favouring parasite transmission. We demonstrate that mechanisms driving modulation of the innate and the adaptive immune responses take place in the bone marrow and the secondary lymphoid organs. As such, in the spleen mature IgM+CD1d+ Marginal Zone and IgMIntIgD+ Follicular B cells are rapidly depleted due to apoptosis and terminal differentiation into CD138+ Plasma cells. At the same time progressing ablation of the bone marrow immature CD93+ and Vpreb3+Ly6d+IghM+ transitional spleen B cells leads to the depletion of peripheral mature B cells. As a consequence, reduced number of new B cells can be generated, preventing the host from responding to newly emerging antigenic variants or remembering already encountered trypanosomes. In this context, the gradual infection-induced decrease in memory B cells was also demonstrated in the

spleen. Strikingly, this B cell depletion coincided with large expansion the neutrophil population carrying cytotoxic load of metalloproteinases MMP-8 and MMP-9. The subsequent degranulation of these enzymes caused remodelling of the extracellular matrix (ECM), leading to severe damage of the spleen architecture, in the absence of a sufficient TIMP inhibitory activity. The scRNA-seq transcriptomic profiling indicated that expanded neutrophil population contained proliferating precursors, two immature subpopulations, and inflammation reprogrammed mature neutrophils. While usually neutrophils are associated with rapid and efficient pathogen removal, this seems not to be the case in trypanosome infections. Curiously, neutrophil depletion coincided with prolonged host survival, reduced organ damage, increased Plasma cell numbers, and improved systemic parasite control. Hence, the neutrophil cytotoxic signature genes constitute indicators of trypanosomiasis associated inflammation and pathology, seen also in *T. b. rhodesiense* sleeping patients.

10:15 10 (20 mins)

Trypanosomatid virulence factors and new perspectives in vaccine development for leishmaniasis and Chagas disease

Prof Santuza Maria Teixeira, *Federal University of Minas Gerais*

S Teixeira[1]; G Burle-Caldas[1]; RS Fernandes[1]; JT Castro[1]; I Vieira[1]; AF Braz[1]; NS Hojo-Souza[1]; NA Santos[1]; AP Fernandes[1]; RT Gazzinelli[1];
[1] Departamento de Bioquimica e Imunologia and Centro de Tecnologia de Vacinas, Universidade Federal de Minas Gerais, Brazil

Genomic studies allowed us to investigate virulence factors that are essential for survival of intracellular parasites, among them, several surface proteins from *Trypanosoma cruzi* and *Leishmania spp.* Using RNAi, we have shown that amastins are essential factors involved in amastigote interaction with the parasitophorus vacuole (PV) within infected macrophage. Using CRISPR-Cas9, we generated *T. cruzi* knockout (KO) mutants in which genes encoding active Trans-sialidases (TS) were disrupted and showed that the transfer of sialic acid from the host to the parasite surface is essential for parasite survival in mice. TS are encoded by the largest *T. cruzi* gene family with more than 1,000 genes, but only 16 of them encode proteins with an active catalytic site. Disruption of active TS genes does not affect epimastigote growth in the insect vector or the parasite's capacity to invade cells but resulted in impaired differentiation of intracellular amastigotes into trypomastigotes and parasite egress. *In vitro* infection of HeLa cells with TS KO mutants resulted in significantly lower levels of IL-1-β, IL-1-α and IL-6 compared to cells infected with WT parasites. Importantly, when inoculated into mice, TS KO parasites were unable to establish infection even in immunodeficient animals. Like other *T. cruzi* surface proteins expressed in the mammalian stage, TS are anchored at the parasite membrane through a GPI anchor that contains inositol-phosphorylceramide (IPC). Like TS mutants, parasites with disrupted IPC synthase gene have no growth defect but have impaired capacity to differentiate into metacyclic trypomastigotes and are unable to infect mice. Mice immunized with these attenuated *T. cruzi* strains are fully protected against a challenge infection with a virulent strain. Besides using genetically attenuated parasites as vaccinal strains we showed that mice can be also protected against infection with *T. cruzi* and *L. amazonensis* if they are immunized with recombinant proteins containing sequences from TS genes or from a conserved Leishmania antigen. Finally, we have also tested lipid nanoparticle-encapsulated RNAs encoding TS as well as RNA encoding a *L. amazonenis* antigen and showed that the LNP-RNA immunization protocol represents a promising vaccine strategy for preventing leishmaniasis and Chagas disease. Supported by: Conselho Nacional de Desenvolvimento Científico e Tecnológico-CNPq, Fundação de Apoio a Pesquisa do Estado de Minas Gerais-FAPEMIG and Instituto Nacional de Ciencia e Tecnologia de Vacinas-INCTV (Brasil) and The global Challenges Research Fund-GCRF (UK)

(12) Cellular heterogeneity - (Lecture theatre 3)

Chair: Calvin Tiengwe

09:00 (25 mins)

Drugs, sex, and schistosomes: control of female schistosome sexual development by a male-derived non-ribosomal peptide pheromone

Prof Jim Collins, *UT Southwestern Medical Center*

J Collins[1];
[1] *UT Southwestern Medical Center, United States*

Schistosomes cause morbidity and death throughout the developing world due to the massive numbers of eggs female worms deposit into the blood of their hosts. Studies dating back to the 1920s show that female schistosomes rely on constant physical contact with a male worm both to become and remain sexually mature; the molecular details governing this process remain elusive. In this seminar, I will detail our discovery of a novel dipeptide, β-alanyl-tryptamine, that is synthesized by a non-ribosomal peptide synthetase in male worms in response to male:female pairing. Using approaches such as scRNAseq and metabolomics we demonstrate that male worms possess a population of ciliated neuronal cells that secrete β-alanyl-tryptamine where it can act directly on female worms to trigger sexual development and egg-laying. Together, our data suggest new avenues for therapeutic intervention while also uncovering an unexpected role for non-ribosomal peptides as metazoan signalling molecules.

09:25 (20 mins)

From whole worm to single cells: using transcriptomics to understand how a gastrointestinal nematode thrives in its host

Dr James Wasmuth, *University of Calgary*

JD Wasmuth[1]; S Pollo[1]; C Finney[1];
[1] *University of Calgary, Alberta, Canada*

Heligmosomoides bakeri, a parasitic nematode of mice, is closely related to economically important parasites of livestock and hookworm parasites of humans. As a murine parasite it is more amenable to being maintained and manipulated in a controlled laboratory environment than its relatives. The worm enters its host during its third larval stage and develops through another larval stage into adults that reside in the lumen of the small intestine to mate and lay eggs. Unravelling these processes and others critical to *H. bakeri* survival will not only reveal new targets for new drugs but also refine previous predictions of parasite immunomodulatory molecules, which have therapeutic potential in humans as anti-inflammatories. We set out to describe the gene expression of *H. bakeri* during the parasitic phase of its lifecycle.

First, we investigated how the whole worm expression of genes varies across infection time-points. We found that up to 68% of genes were differentially regulated between male and female worms, including genes associated with modulating the host's immune response and potential anthelmintic targets. In comparing tissue-encysted larvae with lumen-dwelling adults, we found an increased importance for anaerobic respiration and hypothesise that aerobic conditions are important for the critical developmental processes of moulting and cuticle synthesis.

To understand gene expression at a finer granularity, we generated single cell RNA-seq data from young adult male and female worms. We used cell type markers from *C. elegans* to putatively identify gamete, embryo, intestine, hypodermis, neuron, and muscle cells. Putative intestinal transcription

profiles suggest compartmentalisation of function along the anterior-posterior axis of the worms, with an emphasis on protein synthesis in the anterior portion. Embryonic profiles are noticeably different from *C. elegans* embryogenesis, particularly with respect to paternal contributions to the early embryo.

Overall, these datasets extend our understanding of how *H. bakeri* survives in its host and provide a public resource for further investigation into host-parasite interactions and anthelmintic discovery. They also lay the groundwork for more comprehensive comparisons with other nematodes.

09:45 (15 mins)

Schistosomes – old questions, new technologies

Dr Gabriel Rinaldi, *Aberystwyth University*

R Pichon[3]; T Attenborough[1]; E Hall[3]; J Bulathsinghalage[3]; M Lotkowska[2]; M Evans[3]; B Hulme[3]; J Forde-Thomas[3]; K Rawlinson[4]; K Hoffmann[3]; M Berriman[1]; G Rinaldi[3];
[1] University of Glasgow , UK; [2] Wellcome Trust Sanger Institute, UK; [3] Aberystwyth University, UK; [4] Marine Biological Laboratory, Woods Hole, United States

Schistosomes are agents of Schistosomiasis, a major Neglected Tropical Disease (NTD) that affects more than 250 million people worldwide. With two separate sexes — a heterogametic female (ZW) and a homogametic male (ZZ) — schistosomes are the exception among flatworms, which are largely hermaphrodites. However, the phenotypic sexual dimorphism of the parasite only becomes apparent by adulthood within the mammalian host, and not during other developmental stages. Male and female worms undergo separate but concurrent sexual differentiation of their gonads and somatic tissues that eventually allows intersexual pairing, a critical step for egg production and life cycle propagation. This is a key but poorly understood process in early intra-mammalian development of schistosomes. To tackle this knowledge gap, we used functional genomics in tandem with cutting-edge molecular tools and focused on two critical developmental transitions: (1) the cercaria–schistosomulum, i.e. from free-living infectious larva to the first intra-mammalian parasitic stage; (2) sexually monomorphic–dimorphic intra-mammalian developmental stages. For transition (1), single cell transcriptomics of female or male cercariae and two-day old (D2) schistosomula revealed sex-biased expression across different cell clusters in both early sexually monomorphic developmental stages. We are currently confirming these findings by droplet digital PCR (ddPCR), that will be followed by spatial validation and functional characterisation of informative genes by *in situ* hybridisation and RNAi, respectively. For transition (2), we accurately determined the timing of sexual dimorphism being established *in vivo*, by morphological analysis and confocal imaging of individual parasites collected from mice infected with either male or female parasites. Preliminary single cell RNA-seq data identified tentative cell populations involved with this sexually monomorphic–dimorphic transition. In parallel, we have been refining culture systems that facilitate the *in vitro* study of schistosome development, including the establishment of dimorphism. We have also been exploring long term gene silencing protocols that include genome editing mediated by CRISPR-Cas, and the long-term preservation of schistosome developmental stages. The latter would positively impact on the 3Rs (i.e. Replacement, Reduction and Refinement) in the use of animals for research. 'Omics' approaches coupled with cutting-edge cellular and molecular technologies, including single cell transcriptomics, *in vitro* long-term culture, and gene perturbation mediated by RNAi and genome editing will shine new light on schistosome biology and help to expose targets for novel control strategies of this major NTD.

10:00 (15 mins)

Capturing motile cells poses challenges to microfluidic encapsulation in scRNAseq

Lara Lopez Escobar, *Instituto de Medicina Molecular*

L Lopez-Escobar[1]; A Nascimento[1]; LM Molecular[1];
[1] Instituto de Medicina Molecular Liboa, Portugal

Single-cell RNA sequencing (scRNAseq) using microfluidic systems, such as 10XGenomics, is a prevailing trend to study cell states and identities. This method involves encapsulation of the single cells in drops of oil, called GEMs (Gel Bead-in-Emulsion). Whether motility of cells, specifically extracellular parasites like *Trypanosoma brucei,* affects encapsulation efficiency remains unknown. We conducted experiments to investigate the effects of adding motile and non-motile parasites to the encapsulation process of 10XGenomics at both the transcriptional and microscopy levels. Following encapsulation, we observed that these parasites did not incur any damage during the encapsulation process and retained their high motility, potentially causing rupture of droplets, and releasing the parasites into the interspaces of the GEMs. The posterior analysis revealed that a significant proportion of immotile parasites persisted in the final data, while the highly motile ones nearly disappeared by the end of the analysis. This data highlights the importance of considering cell motility in microfluidic-dependent setups and the fact that valuable information from highly motile cells may be lost.

10:15 (15 mins)

Can many biomarkers make light work of ovine fasciolosis diagnostics?

Christy Wray, *Queen's University Belfast*

C Wray[1]; D Wells[1]; C Herron[1]; RM Morphew[2]; P McVeigh[1];
[1] Queen's University Belfast, UK; [2] Aberystwyth University, UK;

The liver fluke *Fasciola hepatica* is a widespread threat to farming, with production losses exacerbated by predominantly coprology based diagnostic tools that only detect patent infection. Poor available diagnostics has led to overuse of anthelmintics, driving selection pressure for resistance which is becoming an increasingly important issue. Thus, novel diagnostics methods capable of diagnosing active infection are needed. In human medicine non-invasive "liquid biopsies" are often used to diagnose active diseases as well as advise prognostically. Based on these approaches, we have investigated the small-RNA and protein profiles in the serum of sheep experimentally infected with *F. hepatica*. Time-series analysis of these profiles at 0 days (pre-infection) 28 days (juvenile/acute infection) and 105 days (mature/chronic infection) shows statistically significant differentially expressed host micro (mi)RNAs as well as the presence of parasite miRNAs in the serum of infected hosts. Proteomic profiles also show significant differential expression between timepoints. These data are currently being further analysed with the goal of developing a biomarker panel, made up of the most highly informative biomarkers that will make the base of a diagnostic test. Such a diagnostic test could support sustainable use of anthelmintics by allowing more targeted treatment as opposed to flock-wide anthelmintic use.

(24) Molecular and cellular biology 2 - (Teaching room 4)

Chair: Joana Faria

09:00 (15 mins)

Integrating cell signalling and host-parasite interactions to determine important drivers for schistosome growth, development, and survival in the human host

Prof Anthony Walker, *Kingston University*

S Maharjan[1]; NA Aguoru[1]; RS Kirk[1]; SP Lawton[2]; AJ Walker[1];
[1] Kingston University, UK; [2] Scottish Rural University College, UK

The outer surface tegument layer of schistosomes is thought to be vital for schistosome survival in the mammalian host. However, the importance of this unique organ to schistosome growth and development, and how it interfaces with host molecules to support these processes, is not well understood. Across several projects, we have sought to elucidate how lipid rafts, important cholesterol-rich membrane microdomains, act as cellular signalling hubs in *Schistosoma mansoni*, and how such signalling drives the growth, development, and survival of the parasite, particularly the exponentially growing liver schistosomula stage. We demonstrate that lipid rafts cluster in response to human epidermal growth factor (EGF) in schistosomula, concomitant with the localisation of receptors that bind human EGF (EGFRs) and insulin (IRs). The activation of several protein kinase pathways within the parasite, including protein kinase C (PKC), extracellular signal-regulated kinase (ERK), p38 mitogen-activated protein kinase (p38 MAPK), and Akt were modulated by tegument cholesterol depletion, with effects reversed by cholesterol reloading. We also demonstrate that heat shock protein 90 (HSP90) acts as an important signalling hub to these protein kinases in schistosomes. Lipid raft disruption or blockade of EGFR signalling at the tegument surface reduced somule motility and survival, and blunted stem cell proliferation and somule growth and development, particularly to the liver schistosomula stage. Our findings support a novel paradigm for schistosome growth and vitality in the human host, directed by tegument associated host-parasite interactions, that could be exploitable for developing of new approaches for schistosomiasis control.

09:15 (15 mins)

Plasmodium sporozoite excystation involves local breakdown of the oocyst capsule

Dr Sadia Saeed, London School of Hygiene and Tropical Medicine

S Saeed[1]; JT Dessens[1]; AZ Tremp[1];
[1] London School of Hygiene and Tropical Medicine, UK

Plasmodium oocysts develop on the abluminal side of the mosquito midgut in relatively small numbers. Oocysts possess an extracellular cell wall—the capsule—to protect them from the insect's haemolymph environment. To further maximise transmission, each oocyst generates hundreds of sporozoites through an asexual multiplication step called sporogony. Completion of transmission requires sporozoite egress from the capsule (excystation), but this process remains poorly understood. In this study, we fused the parasite-encoded capsule protein Cap380 with green fluorescent protein in a transgenic *P. berghei* line, allowing live fluorescence imaging of capsules throughout sporogony and sporozoite excystation. The results show that capsules progressively weaken during sporulation ultimately resulting in sporozoite exit through small holes. Prior to formation of the holes, local thinning of the capsule was observed. Our findings support an excystation model based on local, rather than global, weakening of the capsule likely facilitated by local re-orientation of sporozoites and apical secretion.

09:30 (15 mins)

Cryptosporidium remodels host Microvilli through an exported virulence factor

Elena Rodrigues, *The Francis Crick Institute*

E Rodrigues[1]; TT Mkandawire[1]; K Sala[1]; A Sateriale[1];
[1] The Francis Crick Institute, UK

The intestinal parasite *Cryptosporidium* is a leading cause of diarrhoeal disease, contributing to early childhood morbidity and mortality. Like other members of the phylum Apicomplexa, *Cryptosporidium* has secretory organelles containing proteins that are exported into host cells following parasite invasion. For *Cryptosporidium*, the identities and functions of almost all of these proteins are unknown. Using a bioinformatics approach, we first identified a putative host-exported protein with serine repeats, which we epitope tagged at the endogenous locus. With a combination of super-resolution and expansion microscopy we discovered that this protein localises to the parasite's secretory dense granule organelles prior to host-cell invasion, and then within the host microvilli following invasion. To determine the function of this MicroVilli Protein (MVP) we used yeast-2-hybrid screening, detecting interacting partner EBP50; a scaffold protein known to facilitate F-actin recruitment and control microvilli dynamics. Microvilli elongation is commonly seen in *Cryptosporidium* infected epithelial cells, but the mechanism for this was previously unknown. Parasites deficient in MVP have moderately attenuated growth yet show a complete lack of elongated host microvilli during infection. It is known that the *Escherichia coli* virulence factor MAP also interacts with EBP50, driving cell surface membrane protrusions and displacement of the NH3 sodium transporter contributing to diarrhoeal symptoms. While MVP has C-terminal homology with MAP, there does not appear to be evidence of a horizontal transfer event. This suggests a convergent evolution between bacteria and parasite that may contribute to diarrhoeal symptoms during infection.

09:45 (15 mins)

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

Veronica Harris, *University of St Andrews North Haugh*

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 and membrane formation. In the protozoan parasite *Trypanosoma cruzi*—the causative agent of Chagas' disease—*myo*-inositol acts as a precursor to phosphatidylinositol (PI), an essential membrane lipid component. PI in turn is then 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 and scavenged from the environment, however, the proteins involved in both pathways have not been fully studied in *T. cruzi.* Therefore, the aim of this project is to genetically validate and biochemically characterise the putative inositol-3-phosphate synthase (*Tc*INO1) from the *de novo* synthesis pathway as well as the *myo*-inositol transporter (*Tc*MIT) from the extracellular uptake pathway.

Both proteins—*Tc*INO1 and *Tc*MIT—are genetically validated as essential and biochemically characterised. In addition, localisation and phenotyping of *TcINO1* and *TcMIT* genetically altered *T. cruzi* has been completed, which helps establishes how *T. cruzi* differentiate between *de novo* and scavenged *myo*-inositol.

10:00 (15 mins)

Blastocrithidia nonstop mitochondrial genome and its expression are remarkably insulated from nuclear codon reassignment

Prof Vyacheslav Yurchenko, *University of Ostrava*

V Yurchenko[1]; DA Afonin[2]; ES Gerasimov[2]; I Škodová-Sveráková[1]; K Záhonová[1]; Z Paris[4]; J Lukeš[5]; SL Zimmer[3];

[1] University of Ostrava, Czech Republic; [2] Moscow State University, Moscow, Russian Federation; [3] University of Minnesota Medical School, Duluth campus, United States; [4] Institute of Parasitology, Biology Centre, Czech Academy of Sciences, Czechia; [5] Institute of Parasitology, Biology Centre, Czech Academy of Sciences, Czechia

The canonical stop codons of the nuclear genome of the trypanosomatid *Blastocrithidia nonstop* are recoded. Here, we investigated the effect of this recoding on the mitochondrial genome and gene expression. Trypanosomatids possess a single mitochondrion and protein-coding transcripts of this genome require RNA editing in order to generate open reading frames of many transcripts encoded as "cryptogenes". Small RNAs that can number in the hundreds direct editing and produce a mitochondrial transcriptome of unusual complexity. We find *B. nonstop* to have a typical trypanosomatid mitochondrial genetic code, which presumably requires the mitochondrion to disable utilization of the two nucleus-encoded suppressor tRNAs, which appear to be imported into the organelle. Alterations of the protein factors responsible for mRNA editing were also documented, but they have likely originated from sources other than *B. nonstop* nuclear genome recoding. The population of guide RNAs directing editing is minimal, yet virtually all genes for the plethora of known editing factors are still present. Most intriguingly, despite lacking complex I cryptogene guide RNAs, these cryptogene transcripts are stochastically edited to high levels.

10:15 (15 mins) Not presented (Travel problem)

~~Key *Leishmania* trans-regulators are essential for parasite surveillance and infectivity~~

~~Dr Pegine Walrad, *University of York*~~

~~E Parry[1]; N Telos[1]; RP Noish[1]; FS Pais[1]; AA Dowle[1]; K Nowling[1]; J Mottram[1]; PB Walrad[1]; [1] *University of York, UK*~~

~~Like other Kinetoplastids, gene expression in *Leishmania* species is overwhelmingly post-transcriptionally controlled. This elevates the importance of RNA binding proteins (RBPs) in these systems as the primary gene regulators. Building upon the *L. mexicana* RBPome we isolated previously from the 3 main parasite lifecycle stages (Pablos et al. MCP, 2019), 70 non-basal RBPs were selected toward further investigation. An *L. mexicana* barcoded trans-regulator knockout clone library was created using CRISPR-cas9 (Baker et al. Nat Comms, 2021) and screened through lifecycle progression and macrophage or mouse infections. Remarkably, 60% of the RBPs screened are essential for cell viability and 26% contribute to lifecycle progression to human-infectious stages, infectivity and/or virulence. Examination of individual knockout lines verify the screen outcomes of specific RBPs essential for parasite growth, viability and infectivity. 13 RBPs were endogenously tagged, immunoprecipitated and submitted for transcriptomic and proteomic analyses to identify all RNP components. Discrete complexes have been identified that may represent novel virulence factors. Further analyses are underway to map interaction dynamics of these key RNP regulators that drive differentiation and virulence capacity in *Leishmania*.~~

(13) Helminth epidemiology 2 - (Lecture theatre 1)

Chair: Jane Hodgkinson

11:00 (25 mins)

A Tale of Schistosomiasis in Malawi: From Burden to Prevention

Dr Janelisa Musaya, *Malawi Liverpool Wellcome clinical research program*

J Musaya[1];
[1] Malawi Liverpool Wellcome clinical research program, Malawi

In Malawi, schistosomiasis has long been a significant public health concern. All 29 of Malawi's health districts are endemic for schistosomiasis, with both urogenital and intestinal forms present within the country. The Prevalence rate ranges from 1.3% to 25.4%, which are classified as low to moderate prevalence according to the World Health Organization (WHO) endemicity categories. The warm climate and abundant water sources create an ideal environment for schistosomiasis transmission, particularly affecting rural communities living near freshwater bodies like lake Malawi. School-age children are particularly vulnerable due to frequent water contact during daily activities. Over the years our team has implemented various projects from understanding the disease biology, epidemiology to prevention. Here we want to share the schistosomiasis journey of the different findings and challenges encountered and the future work that we are planning to embark on. Our goal is to embark on a one health approach to transform this tale into one of triumph over a persistent adversary, improving the lives of people affected by schistosomiasis.

11:25 (20 mins)

Bovine schistosomiasis in Malawi emerging public health problem: revealing zoonotic haematobium hybrid

Dr Alexandra Juhász,, *Liverpool School of Tropical Medicine*

A Juhász[1]; LJ Cunningham[1]; S Jones[1]; J Archer[1]; L Field[1]; SA Kayuni[1]; EJ La Course[1]; JR Stothard[1]; P Makaula[2]; D Lally[2]; G Namacha[2]; D Kapira[2]; P Chammudzi[2]; J Musaya[2]; E Seto[3];
[1] Liverpool School of Tropical Medicine, UK; [2] Malawi Liverpool Wellcome Trust Programme of Clinical Tropical Research, Malawi; [3] University of Florida, Gainesville, United States

Despite ongoing control, schistosomiasis is widely distributed along Afrika, affecting both the human population and their livestock. Abrupt changes in the genetic makeup of human schistosome worms are known which demonstrates the ability to form viable hybrids with closely related *Schistosoma* species. In April 2022, a total of 153 cattle carcasses were examined, as well as 321 faecal samples and state-of-the-art tracking with remote GPS dataloggers in Malawi. Prevalence of schistosomiasis in cattle was 70% in Mangochi, 63% in Chikwawa, 21% in Nsanje districts and 21% in Blantyre slaughterhouses. Inspecting the molecular data, it showed that not only *Schistosoma mattheei*, which was found for the first time in the country, and the previously known *S. haematobium* worms of human origin, but their introgressive hybrids are also present in domestic cattle in Malawi. Also, praziquantel treatment was given to eight GPS tracked cattle, six weeks post treatment the youngest animal was detected with reinfection, the egg output with age was declined.

11:45 (15 mins)

Evaluation of surveillance-response interventions for *Schistosoma haematobium* elimination on Pemba Island, Tanzania: A 4-year intervention study with repeated cross-sectional surveys

Lydia Trippler, *Swiss Tropical and Public Health Institute*

L Trippler[2]; J Hattendorf[2]; MN Ali[1]; SO Najim[1]; KS Khamis[1]; KR Suleiman[1]; SM Ame[3]; S Juma[3]; F Kabole[3]; SM Ali[1]; S Knopp[2];
[1] Public Health Laboratory - Ivo de Carneri, Tanzania; [2] Swiss Tropical and Public Health Institute, Switzerland; [3] Neglected Tropical Disease Programme, Ministry of Health, Tanzania

Background: The WHO aims to eliminate schistosomiasis as a public health problem worldwide by 2030. Pemba Island, Tanzania, achieved this goal in 2017 and is now targeting interruption of transmission. In most parts of Pemba, the *Schistosoma haematobium* prevalence is below 3%. Mass drug administration of praziquantel no longer seems justified. Instead, we implemented a surveillance-response approach with targeted interventions in low-prevalence areas. Here, we assessed the sensitivity of the surveillance-response approach to identify and treat all infected individuals in the area and its impact for elimination.

Methods: In the 4-year SchistoBreak project, annual cross-sectional surveys in schools and communities were conducted to identify low-prevalence and hotspot areas and implement interventions accordingly. In low-prevalence areas, a surveillance-response approach was implemented, where, in a first step, children in primary and Islamic schools were screened for *S. haematobium* infection. Subsequently, positive-tested children were treated with praziquantel and accompanied to their homes and the water bodies they used. Testing for *S. haematobium* was offered to household members and individuals at water bodies, and treatment to those who tested positive. Snail surveys were conducted at the water bodies to search for *Bulinus*, and if found, niclosamide was applied. To assess the sensitivity of the surveillance-response approach, the number of positive-tested individuals in the interventions was divided by the estimated number of infected individuals in the whole study area, as determined by cross-sectional surveys and population census data.

Results: In 2021, the baseline *S. haematobium* prevalence in 15 low-prevalence areas was 0.5% (7/1552) in schoolchildren. After one year of surveillance-response interventions, the prevalence decreased to 0.4% (6/1653). In 2022, the prevalence of schoolchildren in 16 low-prevalence areas was 0.6% (12/2123) and changed to 0.7% (15/2240) in 2023 after the interventions. In 2023, the prevalence in 17 low-prevalence areas was 0.4% (8/2287) and changed to 0.8% (9/1103) in 2024. In 2021, the baseline *S. haematobium* prevalence in 15 low-prevalence areas was 0.5% (14/2969) in community members. After one year of surveillance-response interventions, the prevalence changed to 0.7% (19/2928). In 2022, the prevalence of *S. haematobium* in 16 low-prevalence areas was 0.6% (18/3175) and dropped to 0.3% (10/2979) in 2023 after the interventions. In 2023, the prevalence in 17 low-prevalence areas was 0.4% (12/3255) and changed to 0.7% (22/3014) in 2024. The sensitivity of the surveillance-response approach to identify and treat all individuals estimated to be infected with *S. haematobium* in the population of the study area was 96.3% for schoolchildren, 3.7% for adults, and 56.0% overall. In 26.2% of the water bodies that were surveyed based on children's use, *Bulinus* were found and niclosamide was applied.

Conclusion: The surveillance-response interventions showed a very high sensitivity in identifying and infected children but not adults. Many water bodies were discovered and treated with niclosamide that serve as habitats for *Bulinus.* However, while surveillance-response interventions maintained the low *S. haematobium* prevalence in the study area, they did not result in transmission interruption within three years.

12:00 (15 mins)

The short-term impact of *Schistosoma mansoni* infection on liver morbidity and health-related quality of life: implications for current elimination policies

Prof Poppy Lamberton*, University of Edinburgh*

R Lim[2]; R Lahoti[2]; AB Pedersen[2]; M Arinaitwe[3]; V Anguajibi[6]; A Nankasi[5]; F Besigye[5]; A Atuhaire[5]; J Webster[4]; P Lamberton[1];
[1] University of Glasgow , UK; [2] University of Edinburgh, UK; [3] Ministry of Health, Uganda; [4] Royal Veterinary College, UK; [5] Vector Control Division, Ministry of Health, Uganda; [6] China-Uganda Friendship Hospital, Uganda

Background: The World Health Organization (WHO) aims to eliminate schistosomiasis as a public-health problem by 2030, with <1% prevalence of heavy infections in school-aged children (SAC) used as a target threshold. However, standard morbidity measures poorly correlate to infection intensities and there is a lack of evidence on Schistosoma's impact on health-related quality of life (HRQoL), with little understanding of what truly constitutes a public-health problem.

Methods: We conducted community-based cross-sectional surveys in moderate and high *Schistosoma mansoni*-endemic communities in Uganda. We measured parasitic infections and used the EQ-5D instrument to estimate HRQoL utilities in 560 participants. Ultrasound examinations were performed on 287 participants from the high endemicity community and the Niamey protocol used to characterise periportal fibrosis (PPF), portal vein dilation (PVD) and left parasternal line (PSL) enlargement. Tobit and linear regression models were used to predict HRQoL determinants and logistic regression models to understand liver morbidity predictors.

Results: Just under half of the 560 participants (58% of those with ultrasound data) were diagnosed with *S. mansoni*. PPF prevalence was 4-9% (depending on image pattern cut offs). PVD and PSL prevalence were 34% and 33% respectively. 11-14 year olds had the highest infection intensity but pre-school aged children (PreSAC) were significantly more likely to have PVD and PSL morbidities than 11-14 year olds. Current *S. mansoni* infection was not associated with the liver morbidity markers. No significant association between HRQoL and *S. mansoni*-infection status/intensity was observed and schistosomiasis associated symptom severity, and socio-economic status, were better predictors of short-term HRQoL.

Conclusions: Our findings add to the growing evidence indicating a lack of association between current *S. mansoni* egg count with current morbidity markers. The notable burden of PVD and PSL in PreSAC, stresses the need to elucidate the impact of subtle and early morbidities and accommodate the young children in community treatment programmes. Morbidity and HRQoL data are key to disentangling the link between infection(s) and short-term health outcomes, and our findings highlight the complexity of correlating current infection(s) with long-term morbidity. Evidence is needed on subtle morbidity, longer-term morbidity, HRQoL, and health and economic outcomes associated with schistosomiasis interventions to inform the economic case for upfront investments in schistosomiasis interventions.

12:15 (15 mins)

Opening a can of worms: Detecting zoonotic *Strongyloides* species within strongyloidiasis

Dr Lucas Cunningham, *LSTM*

LJ Cunningham[1], A Juhasz[1]; S Jones[1]; J Archer[1]; W Nevin[1]; JJ Verweij[3]; J Cracknell[2]; J Quayle[2]; JR Stothard[1];
[1] Liverpool School of Tropical Medicine, UK; [2] Knowsley Safari, UK; [3] Elisabeth-Tweesteden Hospital, UK

Since 2009, the TaqMan real-time PCR developed by Verweij et al. has been the frontline molecular diagnostic for the detection of *Strongyloides stercoralis*, yet it is actually a genus-specific assay. Taking advantage of

newly designed species-specific primers and probes targeting hyper-variable regions of the ribosomal 18S gene, alongside whole-genome sequencing, we have clearly shown the presence of *Strongyloides fuelleborni* within various clinical samples. Indeed, the role of zoonotic *Strongyloides* species has been grossly underestimated, even more so as shortcomings, and failures, in serological detection come to light. Here, we present current refinements in species-specific real-time PCR assays, alongside our exploration of e-DNA typing of soils to identify sites of active transmission of *S. fuelleborni* within a captive population of baboons in a UK safari park.

(14) Parasite-Immune interactions 2 - (Lecture theatre 2)
Sponsored by PCR Biosystems

Chair: Joe Turner

11:00 (25 mins)

Life stage-specific glycosylation of schistosome-derived extracellular vesicles (EV) directs functional interactions of EV with host immune cells

Prof Cornelis Hokke, *Leiden University Medical Center*

C Hokke[1]; EN Nolte-'t Hoen[2]; ME Kuipers[1]; HH Smits[1];
[1] Leiden University Medical Centre, Netherlands; [2] Faculty of Veterinary Medicine, Utrecht University, Netherlands

Glycans play an essential role in pathogen-host interactions. Larval and adult *Schistosoma mansoni* release distinct excretory/secretory (ES) glycoconjugates. ES products also contains extracellular vesicles (EVs). We previously found that schistosomula-derived EVs are glycosylated and bind to human dendritic cells (hDC) via the C-type lectin receptor (CLR) DC-SIGN, leading to increased IL-10 and IL-12 release. Here, we investigated the glycosylation of EVs released by adult worms, compared this to schistosomula EVs, and addressed how glycans affect EV-host cell interactions via CLRs.

EVs were obtained by ultracentrifugation and iodixanol density gradients from cultured schistosomes. Isolated EVs were analysed by NTA and cryo EM showing that adult worm EVs have a different appearance and size distribution than schistosomula EVs. N-glycan and lipid glycan content of EVs was determined by mass spectrometry.

The most abundant glycans on the surface of adult worm EVs contained GalNAcβ1–4GlcNAc (LacDiNAc, LDN) motifs, whereas the Galβ1–4(Fucα1–3)GlcNAc (Lewis X) motif dominated in the surface glycans of schistosomula EV. Other differences in EV glycosylation between the two life stages were observed by Western blot using anti-glycan mAbs. In line with these structural observations, the adult worm derived EV bind to cells that express macrophage galactose-type lectin (MGL), an LDN-binding CLR expressed on hDCs and macrophages, whereas schistosomula EV primarily interact with hDC via DC-SIGN. In addition, we found that EVs from adult worms simulate cytokine responses, including IL-10, in B-cells.

Overall, our observations suggest that specific glycosylation of EVs from helminths plays a critical role in differential recognition of, and response to, helminth EVs by host immune cells.

11:25 (25 mins)

Helminth immunomodulatory protein activity controlled by location and timing

Dr Henry McSorley, *University of Dundee*

H McSorley[1];
[1] *University of Dundee, UK*

Intestinal nematodes are associated with modulation of host immune responses, suppression the development of allergic disease and allowing their persistence in the host. Studying the mechanisms of parasite immunomodulation could lead to both new treatments for allergic diseases, and new targets for vaccination. The intestinal parasitic nematode *Heligmosomoides polygyrus bakeri* secretes an array of immunomodulatory proteins. The HpARI family (HpARI1, HpARI2 and HpARI3) act on the IL-33 cytokine, while the HpBARI family (HpBARI and HpBARI_Hom2) act on the IL-33 receptor, ST2. These 5 proteins have disparate effects on IL-33 responses in models of allergic asthma: while most of these proteins suppress IL-33 responses, HpARI3 alone has the surprising effect of amplifying responses in IL-33-dependent models. Our current work uses vaccination, antibody blockade, protein binding and structural studies to elucidate the effects of these immunomodulatory proteins. We find that *H. polygyrus* co-opts the IL-33 pathway to block its pro-type 2 immune response effects in situ, while amplifying the cytokine's distal regulatory T cell-inducing effects. We further find that administration of specific members of these immunomodulatory families as vaccines can provide effective immunity to the parasite. Our work provides a basis for further studies on immunomodulatory proteins from parasitic helminths, their unexpectedly complex mechanisms of action, and their potential for use in vaccines and therapeutic regimens.

11:50 (10 mins)

Identification of species-specific glycan antigens of *Schistosoma haematobium*

Laudine Petralia, *New England Biolabs*

L Petralia[2]; A Van Diepen[1]; A Kildemoes[1]; T Veldhuizen[1]; L Nguyen[1]; T Zhang[1]; M Wuhrer[1]; JM Foster[2]; C Hokke[1];
[1] *Leiden University Medical Centre, Netherlands;* [2] *New England Biolabs, United States*

Schistosomes are parasitic worms responsible for devastating chronic diseases worldwide. *Schistosoma mansoni* and *S. haematobium* are the major species infecting humans, causing intestinal and urogenital pathologies, respectively. The *S. mansoni* glycome has been studied in detail, revealing complex, immunogenic and life stage-specific glycans crucial in host-parasite interactions. Very little is known, however, regarding the glycosylation and glycan antigenicity for other schistosome species, including *S. haematobium* which is estimated to be responsible for half of the approximately 250 million schistosome infections. Thus, we investigated the glycans expressed on cercariae, worms and eggs of *S. haematobium*. First, protein and lipid-linked glycans were released using enzymatic and chemical techniques and characterized using mass-spectrometry (MS) based approaches. Glycan structures were determined using sequencing techniques including exoglycosidase digestions in combination with MALDI-TOF-MS, and porous graphitized carbon-liquid chromatography-MS for in-depth resolution of complex isomeric structures. Our analysis revealed substantial differences between *S. haematobium* and *S. mansoni* glycosylation. Notably, *S. haematobium* glycosphingolipid (GSL) glycans are built on a trihexosyl core unlike the disaccharide core described in *S. mansoni*, are enriched in terminal acidic residues, but present a lower degree of fucosylation. The protein-linked glycans, on their hand, present core-modifications and terminal motifs identical to *S. mansoni*, although expressed with major quantitative differences. Next, a selection of glycans representative of *S.*

haematobium and *S. mansoni* glycomes including a broad coverage of the differential structures was purified and printed on a glycan microarray. Upon array screening, we observed a strong binding to acidic GSL glycans of IgG in sera from *S. haematobium*-infected individuals compared to *S. mansoni*-infected individuals and uninfected controls. These results indicate that the species-specific glycans characterized in *S. haematobium* are immunogenic and may play a role in *S. haematobium* specific immunobiology and pathology. Additionally, they constitute a potential diagnostic target specific for *S. haematobium* infections.

12:00 (10 mins)

Human immune responses to *Schistosoma mansoni*, lessons from controlled human infection models and natural endemic infection

Emma Houlder, *Leiden University Medical CenterLUMC*

E Driciru[3]; JP Koopman[1]; RA Steenbergen[1]; F Sonnet[1]; KA Stam[1]; JJ Janse[1]; HM Bes-Roeleveld[1]; E Iliopoulou[1]; I Nambuya[2]; JC Sijtsma[1]; YC Kruize[1]; A van Diepen[1]; C Hokke[1]; M Egesa[3]; AS MacDonald[2]; H Mpairwe[3]; M Yazdanbakhsh[1]; A Elliott[3]; M Roestenberg[1]; EL Houlder[1];
[1] Leiden University Medical Centre, Netherlands; [2] University of Manchester, UK; [3] MRC/UVRI and LSHTM Uganda Research Unit, Uganda

Prior studies have revealed mixed Type-1/Type 2 response in early migrating and maturing *Schistosoma mansoni (Sm)* infection, developing to a Type-2 and regulatory response upon egg production. These findings have been mainly derived from animal (murine) models, as longitudinal assessment of how worm-specific immune responses develop in humans has not been possible. Here, we have used a *Sm* controlled human infection model (*Sm*-CHI) to study immune response development over repeat (3x) male-cercariae exposure (Netherlands, n=24), comparing our findings to natural infection (Uganda, n=30). *Sm*-specific cellular and cytokine responses were assessed via spectral flow cytometry and luminex. Clinically, repeated *Sm*-CHI led to reduced symptoms (when compared to single), but did not result in (sterile) protection. In line with this symptom profile, Type-1 responses (serum CXCL10, activated $CD38^{+}HLADR^{+}$ T cells) peaked post exposure one and two, reducing post exposure three. In contrast, *Sm*-specific regulatory and Th2 responses increased with repeat exposure. Five *Sm*-CHI participants were inadvertently exposed to female (instead of male) cercariae during exposure two. This led to a potential mixed-sex infection and one positive *Sm* faecal PCR post exposure three before praziquantel treatment, indicative of low-level egg production. An elevated Type-2 response was observed in mixed-sex exposed individuals, with eosinophilia and *Sm*-specific Th2 cytokine production. *Sm*-specific Th2 responses in mixed-sex *Sm*-CHI were significantly higher than those observed in endemic natural infection, likely attributable to well-described immunoregulation induced by chronic *Sm* infection. Taken together, this data significantly advances our understanding of human immune response development during schistosome infection.

12:10 (20 mins)

Disease susceptibility and gut health in the wild: Determining interactions between diet, gut microbiome, and immunity

Prof Kathryn Else, *University of Manchester*

I Mair[2]; R Bancroft[1]; R Fletcher[1]; L Logunova[1]; AB Pedersen[2]; K Else[1];
[1] University of Manchester, Lydia Becker Institute of Immunology and Inflammation, UK; [2] University of Edinburgh, Institute for Ecology and Evolution, UK

The gastrointestinal dwelling nematode parasite *Trichuris* (whipworm) causes chronic infections, associated with a significant health burden in humans, livestock and wildlife. Understanding host-parasite interactions and adaptations in wild animal systems is important for our understanding of both the host immune response and in assessing the zoonotic potential of such pathogens. Mechanistic insight into the host immune response to whipworm infections, using laboratory mice experimentally infected with the mouse species of whipworm *Trichuris muris*, has established the balance of Type 1 and Type 2 immune responses as the major determinant of susceptibility versus resistance to infection. However, in the lab, single parameters, such as diet, sex, genetics, microbiome composition and age are all known to impact the outcome of infection. How these contribute to the quality of the parasite-specific immune response in a natural, multivariate environment, and how this affects host health, is unknown.

To bridge between laboratory findings and a real-world context, we are studying the adaptive immune response to *Trichuris muris* in a wild, free-living island population of house mice naturally infected with whipworm. Using immunological and ecological data collected from 200 mice on the Isle of May, UK, sampled across 2018-2019 we have previously shown that wild mice harbouring chronic, low-level infections produced lower levels of cytokines in response to *Trichuris* antigen than laboratory-housed C57BL/6 mice and that the local *Trichuris* -specific Th1/Th2 balance is positively associated with worm burden in older wild mice.

Building on these data sets, we are currently experimentally testing whether diet supplementation directly, or indirectly, impacts helminth infection through changes in the immune response and/or in the gut microbiota. To harness the potential of diet supplementation as a possible solution to reduce infection burdens and their associated pathology, it is crucial that we disentangle these complex diet-immune response-gut microbiota interactions. Thus, we are combining dietary and immunological interventional experiments in wild mouse populations to determine what drives diet-mediated improvements in helminth resistance and reductions in host harm. I will describe some of our preliminary parasitological and immunological results following our first field trip for this project.

(15) Subcellular structure - (Lecture theatre 3)

Sponsored by ZEISS/Appleton Woods

Chair: Jack Sunter

11:00 (25 mins)

Resolving the spatial proteome of *Plasmodium falciparum* asexual stages and their interaction with the erythrocyte

Dr Ross Waller, *University of Cambridge*

R Waller[1];
[1] University of Cambridge, UK

Despite *Plasmodium* sharing much common basic biology with other apicomplexans, only half of its genes are shared with the other intracellular parasites of this group. Even amongst *Plasmodium* species there is considerable gene novelty, gene family expansion and contraction, and within species there is further ongoing selection for gene sequence change. To understand the cell and evolutionary biology of

these organisms, including which parts of the cell and/or cell processes are the sites of greatest adaptation or innovation, we need to know the cellular context of the parasite's gene products. LOPIT spatial proteomics enables the steady state locations of thousands of cell proteins to be determined simultaneously and, thus, blueprints of the spatial organisation of cells' proteomes to be determined. We have applied this proteomic method to three asexual stages of *P. falciparum*-infected erythrocytes and provide resolution of the parasite's intracellular organelles, the exported parasite proteins within the host cell, and also the host's proteins and how they are reorganised upon infection.

11:25 (15 mins)

A novel approach to understanding parasite nutrient uptake and metabolism at a subcellular scale using Nanoscale Secondary Ion Mass Spectrometry

Macaulay Turner, *University of Manchester*

M Turner[1]; KL Moore[1]; KJ Else[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 (DALYs). The parasite inhabits the caecum and proximal colon of infected individuals with its anterior end embedded within a unique intracellular epithelial cell niche. Currently we do not know what the parasite feeds on, or the main route of nutrient uptake. Although the parasite has a mouth it lacks a muscular pharynx, arguably making feeding through the mouth unlikely. *Trichuris* spp have a structure termed the bacillary band, which occupies one third of the circumference of its anterior end. The function of the bacillary band is enigmatic, with it ascribed both secretory and absorptive in function. Previous work has shown that fluorescently labelled glucose is taken up by the worm at the bacillary band pores and localises in the central stichocyte cells. Whilst the use of fluorescent tags is an incredibly powerful tool, the addition of a fluorophore can drastically alter a molecule's kinetics and may change uptake mechanisms as well as how it is metabolised within the parasite. Thus, alternative methodologies to localise compounds at a subcellular level are required.

NanoSIMS is a high-resolution secondary ion mass spectrometry instrument (beam size can be focused to 50 nm) 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. NanoSIMS can be used in tandem with stable isotope probing, with this method an organism is exposed to a compound labelled with a stable isotope The NanoSIMS instrument can then detect and localise isotopic enrichment in the sample at the subcellular scale to infer mechanism of uptake, and utilisation of the compound. The use of stable isotope labelling has the advantage that it does not alter the size or structure of the compound, therefore will mirror the uptake mechanisms, and will be metabolised the same as an unlabelled compound.

We have used stable isotope probing with the NanoSIMS to reveal how *T. muris* utilises glucose after uptake. Worms were exposed to 13C labelled glucose for different times and the anterior end of the worms were imaged with NanoSIMS at a high lateral resolution (~90 nm). We have shown that glucose, or glucose metabolites, localise to the stichocyte granules, stichocyte membrane, muscle and in small (150 nm) circular structures within the bacillary band cells. Additionally, we have shown the amino acid alanine localises to the stichocyte granules.

Our data thus (a) reveals a possible novel function of the stichocyte granules as a nutrient store and (b) showcases the potential of NanoSIMS in understanding the biology of large multicellular parasites.

NanoSIMS and stable isotope probing therefore offer a new approach to understanding nutrient uptake and metabolism in the field of parasitology at the subcellular scale.

11:40 (10 mins)

Advanced imaging methods for investigating parasite structure and function

Dr Matt Haley, *Carl Zeiss Ltd*

ZEISS advanced microscope solutions enable users to observe and analyse dynamic processes in living cells. The talk will highlight the latest technological advancements in microscopy, including advanced optical techniques, automation, and software integration. Attendees will learn how ZEISS microscopes can help them to obtain high-resolution multidimensional data of living cells, track cell behaviour over time, and perform quantitative analysis of biological processes. Overall, the talk aims to showcase the capabilities of ZEISS solutions and how they can help researchers to gain new insights into the structure and function of parasites.

11:50 (15 mins)

Characterization of novel and essential kinetoplast components in *Trypanosoma brucei*

Dr Michael Hammond, *Institute of Parasitology Czech Academy of Science*

M Svobodova[1]; LR Cadena[1]; L Chmelova[2]; C Benz[1]; V Yurchenko[1]; V Raskova[1]; J Lukes[1]; M Hammond[1]; I Durante[1];

[1] Institute of Parasitology, Biology Centre, ASCR, Czechia; [2] Life Science Research Centre, Faculty of Science, University of Ostrava, Czechia

The kinetoplast represents the defining feature of kinetoplastid protists. As a singular concentration of mitochondrial DNA, the successful replication and segregation of its intercatenated DNA maxi- and minicircles represent critical processes to cell viability. From the MitoTAG study of *Trypanosoma brucei*, we identified a selection of potential kinetoplast components, for which we use alternative epitope labelling to ultimately validate eight proteins as novel kinetoplast components. RNAi knockdown studies demonstrate the essential nature for several of these proteins, revealing aberrant growth and cell cycle phenotypes, accompanied by reductions in maxi- and minicircle abundance. Furthermore, we note an intriguing mitochondrial DNA accumulation phenotype demonstrated by one particular cell line knockdown, which additionally shows exceptional conservation throughout the trypanosomatid clade. This study represents the single largest discovery of proteins associated with this sub-cellular structure long considered to be an attractive drug target for this group of parasitic protists.

12:05 (25 mins)

Deciphering the conoid of *Toxoplasma gondii*: Insights into ultrastructural components and their functions

Prof Dominique Soldati, *University of Geneva*

AT Puig[1]; R Haase1[1]; N Dos Santos Pacheco[1]; B Ren[1]; B Maco[1]; A Guérin[1]; M Martinez[2]; YW Chang[2]; D Soldati-Favre[1];

[1] Department of Microbiology and Molecular Medicine, Faculty of Medicine, University of Geneva, Switzerland; [2] Department of Biochemistry and Biophysics, Perelman School of Medicine, University of Pennsylvania, United States

Members of the phylum of Apicomplexa are unified by an apical complex consisting of cytoskeletal structures and secretory organelles, tailored for motility, invasion and egress. Gliding is powered by actomyosin-dependent rearward translocation of apically secreted transmembrane adhesins. In the coccidian subgroup of Apicomplexa, the conoid is composed of a cone of spiralling tubulin fibers, preconoidal rings and two intraconoidal microtubules. The conoid is a dynamic organelle that extrudes in motile parasite. Ultrastructure-expansion microscopy applied to known and novel conoid proteins has uncovered the preconoidal rings as hubs for actin polymerization and led to a plausible role of conoid dynamics as gatekeeper for the engagement of F-actin in the glideosome. Rhoptry discharge is vital for invasion and involves docking one or two rhoptries to a macromolecular secretory apparatus within an apical vesicle (AV). *T. gondii* is armed with 10-12 rhoptries and 5-6 microtubule-associated vesicles (MVs) presumably facilitating iterative rhoptry discharge. Cryo-electron tomography combined with functional analysis of intraconoidal microtubule (ICMT)-associated proteins highlights the pivotal role of ICMTs in scaffolding the discharge of multiple rhoptries.

(23) Complex ecology data analysis - (Teaching room 4)

Chair: Andy Fenton

11:00 (25 mins)

Modelling helminth transmission at the wildlife-livestock interface: a difficult relationship with data?

Prof Eric Morgan, *Queen's University Belfast*

E Morgan[1];
[1] Biological Sciences, Queen's University of Belfast, UK

Parasite transmission in multi-host systems involves many moving parts, and to gather comprehensive empirical understanding of its dynamics often faces insurmountable logistical challenges. Yet, guidance is needed on consequences for conservation and agriculture, especially under climate and landscape change. Computer models can help to fill this gap, but themselves require data inputs for parameterisation, calibration and validation. Given that generalist helminth species are the most relevant at the interface, it has been expedient to borrow heavily on knowledge from livestock systems to parameterise these models and to validate them, although this can generate bias and ignore key uncertainties. These issues are explored frankly through a series of case studies that set out to identify key points of nematode cross-transmission in complex ungulate systems, in which host movement - including vertical movements of mountain ungulates and horizontal antelope migrations - and variable weather generate highly discontinuous dynamics. New technologies are improving ability to collect more precise data *in situ* but there is still an ongoing need to determine response norms of key parasite vital rates in natural settings and populations, and to consider indirect as well as direct impacts of climate change on parasite ecology. Ultimately, predictive models are perhaps most useful in this context to generate testable hypotheses and should therefore be constructed with realistic data sources in mind. The skills and resources needed to combine models and data effectively and iteratively towards better predictions and applications will benefit from multi-disciplinary collaboration and an absence of hubris.

11:25 (20 mins)

Quantifying complex outcomes of disease control interventions

Dr Mafalda Viana, *University of Glasgow*

M Viana[1];
[1] University of Glasgow , UK

Control interventions via lethal removal of animals that transmit pathogens between hosts is a widely implemented strategy against many human and animal diseases. These interventions can succeed, fail or have unforeseen consequences, which means long-term control relies on understanding the underling mechanisms leading to the observed response of population reduction. Here, I explore how pairing routinely collected data with ecological or epidemiological models can reveal the ecological pathways to intervention outcomes in two systems. First, for malaria control, the bednet Olyset-DUO trialled in Burkina Faso was hypothesized to reduce mosquito populations through two different routes (reduction of adult survival and fecundity) but how it translated to wild populations was undetermined. Our results confirmed the expected demographic impacts but showed these were transient, leading loss of effectiveness. Next, I explored how culling vampire bats against rabies in Peru caused unintended reductions in another potentially zoonotic virus, H18 influenza. Together, these studies highlight that integrating different types of evidence under the same approach can be essential to dissect the impacts of interventions and that this mechanistic understanding can be harnessed towards evidence-based intervention strategies.

11:45 (15 mins)

Current *Schistosoma mansoni* exposure and infection have distinct determinants: a data-driven population-based study in Uganda

Mr Fabian Reitzug, *Big Data Institute, University of Oxford*

F Reitzug[2]; B Nabatte[1]; A Byaruhanga[1]; F Besigye[1]; NB Kabatereine[1]; G Chami[2];
[1] Vector Control Division, Ministry of Health, Uganda; [2] University of Oxford, Big Data Institute, UK

Background: Exposure to parasitic flatworms causing schistosomiasis depends on complex human-environment interactions. However, as exposure has been predominantly studied as a predictor of current infection, it is currently unclear whether determinants of exposure and infection fundamentally differ.

Methods: We conducted a comprehensive characterisation of exposure (water contact) within the SchistoTrack Cohort for 2,867 individuals aged 5-90 years in 38 fishing villages in Eastern and Western Uganda and collected detailed biomedical, behavioural, socio-demographic, and environmental data. We used generalised additive models (GAMs) to characterise age-dependent trends in having any water contact, water contact frequency, and duration as well as gender differences in water contact over age. We also used GAMs to describe how water contact was influenced by household distance to water bodies. To select variables for our main regression models predicting water contact and *Schistosoma mansoni* infection status, we used Bayesian variable selection (BVS) on a candidate set of 30 variables. All variables with marginal inclusion probabilities ≥ 0.5, corresponding to the marginal probability model, were selected for inclusion. Multivariable logistic regressions with standard errors clustered at the household level were used to account for our sampling design. We evaluated model performance based on the area under the receiver operating curve (auROC) obtained from 10-fold cross-validation.

Results: For every 1km increase in household distance to freshwater bodies, water contact decreased by an absolute 24%. Water contact peaked at age 30; whereas infection peaked at age 15—the same year that gender differences in water contact emerged. Among adults (age 18+), males engaged primarily in occupational water contact and females in domestic water contact, which accounted for 82% and 75% of their total water contact duration, respectively. In multivariable regression models, age, gender, occupation, site contamination, drinking water source, and village-level infection prevalence predicted water contact. Predictors of *S. mansoni* infection status were age, level of education, occupation, type of water site, number of water sites per village, and village-level infection prevalence. Among 12 selected predictor variables of water contact, only five (42%) were also selected as predictors

of current infection status. The predictive performance of the water contact model was significantly higher than the performance of the model predicting infection status (auROC of 0.783 versus 0.695, respectively, $p<0.01$).

Discussion: Trends in age-dependent current exposure did not correspond to age-dependent infection prevalence. Thus, assumptions in mathematical transmission models which stipulate a direct exposure-infection correspondence may not be warranted.

12:00 (15 mins)

Understanding the influence of environmental factors on disease dynamics: Insights from Desert Bighorn Sheep Populations

Dr Diana Meza, *University of Warwick*

D Meza[2]; S Hawkes[2]; AB Pedersen[1]; BR Beechler[3]; AE Jolles[3]; CW Epps[3]; S Carpenter[3]; CM Aiello[3]; EE Gorsich[2];
[1] University of Edinburgh, UK; [2] University of Warwick, UK; [3] Oregon State University, UK

Heterogeneity in host contact patterns, driven by individual and group traits, affects pathogen invasion and persistence. We study how environmental conditions impact disease dynamics in Desert Bighorn Sheep populations. Analysing data from eight populations in the Mojave Desert, we explore the role of environmental variation in contact ecology, individual traits versus population-level conditions, and consequences for disease outbreak size and persistence. By upscaling contact networks based on different temporal scales reflecting three pathogen infectious periods, we find significant effects of rainfall, temperature, and sex-reproductive season interactions on social contacts. Our research quantifies the impact of the interplay between environmental and demographic factors on disease transmission dynamics, offering insights into wildlife ecology and disease management.

12:15 (15 mins)

Quantifying demographic contributions to helminth transmission dynamics in wild sheep

Dr Andrew Dean, *University of Liverpool*

A Dean[1]; A Fenton[1];
[1] Department of Evolution, Ecology and Behaviour, Institute of Infection, Veterinary, and Ecological Sciences, University of Liverpool, UK

The Soay sheep on the Scottish island of Hirta are an unmanaged population that has been extensively studied for a number of decades, and thus provide an ideal system in which to study natural host-parasite dynamics in a large herbivore species. Identifying the key demographics of lambs, reproductive and non-reproductive females, and males, in which we are likely to see between-group variation due to differing seasonal pressures (e.g. giving birth, rutting, etc.), we developed a demographic model of helminth transmission. We then used multi-chain Monte Carlo simulation to fit this model to several years of annual host population counts and seasonal parasite counts from faecal sampling. Thus, we were able to estimate variation in parasite transmission rates between the different demographic groups and across different years, thereby providing insight into the key drivers of infection in the Soay sheep population. More generally, we show that such combined data and modelling approaches are a flexible and effective means by which to understand host contributions to parasite transmission at the population scale.

(16) Control & elimination open session - (Lecture theatre 1)

Chair: Helen Price

14:00 (10 mins)

Does the removal of macroparasites have an effect on the microparasite community in Bighorn sheep?

Alex Morris, *Cardiff University*

A Morris[4]; BR Beechler[5]; A Jolles[5]; EE Gorsich[3]; AB Pedersen[1]; J Lello[2];
[1] University of Edinburgh, UK; [2] Cardiff School of Biosciences, Cardiff University, UK; [3] University of Warwick, UK; [4] Cardiff University, UK; [5] Oregon State University., United States

Concurrent infections with multiple parasite species is common in both domestic and wild animals. Coinfecting parasites can interact in multiple ways, and how parasites interact within the host can affect parasite transmission, disease severity and alter disease control strategies. It is therefore important to understand how parasites interact within the host as this can help us predict how the removal of one target parasite effects the remaining parasite communities and what effect this may have on host health. We performed a parasite perturbation experiment in a population of Bighorn sheep (*Ovis canadensis*) in Southeast Oregon, by using a long lasting anthelmintic to supress nematodes and monitoring the effect this had on non-target parasite species. We found an increase in both the abundance and prevalence of coccidia in response to deworming. We also found sex differences, as males had higher oocyst counts than females following treatment. Our results demonstrate an experimental interaction between helminths and coinfecting parasites in a wild ungulate and show the importance of understanding the wider consequences of drug treatments.

14:10 (10 mins)

How does the composition of mixed wildlife-livestock communities impact ungulate parasite burden and diversity in Botswana?

Isabella Endacott, *University of Liverpool*

I Endacott[2]; A Fenton[2]; J Bro-Jorgensen[2]; N Babayani[4]; J Graham-Brown[2]; K Evans[3]; J Gilleard[1]; E Redman[1]; H Vineer[2];
[1] University of Calgary, United States; [2] Institute of Infection, Veterinary and Ecological Sciences, University of Liverpool, UK; [3] Elephants for Africa, Botswana; [4] Okavango Research Institute, University of Botswana, Botswana

For centuries large populations of livestock and wildlife have coexisted across the African savannah rangelands, with unrestricted seasonal movement up until the latter half of the 20th century. However, in recent decades harmonious coexistence has become increasingly unstable, in part due to concern for the spread of direct and indirect infectious diseases between livestock and wildlife. Wild ungulates are implicated in the spread of prevalent livestock diseases, but transmission is also bi-directional with diseases likewise capable of spreading from livestock into vulnerable wildlife populations. It is now evermore important to study the burden, distribution and movement of parasites from the perspective of multi-host parasite systems, rather than solely one-host (and one-parasite) systems.

To determine the impact of host ecological communities on parasite communities, we characterised gastrointestinal nematode diversity and abundance in three ecosystems in Botswana with differing levels of livestock-wildlife interaction. We hypothesised that parasite prevalence and diversity varies locally and regionally in Botswana due to host community dynamics, with specific host species contributing disproportionately to onwards transmission.

Study sites were characterised by villages bordering national parks with varying levels of boundary permeability, which in turn were further subset according to the level of wildlife occupation, i.e. wildlife-only, livestock-only, and sympatric. Faecal samples from 16 wildlife and livestock species were collected from the ground with a total of 1500 individual faecal egg counts processed. Counts of strongyle eggs ranged widely between species from low mean counts of 31 eggs per gram (epg) in kudu, and 36epg (giraffe) to high mean counts of 695epg (donkey), 586epg (elephant), and 1339epg (zebra). Samples were collected from several infrequently encountered antelope species, potentially representing the first description of gastrointestinal parasites in these host species in Botswana. Spatial analyses of ungulate observations were used to quantify the degree of shared land use by livestock and wildlife at each site. Generalised linear mixed models incorporating these analyses were used to relate the parasitic gastrointestinal nematode (GIN) burden to host and site characteristics. Parasitic L3 larvae samples have been submitted for ITS-2 sequencing, enabling GIN species presence and abundance. We will use these findings to evaluate how host community structure impacts GIN diversity and prevalence using multi host network modelling. The overarching aims of this project are to better understand the specific roles of different ungulate species in parasite transmission, to inform on parasite control strategies and support human-wildlife conflict mitigation actions.

14:20 (10 mins)

Mental health and skin Neglected Tropical Diseases (NTDs) - A participatory mixed method evaluation of integrated mental health and NTDs in Liberia

, *Liverpool School of Tropical Medicine*

C Barrett[1]; E Rogers[3]; S Chowdhury[1]; I Hotopf[1]; H Berrian[3]; W Tate[4]; J Kollie[5]; C Parker[3]; G Zawolo[4]; J Smith Jr[4]; K Kollie[3]; Z Zaizay[4]; R McCollum[1]; L Sempe[2]; S Theobald[1]; L Dean[1];
[1] Liverpool School of Tropical Medicine, UK; [2] Global Health and Development Division, Queen Margaret University, Edinburgh., UK; [3] Neglected Tropical Disease Programme, Ministry of Health, Monrovia, Liberia; [4] Actions Transforming Lives, Monrovia, Liberia; [5] Pacific Institute for Research and Evaluation, University of Liberia, Monrovia, Liberia

Background: Skin Neglected Tropical Diseases (NTDs) cause preventable morbidity, disfigurement and disability due to inability to access timely appropriate care, often related to under-resourced health systems. Persons affected are predisposed to poor mental wellbeing, physical health and stigmatisation; this is often influenced by social dimensions and norms, including gender, poverty and misconceptions about the cause of conditions. REDRESS used participatory mixed-methods approaches to evaluate the integration of mental health support within case management for persons affected by skin NTDs in Liberia, led by the Ministry of Health and co-developed by healthcare staff, informal health providers and persons affected. This intervention included training health workers in mhGAP, training health workers at facility and community levels, informal providers and patient advocates in 'look listen link' to support identification and referral of persons needing mental health support, and establishment of peer support groups for persons affected to share experiences and provide mutual support.
Methods: Mixed-methods were applied to evaluate the intervention processes and outcomes, utilising quantitative and participatory creative methods. Baseline surveys were conducted between October 2022 and January 2023 with a 3-month follow-up survey. Outcome variables, depression and anxiety were assessed using a Patient Health Questionnaires (PHQ-9) and Generalised Anxiety Disorder Assessment (GAD-7), respectively. Linear regression models were conducted to assess individual changes in outcome variables, reporting odds ratios (OR) and p-values. Additionally, photovoice (narratives through photography) and body-mapping (illustrations) documented the impact on mental wellbeing and stigma amongst persons affected pre and post intervention.

Results: Persons affected by skin NTDs illustrated experiences of pervasive stigma and discrimination and the psychosocial impact of this through photovoice and body mapping at baseline, describing feelings of sadness, isolation and in some cases, suicidal ideation. Of 150 baseline surveys, 38.7% (95% Confidence Intervals (CI) 30.9 to 47.0) reported moderate depressive symptoms or above (PHQ-9≥10). For anxiety, 22.7% (95% Confidence Intervals (CI) 16.2 to 30.2) reported moderate or severe symptoms of anxiety (GAD-7≥10). Post-intervention, persons affected expressed improvements in self-confidence, increased community participation and improved mental wellbeing through joining peer support groups; many also described experiencing less stigma within their communities. This is also reflected in the quantitative data through significant reductions in individual depression and anxiety scores compared with three-month follow-up (OR = 4.2, 2.4, p-value = 0.028, 0.048)
Conclusion: To achieve Universal Health Coverage targets, active case finding, and holistic person-centred care is crucial to addressing the physical and psychosocial impacts of skin-NTDs. Delivering mental health and NTD services together through the primary health care system and in collaboration with communities presents a critical opportunity for improved availability, acceptability, and adherence to care, whilst supporting health systems strengthening and promoting equity in healthcare access.

14:30 (10 mins)

Molecular detection of host blood meal and pathogen diversity in bat-associated ticks in Europe

Dr Tamara Szentivanyi, *Pathogen and Microbiome Institute, Northern Arizona University,*

T Szentivanyi[5]; N Takács[1]; AD Sándor[2]; A Péter[1]; SA Boldogh[3]; D Kováts[4]; JT Foster[5]; P Estók[6]; S Hornok[1];
[1] University of Veterinary Medicine, Budapest, Hungary; [2] University of Agricultural Sciences and Veterinary Medicine, Romania; [3] Aggtelek National Park Directorate, Hungary; [4] Hungarian Biodiversity Research Society, Hungary; [5] Pathogen and Microbiome Institute, Northern Arizona University, United States; [6] Eszterházy Károly Catholic University, Hungary

Potentially zoonotic pathogens have been previously detected in bat-associated ticks. Their role in disease transmission, as well as their frequency of feeding on non-bat hosts, is poorly known. We used molecular blood meal analysis to reveal feeding patterns of bat tick species, including *Ixodes ariadnae* (n = 11), *I. simplex* (n = 9), and *I. vespertilionis* (n = 141) collected in Hungary and Romania. About 78% of the samples showed the presence of vertebrate DNA, predominantly revealing bats. We also detected non-bat hosts in these ticks, such as domestic dogs, *Canis lupus familiaris,* wild boar, *Sus scrofa*, and a horse, *Equus* sp. We found the presence of *Neoehrlichia mikurensis* in bat ticks for the first time. Overall, bat-associated ticks may exhibit a broader host range than previously thought. Their role as disease vectors should be re-evaluated in more complex host systems, as they may contribute to pathogen transmission beyond just bat hosts.

14:40 (10 mins)

Reproducibility matters: intra- and inter-sample variation of the point-of-care circulating cathodic antigen test in two *Schistosoma mansoni* endemic areas in Uganda

Elías Kabbas Piñango, *University of Glasgow*

E Kabbas-Piñango[3]; M Arinaitwe[4]; GJ van Dam[2]; M Adriko[4]; A Namukuta[4]; A Nankasi[4]; NK Mwima[4]; F Besigye[4]; JM Prada[1]; P Lamberton[3];
[1] University of Surrey, UK; [2] Leiden University Medical Center, UK; [3] School of Biodiversity, One Health

and Veterinary Medicine; College of Medical, Veterinary and Life Sciences, University of Glasgow, UK; [4] Vector Borne and Neglected Tropical Diseases Control Division, Ministry of Health, Uganda

Over 240 million people are infected with schistosomiasis. Detecting *Schistosoma mansoni* eggs in stool using Kato–Katz thick smears (Kato-Katzs) is highly specific but lacks sensitivity. The urine-based point-of-care circulating cathodic antigen test (POC-CCA) has higher sensitivity, but issues include specificity, discrepancy between batches and interpretation of trace results. A semi-quantitative G-score and latent class analyses making no assumptions about trace readings have helped address some of these issues. However, intra-sample and inter-sample variation remains unknown for POC-CCAs. We collected 3 days of stool and urine from 349 and 621 participants, from high- and moderate-endemicity areas, respectively. We performed duplicate Kato-Katzs and one POC-CCA per sample. In the high-endemicity community, we also performed three POC-CCA technical replicates on one urine sample per participant. Latent class analysis was performed to estimate the relative contribution of intra- (test technical reproducibility) and inter-sample (day-to-day) variation on sensitivity and specificity. Within-sample variation for Kato-Katzs was higher than between-sample, with the opposite true for POC-CCAs. A single POC-CCA per person with a G3 threshold most accurately assesses individual infections and provides a good prevalence estimate. However, to reach the WHO target product profile requirement of 95% specificity for monitoring and evaluation, at least 2 days of urine sampling, 2 POC-CCAs per person, and the less sensitive threshold of G4 are needed.

14:50 (10 mins)

Development of a whipworm vaccine using virus-like particles

Jacob Thompson, *University of Manchester*

J Thompson[1];
[1] *University of Manchester, UK*

Trichuriasis results from infection with the intestinal dwelling parasitic nematode *T. trichiura*, colloquially known as the human whipworm. The disease affects ~465 million people worldwide and causes colitis, mucoid diarrhoea, rectal prolapse, rectal bleeding, abdominal pain/tenesmus, and iron deficiency anaemia, which accumulates in ~232,000 DALYs (Disability adjusted life years) annually. Presently, Trichuriasis is treated via anthelmintic drugs as part of mass drug administration (MDA) campaigns. However, these drugs lack efficacy against *T. trichiura*. This has led to a growing interest in an anti-trichuris vaccine, which would provide long lasting immunity, staving off the potential development of drug resistance and breaking the cycle of reinfection commonly found in endemic communities. Here we describe the development of novel vaccine candidates consisting of two MHC-II T cell epitopes identified *in silico* derived from a *Trichuris* chitin-binding domain-containing protein (CBD) and chymotrypsin-like serine protease (CLSP). These epitopes were genetically fused to the Hepatitis B core antigen (HBcAg), a virus-like particle (VLP) that is well-documented as a vaccine carrier due to its ability to confer high levels of immunogenicity to foreign antigens. We also incorporated the universally immunogenic P2 Tetanus epitope into the vaccine candidates to further boost immunogenicity. Vaccine candidates were shown to induce the production of anti-Trichurid epitope antibodies in C57BL/6 mice. Furthermore, the inclusion of the Tetanus epitope was shown to boost the generation of parasite epitope-specific antibodies in C57BL/6 mice previously primed with the Tetanus toxoid. Despite these correlates of protection, the vaccine candidates did not induce a reduction in worm burden in C57BL/6 mice, within the first 14 days of an acute (150 eggs) *T. muris* challenge infection. Therefore, our modifications to the vaccine candidates increased the parasite-specific IgG antibody response elicited against it in certain contexts, however, this failed to translate into protection against the parasite.
Funded by a BBSRC DTP studentship.

15:00 (10 mins)

A vaccine dose and a worm's host: Malaria vaccination in a schistosome-endemic region of Malawi

Sarah Rollason, *Sarah Rollason*

S Rollason[2]; JR Stothard[1]; J Archer[1]; S Jones[1]; A Juhàsz[1]; J Musaya[3]; P Makaula[3]; SA Kayuni[3]; P Chammudzi[3]; D Kapira[3]; EJ LaCourse[1]; D Lally[3]; B Mainga[3]; G Namacha[3]; J Lello[2];
[1] Liverpool School of Tropical Medicine, UK; [2] Cardiff University, UK; [3] Malawi-Liverpool-Wellcome Trust Clinical Research Programme, Malawi

Malaria is of critical public health importance in sub-Sharan Africa, with a disproportionate burden of malaria infection and mortality in pre-school aged children. A landmark vaccine against malaria caused by *Plasmodium falciparum,* the most pathogenic species of malaria, has recently been developed. We present data from a cohort of pre-school aged children in Mangochi District, Malawi included as part of the vaccination pilot study. Prior work by our group has demonstrated that *Schistosoma mansoni and Schistosoma haematobium* are co-endemic in this age group. Schistosomes have the potential to impact vaccine efficacy due to the changes within the host's immune system during infection. Additionally, the changes in the immune response to the vaccine, could impact schistosome infection. Here we explore two key questions: 1) Is malaria vaccination efficacy affected by schistosome coinfection? and does schistosome infection intensity differ in children pre and post malaria vaccination? The fight to reduce malaria deaths has stalled in recent years, and the vaccine promises a new tool in the fight to save lives. We provide an early report on how useful this tool may be in areas where schistosomes are the norm.

15:10 (10 mins)

Comparative analysis of the immune responses elicited by native versus recombinant *Fasciola hepatica* vaccines

Dr Krystyna Cwiklinski, *University of Liverpool*

R Lalor[3]; A McEvoy[2]; J López Corrales[3]; S Ellis[2]; C De Marco Verissimo[3]; O Keane[2]; JP Dalton[3]; K Cwiklinski[1];
[1] University of Liverpool, UK; [2] Teagasc, Ireland; [3] University of Galway, Ireland

The ongoing effort to develop a liver fluke vaccine has been greatly enhanced by the major advances in our knowledge of liver fluke biology, facilitated by the availability of large sequencing datasets and biochemical analyses. Based on this data, our vaccine candidate selection strategy has focussed on disrupting key biological processes by combining groups of antigens with similar/complementary functional actions into a single vaccine cocktail. In this study, we used size exclusion chromatography to fractionate adult worm excretory-secretory protein products to further interrogate the molecules secreted by *Fasciola hepatica*. The protein composition of each fraction was ascertained by proteomic analyses. The most abundant proteins present in each fraction were selected for recombinant protein expression. Two sheep vaccine trials were carried out over two consecutive years to evaluate the vaccine efficacy of four native fractions versus recombinant protein cocktails that recreated the native protein fraction composition, each formulated in the adjuvant Montanide 61 VG (Seppic). Distinct antibody and cell mediated responses were elicited in both trials by the native fraction vaccines, which were comparable to their recombinant counterparts. This implies that post-translational modifications of the proteins examined were not relevant in the protective immune responses elicited. While no significant reduction

in liver fluke burden was observed for any of the vaccine cocktails, the animals vaccinated with the native fraction composed of glycolytic enzymes displayed a significantly greater weight gain relative to the other vaccine groups and the non-vaccinated control group. The impact on weight loss is consistent with previous sheep vaccine trials performed in our laboratory and highlights the positive impact vaccination can have on animal welfare without statistically reducing parasite burdens. These studies also highlight the need for studies to be repeated in different larger cohorts of animals to obtain significantly robust measures of vaccine efficacy and reproducibility.

(17) Parasite-microbiome interactions - (Lecture theatre 2)
Sponsored by Royal Society Publishing, MDPI and
MicroMolecular Systems

Chair: Laura Peachy

14:00 (25 mins)

Structural and functional analyses of antimicrobial peptides in worm excretory/secretory products

Prof Cinzia Cantacessi, *University of Cambridge*

J Rooney[2]; E Rivera-de-Torre[3]; R Li[4]; K Mclean[1]; DR Price[1]; AJ Nisbet[1]; A Hofmann[6]; S Bakshi[4]; A Zarkan[5]; C Cantacessi[2];
[1] Moredun Research Institute, UK; [2] Department of Veterinary Medicine, University of Cambridge, UK; [3] Department of Biotechnology and Biomedicine, Technical University of Denmark, Denmark; [4] Department of Engineering, University of Cambridge,, UK; [5] Department of Genetics, University of Cambridge, UK; [6] Max Rubner-Institut, Federal Research Institute of Nutrition and Food, Germany

The complex relationships between gastrointestinal (GI) nematodes and the host gut microbiota have been implicated in key aspects of helminth disease and infection outcomes. Nevertheless, the direct and indirect mechanisms governing these interactions are, thus far, largely unknown. This presentation will describe the results of our recent experiments demonstrating that the excretory-secretory products (ESPs) and extracellular vesicles (EVs) of key GI nematodes contain peptides that, when recombinantly expressed, exert antimicrobial activity *in vitro* against *Bacillus subtilis*. In particular, using time-lapse microfluidics microscopy, we show that exposure of *B. subtilis* to a recombinant saposin-domain containing peptide from the 'brown stomach worm', *Teladorsagia circumcincta* , and a metridin-like ShK toxin from the 'barber's pole worm', *Haemonchus contortus,* results in substantial membrane damage, membrane blebbing , and cell lysis. Data from our study support the hypothesis that GI nematodes may modulate the composition of the vertebrate gut microbiota directly via the secretion of antimicrobial peptides, and pave the way for future investigations aimed at deciphering the impact of such changes on the pathophysiology of GI helminth infection and disease. However, it is highly likely that worm-microbiota crosstalk occurs through a complex network of direct and indirect mechanisms that act synergistically to enhance parasite survival in a hostile environment. Unravelling the complexities of helminth-host microbiome relationships may lead to a better understanding of helminth biology and to the discovery and development of novel and sustainable parasite control strategies.

14:25 (20 mins)

Not only *Orientia* and scrub typhus? New frontiers in microbiome research for chiggers, the world's tiniest vectors

Prof Ben Makepeace, *University of Liverpool*

B Makepeace[1];
[1] *University of Liverpool, UK*

Trombiculid mites are highly diverse, globally distributed arachnids with parasitic larval stages ("chiggers") that typically are only 0.25 mm in length. Chiggers feed primarily on terrestrial vertebrates, whereas subsequent stages in the trombiculid lifecycle are free-living predators in soil ecosystems. The main impact of chiggers is twofold: they cause irritation and potentially severe allergic reactions to their bites in humans and other animals (i.e., trombiculiasis); and a small number of species are vectors of a neglected zoonosis, scrub typhus, which is caused by *Orientia spp.* bacteria. Scrub typhus has a median mortality rate of 6% if untreated and is very challenging to diagnose, with major clinical impact across the Indian subcontinent, China, Southeast Asia, the Korean Peninsula, and Oceania. Endemic disease has also emerged in recent decades in the Middle East and South America, and is suspected to circulate in Africa. Here, I explain how research on chiggers has lagged behind that of ticks but recent data from chigger microbiome studies suggest overlapping features with their larger, distant ixodid cousins, as well as distinct characteristics that may reflect different modes of parasitism. Chiggers are frequently infected with what are considered to be tick or flea-borne pathogens, as well as with viruses that are believed to be transmitted directly without the involvement of a vector. However, overlooked data from China challenge these assumptions, and evidence for vertical transmission of multiple microorganisms (not only *Orientia*) has been obtained. Recent metagenomic studies on chiggers from the Arabian Peninsula have uncovered a unique, divergent clade of the *Wolbachia* symbiont and a rodent-associated spirochaete that has no known vector. Overall, the surprising complexity of the chigger microbiome suggests that the ecological impact of these vectors on wildlife hosts and in pathogen transmission has been underestimated, with implications for our understanding of zoonoses beyond scrub typhus.

14:45 (20 mins)

Trichomonas – Bacteria interactions: A Laterally Acquired Molecular Toolkit to Target the Microbiota and Potentially Enable Zoonotic Events

Adam Hart, *Newcastle University*

A Hart[1]; J Biboy[1]; J Grey[1]; W Vollmer[1]; RP Hirt[1];
[1] *Newcastle University, UK*

Species members of the *Trichomonas* genus are responsible for disease in several animal species including Humans. Most notably *Trichomonas vaginalis*, the most common non-viral STI for humans, causes trichomoniasis, which is strongly associated with increased susceptibility to other important pathogens such as HIV and HPV.

Trichomonas species live at various mucosal surfaces characterised by complex microbiota amongst a wide range of animal hosts. *Trichomonas* infections are associated with significant changes in the microbiota's taxonomic composition commonly referred to as dysbiosis when associated with diseases. Notably, infections with *Trichomonas vaginalis* and *Trichomonas gallinae* (a bird parasite) result in the depletion of the mutualist bacteria *Lactobacillus* spp., known to play numerous important roles in female urogenital defence against pathogens. However, the molecular and cellular basis of interactions between *Trichomonas* and members of the microbiota are poorly understood.

We exploited *Trichomonas gallinae* in co-culture with *Escherichia coli* as a model system in combination with comparative genomics and transcriptomics (RNAseq) to identify candidate enzymes and peptides targeting bacteria. A number of genes encoding homologues of bacterial cell wall targeting enzymes, including candidate lysozymes, and anti-microbial peptides, both of which are conserved throughout *Trichomonas* spp., were identified and with a number of them shown to be significantly upregulated within the co-culture system. We also observed changes in bacterial and parasite behaviour and morphology.

Integrating comparative genomics between species, transcriptomics within our model and already known interactions between *Trichomonas* and bacteria; involving for example the peptidoglycan targeting NlpC/P60 endopeptidases; illustrates a number of mechanisms for the parasites to potentially out-compete neighbouring bacteria, with a likely important role of deconstructing bacterial cell wall peptidoglycans. This could be to extract nutrients from the microbiota and/or alter the microbiota to produce a more hospitable host environment for the *Trichomonas*. This could lead to the promotion of *Trichomonas* species growth within an infected individual and through dysbiosis, could also contribute at promoting damaging host tissue inflammations. Furthermore, the conserved ability to target the bacteria from the microbiota could contribute to enabling zoonosis.

Together these findings bring new insights into the molecular and cellular basis of *Trichomonas*-Bacterial interactions and how these evolutionary conserved interactions, gained through several lateral gene transfers from bacteria, can potentially influence the zoonotic ability of *Trichomonas*.

15:05 (25 mins)

Towards the Use of Novel High Density *Anopheles*-Specific *Wolbachia* Strains for Anopheles Vector Control

Dr Grant Hughes, *Liverpool School of Tropical Medicine*

G Hughes[1];
[1] Liverpool School of Tropical Medicine, UK

The use of *Wolbachia* as a novel vector control strategy has been highly successful, demonstrating a significant impact on disease prevalence in field trials targeting *Aedes* mosquitoes. Long thought to be absent from natural populations of *Anopheles* mosquitoes, which are highly effective malaria vectors, *Wolbachia* based interventions have proven challenging. Our recent discovery of natural high density *Wolbachia* strains in populations of *Anopheles moucheti* and *Anopheles demeilloni* has reinvigorated efforts to create transinfections in medically relevant *Anopheles* mosquitoes. Here we present our work demonstrating high density maternally transmitted strains of *Wolbachia* in *An. moucheti* and *An. demeilloni* from sub-Saharan Africa, providing concrete evidence for resident *Wolbachia* strains in this genus. In addition, we report on our recent endeavours work with these novel strains of *Wolbachia* in the lab, thereby providing a tractable source of *Wolbachia* for further experiments. We discuss our findings in the context of developing novel *Wolbachia*-based control approaches in *Anopheles* to reduce the burden of malaria and our recent insights on the molecular mechanisms induced by *Wolbachia* to interfere with pathogens.

(18) Life-cycle interfaces - (Lecture theatre 3)

Chair: Juan Quintana & Mathew Sinton

14:00 (25 mins)

From Receptors to Lipolysis: Tracing *T. brucei's* Route in Host Fat Tissue

Dr Luisa Figueiredo, *Instituto de Medicina Molecular*

L Figueiredo[2]; S Trindade[2]; M De Niz[3]; H Machado[2]; L Lopez-Escobar[2]; R Zechner[4]; T Smith[1]; C Franco[5];
[1] University of St Andrews, UK; [2] Instituto de medicina Molecular, Faculdade de Medicina, Universidade de Lisboa, Portugal; [3] Center for Advanced Microscopy and Nikon Imaging Center, Northwestern University, United States; [4] Institute of Molecular Biosciences, University of Graz, Austria; [5] Catolica Biomedical Research Centre, Lisboa, Portugal

Trypanosoma brucei crosses blood vessels and colonises multiple tissues. Adipose tissue is one of the largest reservoirs in mouse models. In this talk, I will discuss the molecular basis for this preferential colonisation, the consequences for the host, and potential benefits for the parasite. Using tools like intravital microscopy and mouse genetic models, we have identified key host surface receptors required for colonisation and shown that adipocyte lipolysis activation is in part responsible for loss of fat mass during infection. Proteomics and novel methods to quantify parasite proliferation *in vivo* have revealed that parasites adapt to the tissue microenvironment and may enter a persistence stage. These insights enhance our understanding of the evolutionary dynamics of host-parasite interactions and the mechanisms underlying disease relapse.

14:25 (15 mins)

Mechanisms of life cycle simplification in field-derived and laboratory-selected African trypanosomes

Dr Guy Oldrieve, *University of Edinburgh*

G Oldrieve[2]; F Venter[2]; M Cayla[1]; KR Matthews[2];
[1] Department of Biology, University of York, UK; [2] Institute for Immunology and Infection Research, University of Edinburgh., UK

African trypanosomes undergo development to transmissible stumpy forms in their mammalian host to favour uptake by their tsetse fly vector. However, *Trypanosoma brucei evansi* and *Trypanosoma brucei equiperdum* have simplified their lifecycle by escaping dependence on tsetse allowing an expanded geographical range, with direct transmission achieved via biting flies or through sexual transmission between animals. Concomitantly, stumpy formation is lost, and the isolates are described as monomorphic. Through genomic analysis of distinct field isolates we identified and functionally confirmed molecular changes that reduce stumpy formation. Further, by laboratory selection for reduced stumpy formation, we identified reversible steps in the initial development to monomorphism. This identifies a trajectory of events that simplify the trypanosome life cycle with impact on disease spread, geographical range and virulence.

14:40 (15 mins)

Cytoadhesion of *Trypanosoma congolense* to bioengineered 3D bovine microvessels

Dr Sara Silva Pereira, *Universidade Católica Portuguesa*

S Silva Pereira[3]; T Porqueddu[2]; S Sanz Sender[4]; M Bernabeu[4]; L Figueiredo[1];
[1] Instituto de Medicina Molecular Liboa, Portugal; [2] iMM - Instituto de Medicina Molecular João Lobo Antunes, Portugal; [3] Universidade Católica Portuguesa; [4] EMBL Spain

African trypanosomes are extracellular parasites of a plethora of mammals, causing a range of lethal diseases collectively known as African trypanosomiasis. One of the greatest difficulties in trypanosomiasis control is the complexity of the trypanosome interaction with the mammalian

host. *Trypanosoma congolense*, one of the most pathogenic and prevalent African trypanosome species for African livestock, cytoadheres to the vascular endothelial cells, in a process known as sequestration.

Previously, we showed that sequestration in the brain determines acute cerebral disease, but we lacked a robust method to investigate how sequestration is governed. Therefore, we have developed two bioengineered 3D microvessel models composed by either primary bovine brain or aorta microvascular endothelial cells to directly assess how sequestration of two clinically-distinct parasite strains is affected by blood flow properties and endothelial cell activation in two organotypic vascular beds.

This physiologically-relevant platform allows direct assessment of cytoadhesion in a controlled environment, thus being ideal to identify parasite ligands and host receptors of sequestration. This knowledge is important for the successful development of therapeutic strategies that interfere with parasite survival in the mammalian host, thus abrogating disease and/or reducing disease severity.

14:55 (15 mins)

Bottling it all up: Using parasite population biology to identify susceptibility pathways in leishmaniasis

Ciara Loughrey, *University of York*

C Loughrey[1]; J Carnielli[2]; N Brown[1]; H Ashwin[1]; S Dey[1]; P Kaye[1]; J Mottram[1];
[1] University of York, UK; [2] York Biomedical Research Institute, UK

Leishmania donovani causes the systemic multi-organ disease 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.

We have combined CRISPR genome editing, high-throughput sequencing and Sequence Tag-based Analysis of Microbial Population Dynamics (STAMP) to determine dissemination patterns within a mouse model using an isogenic library of 102 *L. donovani* lines, each with a unique barcode. Using STAMP, we have assessed where bottlenecks occur in dissemination by calculating the 'Founder Population' (FP) in multiple tissues at different time-points post-infection. Additionally, we have measured the genetic distance between parasite populations in different tissues, to understand the potential mechanisms underlying dissemination patterns.

To investigate changes in dissemination over time, we infected male and female C57BL/6J mice with the promastigote barcoded library for 2 days, 14 days or 28 days, and assessed the parasite diversity in multiple tissues to calculate FP sizes. We have found that the liver is permissive to parasite colonisation and its diversity is relatively stable over time, whilst lymph nodes show a highly restrictive bottleneck, resulting in a small FP that increases significantly over a four-week infection. Spleen, lung, gut and bone marrow show intermediate FP sizes.

To understand the mechanisms for the earliest bottleneck events, we used a complement-deficient (B6.129S4-C3tm1Crr/J) mouse model; we found that complement knock-out increases the permissiveness of the bottleneck in multiple tissues at 2 days post-infection, with males exhibiting a more significant impact than females.

Using genetic distance measurements, we have additionally generated data supporting the hypothesis that the liver is the key source of parasites for the colonisation of other tissues. Overall, our findings provide an insight into the mechanisms regulating population bottlenecks and colonisation dynamics, which could have important implications for parasite and host evolution and in disease outcomes.

15:10 (20 mins)

Stuck in the throat: Dissection of *Leishmania* parasite adhesion in the sand fly vector

Dr Jack Sunter, *Oxford Brookes University*

RY Yanase[3]; K Pruzinova[1]; F Moreira-Leite[3]; E Rea[3]; J Sadlova[1]; P Volf[1]; JD Sunter[2];
[1] Charles University, Czechia; [2] Department of Biological and Medical Sciences, Oxford Brookes University, Oxford, UK; [3] Oxford Brookes University, UK

Within its 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 adhesion is critical to understanding parasite transmission. We have previously generated high-resolution 3D models of haptomonads attached to the stomodeal valve using volume electron microscopy. This showed that the adhesion complex consisted of filaments that run through the flagellum to an electron-dense plaque, with connections across to the surface of the valve. Using comparative proteomic approaches, we identified three Kinetoplastid-Insect Adhesion Proteins (KIAPs) that locate to different regions of the attachment complex. These proteins are present in other kinetoplastid parasites, suggesting a common mechanism of adhesion in the kinetoplastid parasites. Deletion analysis compromised *Leishmania* adhesion both *in vitro* and in the sand fly, confirming that we have identified the first critical components of the adhesion complex. Infection of sand flies with *Leishmania* parasites results in damage to the cuticle surface of the valve and distension of the midgut by secretion of the promastigote secretory gel, enhancing parasite transmission. Interestingly, we found that loss of parasite adhesion in the sand fly caused reduced distension of the midgut, with no observable damage to the cuticle surface of the valve. Overall, our study provides the first molecular insights into a kinetoplastid parasite vector adhesion interface and highlights the importance of *Leishmania* adhesion for the modification of the sand fly gut environment.

(19) Veterinary vaccines - (Teaching room 4)

Sponsored by Elsevier

Chair: Ben Makepeace

14:00 (25 mins)

Developing subunit vaccines for Animal African Trypanosomiasis

Prof Gavin Wright, *University of York*

D Autheman[1]; C Crosnier[1]; L Morrison[2]; A Evans[3]; H Davies[1]; AP Jackson[4]; G Wright[1];
[1] Department of Biology, Hull York Medical School, UK; [2] Roslin Institute, Royal (Dick) School of Veterinary Studies, UK; [3] Clinglobal, Mohammedia, Morocco; [4] Department of Infection Biology, University of Liverpool, UK

Trypanosomes are protozoan parasites that cause infectious diseases including human African trypanosomiasis (sleeping sickness), and nagana in economically-important livestock animals. An effective vaccine against trypanosomes would be an important control tool, but the parasite has evolved sophisticated immuno-protective mechanisms including antigenic variation that present an apparently insurmountable barrier to vaccination. Using a systematic genome-led reverse vaccinology approach and murine infection models of trypanosome infection, we show that protective invariant subunit vaccine antigens can be identified. Vaccination with a single recombinant protein comprising the extracellular region of a conserved cell surface protein induced long-lasting protection. Immunity was passively transferred with immune serum, and recombinant monoclonal antibodies could induce sterile protection

and revealed multiple mechanisms of antibody-mediated immunity, including a major role for complement. To translate this research, we are developing livestock infection models of both *Trypanosoma congolense* and *Trypanosoma vivax* that are suitable for testing subunit vaccines. Our discovery identifies a vaccine candidate for an important parasitic disease that has constrained the socioeconomic development of sub-Saharan African countries and challenges long-held views that vaccinating against trypanosome infections cannot be achieved.

14:25 (25 mins)

Controlling parasitic nematodes of sheep with sub-unit vaccines – the state of play

Prof Alasdair Nisbet, *Moredun Research Institute*

A Nisbet[1];
[1] Moredun Research Institute, UK

Developing recombinant sub-unit vaccines against parasitic nematodes in livestock has been a long-term goal for a number of researchers over the last few decades and has not yet yielded a commercial product. Of the parasitic diseases considered to be most impactful on sheep health and welfare – sheep scab, fascioliasis and parasitic gastroenteritis (PGE), there is only one vaccine currently available (Barbervax) and that is specific for *Haemonchus contortus*. PGE in temperate regions, however, is caused by the major endemic gastrointestinal nematodes (GIN) of sheep (*Teladorsagia circumcincta, Trichostrongylus* spp. and *Nematodirus battus*) and we have now developed a prototype recombinant sub-unit vaccine against the most common and important of these, *T. circumcincta*. This vaccine induced significant (up to 75% reduction in worm numbers) protection against challenge in lambs, was effective in periparturient ewes and a simplified prototype also gave good levels of protection. However, this novel vaccine has variable efficacy and can only target a single species of GIN, whereas adequate worm control requires multiple species to be repeatably targeted if the vaccine is to complement anthelmintic treatments. We have therefore developed strategies to improve efficacy and to reduce variability through understanding host:parasite interactions, developing novel delivery systems to stimulate the appropriate immune responses and we have begun to identify common protective antigens and/or peptide epitopes from multiple GIN and will, in future, test the efficacy of these "multivalent" vaccines delivered in a range of ways.

14:50 (15 mins)

Combined mucosal and systemic immunisation strategy against bovine neosporosis

Dr Alexandra Correia, *University of Porto -– ICBAS*

A Correia[1];
[1] University of Porto -– ICBAS, Portugal

The apicomplexan parasite *Neospora caninum* is a major causative agent of abortions and stillbirths in cattle, representing a global economic burden surpassing one billion dollars per year. Vaccination is considered the most cost-effective approach to manage neosporosis; however, no commercial vaccine is currently available to prevent this disease. We previously determined that mucosal immunisation with *N. caninum* membrane proteins plus CpG ODN adjuvant successfully protected mice challenged with this parasite by inducing a strong Th1-type response. Here, we aimed at improving the immunogenicity of the antigenic preparation for use in cattle. In this sense, we included additional adjuvants to the preparation and promoted the host's systemic immune response by combining intranasal and subcutaneous dose administrations. Parasite-specific cellular and humoral responses were evaluated in immunised and sham-immunised Holstein-Friesian female calves. Immunisation

raised *N. caninum*-specific serum and saliva IgG and IgA antibodies. Moreover, it induced the generation of memory CD4+, CD8+, and TCRγδ T cells that responded with extensive proliferation and elevated production of IFN-gamma to the antigenic restimulation. Protection conferred by the refined immunisation procedure was assessed in sham-immunised and immunised calves experimentally infected with *N. caninum*. Preliminary results, using nested PCR and multiple amplification replicas, indicated that 8/8 control animals presented detectable brain parasitic DNA, while consistent parasitic DNA amplification occurred only in 3/8 brain samples from immunised calves. Taken together, these results show that our immunisation strategy was effective in inducing parasite-specific humoral and cellular immunity in the bovine host and encourage testing this strategy on a larger scale and in bovine pregnancy models. Funded by FCT -– PTDC/CVT-CVT/3045/2021.

15:05 (25 mins)

Recombinant anticoccidial vaccines for chickens – how good is good enough?

Prof Damer Blake, *Royal Veterinary College*

D Blake[1];
[1] Royal Veterinary College, UK

Eimeria can cause the disease coccidiosis in livestock and poultry, most notably in chickens where global costs incurred by these parasites exceed £10 billion every year. Current anticoccidial control is underpinned by routine chemoprophylaxis but resistance is widespread, with strong public and legislative pressure to reduce use. Alternatives include a range of live wild-type or attenuated vaccines that can be highly effective but cost more than drugs, have limited production capacity, and require expert management. Demand for novel, cost effective recombinant anticoccidial vaccines is high, but industry requirements are stringent. Host performance, measured as body weight gain (BWG) or food conversion ratio (FCR), must be protected, with parasite replication and infection-associated pathology minimised. For *Eimeria*, extensive efforts including candidate gene and genetic mapping strategies have identified a panel of immunoprotective antigens as priority vaccine candidates. Targets including Apical Membrane Antigen 1 (AMA1), Immune Mapped Protein 1 (IMP1), and Microneme Protein 3 (MIC3) show promise when administered as recombinant proteins or DNA vaccines, but more cost-effective and scalable approaches are required by the poultry industry. Options such as vectored vaccines that can be incorporated into poultry diets or inoculated at scale in the hatchery are needed. Examples include use of *Saccharomyces cerevisiae* to express and delivery recombinant antigens, supplementing the widespread use of this yeast as a feed additive in poultry diets with vaccine delivery. Killed *S. cerevisiae* spores, even those that have been genetically modified to express vaccine antigens, are Generally Regarded As Safe (GRAS) from a regulatory perspective, minimising challenges posed from use of Genetically Modified Organisms (GMOs). Alternatives include genetic modification of *Eimeria* vaccine strains to express antigens representative of different species to create a streamlined, multivalent anticoccidial vaccine. This approach has also been evaluated to express and deliver anti-*Campylobacter* vaccine candidates, offering broader appeal to industry. Persistent challenges include fine tuning vaccine formulations for optimal efficacy and economic margin when compared to industry staples such as ionophore prophylaxis. It is likely that legislative changes to reduce or remove chemoprophylaxis will be significant for the future of recombinant vectored anticoccidial vaccines.

President's Medal - (Brett Lecture Theatre)

Chair: Joanne Hamilton

~~16:00 (30 mins)~~ not presented

~~Using trypanosomes to understand the inner workings of the brain~~

~~Dr Juan Quintana, *University of Manchester*~~

~~J Quintana[1];~~

~~[1] *University of Manchester, UK*~~

~~One fascinating aspect of Human African Trypanosomiasis, also known as sleeping sickness, is the profound effects this infection has on the brain, which in turn affects a wide range of behaviours, from feeding to sleep. However, the basis of such behavioural disturbances is far from being fully understood. This could in turn teach us how the brain operates under stressful conditions such as inflammation. In this talk, I will cover the work my team and I have conducted to address this gap in knowledge from an immunological point of view. In a nutshell, I discovered that brain-resident B cells play opposing roles locally during infection, from limiting brain inflammation via anti-inflammatory cytokines (Quintana et al, Nat comms, 2022) to inducing autoimmunity (Quintana et al. PLoS Biology, 2023), indicating that sleeping sickness is much more complex than previously anticipated. Serendipitously, my preliminary studies show that mice devoid of a particular type of B cells, known as regulatory B cells, show more severe neuroinflammation and are unable to sustain normal behaviour under homeostasis and during infection compared to littermate controls, highlighting the importance of these cells in supporting brain homeostasis and resilience. Together, these observations indicate that brain-derived B cells are not only functionally heterogeneous (e.g., regulatory vs. pathological) but essential to control how the brain works. Moving forward, my laboratory will investigate the origin and functional diversity of B cells in the brain, how these cells control the immunological landscape in this organ during infection, and the consequences of such neuroimmune interactions for behaviour, placing brain-resident B cells as guardians of the brain's integrity and function.~~

~~Selected publications: 1. Quintana JF, et al. Single-cell and spatial transcriptomics analyses reveal microglia-plasma cell crosstalk in the brain during *Trypanosoma brucei* infection. Nat commun. 13, 5752 (2022). 2. Quintana JF, et al. The murine meninges acquire lymphoid tissue properties and harbour autoreactive B cells during chronic *Trypanosoma brucei* infection. PLoS Biol. 21(11): e3002389 (2023).~~

Wright Medal - (Brett Lecture Theatre)

Chair: Joanne Hamilton

16:30 (60 mins)

Seeking simplicity in the complexity: the community epidemiology of multihost/multiparasite systems

Prof Andy Fenton, *University of Liverpool*

A Fenton[1];

[1] *University of Liverpool, UK*

As we are only too aware, many parasites that cause infectious diseases of human, livestock or conservation importance, circulate within diverse ecological communities comprising many potential host species and many other parasite species. Interactions across these communities can severely hamper our ability to manage the impacts these parasites have. Interactions between co-circulating parasites can alter host susceptibility to, and impact of, the various parasite species involved. And variable abundances and susceptibilities among different host species can alter parasite transmission,

maintenance and spread across the community. Given these multiple interacting processes it is easy to become overwhelmed by the apparent complexity of these systems, making it very hard to understand the key processes driving parasite emergence, spread and impact. Perhaps though there are 'covert simplicities' in many systems which, if we can identify them, can help us see past the apparent complexity, and focus on the key aspects that dominate parasite transmission dynamics. I will describe our attempts to identify these key aspects through the combination of conceptual and theoretical frameworks, and large-scale field experiments, to help tackle the apparent complexity of natural multihost – multiparasite systems.

Posters

Poster 1 : An integrated bioinformatics/cheminformatics drug repurposing pipeline to identify novel anti-schistosomal compounds

Bismark Dankwa, *Aberystwyth University*

B Dankwa[2]; J Forde-Thomas[2]; K Lees[2]; A Coghlan[3]; M Berriman[1]; K Hoffmann[2];
[1] University of Glasgow , UK; [2] Aberystwyth University, UK; [3] Wellcome Sanger Institute, UK

Schistosomiasis, a major neglected tropical disease, is responsible for thousands of human deaths annually. Currently, praziquantel is the only approved chemotherapy for treatment. However, concerns over praziquantel resistance, the need for repeat dosing and the lack of efficacy against juvenile worms threaten the long-term sustainable control of the disease. In the absence of an effective vaccine, there is the pressing need to develop new drugs to complement or replace the existing therapy. However, *ab initio*, whole-organism screening of druggable gene sets for early anti-schistosomal leads using high-throughput *ex vivo* phenotypic assays is expensive, laborious, and time-consuming. To fill this gap, we have developed an advanced bioinformatics/cheminformatics drug repurposing pipeline that has identified schistosome drug targets and hit to lead compounds based on associated human protein targets in the ChEMBL database. The approach combined top BLAST hits (E-value <= 1e-10) between *Schistosoma mansoni* protein coding genes (9896; version 10; PRJEA36577) and ChEMBL single protein targets, together with datasets comprised of bulk transcriptomes (RNA-seq), functional phenotypes (RNAi or gene knockout), compara families, multi-species homologues, protein structural information, sequence alignment conservation, toxicity targets, phylogenetics and chokepoint enzymatic predictions to rank 1921 probable targets (out of 9896). Focusing on this subset of probable targets, compounds linked to their counterpart ChEMBL proteins were extracted and screened through filters such as Lipinski rule of five, toxicity, safety warnings, pan-assay interference, binding affinities and ADMET properties to remove potentially risky compounds. A favourable library of 4916 purchasable compounds consisting of 11% phase IV drugs, 4% phase III compounds and 85% hit-to-lead medicinal chemistry compounds was retained. Within-library similarity clustering (≥ 90%) using SkelSpheres descriptors as well as clustering with 2828 anthelminthic compounds curated from the literature identified closely related compounds that limited library diversity. Removing these and previously screened in-house compounds left us with 2077 diverse compounds against the 1921 *S. mansoni* targets awaiting experimental validation in our diverse *ex vivo*, whole organism assays.

Poster 2* : Role of thrombospondin type 1 repeat (TSR) domain proteins in motility and virulence of *Babesia* parasites

Mr Alex Grannell, *Royal Veterinary College*

A Grannell[1]; E Knuepfer[1]; L Eyssen[2]; R Owens[2];
[1] Royal Veterinary College, UK; [2] The Rosalind Franklin Institute, UK

Invasion of host erythrocytes by apicomplexan parasites is a promising field for vaccine development and one that is well studied for *Plasmodium* species. *Babesia divergens* is a tick-borne parasite causing disease of human and veterinary importance, but our knowledge surrounding molecular mechanisms of how *B. divergens* invades host erythrocytes is limited. Transcriptome analysis showed that *Babesia* expresses genes encoding thrombospondin type one repeat (TSR) domain containing proteins in multiple life cycle stages. Orthologues of these proteins in *Plasmodium* are vital for parasite motility and host cell invasion. Using orthologous knowledge, we hypothesise that TSR domain containing proteins are equally important for parasite

mobility and ability of host cell invasion of *B. divergens* both in vertebrate life cycle stages and during tick development of the parasite.

To investigate this, TSR domain containing proteins from *B. divergens* were expressed recombinantly to generate antibodies. Polyclonal antibodies and nanobodies specific to our target proteins will next be tested *in vitro* for efficacy in inhibiting erythrocyte invasion. This will provide critical information whether these target proteins could be used as vaccine candidates to prevent babesiosis both in cattle and humans.

Poster 4* : Generating evidence for antibiotic stewardship through NTD control

Gabrielle Thompson, *St Andrews University*

G Thompson[1]; MM Moore[2];
[1] St Andrews University, UK; [2] IIIR, UK

Introduction: Neglected tropical diseases (NTD) affect over 1.7 billion people globally. Of the 20 recognized NTDs, 6 are antibiotic-treated infectious diseases: Buruli ulcer, leishmaniasis, leprosy, onchocerciasis, trachoma, and yaws.

Context and Aim: The WHO 2021-2030 NTD roadmap recommends antibiotic use for mass drug administration (MDA) and/or case management for these 6 diseases. We aim to generate evidence on antibiotic use and resistance to justify NTD elimination as an antibiotic stewardship strategy.

Method: We carried out a comprehensive review of countries' NTD plans and WHO standard practice guidance to identify the antibiotics used, along with a literature review to measure resistance in NTDs and other diseases.

Findings: Rising antibiotic resistance poses a threat to NTD elimination and basic standards of care. Of the 6 antibiotic-treated NTDs, 4 demonstrate resistance. Further, of the 14 antibiotics used, 6 show resistance for both the corresponding NTD and one or more infectious diseases such as tuberculosis, gonorrhoea, and pneumonia.

Innovative contribution to policy, practice and/or research: Global progress in NTD elimination is evident. However, there remains a paucity of data for policy makers to prioritize NTD elimination as an antibiotic stewardship strategy. Presenting evidence for decreasing resistance development through reducing antibiotic use, particularly through MDAs, is a valuable political and economic argument for strengthening NTD efforts.

Poster 5* : Drug-resistant trypanosome isolates populations in dogs in Enugu North Senatorial Zone, Southeastern Nigeria

Dr Chukwunonso Obi, *University of Nigeria, Nsukka*

CF Obi[1]; IO Ezeh[1]; MI Okpala[1]; A Onyeabo[2]; RC Ezeokonkwo[1];
[1] University of Nigeria, Nsukka, Nigeria; [2] Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria

African trypanosomiasis is an important wasting and an endemic protozoan disease-causing morbidities and mortalities in humans and animals in sub-Saharan Africa. Currently, chemotherapy is the widely practised African trypanosomiasis control method in dogs. However, their efficacy is threatened by the emergence of drug-resistant trypanosomes owing to extensive use and misuse over several decades. Thus, this study focused on the sensitivity of *Trypanosoma* sp. isolated from dogs in Southeastern Nigeria to trypanocides. *Trypanosoma brucei* (n = 44) and *T. congolense* (n = 4) isolated from naturally infected dogs in Southeastern

Nigeria, between January and August 2016 were subjected to single-dose tests to assess their sensitivity to trypanocides - diminazene aceturate (DA) and isometamidium chloride (ISM). Subsequently, DA and multidrug-resistant isolates were further subjected to DA multi-dose test and CD50 was determined. Clones were derived from a randomly selected multidrug-resistant isolate and their sensitivity was also assessed. 100% and 83.3% of *T. congolense* and *T. brucei* respectively were resistant to the trypanocides. Amongst the drug-resistant isolates, 50%, 16.7% and 33.3% were resistant to DA, ISM and both trypanocides respectively with CD50 ranging between 14.26 – 34.69 mg/kg. Drug-resistant trypanosomes were characterized into highly resistant (CD50 = 11–24.99 mg/kg) and very highly resistant (CD50 = >25 mg/kg) trypanosome isolates. Clones also expressed high levels of resistance to both DA and ISM with CD50 values between 35.19 and 37.19 mg/kg. Trypanocidal resistance was thus, confirmed and appears to be widespread in dogs in Southeastern Nigeria. The adoption of an integrated trypanosomiasis control strategy is most desirous.

Poster 6* : Albendazole efficacy against gastrointestinal nematodes of pigs in Nsukka Local Government Area of Enugu State, Nigeria

Dr Chukwunonso Obi, *University of Nigeria, Nsukka*

IK Idika[1]; CF Obi[1]; TA Nzeakor[1]; S1 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 farms and examined for GINs. Six out of the 13 pig farms 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 worms to albendazole was suspected in three farms 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 7* : Comparative serum biochemical changes in Nigerian local dogs following single infections of drug-sensitive and multidrug-resistant *Trypanosoma congolense* and *Trypanosoma brucei*

Dr Chukwunonso Obi, *University of Nigeria, Nsukka*

CF Obi[1]; MI Okpala[1]; NT Emejuo[1]; IO Ezeh[1]; DN Onah[1]; RC Ezeokonkwo[1];
[1] University of Nigeria, Nsukka, Nigeria

Animal trypanosomiasis is an important endemic disease in sub-Saharan Africa. Its control relies on chemotherapy, and resistance to trypanocides has been widely reported. A paucity of information exists on the pathogenicity of drug-resistant canine trypanosomes. Thus, this study compared the serum biochemical changes in Nigerian local dogs infected with either drug-resistant or drug-sensitive *Trypanosoma brucei* or *Trypanosoma congolense*. Twenty Nigerian local dogs were used for this study and were randomly assigned into five groups (I - V) of four dogs each. Group I served as the uninfected control group, while groups II and III were infected intraperitoneally with 106 drug-sensitive *T. congolense* and *T. brucei* respectively. Groups IV and V were inoculated intraperitoneally with 106 multidrug-resistant *T. congolense* and *T. brucei*, respectively. The serum total protein (TP), serum albumin (ALB), serum globulin (SG), aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), bilirubin, urea, creatinine, total cholesterol (TCHOL), triglycerides, very low-density lipoprotein cholesterol (VLDL-C), high-density lipoprotein cholesterol (HDL-C), malonaldehyde (MDA), superoxide dismutase (SOD), mean fasting blood glucose levels (FBG), and serum testosterone levels (TEST) were assessed. The mean pre-patent period of groups II-V were 4.25, 3.5, 5.2, and 10.3 days respectively. Significant variations were observed in the serum biochemical parameters of the infected groups. Group V dogs had lower ($P<0.05$) mean AST, ALT, ALP, bilirubin, urea, creatinine, MDA, and higher ($P<0.05$) mean TP, SOD, FBG, and TEST than group III dogs. However, these parameters did not differ statistically ($P>0.05$) amongst groups II and IV dogs. It was concluded that drug-sensitive *T. brucei* was more virulent than the multidrug-resistant *T. brucei* while multidrug-resistant and drug-sensitive *T. congolense* had comparable virulence which were higher than that of multidrug-resistant *T. brucei*.

Poster 8 : *Toxoplasma gondii* Antibodies in Tropical Seabirds from the Rocas Atoll Biological Reserve, Brazil

Prof Solange M Gennaril, *University of Sao Paulo*

SM Gennari[1]; DB Mariani[7]; HS Soares[2]; R Hurtado[3]; VC Galizia[6]; MB Silva[8]; EC Macedo[4]; RA Dias[1]; JC Ramos Silva[5];

[1] University of Sao Paulo, Brazil; [2] Santo Amaro University, Brazil; [3] Southern African Foundation for the Conservation of Coastal Birds, Brazil; [4] Instituto Chico Mendes de Conservação da Biodiversidade, Brazil; [5] Universidade Federal Rural de Pernambuco, Brazil; [6] Self-Employed Veterinarian, Brazil; [7] Federal University of Pernambuco, Brazil; [8] Chico Mendes Institute for Biodiversity Conservation, Brazil

Toxoplasma gondii is a coccidian parasite that infects almost all warm-blooded animals, including birds. The Rocas Atoll Biological Reserve, located in the northeast region of Brazil, in the state of Rio Grande do Norte, is the only atoll in the South Atlantic Ocean, and is home to the largest population of tropical seabirds in the western Atlantic Ocean. The objective of this study was to verify the occurrence of anti-*Toxoplasma gondii* antibodies in seabirds from the Rocas Atoll. The seabirds were captured in three expeditions carried out from June to November 2017. A clinical examination was carried out on each bird, blood samples were taken, and the individual was identified through the placement of metal rings with subsequent release of the bird in the same capture area. For the detection of anti-*T. gondii* antibodies, the Modified Agglutination Test was used. The serum samples were first screened at 1:5 dilution (cut-off point) and the positive samples were titrated at a two-fold serial dilution. In total, 267 birds of seven species belonging to three families Sternidae, Fregatidae and Sulidae and two orders, Charadriiformes and Suliformes, were sampled. Of the 267 samples tested, 20 (7.3%) were seropositive for *T. gondii* antibodies being: 9 of the 48 Brown Noddy (*Anous stolidus*), 1 (26 birds) Black Noddy (*Anous minutus*), 3 (20 birds) Magnificent Frigatebird (*Fregata magnificens*), 5 (95 birds) Sooty Tern (*Onychopriom fuscatus*) and 2 (20 birds) Red-footed Boody (*Sula sula*). None of the 33 Masked Boody (*Sula dactylatra*) and 25 Brown Boody (*Sula leucogaster*) samples presented antibodies against *T. gondii*. The antibody titres were 5 (n=6), 10 (n=4), 20 (n=3), 40 (n=6) and 160 (n=1). Due to the uniqueness of this island

environment, continuous and systematic monitoring of these seabirds is suggested, aiming to promotion of unique health in the Conservation Unit.

Poster 9* : Molecular epidemiology of tick-borne bovine blood protozoa deciphering the emerging and new variants in Bangladesh

Dr MD Hasanuzzaman Talukder, *Bangladesh Agricultural University*

M Ahmed[1]; BC Roy[1]; N Ahmed[1]; MM Zim[1]; MM Sajib[1]; MR Rabbi[1]; MK Rahman[1]; MM Hasan[1]; H BISWAS[1]; MH Talukder[1];

[1] *Bangladesh Agricultural University, Bangladesh*

Objective: Tick-borne blood protozoan parasitic diseases have significant impacts on livestock and dairy production. The global warming and geo-climatic condition of Bangladesh is very conducive to a wide variety of ticks which increases the incidence of the diseases. The present study was aimed to determine the seroprevalence of *Anaplasma* infection and molecular epidemiology of major tick-borne blood protozoan parasites of cattle from selected districts.

Materials and Methods: A total of 384 blood and sera samples were randomly collected from different commercial cattle farms as well as small stakeholder farms of producer groups (PG) of Livestock and Dairy Development project (LDDP) of the selected regions of Bangladesh. All sera samples (384) were analyzed using a commercially available competitive enzyme linked immunosorbent assay (cELISA) kit. For molecular epidemiology, after DNA extraction of the blood samples, initially multiplex PCR was performed for the presence of the infections. Then PCR was done by specific genes of the parasites and subsequently sequenced for bioinformatics and phylogenetic analysis.

Results: The cELISA tests reveals high seroprevalence of *Anaplasma* species in dairy cattle (46.39%) followed by calves (41.08%) and breeding bulls (40.0%). Significantly ($p<0.05$) high seroprevalence (52.76%) was found in cattle (aged>1 year) than cattle (aged<1 year) (32.97%); females (47.87%) than the males (30.39%) and crossbreed cattle (46.05%) than indigenous cattle breed (34.04%), respectively. The study reveals that calves are also susceptible to *Anaplasma* infections due to transplacental transmission. The multivariable logistic regression analysis identified the age (>1 year), sex (female) and breed (crossbreed) of cattle as potential risk factors for bovine anaplasmosis. The molecular prevalence of *Theileria*, *Anaplasma* and *Babesia* species revealed 40.35%, 17.5% and 0.35% respectively. The highest prevalence of co-infections of *Anaplasma* and *Theileria* species(8.92%) and *Babesia* and *Theileria* species (0.35%) have been found. The sequenced analysis revealed, *A. marginale* (44.5%) followed by *A. bovis* (33.3%), *A. centrale* (11.1%) and *Candidatus A. cinensis* (11.1%) and *T. orientalis* (40.35%) indicate an emerging bovine anaplasmosis and theileriosis in Bangladesh. Further bioinformatics analysis is going on and interestingly, the combined phylogenetic analysis (ML) of 16S rRNA and groEL sequences demonstrated that *Anaplasma* sp. (Mymensingh proposed) is closely related to *A. platys* and 18S rRNA sequences revealed that *Babesia* sp. (Mymensingh proposed) is closely related to *B. bigemina* and Theileria orientalis BR-BDH3 clustered together with *T. orientalis* type buffeli from Australia and *Theileria buffeli* from China, respectively.

Conclusions: Genetic characterization and whole genome sequencing of *T. orientalis* and unknown *Anaplasm* sp. (Mymensingh) and *Babesia* (Mymensingh) will help to deciphering the genetic variants and new species relevant to virulence as well as vaccine or new drug development. Acknowledgments Research and Innovation sub-project (grant awarded to Prof. M H Talukder; October 2022) of the Livestock and Dairy Development project (LDDP), Department of Livestock Services (DLS), funded by World Bank and Govt. of the People's

Republic of Bangladesh

Poster 10* : Targeting LmxMKKK19 for better understanding of Mitogen-Activated Protein Kinase cascades in *Leishmania mexicana*

Anil Ata, *The University of Strathclyde*

A Ata[2]; R Burchmore[1]; M Wiese[2];
[1] School of Infection and Immunity, University of Glasgow, UK; [2] Strathclyde Institute of Pharmacy and Biomedical Sciences, University of Strathclyde, UK

Little is known about *Leishmania* MAP kinase kinase kinases (MAPKKK). LmxMKKK19 has been annotated in TriTrypDB as a putative MAPKKK in *Leishmania mexicana.* To understand MAP kinase cascades in *Leishmania,* we targeted LmxMKKK19 for deletion and tagging using CRISPR-Cas9. Single and double allele knockout clones were generated by electroporation utilising two different resistance markers. Add-back clones were obtained implementing homologous recombination of *LmxMKKK19* into the ribosomal RNA gene locus. The generated clones were screened for changes in morphology, growth and their potential to infect Balb/c mice. To unveil the localisation of LmxMKKK19 in promastigotes, the protein was tagged with mNeonGreen (mNG) using CRISPR-Cas9 at either its N- or C-terminus. Furthermore, in a CRISPR-Cas9 cell line, expressing FKBP-miniTurbo biotin ligase, a component of a 2C-BioID system, FRB, the second component, was fused to LmxMKKK19 for identification of possible interaction partners utilising biotin proximity labelling, tryptic digest of biotinylated proteins isolated using streptavidin-agarose, and mass spectrometry. Proteins encoded by LmxM.33.2090 and LmxM.07.0900 were determined as putative interaction partners of LmxMKKK19. Using CRISPR-Cas9 on a cell line already expressing mNG-tagged LmxMKKK19, monomeric red fluorescent protein (mRFP) was fused to each of these proteins and co-localisation studies were carried out employing fluorescence microscopy. Further experiments to confirm the interaction of the protein kinases are under way.

Poster 11 : T-helper cell phenotype in wild Soay sheep: patterns of (co)variation and associations with parasites and fitness

Dr Adam Hayward, *Moredun Research Institute*

Y Corripio-Miyar[1]; A Hayward[1]; H Lemon[4]; X Bal[3]; J Pilkington[2]; JM Pemberton[3]; D Nussey[2]; TN McNeilly[1];
[1] Moredun Research Institute, UK; [2] University of Edinburgh, Institute of Evolutionary Biology, UK; [3] University of Edinburgh, UK; [4] University of Edinburgh, Institute of Ecology and Evolution, UK

T-helper (Th) cells co-ordinate the vertebrate immune response to ensure that infections with a diverse array of parasites are met with appropriate and effective responses. Helminths are typically associated with a T-helper type 2 (Th2) cell-mediated immune response, while intracellular parasites such as protozoans are associated with a Th1 response. Laboratory models have typically reported that Th1 and Th2 responses are antagonistic, such that a strong Th2 response is associated with a downregulated Th1 response. These results, however, have been challenged by studies of natural infections that suggest much less clear-cut results in resource-limited, genetically diverse, hosts infected with multiple parasite species. Since 1985, a wild population of Soay sheep living in the St Kilda archipelago, NW Scotland, has been the subject of an individual-based study, with animals monitored from birth until death and repeatedly captured and sampled. The sheep are infected with gastrointestinal nematodes (GIN) and coccidia, which are highly prevalent and known to influence host body weight and survival. GIN and coccidian infections have been monitored for >30 years, and for the last 4 years we have measured immune phenotypes associated with different aspects of cell-mediated and humoral

immunity. We found that counts of Th cells of different phenotypes were generally positively correlated with each other, as were levels of different cytokines and different antibodies, but there were no strong positive associations among cell counts, cytokines or antibodies. Cell counts generally had low repeatability (among individual variation) across the four years, while antibodies were highly repeatable; the Th1 cytokine IFN-γ and the Th2 cytokine IL-4 were moderately repeatable. IFN-γ and IL-4 were positively correlated at both the between- and within-individual levels, suggesting individuals with high levels of one cytokine tended to have high levels of the other, although this result was not apparently mediated by body condition or parasite exposure. IL-4 was negatively associated with GIN faecal egg count, while IFN-γ was negatively associated with coccidian faecal oocyst count, suggesting that these cytokines reflect resistance to these respective parasites. None of our immune markers were strongly associated with lamb survival once body weight was accounted for. Our results provide some glimpses of how different aspects of immune function interact to produce effective responses to complex infection, but more data will enable these interactions to be dissected.

Poster 12* : A Lateral Flow Assay for Schistosome cfDNA Detection in Urine

Rory Barnes, *University of Glasgow*

R Barnes[1]; J Reboud[1]; JM Cooper[1];
[1] University of Glasgow, UK

The current diagnostic toolkit for schistosome infections does not offer the required sensitivity in low prevalence settings to support elimination campaigns. Nucleic acid amplification tests (NAATs) offer an opportunity to fill this requirement. However, current NAAT methods are too cumbersome and costly for high-throughput, point-of-care use required by schistosomiasis monitoring and evaluation campaigns. Two of the key barriers to current NAAT use in these settings are complex sample preparation, and the advanced equipment required for amplification and result readout. Here we present a simple filter-based sample preparation method, and sensitive isothermal nucleic acid amplification assays with results presented on a lateral flow strip, to detect schistosome infection in urine. During infection, parasite DNA fragments are released into the circulation in extracellular vesicles and by dying parasites. This cell-free DNA (cfDNA) passes through the kidneys and into the urine, thus presenting a non-invasive diagnostic target that can be used to detect multiple schistosome species in a single sample. We leveraged the charge-switching properties of a chitosan-functionalised filter to rapidly enrich DNA in large volume samples, such as urine, to microlitre volumes that can be amplified by loop mediated isothermal amplification (LAMP). We modified LAMP primers for *S. haematobium* and *S. mansoni* specific gene targets with labels that enable binding of the amplicons to gold nanoparticles, which are then immobilised on an antibody-coated test line of a lateral flow strip to indicate a positive result. The analytical sensitivity (LoD) of these assays for *S. haematobium* and *S. mansoni* was $4.9x10^3$ and $2.5x10^3$ target DNA copies per reaction, respectively. We will combine these assays onto a single lateral flow device, to be used alongside the cfDNA enrichment method, as a proof-of-concept that *S. haematobium* and *S. mansoni* can both be detected in a urine sample. Ultimately, a setup providing rapid, equipment-free DNA enrichment followed by an isothermal amplification with a lateral flow readout will move us one step closer to overcoming the logistical and cost challenges associated with getting NAATs to the point-of-care, where they are required.

Poster 13* : Unravelling the epidemiology of ticks and tick-borne infections in Benue state, Nigeria

Raymond Tersoo Ada, *Student, University of Salford*

R Ada[1]; RJ Birtles[1]; K Bown[1]; V Ndolo[1];
[1] University of Salford, UK

Background: Ticks on cattle continue to be a problem in the Nigerian livestock industry as transhumance has aided the introduction of new tick species via cross-boundary movement into regions where they were absent. These ticks act as carriers of pathogens like *Anaplasma, Babesia*, and *Theileria* with veterinary and economic importance. To develop effective control strategies, it is crucial to understand the distribution and factors influencing tick populations and cattle providence through epidemiological studies. This research aimed to assess tick diversity and cattle provenance in Benue State.

Methods: This study focused on a tick survey using simple tools (compound and dissecting microscope) along with identification keys, three hundred and ten (310) questionnaires were used to understand the provenance of the cattle in the study area to highlight the first step in understanding the tick-borne pathogen transmission pathways and disease epidemiology. Tick samples were collected from 281 cattle (1st survey) from January to February 2022, and 310 cattle (2nd survey) from August to September 2023.

Results: A total of 185 cattle from the 1st survey were found to be infested with ticks. 658 ticks were recovered and identified from these animals and were found to belong to eight species, belonging to three (3) genera, *Hyalomma, Amblyomma* and *Rhipicephalus* including subgenus *Boophilus. Hyalomma impeltatum* (27.5%) was the most encountered tick but *Amblyomma variegatum* (24.8%), R. (B) geigyi (23.7%) and *R. (Boophilus) microplus* (17.3%) were also frequently found. *Hyalomma truncatum* (5.0%), *Rhipicephalus turanicus* (0.6%), *R. (Boophilus) decoloratus* (0.9%) and *Hyalomma rufipe*s (0.2%) were rarely encountered. The observed tick diversity aligns with previous studies in Nigeria, indicating a dominance of three genera: *Amblyomma*, *Hyalomma,* and *Rhipicephalus*. However, the significant presence of *Hyalomma* ticks in this study suggests a shift in tick diversity within the North Central region, possibly due to climate variability, including higher temperatures and unpredictable rainfall patterns. *Rhipicephalus turanicu*s and *R. (B) geigyi* ticks not associated with the study area in past years were identified. The number of ticks per animal ranged between 1 to 15, with a median of 7. Ticks collected in the 2nd survey are yet to be identified but questionnaires (310) completed by stakeholders in the local livestock industry in Benue State indicate that most cattle in Benue were reared outside the state in locations further northern, either in Nigeria or in neighbouring Cameroon. Typically, cattle only remain in Benue for less than a month before being slaughtered or moved further south.

Conclusion: The presence of the diversity and dynamics in ticks and cattle markets underscores the need for proper mo

Poster 14 : Phylogenetic framework to study the evolution of traits in trypanosomatids

Dr Alexei Kostygov, *University of Ostrava*

AY Kostygov[2]; AT Albanaz[2]; A Butenko[1]; E Gerasimov[3]; J Lukeš[1]; V Yurchenko[2];
[1] Institute of Parasitology, Biology Centre, Czechia; [2] University of Ostrava, Czechia; [3] Lomonosov Moscow State University, Russian Federation

The family Trypanosomatidae is an extensively studied group of flagellates parasitizing a wide range of hosts, including vertebrates, arthropods, leeches, plants, and even ciliates. The interest to this taxon is stipulated by the significant impact of human pathogens, such as *Trypanosoma brucei*, *T. cruzi*, and *Leishmania* spp. Trypanosomatids are categorized into monoxenous (have a single host) and dixenous (alternate between two hosts, one of which is termed a vector). Insects are the most frequent hosts of monoxenous species and commonly act as vectors for dixenous ones. In addition to practical implications, these flagellates exhibit a

multitude of traits garnering research interest: polycistronic gene transcription, trans-splicing, extensive RNA editing, glycosomes, etc. Some members of the family feature additional peculiarities such as an unconventional genetic code, intracellular bacterial symbionts, or two flagella attached to each other.

The growing role of genomics has significantly influenced the advancement of the trypanosomatid studies in the last two decades. Starting from the human-pathogenic species such studies soon expanded into the area of monoxenous flagellates and dixenous plant-infecting *Phytomonas* spp., which allowed comparative analyses highlighting numerous lifestyle-related changes in gene repertoire.

Recently the list of the available genomic sequences has significantly increased so that at least one species from each trypanosomatid genus as well as most subgenera of trypanosomes. Based on this nearly comprehensive dataset we performed a phylogenomic inference, resulting in the clarification of previously uncertain phylogenetic relationships for a number of trypanosomatid lineages. For example, we demonstrated the paraphyly of the genus *Crithidia* in respect to *Leptomonas* and *Lotmaria*, relatedness of *Vickermania* and *Jaenimonas,* sister relationship of African salivarian trypanosomes with the poorly studied subgenus *Squamatrypanum*, parasitizing squamate reptiles and small mammals.

We offer the obtained tree as a framework to address questions on the evolution of various traits in trypanosomatids and provide examples of using it for this purpose (evolutionary distribution of the hydrogen peroxide -decomposing enzyme catalase and patterns of kinetoplast cryptogene editing).

Poster 15 :

Poster 16 : Deploying a new reagent and method for detecting dormant and dead malaria parasite in the sample analysis

Porntida Kobpornchai, *Mahidol University, Faculty of Medicine, Siriraj hospital, Department of Parasitology*

P Kobpornchai[2]; K Kulkeaw[1];
[1] Siriraj Integrative Center for Neglected Parasitic Diseases, Department of Parasitology, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, Thailand; [2] Siriraj Integrative Center for Neglected Parasitic Diseases, Department of Parasitology, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, Thailand

Artemisinin remains effective as the first line drug; however, according to epidemiological studies in Southeast Asia, delay clearance of the parasitized erythrocytes has been documented, emphasizing artemisinin resistance surveillance. Under a light microscope, artemisinin-exposed *Plasmodium falciparum* form a dead-like morphology but capable of re-entering the intraerythrocytic growth, establishing a hypothesis of dormant parasites. However, the existence of dormant form of *P. falciparum* in erythrocytes remain controversial in part due to morphological similarity between dormant and pyknotic parasites. A lack of tools to discriminate both stages further limit our ability to monitor the dormancy of *P. falciparum*. Here, we demonstrated the efficacy of a new laboratory assay that is compatible with the artemisinin susceptibility test. This research brings a concept of cell permeability to develop a trio fluorochrome alternative to the ring-stage survival assay (RSA). As a result of the use of two different types of DNA-binding fluorophores, the parasite membrane loss selective activity after artemisinin exposure, allowing enter of cell impermeant fluorophore. By contrast, the membrane of the infected erythrocyte at the ring-stage retains a selective cell membrane regardless of the parasite survival status. Together with mild detergent, we could identify nonviable parasites would lose such selective membrane properties similar to the process of cell apoptosis or necrosis. This approach clearly confirmed that the dormant

parasites retained an intact membrane and resumed growth within 6 days, whereas those that lost the parasite membrane failed to regrow, ensuring an indicator for truly dormant parasites. Therefore, the use of this proposed method effectively discriminated between dormant and dead parasites in an RSA. In conclusion, this assay is a simple and rapid technique which makes a significant contribution to advancing diagnosis and treatment of malaria.

Poster 17* : Immunological profile indicates diminished health in hybrid mice regardless of infection status

Fay Webster, *PhD Candidate, Humboldt-Universität zu Berlin Faculty of Life Sciences*

F Webster[1]; LL Bednář[1]; S Rausch[2]; E Heitlinger[1];
[1] Humboldt University of Berlin, Germany; [2] Freie Universität Berlin, Germany

We study host-parasite coevolution in the European house mouse hybrid zone, where interbreeding of *Mus musculus domesticus* and *Mus musculus musculus* can lead to extreme transgressive phenotypes in hybrids. Increased resistance to various parasites, including the protozoan parasite Eimeria spp., is a transgressive phenotype that attracted interest because it might be linked with differences in fitness. However, it is questionable whether resistance is a fitness component per se, as host health might be correlated with the ability to reduce acute infection load in any direction. It is thus essential to evaluate not only resistance but the ability of hybrids to maintain health in general and during infection.

Our study aimed to quantify the health impacts of infections with Eimeria spp. in the house mouse hybrid zone. The cross-sectional design of our established field study does not allow us to measure health impacts directly. We therefore developed an approach to infer and extrapolate health impact from laboratory infections. In a longitudinal, experimental setup, we developed a random forest model trained on immune gene expression data to predict the maximum weight lost during an infection as a proxy for the host's health. Trained on experimental laboratory infections closely replicating field infections, our model achieved high accuracy and recall in cross-evaluation. We then applied the model to a dataset of 336 mice collected in the field and inferred the health impacts of *Eimeria* spp—infections on wild mice.

When infected with *Eimeria*, mice from the natural environment show the inflammatory phenotype indicative of weight loss in the laboratory. Hybrid mice display an inflammatory phenotype indicative of a more detrimental health impact than their pure conspecifics. Interestingly, the hybrids' more inflammatory immune status is not dependent on infection but is primarily observed in uninfected individuals.

Hybrid mice show an increased baseline of inflammatory responses, which might lead to higher resistance and the detection of fewer parasites. The fitness consequences of this elevated immune reaction, if any, are unclear but likely rather negative than positive. The system offers the opportunity to improve our understanding of the impact of genetic diversity and genetic incompatibilities on host-parasite interaction.

Poster 18* : Nematode-virus co-infection has a variable impact on the resistance and tolerance to *H.polygyrus* in genetically diverse mice

Insani Hubi Zulfa, *SRUC*

IH Zulfa[3]; M Chase-Topping[2]; I Dry[1]; A Doeschl-Wilson[1]; J Houdijk[3]; S Athanasiadou[3];
[1] The Roslin Institute, University of Edinburgh, UK; [2] Royal (Dick) School of Veterinary Studies and The Roslin Institute, University of Edinburgh, UK; [3] SRUC, UK

To combat infection, hosts develop two defence strategies: resistance, which is their ability to clear out the pathogen and tolerance, their ability to reduce the impact of the pathogen on their fitness. The aim of the study was to characterise the phenotypic variation of host resistance and tolerance to *Heligmosomoides polygyrus* in genetically diverse mice during co-infection (*H. polygyrus* and Theiler's murine encephalomyelitis virus). Three strains of mice were used: SJL mice (resistant to *H. polygyrus* but susceptible to TMEV), BALB/c mice (intermediate susceptible to both *pathogens)*, and C57BL/6 (susceptible to *H. polygyrus* but resistant to TMEV). Mice of each strain were infected with either a single (*H. polygyrus*) or two pathogens (*H. polygyrus* and TMEV) or were sham infected (n=15). Both pathogens were administered at the subclinical level (200 L_3 *H. polygyrus* in 0.2ml water and an avirulent TMEV at 10^6 pfu in 0.2 ml DMEM). Mice were euthanised at 14 dpi and 42 dpi to represent nematodes establishment period and nematodes clearance period. In the susceptible to *H. polygyrus* C57BL/6 mice, average daily gain (ADG 0.08 g/day) and FI (0.07g/day) was higher in Co-inf compared to Par, Vir, and Sham at 14 dpi (P<0.001). However, in the BALB/c mice, co-infection treatment resulted in 20% lower ADG compared to mice receiving *H. polygyrus*-only (P=0.034); 10% of ADG loss was observed in the resistant to *H. polygyrus* SJL mice (P<0.001). When compared to *H. polygyrus*-only infected C57BL/6 mice, co-inf resulted in 15% lower EIC (P<0.001), and 20% worm counts (P<0.05), whereas Co-inf BALB/c mice showed 5% elevated EIC (P<0.001) and 10% worm counts (P<0.05) compared to *H. polygyrus*-only mice. Co-inf did not have any impact on resistance traits in SJL mice. The trend was the same for all parasitological measurements at 42 dpi. Compared to *H. polygyrus* only SJL mice, Co-inf mice were 5% more tolerant (P<0.05). Co-inf C57BL/6 mice tended to be more tolerant (P=0.058) whereas Co-inf BALB/c mice tended to be less tolerant than their *H.polygyrus* only counterparts (P=0.051). Our data showed that the impact of co-infection with two intestinal pathogens resulted in significant variation on host resistance and tolerance to *H.polygyrus* in three inbred mouse strains. Contrary to expectation, mice susceptible to *H.polygyrus* benefited most from co-infection, as their resistance was improved compared to *H.polygyrus*-only counterparts. On the other hand, mice already resistant to *H.polygyrus* improved their tolerance following co-inf, compared to *H.polygyrus* only mice. The underlying mechanisms of these co-infection phenotypes are currently

Poster 19 : Restless nights when sick: ectoparasite infections alter rest-activity cycles of diurnal fish hosts

Dr Elissavet Arapi, *OneZoo CDT Team, Cardiff University*

EA Arapi[3]; M Reynolds[1]; AR Ellison[2]; J Cable[3];
[1] UK Health Security Agency, UK; [2] School of Natural Sciences, Bangor University, UK; [3] School of Biosciences, Cardiff University, UK

Circadian rhythms are timekeeping mechanisms responsible for an array of biological processes. Disruption of such cycles can detrimentally affect animal health. Circadian rhythms are also critical in the co-evolution of host-parasite systems, as synchronization of parasite rhythms to the host can influence infection dynamics and transmission potential. This study examines the circadian rhythms in behaviour and activity of a model fish species (*Poecilia reticulata*) in isolation and in shoals, both when uninfected and infected with an ectoparasite (*Gyrodactylus turnbulli*). Additionally, the rhythmical variance of parasite activity under different light conditions as well as rhythmical variance in parasite transmissibility were explored. Overall, infection alters the circadian rhythm of fish, causing nocturnal restlessness. Increased activity of gyrodactylids on the host's skin at night

could potentially contribute to this elevated host activity. Whilst migration of gyrodactylids across the host's skin may have caused irritation to the host resulting in nocturnal restlessness, the disruption in guppy activity rhythm caused by the expression of host innate immunity cannot be excluded. We discuss the wider repercussions such behavioural responses to infection might have for host health, the implications for animal behaviour studies of diurnal species as well as the application of chronotherapeutic approaches to aquaculture.

Poster 20 : Novel cystatin of *Trichinella spiralis* (TsCstN) ameliorates ovalbumin (OVA)-induced lung inflammation in asthmatic mouse model

Nipa Thammasonthijarern, *Faculty of Veterinary Medicine, Kasetsart University, Bangkok, Thailand*

N Thammasonthijarern[2]; P Adisakwattana[1];
[1] Mahidol University, Thailand; [2] Faculty of Veterinary Medicine, Kasetsart University, Bangkok, Thailand, Thailand

Asthma, a chronic disease affecting humans and animals, has recently become increasingly prevalent and steadily widespread. The alternative treatment of asthma using helminth infections or helminth-derived immunomodulatory molecules have been evaluated and demonstrated significant amelioration of disease severity index *in vitro* and *in vivo*. *Trichinella spiralis*, a parasitic nematode and its immunomodulatory molecules elicit a potential to relieve asthma and other immune-related disorders. Herein, we investigated the immunomodulatory function of recombinant *T. spiralis* novel cystatin (rTsCstN) in ameliorating acute inflammatory asthma disorders in murine model. Female BALB/c mice were sensitized using intraperitoneal injection of ovalbumin (OVA)/alum and subsequently challenged with intranasal administration of OVA alone or OVA + rTsCstN for three consecutive days, producing OVA-induced allergic asthma models. To evaluate the therapeutic efficacy of rTsCstN, the inflammatory cells and cytokines in bronchoalveolar lavage fluid (BALF) and OVA-specific Immunoglobulin E (IgE) levels in serum, were assessed. Histological alterations in the lung tissues were determined by hematoxylin/eosin (H&E) staining and eventually scored for the extent of inflammatory cells infiltration. The asthma mouse models challenged with OVA + rTsCstN demonstrated a significant reduction of eosinophils ($p < 0.01$), macrophages ($p < 0.05$), and cytokines tumor necrosis factor (TNF)-α ($p < 0.05$) and interferon (IFN)-γ ($p < 0.05$) in BALF when compared with the mice challenged with OVA alone. However, the levels of interleukin (IL)-4 and IL-10 remained unaltered. Histological examination revealed that the mice administered OVA + rTsCstN were less likely to have inflammatory cell infiltration in their perivascular and peribronchial lung tissues than those administered OVA alone. rTsCstN demonstrated immunomodulatory effects to reduce severe pathogenic alterations in the asthma mouse models, encouraging a viable alternative treatment for asthma and other immunoregulatory disorders in humans and animals in the future.

Keywords: asthma; novel cystatin (TsCstN); *Trichinella spiralis*; immunomodulatory molecule

Poster 23* : Elucidation of the life cycle of the trematode *Curtuteria arguinae* using molecular techniques, with insights into ecological relationships

Leslie Stout, *University of Bordeaux*

L Stout[1]; A Chambouvet[4]; G Daffe[3]; A de Montaudouin[2]; F Daramy[1]; X de Montaudouin[1];
[1] University of Bordeaux, France; [2] SEPANSO Aquitaine, France; [3] University of Bordeaux, CNRS, OASU, France; [4] CNRS, Sorbonne University, France

Knowledge of the life cycle of parasites is crucial for numerous reasons such as integrating them into food webs or for studying parasite transmission pathways. The common cockle (*Cerastoderma edule*) is parasitized by a diverse community of digenean trematodes of which the life cycles are elucidated, except for one. Indeed, the trematode *Curtuteria arguinae* can be found as metacercariae infecting cockles as their second intermediate host in southern Europe. Cockles and trematodes in the National Nature Reserve of Banc d'Arguin (Atlantic coast of France) were monitored for more than 20 years, revealing the constant presence of *C. arguinae* in cockles, sometimes reaching very high abundances (up to 850 metacercariae per cockle). However, despite previous efforts to identify its first intermediate host, *C. arguinae*'s life cycle remained unelucidated. Indeed, the typically low prevalence at this stage makes sampling several species of potential intermediate hosts and the recovery of trematode juvenile stages from these samples a massive undertaking with traditional methods (*i.e.* cercarial emission, dissection). Similarly, recovering adult stages in birds (potential final hosts) can be ethically problematic, especially when it concerns protected species.
Therefore, we used a non-invasive eDNA-type approach to detect *C. arguinae* in different samples collected in Banc d'Arguin. On the one hand, the investigation of the first intermediate host consisted in massively collecting the five dominant marine gastropod species in an infestation hotspot. Individuals were kept separately per species in a container with seawater for cercarial emission and DNA was extracted from the remaining fraction of the filtered seawater. On the other hand, we collected feces of individual seabird taxa to investigate the presence of eggs as a proxy of the birds' infestation as final hosts by adult worms. Using newly developed species-specific cox1 primers, we conducted quantitative PCR assays to test the presence of *C. arguinae* DNA in the samples. Finally, in parallel, we sampled the benthic macrofauna along the Banc d'Arguin to study its spatial distribution.
Out of 25 water samples containing five potential host species, all five replicates of one gastropod species tested positive for *C. arguinae*, while the other 20 samples remained negative. Then, the putative first intermediate host was tested by cercarial emission technique, which resulted in an average *C. arguinae* prevalence of 1,6 %. The molecular sequences obtained from cercariae were identical to those isolated from *C. arguinae* metacercariae in cockles. Concerning birds, out of 167 analyzed feces, 30% tested positive for *C. arguinae*, all positive samples (except one) belonging to a unique seabird species. Our results provided new essential information that allowed us to identify the first intermediate host and the final host of *C. arguinae*, hereby elucidating its complete life cycle for the first time. Our results demonstrate the usefulness of molecular techniques to identify potential host species in a non-destructive way before confirming with an integrative molecular and morphological approach. In addition, the benthic macrofauna sampling revealed that the first intermediate host of *C. arguinae* was most present and abundant in the same area which constituted the infestation hotspot in cockles. This shows that the spatial proximity between the first and the second intermediate hosts is of the utmost importance for parasite transmission, even for free-living larval stages, and supports the hypothesis that the spatial distribution of an upstream host strongly influences the spatial distribution of the parasite in a downstream host.

Poster 24 : Next generation sequencing reveals a single species is responsible for the first reported case of macrocyclic lactone resistant cyathostomins in the UK
Katie Bull, *University of Bristol*

K Bull[2]; J Hodgkinson[1]; KJ Allen[2]; J Poissant[3]; L Peachey[2];
[1] University of Liverpool, UK; [2] University of Bristol, UK; [3] University of Calgary, United States

Background: In recent years, resistance to the benzimidazole and tetrahydropyrimidine (PYR) anthelmintics in global cyathostomin populations, has led to reliance on the macrocyclic lactone drugs to control these parasites. Recently, the first confirmed case of resistance to both ivermectin (IVM) and moxidectin (MOX) was reported in the USA in yearlings imported from Ireland [1].

Methods: Faecal egg count reduction tests (FECRT) were performed to determine anthelmintic efficacy and egg reappearance periods (resistance= FECR < 95% lower credible interval (LCI) < 90%) in yearlings on four Thoroughbred studs. Next generation sequencing of the ITS-2 region was used to look at cyathostomin species composition pre and post treatment.

Results: Stud A yearlings had FECRs of 36.4-78.6% (CI:15.7-86.3) after 3 IVM treatments, 72.6% (CI: 50.8-85.2) after MOX, and 80.8% (CI: 61.9-90.0) after PYR. Mares on stud A had a FECR of 97.8% (CI: 93.3-99.9) and 98% (95.1-99.4) after ivermectin and moxidectin treatment, respectively. Resistance to MLs was not found in yearlings or mares on studs B, C or D with FECR after MOX OR IVM treatment ranging from 99.8-99.9% (95.4-100); although yearlings on studs B, C and D all had an egg reappearance period (ERP) of six weeks for MOX and stud C had a four-week ERP for IVM. *Cyathostomum catinatum* was found to be responsible for treatment failure on stud A to IVM and MOX. *Cylicocyclus nassatus* was found to be responsible for treatment failure on stud A to PYR.

Conclusions: In this study specific cyathostomin species were found to be responsible for resistance, highlighting the need for stringent parasitological quarantine procedures, and extensive surveillance of ML efficacy against cyathostomin populations in the UK to gauge the extent of the problem.

[1] Nielsen, M.K., Banahan, M., Kaplan, R.M., 2020. Importation of macrocyclic lactone resistant cyathostomins on a US thoroughbred farm. Int J Parasitol Drugs Drug Resist 14, 99-104.

Poster 25 : Warming effects on the life cycles and ecological impacts of two invasive parasitic copepods infecting native blue mussels

Dr Elli Rosa Jolma, *Royal Netherlands Institute for Sea Research*

E Jolma[1]; KM Wegner[3]; A Born-Torrijos[1]; H Heesterbeek[2]; A van Leeuwen[1]; D Thieltges[1];
[1] NIOZ Royal Netherlands Institute for Sea Research, Netherlands; [2] Utrecht University, Netherlands; [3] Alfred Wegener Institute – Helmholtz Centre for Polar and Marine Research, Coastal Ecology, Wadden Sea Station Sylt, Germany

The success and ecological impact of parasite invasions may be modulated by another anthropogenic stressor, climate change. Temperate coastal ecosystems are disproportionately affected by both and therefore at an elevated risk for the combined effects. Here, we study parasite dynamics in a temperate coastal ecosystem, using temperature experiments and two parasite species that share the blue mussel (*Mytilus edulis*) as their host. In controlled laboratory experiments in a range of temperatures (10°C-26°C), we investigated warming effects on the lifecycles of two species of invasive parasitic copepods (*Mytilicola intestinalis, Mytilicola orientalis*), and on the survival, growth, condition, and reproductive status of infected and uninfected native mussel hosts. The two species of parasites are closely related, but have different invasion histories in Northwestern Europe, including the Dutch Wadden Sea; *M. intestinalis* invaded the system 90 years ago while *M. orientalis* arrived only 20 years ago, originally inhabiting warmer climate regions. Our experiments

showed that in both parasites, an increase in temperature accelerated the development speed of both free-living and parasitic life stages, with the effect being most pronounced in the lower temperature range (10°C-14°C). Only the highest temperature (26°C) limited the egg development success of *M. intestinalis* and host entry success of both parasite species. Mussel survival decreased at 26°C but infection with either parasite species did not affect it. Infection with *M. orientalis* decreased mussel shell growth and infection with *M. intestinalis* lowered mussel condition indices, with these infection effects remaining uniform across temperatures. Mussel reproductive status was highest at the lowest temperatures (10°C-14°C) and infection with either parasite did not affect reproduction. These findings indicate that an increase in temperature enables a higher number of parasite life cycles to be completed per year, which can result in a higher infection pressure on hosts. Because infections affect host growth and condition, the increase in infection pressure is expected to lead to fewer resources for physiological functions such like growth, reproduction, and immune responses. Heatwave temperatures are likely to be harmful for both the host and *M. intestinalis*, while the more recent invader *M. orientalis* will be less impacted. These results foster our understanding of climate change impacts on invasive parasites and can inform ecosystem models that evaluate the impact of nonlethal parasites.

Poster 26 : Glycoengineering of *ex vivo* cultured *Schistosoma mansoni* adult worms using chemical mannosidase inhibitors

Noor Kuhlemaijer, *Leiden University Medical Centre*

E Kuhlemaijer[2]; A Van Diepen[2]; T Veldhuizen[2]; B Hulme[1]; J Forde-Thomas[1]; G Rinaldi[1]; K Hoffmann[1]; C Hokke[2];
[1] Aberystwyth University, UK; [2] Leiden University Medical Centre, Netherlands

Schistosomiasis is a neglected tropical disease (NTD) caused by blood flukes of the genus *Schistosoma*. Worldwide, at least 250 million people are infected with *Schistosoma* parasites and an estimated 779 million people are at risk of infection. Although the infection can be treated with praziquantel (PZQ), this drug is ineffective against juvenile stages and does not prevent reinfection. Hence, there is an urgent need for prophylactic vaccines and to identify new drugs that target different life-stages of the parasite.
In order to develop new vaccines and drugs, understanding of the intricate parasite-host interaction is crucial. The parasite is known to modulate the host's immune response by expressing a variety of antigens and releasing specific molecules. Several of the parasite-derived molecules are glycoconjugates consisting of glycans covalently linked to proteins or lipids. Previously, it has been shown that the glycan component of these glycoproteins and glycolipids may be crucial in provoking a polarised host immune response. Additionally, glycans play essential roles in diverse eukaryotic cell processes such as protein folding and intercellular communication. Taken together, glycans could be pivotal in shaping parasite-host interactions and parasite development.

Our research aims to develop glycoengineered living adult *Schistosoma mansoni* worms and subsequently employ these to study how different glycans might contribute to the mammalian immune responses against parasite-derived molecules. Glycoengineered worms were generated in *ex vivo* cultures using kifunensine and swainsonine, chemical compounds that inhibit specific α-mannosidases involved in the N-glycosylation pathway. We show that the compounds alter the N-glycosylation profile from complex type glycans to hybrid and oligomannosidic N-glycan forms, as measured by MALDI-TOF MS. Notably, the shift in N-glycan forms appeared to gradually increase over time. Additionally, we used phenotypic analyses to assess worm motility and morphology in glycoengineered worms. WHO-TDR motility scoring confirmed that these worms exhibited normal movement when compared to untreated worms. Furthermore, we stained glycoengineered worms with live dyes selective for cell nuclei, plasma membranes and F-actin to study worm morphology. Confocal

fluorescence microscopy revealed no apparent changes in the oral and ventral sucker, oesophagus, testes or ovary and tegument.

Overall, these results show that the N-glycosylation profile of live *S. mansoni* worms can be altered *ex vivo*, without apparent changes in worm motility or morphology. Hereupon, glycosylated extracts of the glycoengineered worms could be used to stimulate immune cells and study the induced immune response. Hence, we conclude that glycoengineered worms are a promising tool to further elucidate the mammalian immune response against worm-derived molecules.

Poster 27 : Evaluation of multiple tegument proteins and FhTLM as vaccines against *Fasciola hepatica* in cattle

Prof Terence (Terry) William Spithill, *La Trobe University*

TW Spithill[1]; H Toet[1]; V Rathinasamy[1]; G Zerna[1]; G Anderson[2]; R Dempster[2]; T Beddoe[1];
[1] *La Trobe University,* Australia; [2] *Virbac (Australia) Pty Ltd, Australia*

Multiple studies have attempted to develop a vaccine to control *Fasciola hepatica* infection in cattle and sheep, evaluating a number of *F. hepatica* proteins. Despite being a logical vaccine target, surface tegument proteins have not yet been studied. Here we report results from 5 trials in cattle evaluating 9 recombinant tegument proteins (Tetraspanins (TSP) 2 and 3; Annexins (Anx) 2, 3 and 8; novel Tegument proteins (Teg) 1, 5, 22 and 25, as well as the Transforming growth factor beta (TGF-β) like homologue (termed FhTLM) protein from *F. hepatica*, as vaccines, both alone or as double/triple/quadruple combinations. Tetraspanin 2 was also evaluated fused to the *E. coli* heat-labile entero-toxin B subunit LTB adjuvant (termed LTB-TSP2) using intranasal vaccination of cattle. Glutathione S-transferase (GST), a known efficacious vaccine protein, was also tested in combination with TSP2, Anx2 and Anx3/Anx8. The FhTLM protein was evaluated in combination with GST only. In total, 17 different vaccine groups were assessed.

Groups of female Angus/Angus cross cattle (n=6-7/group; age 6-18 months) were vaccinated with proteins formulated in Freund's adjuvant as a means to screen a large number of proteins, in an attempt to identify an optimal vaccine combination. In one trial, a nanoparticle commercial adjuvant was tested. After experimental infection with metacercariae, we analysed total IgG and IgG1/IgG2 subtype antibody responses, faecal egg counts and adult fluke numbers recovered from livers at completion of the trial. We also assessed the fraction of animals which showed the lowest fluke numbers (ie. less than the lowest value in the Control group) as another indicator of protection since economic loss is associated with low fluke counts (<30 flukes). A high % of animals with low fluke counts will result in lower herd production losses which is the key parameter for a commercial vaccine.

There was considerable variation in vaccine efficacy between the various proteins tested, using single or combination vaccines. There were no reproducible negative associations between IgG1/IgG2 responses to individual vaccine proteins and low fluke counts. Some reductions in FEC values were observed but were variable between groups. Neither LTB-TSP2 or TSP2 alone, nor combinations of Teg1/5/22/25, induced significant protection. Significant reductions in mean fluke numbers/group (38-48%) were observed in 4 vaccine groups with various combinations of TSP3, Anx2, Anx3 or Anx8 as well as with FhTLM+ GST. However, there was variation between trials in vaccine efficacy with certain combination vaccines. Vaccine efficacy, based on % reductions in mean fluke counts, was closely associated ($r^2 = 0.915$) with the fraction of animals in each group showing fluke counts less than the lowest value in control groups. The data suggest that, under our experimental conditions, 90-100% of animals show relatively low fluke counts with a vaccine efficacy of only 43-48%.

The results indicate that tegument proteins and FhTLM+ GST are potential candidates for a commercial fluke vaccine in cattle and the data will be discussed in the context of how best to assess fluke proteins as vaccines going forward.

Poster 28 : Evolutionary analysis of *Fasciolopsis buski* isolated from a human in India

Dr Aradhana Singh, *University of Edinburgh*

A Singh[1]; S Chaurasiya[2]; T Banerjee[2]; A Tiwari[2];
[1] University of Edinburgh, UK; [2] Banaras Hindu University, India

Background: Fasciolopsiasis, caused by the giant intestinal fluke, *Fasciolopsis buski* (*F. buski*), is a significant cause of morbidity and mortality in South and South-east Asia. This parasite has been widely reported, often in case reports based on morphometric identification or incidental identification of endemic foci. However, genetic analysis of this parasite is limited. Importantly, *F. buski* is found in multiple hosts species, including pigs, and the genetic diversity of this parasite isolated from these hosts is largely unknown.

Methodology: Following endoscopy, worms were isolated from the duodenum of a 50-year-old male in Uttar Pradesh, India. Morphological analysis presumptively identified these worms to be a giant intestinal fluke; leaf-like structure, 3-4cm in length, anterior end broad without conical projection, and ventral sucker close to the oral sucker. DNA was then extracted for 28s rDNA and ITS2 amplification and sequencing. Consensus sequences were generated using Bioedit software and species identification performed using blastn. Multiple sequence alignment with query-anchored dots for analysis was done for *F. buski* species to understand intra species and interspecies homology. Phylogenetic analysis was done using clustalW program of MEGA software and the evolutionary history was inferred using the Neighbor-Joining method.
Results: The parasite showed close similarity to an isolate from pig in one of the north-eastern states in India, Meghalaya, thus affirming the zoonotic cross-transmission of this parasite. The intra species genetic variation for 28S rDNA of *F. buski* was 1-3% from the isolates of India, 5% from Vietnam and 8-11% with other related trematodes. For ITS2 the intra-species genetic variation was 1-2% from isolates of India while 17% from Vietnam and 3-28% among other trematodes. On phylogenetic analysis, *F. buski* isolated from humans or pigs from India were closely related, as compared to those from Vietnam and China, which were grouped into separate clades.

Conclusions: Using a fluke isolated from a human patient, we found that this parasite matched the sequence of *F. buski* found in Indian pigs and that these fluke in India were genetically distinct from those found in Vietnam, at least by 28S rDNA and ITS2 region. Therefore, *F. buski* from India may represent a distinct clade different than those Vietnam and China, which are more closely related. Future studies are needed to elucidate the mechanisms and consequences of zoonotic transmission of *F. buski* in India.

Poster 29* : Filarids with zoonotic potential in non-coastal areas: first epidemiological surveillance in Acre, northern Brazil

Marianna Laura Elis Chocobar, *Faculdade de Medicina Veterinária e Zootecnia*

ML Chocobar[3]; LG Zanfagnini[2]; LC Xavier[2]; R Panarese[1]; W Weir[1]; EM Schmidt[3]; AD Pacheco[2];
[1] University of Glasgow , UK; [2] Federal University of Acre, Brazil; [3] São Paulo State University, Brazil

Canine dirofilariasis, commonly known as heartworm disease, is a zoonotic mosquito-borne affliction found in many parts of the world. It is caused by *Dirofilaria immitis* and *Dirofilaria repens* and is transmitted by

mosquitoes of the genera *Aedes*, *Culex* and *Anopheles*. Relatively few studies have been conducted to date to evaluate the prevalence and distribution of these parasites in Brazil, particularly in the interior of the country. For this reason, it is currently unknown which filarial species circulate among the canine populations in Acre, a non-coastal state in northern Brazil. To address this knowledge gap, the present study aims to characterize the zoonotic filarial species present in a large cohort of dogs domiciled in Rio Branco, the capital of Acre state. Four hundred and forty-four dogs were enrolled in the study, which was conducted at the Veterinary School Clinics of the Federal University of Acre (CVE-UFAC) and the Department of Zoonosis Control of Rio Branco (DCZ). Each dog underwent a clinical examination at which time blood and serum samples were collected. Whole blood samples were analysed using the modified Knott's test and Woo's test. Microfilaeremic dogs' sera were screened using the Snap 4Dx Plus® rapid test (IDEXX Laboratories®). Of the 444 dogs, 59 (13.28%) were found to be microfilaraemic. The microfilariae (mfs) showed an average length and width of 247.53 ± 16.32 and 4.80 ± 0.48 μm, respectively, and were morphologically identified as *Acanthocheilonema reconditum*. Among the positive dogs, only three (5.08%) tested positive for *D. immitis* using the Snap 4Dx Plus® test. Further analyses will be undertaken on all blood samples using molecular methods (i.e. qPCR and Sanger sequencing) to detect *Dirofilaria* spp. and *Wolbachia* DNA. It is anticipated that these molecular tests will reveal a higher prevalence of infection compared to the gross parasitological methods. Importantly, this study reveals the for the first time, the presence of potentially zoonotic filarial species circulating in the Acre dog population, thus highlighting the need for ongoing epidemiological surveillance across under-studied areas of the country. Only with this information can the disease threat be accurately assessed and interventions designed to mitigated the risk to animal and human health.

Poster 30 : The RNA Virome of human and animal parasitic nematodes

Shannon Quek, *Liverpool School of Tropical Medicine*

S Quek[1]; A Hadermann[3]; Y Wu[1]; L DeConinck[2]; S Hegde[1]; A Steven[1]; EJ Lacourse[1]; SA Ward[1]; S Wanji[4]; G Hughes[1]; I Patterson[5]; S Wagstaff[1]; J Turner[1]; E Heinz[1]; J Mathijnssens[2]; R Colebunders[3]; MJ Taylor[1];
[1] Liverpool School of Tropical Medicine, UK; [2] KU-Leuven, Belgium; [3] University of Antwerp, Belgium; [4] University of Buea, Cameroon; [5] Brock University, Canada

From screening published transcriptome data, we have identified 83 different virus genomes and virus-like sequences within 28 species of parasitic nematodes. As these parasites infect >1.5 billion people and animals globally, this finding may have broad-ranging impacts for almost a quarter of humanity and economically important livestock. Our analysis shows extensive diversity and a conserved global spread of virus/nematode associations across multiple continents suggesting an ancestral acquisition event and host-virus co-evolution. Focussing on the viruses of the filarial parasites *Brugia malayi* (Togaviridae, related to Alphaviruses) and *Onchocerca volvulus* (Rhabdoviridae, related to Lyssaviruses) reveals an intimate relationship, with the viruses localising within the reproductive tracts of both species, and being detectable in all laboratory isolates (*B. malayi*) as well as multiple isolates across Sub-Saharan Africa (*O. volvulus*). Viruses of *B. malayi* were also found to localise within cuticular inflations, or 'bosses' that were previously described in the literature, but with no known purpose. Additionally, we were able to show that the final mammalian host of these filarial parasites elicit antibody responses against the viruses demonstrating exposure to host immunity. This observation, as well as the intimate, chronic and life-long exposure to vertebrate tissues that typically characterise parasitic nematode infections, raise questions as to the role this previously hidden virome plays in modulating anti-parasite immunity, and their potential for influencing parasite biology and disease pathogenesis.

Poster 31* : Bayesian network analysis to determine the potential causal association between helminth infection and childhood stunting

Isobel Gabain, *Royal Veterinary College*

I Gabain[1]; J Webster[1]; TR Rosenstock[2]; BY Yet[3];
[1] Royal Veterinary College, UK; [2] Alliance Bioversity International and CIAT, France; [3] Middle East Technical University, Turkey

Childhood stunting is defined by the World Health Organization as falling more than -2 standard deviations below the height-for-age Child Growth Standards median and affects approximately 149.2 million children under five years globally. Soil-transmitted helminths (STH) and schistosomes are considered potentially causative in the mechanistic pathway to stunting. Our research aims to identify potential causal associations between maternal helminthic infection during pregnancy and low birthweight (LBW) babies, and child helminthic infection and physical stunting. Identification of risk factors for stunting mainly relies on logistic regression models, which often assume independence between variables and cannot demonstrate causality. Directed Acyclic Graphs (DAGs) are visual tools for representing probabilistic relationships between variables (exposures, outcomes, and confounders) explicitly. Here, we built a DAG using the systematic Evidence synthesis for constructing directed acyclic graphs (ESC-DAGs) method. Bayesian Network Analysis (BNA) is a method used to infer causal relationships via specification of conditional probability distributions for variables in a DAG based on observed data. We are using BNA to explore the potential aforementioned associations using open-access Demographic Health Survey (DHS) data from selected countries within Africa and Asia. Our analysis involves pooling data across multiple years within individual countries, and collectively across sub-Saharan countries. Further, we are conducting analyses to determine potential improvements in outcomes (LBW and stunting) before and after the conception of mass drug administration programmes during the 2000s. Results to date are discussed in terms of their theoretical and applied implications, primarily in terms of potential actionable insights regarding future deworming recommendations.

Poster 32 : Molecular prevalence of *Sarcocystis* spp. and *Toxoplasma gondii* in slaughtered equids in North Tunisia

Safa Amairia, *National school of veterinary medicine of Sidi Thabet*

S Amairia[4]; M Jbeli[1]; S Mrabet[2]; LM Jebabli[3]; M Gharbi[4];
[1] Health and Veterinary Control Division, Ministry of Defense, Ksar Saïd military base, 1029 Tunis, Tunisia; [2] Department of Hygiene, Municipality of Bizerte, Tunisia; [3] Tunis Abattoir, Health, Hygiene and Environment Service, Ministry of Interior, 1089 Montfleury, Tunisia; [4] Laboratoire de Parasitologie, Univ. Manouba, Institution de la Recherche et de l'Enseignement Supérieur Agricoles, École Nationale de Médecine Vétérinaire de Sidi Thabet, 2020 Sidi Thabet, Tunisia

Background: *Sarcocystis* spp. and *Toxoplasma gondii* are obligate protozoan parasites infecting a large range of wild and domestic animals including equids. Although these pathogens have a wide host range among vertebrates, there is limited understanding regarding their infection in equids. This study aimed to gain knowledge about the public health risk related to these parasitosis by investigating the infection prevalence in slaughtered equids with *Sarcocystis* spp. and *T. gondii* in the slaughterhouses of Tunis and Bizerte, located in Northern Tunisia.

Methods: A total of 184 slaughtered equids from slaughterhouses of Bizerte and Tunis located in Northern Tunisia, were examined for meat infections with *Sarcocystis* spp. and *T. gondii* by PCR. The infection

prevalences were compared using the Chi-square Mantel-Haenszel test (Epi Info). Risk factors were evaluated using stratified odds ratio.

Results: Infections prevalence with *Sarcocystis* spp. and *T. gondii* were 38% (95%CI: 31-45) and 39.7% (95% CI: 32.6-46.7), respectively. The highest infection prevalence of *Sarcocystis* spp. was observed in donkeys (48.6%; 95%CI: 37.3-60) followed by mules (32.8%; 95%CI: 21.3-44.3), and horses (28.3%; 95%CI: 15.2-41.2) (p = 0.04). Similarly, the highest infection prevalence of *T. gondii*, was observed in donkeys (66.2%; 95%CI: 55.4-77), followed by mules (18.7%; 95%CI: 9.2-28.3), and horses (26.1%; 95%CI: 13.4-38.8) (p<0.001). The co-infection prevalence was estimated to 17.4% (95%CI: 11.9-22.9).

Conclusion: Taking into consideration that humans can be infected following consumption of infected equid's meat with *T. gondii* and/or some *Sarcocystis* species, it is of major importance to assess the risk of human infection. Thus, further studies are needed for a better understanding of the epidemiology of these zoonoses.

Poster 33 : Experimental infection of mice with *Trichobilharzia franki*, the major causative agent of swimmer's itch in Europe

Dr Tomas Machacek, *Assistant Professor, Charles University, Prague*

T Macháček[1]; J Procházka[1]; A Revalová[1]; M Majer[1]; B Šmídová[1]; R Leontovyč[1]; P Horák[1];
[1] *Charles University, Prague, Czechia*

Cercarial dermatitis (CD), also known as swimmer's itch, is a waterborne allergic disease caused by avian schistosome larvae penetrating mammalian skin. *Trichobilharzia franki* has been identified as a major contributor to human CD cases in Europe. However, little is known about its interactions with mammals, as previous research focused on less prevalent species like *T. regenti* and *T. szidati*. To address this gap, we infected C57BL/6J female mice with *T. franki* cercariae collected during human CD outbreaks in Czechia in July–August 2023. Mice were sacrificed at 2 and 7 days post-infection (dpi), and parasitological, histopathological, and immunological analyses were performed. In infected pinnae, migrating schistosomula and extensive epidermal lesions were observed at both time points. Additionally, the parotid lymph nodes exhibited significant enlargement. Surprisingly, schistosomula were not detected in the lungs, where visceral schistosomes typically lodge; instead, haemorrhages were observed at 2 dpi, followed by eosinophil-rich pulmonary lesions at 7 dpi. Infected mice displayed spleen enlargement, and splenocytes, when restimulated with *T. franki* cercarial homogenate, secreted a mixture of Th1/Th2/Treg cytokines robustly. Notably, splenocytes also reacted to protein lysates from other avian schistosome species, indicating significant cross-reactivity. No parasite-specific serum IgG was detected at 7 dpi, suggesting limited utility of serological diagnostics in early infections. In summary, our study provides novel insights into the migration, pathogenicity, and immunobiology of *T. franki* in mice. These findings contribute to a better understanding of the implications of cercarial dermatitis caused by this species.

Acknowledgment: This study was supported by the Czech Science Foundation (GA24-11031S): "Swimmer's itch: From detection to protection".

Poster 34* : Assessing anthelmintic resistance in gastrointestinal nematodes of Scottish dairy calves

Paul Campbell, *University of Glasgow*

P Campbell[1]; A Forbes[1]; J McIntyre[1]; R Laing[1]; K Ellis[1];
[1] School of Biodiversity, One Health and Veterinary Medicine, University of Glasgow, UK

Anthelmintic resistance is increasingly reported in cattle worldwide, although reports from Europe and the UK are more limited. This study aimed to evaluate the efficacy of three of the most widely used anthelmintics in cattle (fenbendazole, ivermectin, and moxidectin) against naturally acquired gastrointestinal nematode (GIN) infections using the faecal egg count reduction test.

A total of 210 first grazing season spring-born calves were enrolled from four Scottish farms. Each treatment group consisted of 15-19 individuals. Post-treatment faecal egg counts were performed 14-15 days after treatment. The resistance status was evaluated based on the reduction in arithmetic mean faecal egg count and the lower and upper 95% confidence limits. Reduced efficacy where the criteria for susceptible was not met was observed in 9/11 treatment groups, of which four were classed as resistant. The remaining five groups were classed as inconclusive where neither criterion for susceptible nor resistant were met.

All enrolled farms exhibited reduced efficacy of ivermectin and moxidectin. Ivermectin resistance was confirmed on two farms, one of which was also confirmed to be moxidectin resistant, and the other fenbendazole resistant. The ivermectin and moxidectin resistant farm also harboured bovine lungworm where macrocyclic lactone treatment was ineffective.

Reduced efficacy to anthelmintics was widespread on the sampled dairy farms, with resistance to fenbendazole, ivermectin, and moxidectin detected. British cattle rearing relies on pasture-based production, maximising the contribution of grazing to the diet. Effective GIN control is critical in these systems and to-date has been dependent on efficacious anthelmintics.

Poster 35 : Heterogeneous glycosylation of proteins from *Fasciola hepatica* invasive stage reveals higher complexity in parasite-host interactions

Dr Carolina De Marco Verissimo, *National University of Ireland Galway*

C De Marco Verissimo[2]; K Cwiklinski[1]; J Nilsson[3]; E Mirgorodskaya[3]; C Jin[3]; NG Karlsson[4]; JP Dalton[2];
[1] Institute of Infection, Veterinary and Ecological Sciences, University of Liverpool, UK; [2] Centre for One Health and Ryan Institute. University of Galway, Ireland; [3] Proteomics Core Facility, Sahlgrenska Academy of Science, University of Gothenburg, Gothenburg, Sweden; [4] Department of Life Science and Health, Faculty of Health Science, Oslo Metropolitan University, Oslo, Norway

Fasciola hepatica is a parasitic trematode that uses glycosylated excreted-secreted (ES) and surface molecules to interact with host cells and tissues, and to evade damage caused by cellular and immune responses during host invasion. Despite the unknown glycosylation state of many of the ~100 different proteins found in the ES of the immature invasive stage of *F. hepatica* (NEJs), several are extensively used as diagnostic and vaccine targets. To develop more effective strategies against fascioliasis, information on the glycosylation profile of individual NEJs proteins is critical. In this study, we used a combination of glycan, glycopeptide, and proteomic analyses, along with bioinformatics tools, to identify the glycosylation status of individual *F. hepatica* NEJs proteins. Our results identified 123 glycoproteins in NEJs extracts, 71 of which were in the ES. We mapped 367 glycopeptides and all the 1,696 *N*-glycan forms and 37 *O*-glycan forms to their respective protein and glycosites, revealing a high degree of heterogeneity in the glycosylation of *F. hepatica* NEJs proteins (i.e., in average, 14 different glycan forms can be used to modify each glycoprotein). Unique glycan motifs, such as PC and multi-PC terminals, and xylosylated *O*-glycan cores, were found in 25 distinct NEJs glycoproteins, including cathepsin peptidases B and L, which are well-known vaccine and diagnostic targets. Furthermore, many parasite proteins carried highly truncated *N*-glycans and structures with undefined linkages that could not be

assigned (i.e., $HexNAc2Hex_4dHex_1$), and the roles of which in parasite infection are largely unknown. These structures modify glycoproteins that are excreted-secreted or predicted to be membrane-bound, suggesting that they play key roles in NEJs interactions that command host invasion. Our findings demonstrate that *F. hepatica* NEJs generate great protein variability via glycosylation and highlight that the larvae extracts are far more complex than anticipated by proteomic analysis. This data provides a foundation for improving diagnostics and vaccine development to control fascioliasis.

Poster 36 : Endoparasitic rotifers in UK earthworms: new host and locality records for *Albertia vermiculus* Dujardin, 1838 and *Balatro calvus* Claperéde, 1867 (Monogononta: Dicranophoridae)

Dr Zikmund Bartonicek, *Natural History Museum London / Institute of Parasitology, Biology Centre CAS*

Z Bartonicek[2]; I Martinek[2]; EM Shilland[1]; ME Spencer Jones[1]; JS Hernández-Orts[2];
[1] Natural History Museum, UK; [2] Institute of Parasitology Czech Acadamy of Science, Czechia

Introduction: Rotifers, or wheel animals, are a paraphyletic assemblage of ~2,000 species with remarkably diverse morphologies, ecologies and reproductive modes. Most rotifer species have a predominantly free-living lifestyle in aquatic and terrestrial environments. Strikingly, an endoparasitic lifestyle has been reported for a few species, mostly belonging to the genera *Albertia* and *Balatro* (Monogononta: Dicranophoridae). These parasitic rotifers live in the guts of earthworms or freshwater oligochaetes. In the UK, a single record of an endoparasitic rotifer, *Albertia vermiculus* Dujardin 1838, was reported from the earthworm *Allolobophora caliginosa* (Savigny) in Cardiff more than sixty years ago, with no more parasitic rotifers recorded since. In this study, we provide new data on endoparasitic rotifers of earthworms in the UK.

Methods: In total, 289 earthworms were collected between March 2023 and February 2024 across two sites in Newport, Wales (n = 209) and two sites near Cudham, London (n = 80). Worms were examined for rotifer infections by dissection under a binocular microscope. All encountered parasitic rotifers were identified using light microscopy. High-resolution microphotographs of rotifers were taken, with samples retained for later morphological and molecular analysis.

Results: Two species of endoparasitic rotifers were identified. In Wales, *Albertia vermiculus* was found in six earthworms (prevalence: 3.6%, mean intensity: 5.2 rotifers per infected worm). Here, all rotifers were collected from *Aporrectodea longa* (Ude). In earthworms from London, the rotifers *Balatro calvus* Claperéde, 1867 (prevalence: 7.5%, mean intensity: 5.5 rotifers per infected worm) and *A. vermiculus* (prevalence: 5%, mean intensity: 5.8 rotifers per infected worm) were found. *Eiseniella tetraedra* (Savigny) served as host for *B. calvus,* and *Aporrectodea longa* and *A. caliginosa* (Savigny) were the hosts of *A. vermiculus* in London.

This provides the first UK record for *B. calvus.* Furthermore, *A. longa*, *A. caliginosa* and *E. tetraedra* represent new host records for parasitic rotifers.

Conclusions: The results and samples generated here not only inform our understanding of the parasitic rotifer diversity in the UK but will also be crucial for subsequent planned evolutionary studies into the evolution of parasitism within the Rotifera.

Poster 38* : Effect of an infected blood meal on the survival of mosquitoes

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

Despite considerable efforts to control malaria transmission across the world, it continues to be a serious burden in many communities. Mosquito survival beyond the parasite's incubation period is crucial to sustain transmission. However, there is a substantial overlap between the extrinsic incubation period of *Plasmodium falciparum* and the lifespan of its vector, both of which are known to be affected by external cues like temperature. This suggests that many mosquitoes die before becoming infectious.

Many studies have investigated the effect of malaria parasites on mosquito lifespan. However, while some report parasite-induced mortality, some others do not. Many of these studies ran for limited time periods or explored unnatural parasite-vector combinations. Moreover, most studies investigating the effect of temperature on mosquito survival use different constant temperatures. Some authors have proposed that these parts of the experimental design may produce biased results that are not representative of the dynamics in wild mosquito populations.

Here, we use parametric survival models to describe the effect of an infected blood meal, mosquito species, and temperature oscillations on mosquito survival. Describing how these factors interact to affect mosquito lifespan could improve the ability of transmission models to make estimations of disease incidence under different climate change scenarios.

Poster 39 : The genetic basis of Drosophila-trypanosomatid interaction

Dr Venera Tyukmaeva, *University of Liverpool*

VI Tyukmaeva[2]; H Al Ghafli[1]; AJ Betancourt[2]; SM Barribeau[2];
[1] University of Glasgow , UK; [2] Department of Evolution, Ecology and Behaviour, Institute of Infection, Veterinary, and Ecological Sciences, University of Liverpool, UK

The overwhelming majority of trypanosomatid research focuses on the few species of medical relevance, however, the vast majority of trypanosomatids infect wild animal populations only. Recently, trypanosomatids have been discovered to be common insect parasites in the wild, including different species of *Drosophila*, which presents a great opportunity for insect-trypanosomatid research. *Jaenimonas drosophilae*, is a natural and virulent trypanosomatid parasite of *Drosophila*, that decreases fecundity and causes pupal mortality in *D. melanogaster.* Here, we investigate the genetics of diptera-trypanosomatid infection studied from both the host and parasite sides. To unveil the host response to the infection, we performed screening of ~140 *Drosophila* Genetic Reference Panel lines for susceptibility to the *J. drosophilae* which showed a wide variation in the parasite susceptibility, and further performed GWAS to investigate the genetic basis of the host response. To reveal the how the parasite overcomes *Drosophila* gut defences and establishes in the host, we sequenced *J. drosophilae* genome with PacBio and performed a transcriptome study to compare the parasite gene expression between the parasite during establishment in *Drosophila* gut and *in vitro*. The analysis of differentially expressed genes of parasite in the gut compared to *in vitro* log growth phase showed GO terms enrichment for metal ion binding pathway, and other genes involved in various processes such as ATP-binding and microtubule movement. Our study shows that the *Jaenimonas-Drosophila* system has a potential to be a powerful model for investigating the effects of trypanosomatids on insect hosts, and for further understanding insect immunity and host-parasite co-evolution.

Poster 40 : Occurrence of hybrid and mixed genital schistosomiasis with associated infections in men and women of Nsanje and Mangochi districts in Southern Malawi

Dr Sekeleghe Kayuni, *HUGS project, Malawi-Liverpool-Wellcome Programme*

SA Kayuni[2]; L Cunningham[1]; D Kumwenda[4]; B Mainga[5]; D Lally[2]; P Chammudzi[2]; D Kapira[2]; G Namacha[2]; A Chisale[2]; T Nchembe[2]; L Kinley[6]; E Chibwana[6]; J Archer[1]; A Juhasz[1]; S Jones[1]; J Chiphwanya[3]; P Makaula[2]; EJ LaCourse[1]; J Musaya[2]; JR Stothard[1];

[1] Liverpool School of Tropical Medicine, UK; [2] Malawi-Liverpool-Wellcome Trust Clinical Research Programme, Malawi; [3] National Schistosomiasis and STH Control Program, CHSU, Ministry of Health, Malawi; [4] Obstetrics & Gynaecology Department, Queen Elizabeth Central Hospital, Ministry of Health, Blantyre, Malawi; [5] Laboratory Department, Mangochi District Hospital, Ministry of Health, Mangochi, Malawi; [6] Radiology Department, Queen Elizabeth Central Hospital, Ministry of Health, Blantyre, Malawi

Background*:* Genital schistosomiasis remains an ignored chronic consequence of urogenital schistosomiasis, affecting the genital system of afflicted men and women. So far, this has not been fully described in hybrid infection, an emerging public health concern in endemic areas. As part of a 2-year longitudinal study on Hybridization of UroGenital Schistosomiasis (HUGS), we are conducting a sub-study to describe the morbidity associated schistosome hybrid infections in the male and female genital systems.

Objectives*:* To assess the prevalence of male and female genital schistosomiasis (MGS and FGS) associated with schistosome hybrids and mixed infections among adults in two communities of Nsanje and Mangochi districts. Also, the study described the associated infections including sexually transmitted infections (STIs) among the participants.

Methods*:* Urine, cervicovaginal fluid and swabs, as well as semen samples were collected from study participants. These were analysed to determine schistosome infection by filtration and microscopy of the 10ml urine, direct microscopy of the cervicovaginal lavage and semen and thereafter their sediments after centrifugation, to visualise the *Schistosoma* eggs. Samples also underwent molecular analysis using novel qPCR assay for detection of human, zoonotic and hybrid schistosomes and other co-infections including STIs. Portable colposcopy and histopathology were also conducted.

Results*:* 22 men and 87 women were recruited into the MGS-FGS sub-study. MGS was detected in 50.0% men on microscopy and 72.7% on qPCR while FGS was detected in 18.2% women on microscopy, 47.1% on colposcopy and 54.0% on on qPCR. In Nsanje, 50% men (n=8) and 65.5% women (n=29) had MGS and FGS on qPCR respectively while in Mangochi, it was in 85.7% men (n=14) and 48.3% women (n=58).

Five men with MGS had *S. mattheei,* 3 had *S. mansoni* while 1 had a mixed infection of *S. mansoni* and possible *S. haematobium-S. mattheei* hybrid. Only 5 women with FGS had *S. mattheei* co-infections. Among the women, 73.3% were co-infected with an STI, *Trichomonas vaginalis* detected by qPCR among others while 42.5% had detectable Human papilloma virus (HPV) with 27.0% having high-risk HPV 16 and 18 for invasive cervical cancer.

Conclusions: Our findings indicate that genital schistosomiasis from human, zoonotic and hybrid schistosomes are prevalent in endemic areas, with significant burden of STIs which could pose a further challenge in control interventions being implemented by the National Control Program in the Ministry of Health. In addition, it could increase the risk of other infections like STIs and HIV in the country, and potential complications on women health.

Poster 41* : First record in Algeria of *Derogenes minor* Looss 1901 (Digenea; Derogenidae) from a new host *Xyrichthys novacula* Linnaeus,, 1758 (Labridae): Morphological description

Fatima Zohra Zedam , *USTHB*

F Zedam[1]; F Tazerouti[1];
[1] University of Science and Technology Houari Boumediene , Algeria

Our Study, based at the Laboratory Biodiversity and Environment aims to significantly increase the number of digeneans species known from Algeria. As part of a continuing effort to explore the diversity of Digeneans flatworm parasites of fishes off Algeria, 30 specimens of *Xyrichthys novacula*Linnaeus, 1758 collected off Algeria, southwestern Mediterranean. The digestive tube was carefully examined for the presence of parasitic Digeneans. The species belongs to the family Derogenidae Nicoll, 1910, a taxonomic study of Digenea of the genus *Derogenes Lühe*, 1900 include the species *Derogenes minor* Looss, 1901 based on morphological data. *Derogenes minor* Looss, 1901 is reported from the gall bladder of a new host Xyrichthys novacula Linnaeus, 1758 the first records species off the Algeria coast

Poster 42* : Molecular diagnosis of intestinal schistosomiasis: An overview of current protocols and what is needed, highlighted using data from a *Schistosoma mansoni* and *Schistosoma haematobium* co-endemic area

John Archer, *Liverpool School of Tropical Medicine*

J Archer[1]; S Mesquita[2]; LJ Cunningham[1]; A Juhàsz[1]; S Jones[1]; B Mainga[4]; P Chammudzi[5]; DR Kapira[5]; D Lally[5]; G Namacha[5]; P Makaula[5]; JE LaCourse[1]; SA Kayuni[5]; B Webster[3]; J Musaya[5]; JR Stothard[1];
[1] Liverpool School of Tropical Medicine, UK; [2] Natural History Museum, UK; [3] Natural History Museum, London, UK, UK; [4] Laboratory Department, Mangochi District Hospital, Malawi; [5] Malawi-Liverpool-Wellcome Trust Clinical Research Programme, Malawi

Intestinal schistosomiasis is typically diagnosed using Kato-Katz faecal-egg microscopy. Whilst this method is relatively inexpensive and can be carried out at the point-of-care, it is considered low-throughput and lacks sensitivity, particularly when attempting to diagnose individuals harbouring low-intensity infections. Reliably measuring the prevalence of *S. mansoni* infections in areas of low disease endemicity can therefore be extremely difficult using this method alone. For these reasons, a variety of immunological rapid diagnostic lateral flow tests (RDTs) have also been developed to diagnose infection with *S. mansoni*, of which the most widely used is the urine-based point-of-care circulating cathodic antigen RDT (CCA-RDT). This assay, however, can also lack sensitivity, as well as specificity, when attempting to diagnose individuals harbouring low-intensity infections or when assessing individuals co-infected with both *S. mansoni* and *S. haematobium*.

Whilst both Kato-Katz and CCA-RDT assays are valuable diagnostic tools in certain settings, highly sensitive molecular assays, such as endpoint and real-time/quantitative PCR, are also needed for effective and impactful disease diagnosis, monitoring, and surveillance. PCR, however, is currently unsuited for use in most schistosomiasis-endemic settings. Both PCR itself, as well as the essential preliminary steps needed to isolate DNA from faecal material, require expensive and sophisticated equipment, specialised personnel, and reliable laboratory infrastructure seldom available in endemic areas. In addition, whilst a standardised diagnostic real-time PCR protocol exists to detect a genus-specific locus of the *Schistosoma* internal transcribed spacer 2 (ITS2) region, there is currently no standardised diagnostic PCR assay that is routinely used to differentiate

between the various human-infecting *Schistosoma* species. As such, in areas where multiple *Schistosoma* species are endemic, molecular assays capable of detecting and distinguishing these species are needed.

Isothermal (single and constant temperature) DNA amplification methods offer an alternative to PCR-based amplification and are better suited for use in resource-poor settings as they require minimal equipment and can be highly portable and user-friendly. The most widely used isothermal DNA amplification method is loop-mediated isothermal amplification (LAMP). Again, however, no standardised and routinely used *S. mansoni*-specific LAMP assay capable of reliably diagnosing intestinal schistosomiasis is currently available. In addition, an isothermal and portable *S. mansoni*-specific Recombinase Polymerase Amplification (RPA) assay has also been recently developed, however, this has not yet been fully validated using human faecal material.

Here, we present an overview of current protocols available for the molecular diagnosis of intestinal schistosomiasis, inclusive of methods needed to isolate DNA from faecal material, PCR, and isothermal DNA amplification approaches. We also outline what is currently needed to develop, standardise, and deploy highly sensitive and specific molecular diagnostic tools that can be used at the point-of-care in low-endemicity areas, highlighted using data recently generated from a *S. mansoni* and *S. haematobium* co-endemic area.

Poster 43 : The complete mitogenome of *Tristoma* Diesing, 1850 (Monopisthocotylea, Capsalidae), a gill parasite swordfish *Xiphias gladius*

Dr Chahinez Bouguerche*, Swedish Museum of Natural History*

C Bouguerche[1]; R Gastineau[2]; JL Justine[3];
[1] Swedish Museum of Natural History, Sweden; [2] University of Szczecin, Poland; [3] Muséum National d'Histoire Naturelle, France

Capsalids are monopisthocotylean flatworms parasites found on the skin and gills of fish. Capsalines (subfamily Capsalinae) are large-sized capsalids, parasitic on highly prized gamefish, and species of *Tristoma* parasitise only the gills of swordfish (*Xiphias gladius*). We obtained specimens of *Tristoma integrum* Diesing, 1850 from swordfish collected off Algeria in the Mediterranean Sea. Here, we describe the specimens, including the key systematics characters of dorsolateral body sclerites. One specimen was used for a next generation sequencing analysis but a part of it, including the sclerites, was mounted on a permanent slide, drawn, and deposited in a curated collection. We characterised the complete mitogenome, the ribosomal cluster (including 18S and 28S) and additional genes such as Elongation factor 1 alpha (*EF1α*) and Histone 3. We also retrieved molecular information from the host tissue present in the gut of the monogenean and provide the sequence of the complete rRNA cluster of the host, *X. gladius*. The mitogenome of *T. integrum* is 13 968 bp in length and codes for 12 protein, 2 rRNA and 22 tRNA. Phylogenies of capsalids were generated from 28S sequences and concatenated mitochondrial protein-coding genes, respectively. In the 28S phylogeny, most subfamilies based on morphology were not found to be monophyletic, but the Capsalinae were monophyletic. In both phylogenies, the closest member to *Tristoma* spp. was a member of the *Capsaloides*. In an Appendix, we report the complex nomenclatural history of *Tristoma* Cuvier, 1817 and its species.

Poster 44 : Establishing an *in vitro* culture method and genetic approaches to investigate the infection biology of the parasitic nematode *Teladorsagia circumcincta*

Dr Samuel Duncan, *University of Glasgow*

S Duncan[1]; R Brinzer[1]; D Price[1]; TN McNeilly[1]; AJ Nisbet[1]; C Britton[2];
[1] Moredun Research Institute, UK; [2] University of Glasgow , UK;

The Trichostrongylid scour worms *Trichostrongylus* and *Teladorsagia* infect the gastrointestinal tract of their ruminant hosts, causing tissue damage and significant weight loss. Some livestock acquire protective immunity to scour worms, indicating that vaccination is a promising therapeutic approach. The precise mechanisms of immunity are poorly understood and development of an effective vaccine to protect livestock is needed. Effective vaccine design requires knowledge of how scour worms establish infection and mediate host-parasite interactions during infection. Ability to culture larval stages as they develop, together with the application of reverse genetics approaches such as RNA interference (RNAi) or CRISPR-Cas9 should aid vaccine design. We aim to better understand the biology of scour worms by modulating expression of genes encoding putative virulence factors and genes essential for worm development.

We have developed a culture system to grow parasitic stages of *Teladorsagia circumcincta in vitro*. Infective L3 larvae were ex-sheathed and grown in medium formulated from egg yolk (Rose, 1973), resulting in ~30% of xL3 moulting and developing to L4 stage. L4 larvae that have undergone ecdysis are viable *in vitro* for over 4 weeks and display a significant increase in worm length. We observed sexual dimorphism of L4 stages, with the development of male or female tail morphology indicative of differentiation to late-stage L4. Initial studies using ovine abomasal organoids to co-culture *T. circumcincta* larvae will also be discussed.

Using our *in vitro* larval culture system, we aim to modulate the expression of putative parasite virulence factors and determine any phenotypic effects. These initial advances in our ability to culture *T. circumcincta in vitro* hold promise for application of genetic manipulation and ultimately to elucidate the role of parasitic virulence factors in establishing infection. This knowledge will contribute to our understanding of disease progression and antigen discovery for the development of a scour worm vaccine.

Poster 45 : Higher blood concentrations of the main metabolite of praziquantel, R-trans-4-OH-PZQ, is associated with higher *Schistosoma mansoni* egg reduction and lower reinfection rates

Dr Rachel Francoeur, *University of Chester*

R Francoeur[5]; J Tetteh[2]; M Alzahrani[2]; C Faust[4]; A Atuhaire[1]; M Arinaitwe[1]; M Adriko[1]; D Ajambo[1]; A Nakasi[1]; F Besigye[1]; C Rowel[1]; A Wamboko[1]; LV Carruthers[4]; A Thomson[2]; S Babayan[3]; H Torrance[6]; DG Watson[2]; P Lamberton[4];

[1] Vector Control Division, Ministry of Health, Uganda; [2] Strathclyde Institute of Pharmacy and Biomedical Science, University of Strathclyde, Glasgow., UK; [3] School of Biodiversity, One Health, and Veterinary Medicine, University of Glasgow, Glasgow, UK; [4] School of Biodiversity, One Health and Comparative Medicine, University of Glasgow, UK; [5] School of Natural Sciences, University of Chester, UK; [6] School of Medicine, Dentistry & Nursing, Forensic Medicine & Science, University of Glasgow, UK

Schistosomiasis is a severe disease caused by blood flukes acquired from contaminated water sources. Guidelines set out by the World Health Organization recommend mass drug administration (MDA) with praziquantel as a measure to reduce prevalence and control morbidity. Despite this practice, hyper-endemic areas continue to endure in various regions of Uganda and cure rates and reduction rates post-treatment are highly variable. Poor cure rates may be due to either parasite factors such as drug resistance or host factors such low drug absorption. Cure rates vary between individuals, but despite over a decade of MDA, drug resistance has not spread. Drug absorption levels were assessed in a pharmacokinetic study, as an alternative cause for variable drug efficacy in a hyper-endemic area. Finger prick dried blood spots (DBS) were collected from 197 school-aged children pre-treatment with 40mg/kg praziquantel and at 30 minutes, 1, 2, 3, 5, 7, 10, 17 and 24 hours post-treatment. A population level approach was used where each child provided a maximum of four post-treatment DBS samples each at different time points. Drug absorption and metabolite levels were measured using an offline extraction method. The influence of drug and metabolite levels on parasite clearance

and reinfection rates were assessed using a generalised linear mixed model. There was a positive correlation between the main metabolite R-trans-4-OH-PZQ and higher parasite clearance and lower reinfection rates measured by Kato-Katz. Host biometric parameters were also considered in this study identifying increasing age as a predictor of higher clearance and lower reinfection rates.

Poster 46* : Tick, tick, boom! Trends in *Anaplasma marginale* seroprevalence in, Mojave desert, bighorn sheep

Sophie Hawkes, *University of Warwick*

SF Hawkes[1]; EE Gorsich[1];
[1] University of Warwick, UK

Desert bighorn sheep (*Ovis canadensis nelsoni*) are a bighorn sub-species distributed in fragmented populations across the southwestern US. Parasites in bighorns may be influenced by the distribution of hosts, variation in susceptibility, or the distribution of vectors. We studied the effects of demographic and ecological factors on the seroprevalence of tickborne, bacterial parasite, *Anaplasma* sp. in DBHS. We performed competitive enzyme-linked immunosorbent assays on >1000 serum samples collected from Mojave Desert bighorns from 1983-2022. Then, we used generalised additive models to describe potential linear and non-linear relationships between infection risk and environmental, heath, and demographic covariates. Models representing yearly fluctuations with increasing recent prevalence and ecological conditions favourable to tick populations were strongly supported. Specifically, ecological conditions involving the low/high rainfall and low temperatures were associated with greater *Anaplasma* seroprevalence. These results suggest that environmental conditions relevant for tick ecology are structuring tickborne parasite prevalence with other studies where increased global spread and susceptibility to *Anaplasma* is seen/predicted. Given the link demonstrated between tickborne disease, year, and environmental conditions favourable to ticks, it is assumed that with climate change the distribution of *Anaplasma* in bighorns could shift. Continued monitoring is highly recommended.

Poster 47 : Highly multiplexed ddPCR amplicon sequencing reveals persistent *Plasmodium falciparum* and *Plasmodium vivax* transmission in the Ethiopian highlands

Cristian Koepfli, *University of Notre Dame*

C Koepfli[1]; Y Ewnetu[2]; A Holzschuh[1]; G Da Silva[1]; H Kamugisha[1]; A Lerch[1]; W Lemma[2]; N Berhane[2];
[1] Department of Biological Sciences, University of Notre Dame, United States; [2] University of Gondar, Ethiopia

The Ethiopian highlands are earmarked for malaria elimination yet clinical cases are frequently observed. The epidemiology of *Plasmodium falciparum* and *Plasmodium vivax*, and, in particular, the role of importation by human migration from the highly endemic lowlands is not well studied. We collected over 5000 blood samples from febrile patients presenting to health centres, through cross-sectional surveys conducted at an altitude range from 1800 to 2700 m, and at bus terminals from travellers arriving from the lowlands. We screened samples by microscopy and qPCR. We observed high test positivity of up to 31% among clinical patients, and asymptomatic prevalence ranging from 16-49% for *P. falciparum*, and 17-36% for *P. vivax*. We develop and applied a highly multiplexed droplet digital PCR (ddPCR)-based amplicon sequencing method targeting 35 markers for *P. falciparum* and 60 markers for *P. vivax*. In this assay, PCR amplification in microdroplets enables sequencing of many markers in parallel even in case of low-density infections (i.e., 1 parasite/μL). We

sequenced >400 *P. falciparum* and >200 *P. vivax* infections to study population structure in space and time. Parasite genetic diversity was moderate, and infection complexity was low. Little population structure across a transect of 150 km corroborated local transmission across the highlands. We identified multiple clusters of clonal or near-clonal infections, highlighting transmission of closely related parasites across multiple years. Only a minority of those infected reported travel to the lowlands. Infections collected from travellers did not form a genetically distinct population from those collected from non-travellers, suggesting frequent parasite gene flow between the highlands and lowlands. Yet, in clonal or near-clonal clusters, infections of travellers were frequently observed first in time, indicating that imported parasites were transmitted locally. The frequency of several known mutations conferring drug resistance was high. In conclusion, our epidemiological and genomic data contrasts the official status of the Ethiopian highlands as bearing a very low risk for malaria. Sequencing of closely related parasites enables in-depth studies into the sources and sinks of transmission.

Poster 48 : Improving genomic tools to investigate ivermectin resistance in *Teladorsagia circumcincta*

Dr Jennifer McIntyre, *Research Associate, University of Glasgow*

J McIntyre[4]; K Maitland[4]; D Berger[3]; A Morrison[1]; D Bartley[1]; K Bull[2]; E Devaney[4]; R Laing[4]; SR Doyle[3];
[1] Moredun Research Institute, UK; [2] University of Bristol, UK; [3] Wellcome Sanger Institute, UK; [4] School of Biodiversity, One Health and Comparative Medicine, University of Glasgow, UK

Ivermectin is a powerful endectocide, used to control infections in both humans and animals. However, widespread resistance to this anthelmintic has arisen, particularly in helminths of importance to the livestock industry. The development of anthelmintic resistance by the small ruminant nematode *Teladorsagia circumcincta*, a significant pathogen of growing lambs in the UK and in other temperate climates, threatens food security. We seek to identify the genetic causes of ivermectin resistance in *T. circumcincta*. To do so, we sought to improve the primary tool available to us for this work: the genome annotation and assembly of *T. circumcincta*, which is now largely contained within six large scaffolds corresponding to the five autosomes and the sex chromosome. Using whole genome sequencing of larvae sampled pre- and post-ivermectin treatment on UK farms, together with a reanalysis of a genetic cross, I will show how this new assembly has enabled us to identify a locus under selection by ivermectin on Chromosome 5, and compare this with that found in the related abomasal nematode *Haemonchus contortus*. I will consider some of the key findings and the outstanding questions still remaining.

Poster 49 : Studies on the toxicity and anti-plasmodial activity of *Hymenocardia acida* in albino mice

Dr Ruqayyah Hamidu Muhammad, *Home*

R Muhammad[1];
[1] Home, Nigeria

Malaria is a severe infectious disease caused by *Plasmodium* parasites. Over 90% of malaria deaths occur in Africa, with a high prevalence among children. Nigeria has a significant malaria burden, contributing to a substantial portion of global malaria-related deaths. *Hymenocardia acida* (Jan Yaro), an indigenous plant used by the Hausa tribe in Northern Nigeria for treating various diseases, including fever. The research aims to contribute to the development of a novel and effective treatment for malaria using *Hymenocardia*

acida, considering both its efficacy and safety. Collection, preparation, and extraction of *H. acida* plant materials were carried out. Phytochemical screening to identify various chemical constituents was conducted. *In-vivo* toxicity assessment using acute and chronic tests in mice was also conducted. *In-vivo* anti-plasmodial activity assay to evaluate the extracts' effectiveness in treating malaria. The extracts were found to contain tannins, saponins, flavonoids, steroids, terpenes, carbohydrates, and alkaloids while cardiac glycosides were present only in the leaf extract. Acute toxicity tests showed that LD50 (lethal dose) for both extracts was greater than 5,000mg/kg, indicating safety. Chronic toxicity tests (90 days) showed an increase in body weight, with dose-dependent effects. Liver, kidney, and spleen of the mice treated with extracts were examined. Abnormalities were observed in the liver and kidney, particularly at higher doses. The spleen showed no abnormalities. Percentage parasitaemia was reduced in treated mice compared to untreated infected mice, both leaf and stem-bark extracts exhibited anti-malarial activity, by increasing the survival time of *P. berghei* infected mice and maintain body temperature in infected mice. The stem-bark extract showed higher percentage inhibition of parasites compared to the leaf extract. Extracts demonstrated potential anti-malarial activity. The study suggests that *Hymenocardia acida* extracts showed potential medicinal properties, including anti-malarial effects. Toxicity was observed at higher doses, indicating the need for careful consideration of dosage in medicinal use.

Poster 50 : The RNA-bound Proteome of *Trypanosoma cruzi* Not presented

Dr Regine Walrad, *University of York*

FD Viraque[2], N Teles[1], FS Pais[1], AA Dowle[1], C Robello[2], PB Walrad[1],
[1] *University of York, UK;* [2] *Institut Pasteur Montevideo, Uruguay*

Like other Kinotoplastids, gene expression in *Leishmania* species is overwhelmingly post-transcriptionally controlled. This elevates the importance of RNA-binding proteins (RBPs) in these systems as the primary gene regulators. Building upon the *L. mexicana* RBPome we isolated previously from the 3 main parasite lifecycle stages (Pablos et al. MCP, 2019), 70 non-basal RBPs were selected toward further investigation. An *L. mexicana* barcoded *trans*-regulator knockout clone library was created using CRISPR-cas9 (Baker et al. Nat Comms, 2021) and screened through lifecycle progression and macrophage or mouse infections.

Remarkably, 60% of the RBPs screened are essential for cell viability and 26% contribute to lifecycle progression to human infectious stages, infectivity and/or virulence. Examination of individual knockout lines verify the screen outcomes of specific RBPs essential for parasite growth, viability and infectivity. 13 RBPs were endogenously tagged, immunoprecipitated and submitted for transcriptomic and proteomic analyses to identify all RNP components. Discrete complexes have been identified that may represent novel virulence factors. Further analyses are underway to map interaction dynamics of these key RNP regulators that drive differentiation and virulence capacity in *Leishmania*.

We present the first quantitative RBPome and Whole Cell proteome comparison between the 3 main lifecycle stages of *Trypanosoma cruzi* parasites. Lifecycle progression and bespoke adaptation to distinct host environments demand precise gene expression that is predominantly dependent upon post-transcriptional control. Constitutive transcription elevates the importance of RNA-binding proteins for gene regulation in these parasites, yet strikingly few *T. cruzi trans*-regulators are characterized relative to other kinotoplastids. Using optimized crosslinking and deep, quantified mass spectrometry, we present a comprehensive analysis of the stage-specific RBPomes and whole cell proteomes of the main *T. cruzi* lifecycle stages. Remarkably, while the whole cell proteomes display characteristically distinct surface proteins between these lifecycle stages as expected, the RBPomes of the human infectious trypomastigote and amastigote stages bear striking similarities.

The presence of basal translational machinery and expected markers and near-identical proteomes derived from crosslinked and non-crosslinked cells in all sample sets support the validity of these proteomes. These represent the most in-depth *T. cruzi* proteomes to date with outstanding coverage. Comparisons between these datasets provides unique insight into key candidate regulators that may prove essential to parasite survival, longevity and virulence. These proteomes can further enable in-depth comparisons between Kinetoplastid species toward common essential regulators and host-adaptative mechanisms. Outcomes provide novel insight into the trans-regulatory mRNA:Protein (mRNP) complexes that drive *T. cruzi* parasite lifecycle progression and human infection.

This work was funded by: The UKRI via the Medical Research Council, NTD Network (MRC-GCRF), Horizon's Global Health EDCTP3

Key words: Trypanosoma cruzi; RBP; RNA-Label-free-quantification; Proteome; RBPome/mRNA-binding proteome; Ribonucleoproteins; kinetoplastid; mRNP/trans-regulator.

Poster 51 : Heterogeneous elongation of RNA polymerase I transcription at the active VSG expression site in *Trypanosoma brucei*

Dr James Budzak, *Ludwig-Maximilians-Universität (LMU)*

J Budzak[2]; I Goodwin[1]; TN Siegel[2]; C Tiengwe[1]; G Rudenko[1];
[1] Imperial College London, UK; [2] Ludwig-Maximilians-Universität (LMU), Germany

A Variant Surface Glycoprotein (VSG) coat protects bloodstream form *Trypanosoma brucei* within the mammalian host. VSG is the most abundant mRNA in the cell, with the active VSG gene expressed from one of ~15 VSG bloodstream-form expression sites (BES). The active BES is exclusively transcribed by RNA Polymerase I which is thought to provide continuously high levels of VSG mRNA production. However, using different microscopy approaches we have consistently observed that transcription of the active BES may be heterogeneous. To investigate this further, we used an eGFP reporter assay and directly measured nascent RNA levels across the active BES using single molecule RNA-FISH and 4sUTP nascent RNA labelling.

Interestingly, we find a decrease in pre-mRNA production along the active BES with the highest pre-mRNA levels at active BES promoter region, and the lowest pre-mRNA levels near the telomeric VSG. We find a similar effect at both long and short BESs indicating that reduced nascent RNA production at the active BES telomere is independent of BES length. In addition, previously published RNA Pol I ChIP-qPCR experiments show that RNA Pol I occupancy is higher at the active BES promoter region than at the active telomeric VSG. These data point to a model whereby RNA Pol I elongation is impaired as proximity to the telomere increases, resulting in generation of lower levels of nascent transcripts from the active BES telomere compared to the promoter. These unexpected observations challenge the paradigm that RNA Pol I provides continuously high levels of transcription throughout the active BES.

Poster 52 : *In vitro* exploration of interactions and stressors in Caco-2 cell cultures versus the infective L3 larva stage of *Toxocara canis* and *Parascaris univalens*

Christopher Collins, *Swedish University of Agricultural Sciences*

C Collins[1]; E Tydén[1]; M Åbrink[1];
[1] *Swedish University of Agricultural Sciences, Sweden*

Background: The recent increase in anthelmintic resistance of parasitic nematodes, for example in the equine roundworm *Parascaris univalens,* calls for further research to understand the complex interactions between host and parasite. Although host immune modulation via parasite excretory-secretory products have been described in different experimental models, most host-parasite species-specific and zoonotic interactions remain unexplored. This is largely due to the complex and species-specific infective life cycle and a lack of proper *in vitro* models to effectively test these interactions.

In vitro model: To this end, we have begun investigating the feasibility of cell culture models during interactions with the infective L3 stages of *Toxocara canis* and *Parascaris univalens*. To help quantify our methods, we used the resazurin-resorufin assay. Resazurin is a low molecular weight, non-toxic dye, which during normal metabolism in the cell is non-reversibly reduced to resorufin. Under excitation, resorufin produces a distinct red fluorescence signature, giving a direct readout of metabolic levels. The resazurin-resorufin assay gives robust and repeatable results in a variety of cell types and under a variety of cellular stressors. In addition to metabolic readout, thrashing assays via the WormAssay program, were also performed to judge the effect of stressors on the L3 larva in a non-biased way. Our main stressor was thiabendazol, an actively used anthelmintic of the benzimidazole family know to selectively target beta-tubulin.

Results: When infective L3 larva of *Toxocara canis* or *Parascaris univalens* were co-cultured for 48-hours with adherent human intestinal epithelial cells (Caco-2) we have seen significant changes in overall cellular metabolism (change in resorufin fluorescence), in comparison to control incubations with either L3 larva or adherent cells. When in contact with host cells a significant decrease in thrashing rates in *Toxocara canis* L3 larva, as well as a shift in thrashing behaviour was also observed. Furthermore, we observed a significant decrease in overall metabolic rate in co-cultures of *Toxocara canis* and Caco-2 when exposed for 48-hours to 100μM thiabendazol, a decrease that was not seen in control Caco-2 cells when they were cultured alone under the same thiabendazol concentration. Our results indicate that the early phase *in vitro* co-culture model is resulting in metabolic changes for both host and parasite and parasite behavioural changes which indicates a potential model for further research.

Poster 53* : Characterization of the *Schistosoma mansoni* Bromodomain containing protein 3 (SmBRD3)

Shashika Abeysekara, *Aberystwyth University*

S Abeysekara[1]; J Forde-Thomas[1]; K Hoffmann[1];
[1] *IBERS, Aberystwyth University, UK*

Schistosomiasis, also known as Bilharzia, is a severely neglected tropical disease that ranks second only to Malaria in causing human disease and death. Despite its severity, Praziquantel (PZQ) remains the sole major therapeutic agent in use for over a generation. This has led to growing concerns about potential resistance development, underscoring the need for new treatment strategies. This study explores the complex molecular mechanisms that regulate gene expression in *Schistosoma mansoni,* one of the three predominant species that causes schistosomiasis. Our primary focus is on the Bromodomain-containing protein 3 (SmBRD3), a gene product highly expressed during the miracidia stage of the *S. mansoni* life cycle and likely involved in epigenetic-mediated regulation of gene activity. Building on our previous studies that have identified small molecule inhibitors of SmBRD3 function, here we are characterizing an anti-SmBRD3 antibody that may assist in our further understanding of this epigenetic regulator. To date, western blot analyses of miracidial samples

have revealed additional immunoreactive bands beyond our predicted target that may indicate loose specificity, cross-reactivity with other SmBRD members or SmBRD3 instability. These immunoreactive proteins underwent mass spectrometry for precise protein identification and optimization. Results, however, were unable to identify any peptides belonging to SmBRD3. Therefore, future plans involve further characterization of this antibody using Immunoprecipitation. The findings from this research could pave the way for the development of innovative strategies that target epigenetic regulators, offering a promising approach to combat parasitic infections.

Poster 55 : Association of bovine leukocyte antigen DRB3*007:01 and *009:02 to host resistance to Candidatus *Mycoplasma haemobos* infection in Kedah-Kelantan x Brahman cattle

Dr Onyinyechukwu Agina*, University of Nigeria, Nsukka*

OA Agina[1]; MS 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 bovine leukocyte antigen (BoLA) gene is a significant genetic part of the immune system and has been used as a disease marker in cattle. The 16SrRNA gene of Candidatus *Mycoplasma haemobos* was detected in 37 out of 85 (43.5%) Kedah-Kelantan x Brahman (KKB) cattle and allelic association of the BoLA-DRB3 gene to C. *M. haemobos* infection was evaluated. The association between an allele and *T. orientalis* were evaluated by Fisher's exact and Cochran Mantel Haenszel (CMH) test. The odds ratios (OR) and their 95% confidence intervals for susceptibility or resistance were calculated for each allele. The amplification of the BoLA-DRB3 gene produced clear single bands of 281 bp by the single-step PCR analysis. Sequencing of the PCR amplicons yielded 279 - 320 nucleotides. The PCR-sequence based typing of BoLA-DRB3.2 gene from KKB cattle revealed that the gene is highly polymorphic. Ten novel alleles were detected (BoLA-DRB3*012:04, *015:08, *015:09, *015:11, *015:12, *017:05, *017:07, *024:33, *107:04, *168:01), and these alleles shared about 90.7-95.8% and 85-92% nucleotide and amino acid identities respectively, with the BoLA-DRB3*016:01 cDNA clone NR-1. Five alleles were detected in the C. *Mycoplasma haemobos* infected cattle namely: DRB3*012:01, *015:01, *007:01, *018:01, *009:02. The alleles with the highest frequencies were DRB3*009:02 (50%) and *007:01 (34.2%) in the C. *Mycoplasma haemobos* positive cattle and DRB3*018:01 (41.2%) and *015:01 (35.3%) in the C. *Mycoplasma haemobos* negative cattle. The associated alleles of C. *Mycoplasma haemobos* infection resistance was DRB3*007:01 (OR = 0.161; PCMH = 0.020) and *009:02 (OR = 0.084; PCMH = 0.000). No susceptibility alleles were detected following the Bonferroni correction of p-value, p > 0.0125. Therefore, we presented BoLA-DRB3.2 alleles associated with resistance to C. *M. haemobos* infection and suggests that during breeding, genetic selection of resistant animals could be a natural strategy for tick-borne disease control, particularly when there is no available global vaccine for the prevention and control of this infections. Keywords: bovine leukocyte antigen; alleles; Candidatus *Mycoplasma haemobos*; Kedah-Kelantan x Brahman cattle; PCR-Sequence based typing.

Poster 56 : The nucleotide triphosphohydrolase HD82 maintains genome integrity and replication stability through dNTP homeostasis control in *Trypanosoma brucei*

Pablo Antequera, *Instituto de Parasitología y Biomedicina López Neyra*

P Antequera-Parrilla[1]; VM Castillo-Acosta[1]; LM Ruiz-Pérez[1]; D González-Pacanowska[1];
[1] Instituto de Parasitología y Biomedicina López-Neyra (IPBLN-CSIC), Spain

Agents modulating synthesis and incorporation of nucleotides in DNA are widely used as chemotherapeutics. Processes such as DNA replication and DNA repair depend on the accurate regulation of the synthesis and degradation of nucleotides. In humans, the main dNTPase is SAMHD1, which controls the homeostatic balance of dNTP pools. TbHD82 is a SAMHD1 ortholog identified in *Trypanosoma brucei* and sequence alignments show that the amino acids involved in substrate binding and catalysis are all conserved. While TbHD82 is not essential *in vitro* for proliferation of procyclic and bloodstream form parasites, its absence induces an accumulation of dATP, dCTP and dTTP, suggesting that the protein is a dNTPase. The expression of TbHD82 is cell cycle-dependent and HD82-deficient parasites exhibit a hypermutator phenotype, defects in cell cycle progression with a shortened S-phase and increased fork speed and instability. In addition, TbHD82 null mutants exhibit enhanced activation of the DNA damage response and the enzyme is up-regulated upon genotoxic insult. All these features are in line with the consequences of dNTP imbalances. We suggest that TbHD82 contributes to the maintenance of genome integrity and replication stability by modulating the excessive or unbalanced accumulation of dNTPs.

Poster 57* : Isolating the isolate: proteomic profiling of Triclabendazole-susceptible and resistant *Fasciola hepatica*

Olugbenga Samuel Babatunde, *Aberystwyth University, UK*

OS Babatunde[1]; J Leonard[1]; C Steele[2]; R Cramer[4]; M Fisher[3]; PM Brophy[1]; RM Morphew[1];
[1] Aberystwyth University, UK; [2] Aberystwyth University - IBERS, UK; [3] Ridgeway Research Ltd, UK; [4] University of Reading, UK

Fascioliasis is a disease caused by *Fasciola hepatica*, affecting both livestock and humans, which poses a substantial threat to food security and human health. The control and management of *F. hepatica* relies heavily on triclabendazole (TCBZ) due to the absence of vaccines. Over-dependence on TCBZ for fascioliasis control has led to the emergence of TCBZ-resistant *F. hepatica* strains. Further complications arise from limited diagnostics, making it challenging to differentiate between resistant and susceptible liver fluke infection. Recent work has identified a major locus and the gene content likely responsible for conferring TCBZ resistance. Consequently, there is a pressing need to further confirm potential TCBZ resistance targets, particularly at the protein level. This study aims to utilize an in-depth proteomic approach to confirm the protein profiles from isolates of *F. hepatica* varying in their TCBZ susceptibility. Proteomic profiles were generated from somatic cells and the secretome (encompassing extracellular vesicles and free excretory-secretory proteins) of four *F. hepatica* isolates; comprising two TCBZ susceptible (Aberystwyth and Italian) and two TCBZ resistant (Penrith and Kilmarnock). A GeLC approach (1-D SDS PAGE fractionation prior to mass spectrometry) was conducted on somatic and secretome proteins to provide an in-depth proteome profile. The somatic proteomic profile revealed 1236 proteins from all isolates with high confidence hits. Additionally, 312 proteins were identified from extracellular vesicles having high confidence hits. Within these identifications 8 were identified from 30 proteins that maybe likely for conferring TCBZ resistance. Importantly, the proteomes resolved can successfully delineate TCBZ resistant isolates from TCBZ susceptible isolates.

Poster 58* : A genomic basis for the transition to hematophagy in triatomines, vectors of Chagas disease

Antonella Bacigalupo, *University of Glasgow, SBOHVM*

A Bacigalupo[3]; C Hernández[4]; JD Ramírez[4]; S Pita[5]; C Gyhrs[9]; B Cheaib[10]; AG Villacís[1]; MJ Grijalva[6]; K Brunker[3]; C Botto-Mahan[7]; MA Varas[7]; ML Allende[8]; PE Cattan[2]; KR Elmer[3]; MS Llewellyn[3];
[1] Centro de Investigación para la Salud en América Latina, Pontificia Universidad Católica del Ecuador, Ecuador; [2] Facultad de Ciencias Veterinarias y Pecuarias, Universidad de Chile, Chile; [3] School of Biodiversity, One Health and Veterinary Medicine, University of Glasgow, UK; [4] Centro de Investigaciones en Microbiología y Biotecnología-UR (CIMBIUR), Facultad de Ciencias Naturales, Universidad del Rosario, Colombia; [5] Facultad de Ciencias, Universidad de la República, Uruguay; [6] Infectious and Tropical Disease Institute, Heritage College of Osteopathic Medicine, Ohio University, United States; [7] Facultad de Ciencias, Universidad de Chile, Chile; [8] Millenium Institute Center for Genome Regulation, Universidad de Chile, Chile; [9] College of Medical, Veterinary and Life Sciences, University of Glasgow, UK; [10] Centre for infectious diseases, Universitätsklinikum Heidelberg, Germany

Chagas disease is the most important parasitosis on the American continent, with more than 300,000 new human cases and 12,000 deaths each year. New tools are required to accelerate the interruption of *Trypanosoma cruzi* domiciliary vectorial transmission by triatomines. Here, we provide multiple new genomic resources for triatomine species, including non-hematophagous predatory sister taxa, with the aim of elucidating the process of adaptation to blood feeding within Reduviidae (Hemiptera: Heteroptera).
We gathered samples from species across Latin America, extracted the DNA and performed long-read (ONT) and short-read (Illumina) sequencing, assembly and annotation. The annotation pipeline included a homology-based annotation with Gemoma, using available annotations for *Rhodnius prolixus*, *Triatoma rubrofasciata*, *Cimex lectularius* and *Acyrthosiphon pisum*. The Gemoma annotation was included as input to the GenSAS pipeline, along with available protein and RNA-seq data. Mitogenomes were generated using Novoplasty and annotated with MITOS2 in Proksee, and the phylogeny of triatomine species within Hemiptera was obtained using OrthoFinder.

As results, we produced eight new whole genome assemblies, for six species without previous genomes: *Belminus herreri* (1.1 Gbp, GC 34.1%, N 0.9%), *Mepraia spinolai* (977.1 Mbp, GC 33.8%, N 0.6%), *Panstrongylus geniculatus* (1.2 Gbp, GC 34.7%, N 9.1%), *Psammolestes arthuri* (542.9 Mbp, GC 33.9%, N 1.8%), *Rhodnius brethesi* (550.7 Mbp, GC 33.5%, N 1.8%) and *Rhodnius ecuadoriensis* (583.8 Mbp, GC 33.9%, N 4.2%), and for two species with available but very fragmented assemblies: *R. prolixus* (583.9 Mbp, GC 34.0%, N 3.3%) and *Triatoma infestans* (1.1 Gbp, GC 33.8%, N 2.5%). Furthermore, we also produced the first non-triatomine predatory reduviid whole genome assembly for *Platymeris biguttatus* (909 Mbp, GC 31.9%, N 0.8%), required for genomic comparisons. All of them present high gene completeness (BUSCOs >90%). The mitogenomes show sizes over 15,900 bp, with mostly conserved gene order of the 13 protein-coding genes, two ribosomal RNA genes, 22 transfer RNAs and control region.

Annotated genes related to hematophagy include lipocalins, triabins, odorant binding proteins, ionotropic receptors, and olfactory receptors, among many others. We compare these, focusing on the two main tribes, and the similarities of the non-hematophagous *B. herreri* and *P. biguttatus* with each of them. The preliminary results indicate a polyphyletic origin of hematophagy in Triatominae, reopening the debate on this relevant aspect of Chagas disease vector biology, and stressing the need for increasing the genomic resources for this neglected illness.

This work was supported by: Wellcome [204820/Z/16/Z] (AB), Lister/Bellahouston Fellowship (AB); MR/Y001338/1 (MSL); and Agencia Nacional de Investigación y Desarrollo (ANID):

Poster 59 : Metagenomic surveillance for veterinary and public health relevant bacterial agents carried by blood-sucking arthropods in Chile

Antonella Bacigalupo, *University of Glasgow, SBOHVM*

R Thomas[5]; MC Silva-de la Fuente[6]; A Bacigalupo[3]; N Quiroga[4]; A Santodomingo[5]; M Muñoz[8]; L Vega[2]; N Luna[2]; C Hernández[2]; JD Ramírez[2]; MS Llewellyn[1]; C Botto-Mahan[4]; S Muñoz-Leal[5]; L Moreno[7];
[1] School of Biodiversity, One Health and Veterinary Medicine, University of Glasgow, UK; [2] Centro de Investigaciones en Microbiología y Biotecnología-UR (CIMBIUR), Facultad de Ciencias Naturales, Universidad del Rosario, Colombia; [3] School of Biodiversity, One Health and Comparative Medicine, University of Glasgow, UK; [4] Facultad de Ciencias, Universidad de Chile, Chile; [5] Facultad de Ciencias Veterinarias, Universidad de Concepción, Chile; [6] Facultad de Ciencias Agrarias y Forestales, Escuela de Medicina Veterinaria, Universidad Católica del Maule, Chile; [7] Facultad de Ciencias Naturales y Oceanográficas, Universidad de Concepción, Chile; [8] Institute of Biotechnology, Universidad Nacional de Colombia, Colombia

As vectors of many neglected diseases, blood-sucking arthropods pose an unseen threat until symptomatic cases in humans or animals emerge, or when local epidemics occur. With the current climate change scenario creating an environment conducive to the emergence, re-emergence, and spread of arthropod-borne infectious agents, continuous surveillance, and timely identification of microorganisms with pathogenic potential become permanent tasks. Nanopore sequencing has emerged as a powerful tool for epidemiological surveillance in diverse settings, offering rapid and real-time processing of a relatively large number of samples. Our study utilises long-read sequencing to enhance our understanding of the potential vectorial role that blood-sucking arthropods - fleas, lice, mites, ticks and triatomines - play across Chilean ecosystems regarding Bacteria.

To achieve this goal, we extracted DNA from the guts or the whole body of 186 blood-sucking arthropods. Bacterial DNA was enriched by conventional PCR using universal 16S rRNA primers. The amplicons were sequenced in R10.4.1 flow cells on an Oxford Nanopore MinION MK1C. Bioinformatic analyses were conducted using a custom workflow to analyse metabarcoding data on reads above 900 bp, focusing on finding bacterial agents of medical and veterinary relevance.

As result, 97.85% of the samples yielded 16S rRNA long reads for bacterial DNA, which allowed us to identify potentially pathogenic arthropod-borne bacteria at the genus level. *Orientia* DNA was detected in fleas (1,242 reads) and triatomines (two reads), while *Rickettsia* DNA from the spotted fever group I (SFGI) was found in ticks (51 reads), fleas (29 reads), lice (11 reads), and mites (three reads). Additionally, *Borrelia* DNA from the relapsing fever group (RFG) was detected in mites (2 reads).

Although the detection of *Orientia* DNA in fleas and triatomines was unexpected, the identification of *Borrelia* DNA in mites and *Rickettsia* DNA in fleas, lice, and mites has been previously reported. In this sense, our next steps involve the use of taxa-specific primers to confirm our findings. 16S rRNA gene amplicon sequencing is a promising method for profiling arthropod-borne infectious agents, capturing not only a snapshot of potential pathogens circulating among these arthropods but also establishing the foundation for future genetic analysis.

Funding: Fomento a la Vinculación Internacional para Instituciones de Investigación, Agencia Nacional de Investigación y Desarrollo (ANID): “Fortalecimiento en el análisis metagenómico de patógenos transmitidos por vectores de importancia en salud pública” (FOVI220125); ANID Programa Becas: Doctorado Becas Chile 2019-72200391, Doctorado Nacional 2019-21190078, and 2020-21200182; Fondecyt 1221045, 11220177; Anillo ATE230025.

Poster 60 : A new cell line derived from the tsetse fly *Glossina morsitans morsitans*, vector of trypanosomes of humans and domestic livestock in sub-Saharan Africa

Dr Lesley Bell-Sakyi, *University of Liverpool*

L Bell-Sakyi[2]; LR Haines[1]; C Hartley[2]; A Beliavskaia[2]; JJ Khoo[2]; B Makepeace[2];
[1] Liverpool School of Tropical Medicine, UK; [2] University of Liverpool, UK

Tsetse flies of the genus *Glossina* are important vectors of disease-causing salivarian trypanosomes in sub-Saharan Africa and are major constraints on livestock production, agricultural development, and human health in the region. We have recently established a new cell line, GMA/LULS61, derived from tissues of adult female *Glossina morsitans morsitans*. The tsetse cells are grown at 28 °C in L-15 (Leibovitz) medium supplemented with foetal bovine serum and tryptose phosphate broth. Karyotyping at passage 17 revealed a predominantly haploid chromosome complement. PCR amplification and sequencing of fragments of the COI gene and pan-bacterial 16S rRNA gene confirmed, respectively, species origin and absence of contaminating bacteria. GMA/LULS61 cells supported infection and growth of several insect-derived strains of the intracellular bacterial symbiont *Wolbachia*. The GMA/LULS61 cell line, available from the Tick Cell Biobank at the University of Liverpool, has potential for application in a variety of studies investigating the biology and control of *G. m. morsitans* and its associated pathogenic and symbiotic microorganisms.

Poster 61 :

Poster 62 : Exploring the activity and the essentiality of the putative Δ6-desaturase in the procyclic and bloodstream forms of *Trypanosoma brucei*

Dr Michela Cerone*, University of St Andrews*

M Cerone[1]; TS Terry K Smith[1];
[1] University of St Andrews, UK

Trypanosomatids have an exclusive and finely regulated biosynthetic pathway for *de novo* synthesis of fatty acids (FAs) and particularly of polyunsaturated fatty acids (PUFAs). The key enzymes for the process of unsaturation are known as desaturases. In this work, we explored the activity of the putative Δ6-desaturase in *T. brucei*. *Via* GC-MS analysis of the fatty acids of the cells after genetic manipulation of the level of expression of Δ6-desaturases in both procyclic (PCF) and bloodstream (BSF) forms of *T. brucei*, and *via* supplementation of the media with FA sources, we showed that docosahexaenoic acid (22:6) and/or docosapentaenoic acid (22:5), and arachidonic acid (20:4) and/or docosatetraenoic acid (22:4) are the products and the substrates respectively of this Δ6-desaturases. Surprisingly, we were able to observe, *via* lipidomic analysis with ESI-MS/MS, an increase in inositol-phosphoryl ceramide (IPC) in response to the overexpression of Δ6-desaturases in low-fat media in BSF. The formation of IPC is normally only observed in the stumpy and procyclic forms of *T. brucei*. Therefore, the expression levels of Δ6-desaturases, which varies between BSF and PCF, might be involved in the cascade(s) of metabolic events that cause lipid remodelling and ultimately morphological changes, which are key to the transition between these life-cycle stages.

Poster 63* : Differential transcriptional responses between heterogenous host-*Toxoplasma* interactions

Praveena Chandrasegaran, *University of Edinburgh*

P Chandrasegaran[2]; B Shi[2]; A Gossner[2]; M Hassan[1];
[1] The Royal (Dick) School of Veterinary Studies and the Roslin Institute, The University of Edinburgh, UK; [2] The Roslin Institute, University of Edinburgh, UK

Toxoplasma gondii, a zoonotic apicomplexan that infects over a billion people worldwide, can cause early death in immunocompromised individuals and defects in foetal brain development. *Toxoplasma* is also a major cause of abortion in small ruminants. When *Toxoplasma* encounters host cells, several outcomes are possible, including parasite actively invading the cells, entering the cell via phagocytosis, or failing to enter the cell completely. These heterogenous outcomes occur simultaneously in the same host and likely play significant roles in disease pathogenesis. For example, only phagocytosed parasites are known to induce interleukin 12, which is central to host immune responses against the parasite. Yet, current knowledge of host-*Toxoplasma* interactions is largely based on averaged responses in bulk cell populations. Here, we employed single cell RNA (scRNA) and bulk RNA sequencing to investigate the transcriptional profiles that underpin heterogenous *Toxoplasma* interaction with human peripheral blood mononuclear cells (PBMCs). Our scRNA-seq showed *Toxoplasma* preferentially infects and elicits transcriptional responses in macrophages and dendritic cells in human blood. We also observed the differences in transcriptional responses between infected and bystander cells. Notably, we observed that cell-cell communication between monocytes, macrophages, and dendritic cells, drive transcriptional response to the parasite. Expectedly, using bulk RNA-sequencing data of heterogenous host-*Toxoplasma* interaction outcomes as reference panel, we observed that genes expressed in cells infected via phagocytosis are largely expressed in macrophages and dendritic cell single cell clusters. Overall, by integrating scRNA and bulk RNA sequencing, our study unveils the transcriptional profiles of immune cells in diverse infection outcomes, providing novel avenues for targeted investigations into gene functions and potential implications for therapeutic strategies.

Poster 64 : The Schistosome and Snail Resource (SSR) – Maximising snail and cercariae production by investigating snail-schistosome compatibility

Dr Adam Cieplinski, *Natural History Museum*

A Cieplinski[3]; F Sales Coelho[1]; G Delhaye[2]; C Gustave[3]; V Yardley[1]; A Bustinduy[1]; AM Emery[3]; B Webster[3];
[1] London School of Hygiene and Tropical Medicine, UK; [2] Royal Botanic Gardens Kew, UK; [3] Natural History Museum, UK

Introduction: The Schistosome Snail Resource (SSR) is a Wellcome Trust funded centralised schistosomiasis resource, run through a partnership between the Natural History Museum (NHM) and the London School of Hygiene and Tropical Medicine (LSHTM) in London, UK. Schistosomiasis is a parasitic disease caused by schistosomes and transmitted by freshwater snails. It is one of the most common, of the neglected tropical diseases but the complexity of its lifecycle means few laboratories maintain it, which is a barrier to research. Moreover, schistosome strains maintained in laboratories lack genetic heterogeneity present in natural populations. The NHM's and LSHTM's purpose-built facilities have been working together for over two years providing UK and international researchers with material necessary for conducting research on schistosomiasis. Among other strains, SSR maintains and provides research material from the widely used *S. mansoni-B.*

glabrata NMRI system. As the demand for this standard material increased, so did our need to optimize material production so that we could standardise snail infection rates and also enable highly productive infections leading to the reduction in the numbers of snails needed to be bred and maintained.

Methods: The NHM snail lab has been maintaining different strains of *Biomphalaria glabrata* snails for many years. Although, all these strains have been selected for their compatibility to infections with *Schistosoma mansoni*, we have observed differences in infection efficiency. In order to streamline our culturing efforts and to limit potential bias in future experiments we compared infection rates, cercarial production, shell growth rate and mortality for four strains of *Biomphalaria glabrata* maintained in our facility in order to identify the best host.

Results: We found significant differences in infection rate and in numbers of cercariae produced by different strains. Infected snails also grew much faster, indicating relocation of material for growth. Notably, the highly inbred melanistic strain of *B. glabrata* that was recently derived from one individual snail showed the highest level of infection rate as well as the highest cercarial output.

Conclusions: We decided to focus with our *S. mansoni* infections on the highly inbred strain which in short time resulted in higher infection rates and lowered workload necessary to maintain snail cultures. Our experiment shows the necessity of constant assessment of snail/schistosomes compatibility as the infection rate can easily increase or decrease by random selection of alleles impacting transmission.

Poster 65* : The rumen fluke, *Calicophoron daubneyi*, express an expanded repertoire of pattern-recognition receptors.

Shauna Clancy, *Queen's University Belfast,*

S Clancy[1]; M Robinson[1]; RM Morphew[2]; SA Huws[1];
[1] Queen's University Belfast, UK; [2] Aberystwyth University, UK

The rumen fluke, *Calicophoron daubneyi*, is a parasitic trematode that infects ruminants such as sheep and cattle. Adult parasites reside within the rumen, where they attach themselves to the epithelium using a muscular posterior sucker. This location means that they are in intimate contact with host tissues as well as the rumen microbiome, a unique collection of bacteria, archaea, fungi and protozoa. To date, few studies have investigated the relationship between helminth parasites and the microbiome of domestic livestock. Here, we have exploited multi-omics resources for *C. daubneyi* in order to identify potential homologs of innate immune system molecules secreted by the parasite. These include molecules which may either possess direct antimicrobial activity or function as pathogen recognition receptors (PRRs), innate immune system molecules that recognise pathogen-associated molecular patterns (PAMPs). Whilst a number of innate immune molecule homologs have been identified, two putative PRRs, namely, peptidoglycan-recognition receptors (PGRPs) and DM9-containing proteins (DM9CPs), were found to exist as multi-member families which have undergone significant expansion in *C. daubneyi* compared to other helminth species. A family of saposin-like proteins (SAPLIPs), with putative antimicrobial activity, have undergone similar expansion in *C. daubneyi*. Our data suggest that, due to the unique selection pressures associated with its microbe-rich niche, *C. daubneyi* has have evolved a complex innate immune system which allows the parasite to recognise and respond to microbes within the rumen. This research represents an important first step towards a greater understanding of rumen fluke-microbiome interactions and provides a framework for functional studies of helminth innate immune system molecules.

Poster 66 : Analysis of specific targeting of kinetoplastid ERK8 and GSK3b by kinase inhibitors

Carlos Cordon Obras, *Instituto de Parasitología y Biomedicina López-Neyra (IPBLN-CSIC)*

C Cordon-Obras[1]; R Diaz Gonzalez[1]; G Perez-Moreno[1]; C Bosch-Navarrete[1]; B Martinez-Arribas[1]; LM Ruiz-Pérez[1]; MP Pollastri[2]; AB Dounay[3]; L Ferrins[2]; D Gonzalez-Pacanowska[1];
[1] Instituto de Parasitología y Biomedicina López-Neyra (IPBLN-CSIC), Spain; [2] Northeastern University, College of Sciences, United States; [3] Colorado College, United States

Kinase inhibitors have been described as a tool for rapid compound progression in the discovery of new treatments against parasitic diseases. We present data aimed at target identification for a series of compound classes with anti-kinetoplastid activity and potential kinase inhibitory activity. ERK8 and GSK3b, belonging to the CMGC superfamily of kinases, are known validated targets for therapeutic purposes in kinetoplastids. Existing data point towards these two kinases as potential targets of the selected compound clusters. Recombinant purified TbERK8 and TcGSK3b were obtained and the *in vitro* inhibition profiles of the two enzymes were established. *In vitro* assays were also performed with HsGSK3b for assessment of enzyme selectivity. Moreover, we generated a *T. brucei* line over-expressing TbERK8 and exposed it to a selected set of inhibitors. Kinase over-expression causes a severe growth defect in the parasite upon induction. This toxicity is reverted in a dose dependent manner by certain TbERK8 inhibitors, suggesting that the over-expressed kinase is the main target for this subset of compounds.

Poster 67 : *In vitro* antileishmanial activity of tryptophanol derivatives

Dr Sofia Cortes, *Instituto Higiene e Medicina Tropical, NOVA University Lisbon*

L Braz[2]; MC Almeida[1]; P Furtado[3]; RJ Ferreira[3]; A Tonno[3]; MM M. Santos[3]; S Cortes[1];
[1] Global Health and Tropical Medicine (GHTM), Instituto de Higiene e Medicina Tropical (IHMT), Universidade NOVA de Lisboa, Portugal; [2] Departamento De Medicina Preventiva, Faculdade de Medicina da Universidade de São Paulo (USP), Brazil; [3] Research Institute for Medicines (iMed.ULisboa), Faculty of Pharmacy, Universidade de Lisboa, Portugal

Leishmaniasis is a vector borne disease (VBD) caused by protozoan parasites of the genus *Leishmania* and still one of the Neglected Tropical Disesases (NTDs) causing more than 1 million cases/year worldwide. Current treatments have significant drawbacks in terms of efficacy, safety, compliance, and suitability for use in the field, with growing resistance in clinical isolates, indicating the urgent need for new therapeutic options. In recent years indole-based compounds have been explored as potential antiparasitic agents and indole nucleus has emerged as a privileged molecular scaffold for the generation of new drug candidates.

In this study, a small library of 14 indole compounds - tryptophanol derivatives - was synthetised and explored for its antiparasitic potential against *Leishmania* parasites. *Leishmania infantum* and *L. tropica* promastigote and intracellular amastigote forms, as well as oxidative stress, were used to test the compounds' *in vitro* anti-leishmanial activities. Assessments of cytotoxicity were also conducted on mammal cells (THP1 cell line). The anti-leishmania promastigotes activity produced by these drugs ranged from 100 to 7.3 μM in terms of IC_{50}. Compounds RJ59 and PAF92 induced ROS production, had low cytotoxicity, with good activity in intracellular amastigotes (IC50 20-6 μM). Based on these preliminary results, a new set of derivatives from these two compounds was synthesized by introducing different type of substituents in the main scaffold. Susceptibility of *L. infantum* promastigotes appear to significantly increase with very good safety profiles. More studies are ongoing support the relevance for further investigations of this class of compounds in the context of leishmaniasis therapy.

Acknowledgements: This work was supported by National Funds (Fundação para a Ciência e a Tecnologia) through iMed.ULisboa (UIDB/04138/2020), project PTDC/QUI-QOR/1304/2020 GHTM (UID/04413/2020), LA-REAL (LA/P/0117/2020), PhD fellowship 2022.11539.BD (R. Ferreira) and CAPES/PRINT(Brazil)-project 88887.915934/2023-00 (L. Braz).

Poster 68 : Comparison of molecular markers used for population genetic analyses of *Fasciola gigantica* from Pakistan

Dr Krystyna Cwiklinski, *Liverpool University*

M Komal[2]; K Afshan[2]; S Firasat[2]; JE Hodgkinson[1]; K Cwiklinski[1];
[1] University of Liverpool, UK; [2] Quaid-i-Azam University Islamabad, Pakistan

The helminth parasites, *Fasciola hepatica* and *Fasciola gigantica,* are the causative agents of fasciolosis, an economically important disease of people and their livestock worldwide. Molecular analyses of geographically dispersed *Fasciola* spp. isolates have revealed high levels of genetic diversity within liver fluke populations. These population genetic analyses allow the origins of *Fasciola* spp. isolates to be delineated and are vital for furthering our understanding of how drug resistance genes spread throughout liver fluke populations. Several molecular markers have been developed for analyses of *Fasciola* spp., based on ribosomal, mitochondrial and nuclear regions of the liver fluke genomes. In this study we compared four molecular markers (fatty acid binding protein, fabp; phosphoenolpyruvate carboxykinase, pepck; NADH dehydrogenase, nad1; RAPD) to investigate the *F. gigantica* population substructure across Pakistan. Adult parasites (n=595) were collected from buffalo and cattle across four provinces in Pakistan (Baluchistan, Gilgit and Skardu, Khyber Pakhtunkhwa, Punjab). The four molecular markers confirmed the species classification of all 595 parasites as *F. gigantica*, except for the fabp marker which could not be amplified in 274 parasites (46%), indicating genetic variation within the fabp gene sequence. These 274 samples were collected from across the four provinces, indicating the genetic diversity observed within the fabp gene was not specific to a geographical location. Sequence analysis of the mitochondrial nad1 gene revealed a predominance for single haplotypes within liver fluke parasites infecting individual animals, consistent with other *Fasciola* spp. genetic studies in Pakistan. This study verifies the high level of genetic diversity observed in *F. gigantica* parasites in Pakistan. It highlights the inconsistencies that can be observed between the *Fasciola* spp. molecular markers, indicating that robust analyses of genetic diversity in liver fluke populations requires multi-locus markers.

Poster 69 : Dog genetic background effect is predominant on clinical-immunological traits of the canine visceral Leishmaniasis

Luis Fabio da Silva Batista, *University of Sao Paulo*

LF da S Batista[2]; JL Reis-Cunha[1]; IH Chouman[2]; FM Ferreira[2]; TY Tomokane[2]; JG Mariotti[4]; PS Matsumoto[4]; VM Campriger[4]; VB Pereira[4]; MC Boité[3]; JE Tolezano[4]; E Cupolillo[3]; VL da Matta[2]; MD Laurenti[2]; DC Jeffares[1];
[1] University of York, UK; [2] Universidade de São Paulo, Brazil; [3] Instituto Oswaldo Cruz, Rio de Janeiro, Brazil; [4] Instituto Adolfo Lutz, Brazil

The domestic dog (*Canis lupus familiaris*) is the main reservoir of the visceral leishmaniasis (VL) in urban environment and a model for study of VL. Explore the genetic basis of the *Leishmania infantum* natural infection in domestic dogs can increase the understanding of the balance of different factors that lead to different clinical-

immunological outcomes in visceral leishmaniasis. Here, we examine a set of 234 dogs from Brazil to investigate how much impact the dog genetic background (DGB) has on canine VL (CVL), employing heritability analysis (HA) and genome-wide association study (GWAS). Before the genotype-phenotype association analysis, the genomic DNA from blood samples was genotyped by SNPchip Canine HD (Illumina) with 118,786 reliable SNPs upon filtration. Also, CVL phenotypic traits related to clinical outcome, parasite load, humoral immunity, cell-mediated immunity, and oxidative stress were adjusted with *L. infantum* Mitelfosine Sensitivity Loci (MSL) and epidemiological covariates that may impact exposure to the parasite or the responsiveness to infection (geographic origin, sex, age, repellent collar, anti-visceral leishmaniasis vaccine and treatment) in order to select the best model increasing the accuracy on HA and GWAS. Among the models, the combination of all epidemiological covariates was what most brought the traits closer to the Gaussian distribution, improved the Q-Q plots, increased the accuracy on the HA, and reduced spurious associations on GWAS, except for the traits anti-*L. infantum* IgA, anti-salivary gland lysate (SGL) of *Lutzomyia longipalpis* IgG, leishmanin skin test [LST], IL-10, IFN-g, TNF-a, NO. Inclusion of MSL as covariate in the HA did not significantly change the proportion of clinical-immunological outcomes explained by the DGB. The effect of the DGB was predominant when we compared with the MSL and epidemiological covariates effects, mainly for the traits anti-*L. infantum* IgG (~90% heritability), clinical staging (~40%), and total antioxidant capacity [TAC] (~42%), indicating that many dog genetic signatures contribute to these traits. Candidate genes for anti-*L. infantum* IgG-SNPs are involved in TLR4 inhibition (*PTPN4*) and cytokine receptors (*IL2R* and *IL15R*). *IL6* is one of the candidate genes for clinical staging and *NOS1* candidate gene for TAC. The effect of the DGB was lower but still predominant for PBMC proliferation index [PI] (~28%), superoxide dismutase [SOD] (25%), IgE (~25%), IgM (~24%), clinical outcome (~18%), TGF-b (~17%), total oxidant capacity [TOC] (~15%). For those traits, only for 2 SNPs associated to IgM the significance was above the GWAS cut off. For both, the candidate genes are related to innate immune response (*IL17C* and *MD2* - also involved in TLR4 signalling). Altogether, our findings point to the predominance of the host genome effect on the cli

Poster 70 : Interaction between the dog genetic background and distinct genotypes of *Leishmania infantum*

Luis Fabio da Silva Batista, *University of Sao Paulo*

LF da Silva Batista[2]; JG Mariotti[4]; JL Reis-Cunha[1]; FM Ferreira[2]; VL da Matta[2]; IH Chouman[2]; TY Tomokane[2]; PS Matsumoto[4]; VM Campregher[4]; VB Pereira[4]; E Cupolillo[3]; JE Tolezano[4]; DC Jeffares[1]; MC Boité[3]; MD Laurenti[2];
[1] University of York, UK; [2] Universidade de São Paulo, Brazil; [3] Instituto Oswaldo Cruz, Rio de Janeiro, Brazil; [4] Instituto Adolfo Lutz, Brazil

Genetic variability of Trypanosomatids may impact the outcome of infection, drug resistance or epidemiological traits of diseases. Discrepant geographic distribution of a deletion (DEL) in the Mitelfosine Sensitivity Locus (MSL) on chromosome 31 of *Leishmania infantum* was described in Brazil: Del strain (DEL) was identified in 15 Brazilian states whereas the non-deleted (Non-DEL) in 7. We hypothesized then that a subclinical and less detectable infection caused by DEL in the canine reservoir might persist for long-term in endemic areas, contributing to the greater spread of DEL than Non-DEL strain. To test the hypothesis, we performed a genome-wide association study (GWAS) to better understand the impact of the MSL on the clinical-immunological outcome in dogs naturally infected by *L. infantum*. From a set of 234 dogs, clinical outcome, parasite load, humoral immunity were evaluated, as well as the genomic DNA was genotyped by SNPchip Canine HD (Illumina) with 118,786 reliable SNPs. For the subset of 60 dogs, the MSL was genotyped by qPCR on lymph node aspirates (33 DEL/27 Non-DEL infections) in order to investigate if clinical-immunological traits

of subclinical infection were associated to DEL infection; the presence of dog genetic background (DGB) associated with predominant adaptation of DEL or Non-DEL; and if the inclusion of MSL as covariate change the significance of SNPs associated to clinical-immunological traits. When we regressed clinical-immunological traits onto environmental covariates (origin, sex, age, repellent collar, anti-*Leishmania* vaccine or treatment) and MSL genotypes, only anti-*L. infantum* IgA had a significant association (p = 0.00554) with MSL whereas clinical staging was only suggestively associated (p = 0.09639). Both lower clinical severity and low IgA levels were associated with DEL infection. Infection made by the DEL strain was also more frequent in dogs carrying an allele associated with low levels of anti-*L. infantum* IgG (P < 0,0001), confirming the hypothesis of mild infection caused by DEL strain. No principal component analysis (PCA) cluster, estimated heritability (REML) or SNP significantly associated with MSL were identified in dog genome indicating absence of direct association between the DGB and MSL. On the other hand, the inclusion of MSL as covariate slightly increased the significance of SNPs associated with anti-*L. infantum* IgG and IgM. These data strongly suggest that although there is no dog genotype directly associated to predominant adaptation of DEL or Non-DEL strains during the *L. infantum* infection, distinct MSL genotypes may differently interact with the host's immune system of dogs, leading to different clinical-immunological outcomes and effect of their genetic basis, potentially impacting the epidemiology of the canine visceral leishmaniasis.

Poster 71 : Effect of visceral leishmaniasis on humoral immunity: parasites hopping on the B cell train.

Dr Laura Dirkx, *University of Antwerp*

M Loyens[1]; L Dirkx[1]; M Radwanska[3]; S Magez[2]; G Caljon[1]; S Van Acker[1];
[1] University of Antwerp, Belgium; [2] Vrije Universiteit Brussel, Belgium; [3] Laboratory for Biomedical Research, Department of Environmental Technology, Food Technology and Molecular Biotechnology, Ghent University Global Campus, South Korea

Despite the distinctive significance of B cells in protective immunity and vaccine development for many infectious diseases, their role during intracellular parasitoses is often underexplored. This is also the case for the lethal protozoan disease, visceral leishmaniasis (VL), where the contribution of B cells is largely overlooked. To date, no effective human vaccines are available for VL, underscoring the complexity of the required protective immune response and knowledge gaps in the host-parasite interaction. Information obtained for African trypanosomes demonstrates that related parasites can impair B cell development and vaccine memory.

In this study, *Leishmania infantum* infection in BALB/c mice was found to increase B cell progenitors in the bone marrow and all B cell subtypes analysed in the spleen. This is in line with the clinical manifestation of polyclonal hypergammaglobulinemia and the occurrence of autoantibodies. Using immunization against a fluorescent heterologous antigen it was shown that infection does not impair immune memory, which is reassuring for vaccination campaigns in VL endemic areas. Interestingly, flow cytometric and microscopic examination identified attachment of viable amastigotes to B cells of the bone marrow and spleen, increasing the numbers of activated lysosomes. These extracellularly attached amastigotes could be transferred to infect macrophages.

We speculate that *Leishmania* parasites can hijack B cells to distribute throughout the host body using the haemolymphatic system. Although the underlying interactions and *in vivo* spreading remain to be uncovered, these observations demonstrate that B cells should not be overlooked during VL.

Poster 72 : Exploring the diversity of *Plasmodium falciparum* hypervariable RIFINs and their interactions with human immunomodulatory receptors

Dr Vincent Geoghegan, *University of York*

V Geoghegan[1]; C Crosnier[1]; GJ Wright[1];
[1] University of York, UK

During the intra-erythrocytic replication cycle, *Plasmodium* parasites express members of highly variable, multi-gene families which are exported and displayed upon the surface of the infected erythrocyte. These proteins are thus available to directly interact with and down-modulate the human immune system to promote survival of erythrocytes infected with parasites. With ~150 members, the RIFINs are one of the largest hypervariable multi-gene families in *Plasmodium falciparum* and certain RIFINs have been shown to directly interact with human immunoinhibitory receptors such as LAIR1 and LILRB1. The full immunomodulatory potential of the RIFIN family however is unknown. To directly explore the extent of RIFIN interactions with human immune receptors we have constructed a library of >3,000 unique RIFIN hypervariable domain sequences from *Plasmodium falciparum* lab strains and field isolates. Initial experiments demonstrated that RIFIN hypervariable domains could be expressed and purified from HEK 293 cell supernatants. By also using sf21 insect cells, we could further expand the repertoire of expressed RIFINs. Expressed RIFINs will be systematically tested for direct binding against a panel of ~750 soluble ectodomains representing the human immune receptor repertoire. Interactions detected between our RIFIN library and human immune receptors will provide important molecular insights into how *Plasmodium falciparum* modulates host immunity during malaria progression.

Poster 73* : The role of Fc receptors in the physiopathology of cutaneous leishmaniasis

Ikram Hammi, *University Paris Saclay/ University Hassan II*

HI Hammi[3]; AK Akarid[1]; AD Arnoult[2];
[1] Hassan II University of Casablanca, Morocco; [2] INSERM U1197, France; [3] INSERM U1197, University Paris Saclay/ University Hassan II, France

The problem of leishmaniasis is becoming more urgent as its epidemiology is climate sensitive. Among the 3 forms of leishmaniasis, cutaneous leishmaniasis (CL) is the most spread form worldwide. This form causes skin lesions and ulcerations on different body parts, which can leave long-lasting scars and serious disability. The first signal in the establishment of an effective immune system is the recognition of the intruder by receptors such as Fc Receptors which are found mostly on immune cells. This family of receptors harbours a tyrosine-based activation motifs (ITAM), which initiates several important pathways and which may have a duality in response against intruders, either fighting (ITAM activator) or helping the invasion of the intruder (ITAM inhibitor). In our project we have chosen to study profoundly the interaction between the receptor CD32a or FcRIIA and two different strains of Moroccan *Leishmania* (*L. major* and *L. tropica*). Our experimental setup involved utilizing both murine and human cell lines. Bone marrow-derived macrophages (BMDMs) were isolated from femurs extracted of C57BL/6 Wild type (Wt) and CD32a transgenic (Tg) mice. Additionally, we used the human monocytic cell line THP-1 in which CD32a expression has been knocked down with shRNA. We infected those cells with *L. major* or *L. tropica* and their production of pro-inflammatory cytokines was assessed with ELISA tests. No significant production of IL-1β or TNF-a by THP-1 was noticed. Curiously, western blot of cells infected with both strains revealed a cleavage of CD32a happening with the strain *L. tropica* but not *L. major*. A Kinetic of the infection showed that the cleavage was happening 1h post-infection. The expression of the CD32a was assessed after the infection with both strains through flow cytometry and no significant difference was observed, suggesting that the cleavage was intracellular. We tried several protease inhibitors to prevent

the cleavage of CD32a with no success. Furthermore, we tried to understand the pathways by which the receptor may play a role in the infection by analyzing the expression of different proteins involved in the ITAM pathways after infecting Wt or Tg BMDMs. We first investigated the proteins that play a role in the ITAM activator such as Syk and Fyn. Syk seems to be underexpressed in infected cells of both wt and transgenic mice. Fyn seem to be more underexpressed in BMDM from Tg mice infected with both strains. On the other side, proteins involved in the ITAM inhibitor, SHP1 and LYN seem to be activated as their phosphorylated form were observed in BMDM from Tg mice infected with both strains. It appears that the balance ITAMa/i tends towards the activation of an inhibitory ITAM *in cellulo.* Finally, we infected with both strains wild type and transgenic C57BL/6 mice in the ear. After a first experiment with no lesions, we have conducted a second experiment using *Leishmania freidlin*. Tg mice developed lesions at the second week post-infection, reaching its maximum after 5 weeks and then dropping right after next healing after 8 weeks. Wt mice start developing lesions after 3 weeks and reached a maximum at 5 weeks but still not healed after 8 weeks. The investigation of CD32a receptor interaction with Moroccan *Leishmania* strains sheds light on potential immune mechanisms in combating cutaneous leishmaniasis, with findings suggesting a tendency towards inhibitory ITAM activation in transgenic mice.

Poster 74 : The Tick Cell Biobank: tick and insect cell lines for parasitology research

Catherine Hartley, *University of Liverpool*

JJ Khoo[1]; AC Darby[1]; C Hartley[1]; B Makepeace[1]; L Bell-Sakyi[1];
[1] University of Liverpool, UK

Ticks are not only haematophagous ectoparasites in their own right, but also vectors of a range of disease-causing protozoan and helminth parasites, including species of the genera *Babesia*, *Theileria*, *Hepatozoon* and *Cercopithifilaria*. Haematophagous insects are also notorious for transmitting pathogenic parasites including species of the protozoa *Plasmodium*, *Trypanosoma* and *Leishmania* and the helminth genera *Wuchereria*, *Brugia*, *Loa* and *Onchocerca*. Cell lines derived from tick and insect vectors offer a convenient and accessible approach to many aspects of research on the arthropods and the pathogens they transmit. The Tick Cell Biobank (TCB) at the University of Liverpool is the world's only dedicated culture collection for cell lines derived from ticks and biting insects. We generate, store and supply cell lines and provide training in their maintenance through the parent TCB and our Outposts in Malaysia, Brazil and Kenya. We hold cell lines derived from 20 ixodid and three argasid tick species, including major livestock and human disease vectors, and a growing panel of cell lines from insect vectors including mosquitoes, sand flies, biting midges, tsetse flies and triatomine bugs. Cell lines are distributed to scientists worldwide subject to Material Transfer Agreements; for further information, contact us at tickcellbiobankenquiries@liverpool.ac.uk.

Poster 75* : Development of efficient CRISPR-Cas9 precision editing for *Leishmania* to investigate protein kinase function

Charlotte Hughes, *University of York*

C Hughes[1]; J Carnielli[2]; V Geoghegan[1]; J Faria[2]; J Mottram[1];
[1] University of York, UK; [2] York Biomedical Research Institute, Department of Biology, University of York, UK

Genetic modification has become a staple tool in *Leishmania* discovery research. The CRISPR-Cas9 system has enabled this process to be more flexible and efficient, as it can be used to make large-scale changes such as gene deletion, or small-scale changes, such as mutating individual codons of a protein-encoding gene, known as precision editing. Precision editing allows investigation into the role of specific amino acids in a protein in the parasite e.g. catalytic residues, ATP-binding site gatekeeper residues, and phosphorylation sites of protein kinases. To make precision editing more accessible, we have developed a method for *Leishmania mexicana* that is flexible, affordable, efficient, and selection-free. We use an oligonucleotide-based strategy to produce repair templates designed for synonymous and nonsynonymous mutations in both essential and nonessential genes; a python script aids the design process. We applied this precision editing approach to phosphorylation sites on kinetochore proteins or gatekeeper residues in the ATP-binding sites of protein kinases involved in regulating differentiation. Thirty-five individual precision edits were attempted in *L. mexicana T7Cas9* promastigotes. Up to 24 clones isolated from each of the 35 transfections were assessed by PCR for integration of the repair template, with at least 1 clone for each confirmed by Sanger sequencing to carry the expected homozygous mutation. The efficiency of integration varied for each precision edit. For all integration events (homozygous, heterozygous and "complex"), efficiency varied in each transfection from 8% to 83% (mean of 31%). Of all clones screened, homozygous mutants predominated (24%), with 4.2% heterozygotes and 2.0% "complex". The high efficiency of this precision editing approach allows the dissection of protein kinase signalling events in *Leishmania*.

Poster 76 : The role of *Schistosoma mansoni* MEG proteins during infection

Zaina Ibnahaten, *University of York*

Z Ibnahaten[1]; N Muller-Sienerth[2]; C Crosnier[3];
[1] University of York, UK; [2] Wellcome Sanger Institute, UK; [3] Department of Biology, University of York, UK

Schistosomiasis is a parasitic neglected tropical disease affecting over 240 million people annually, primarily in low to middle income countries. One of the main species of *Schistosoma* responsible for the disease is *Schistosoma mansoni* which is transmitted through infected water. Praziquantel is at present the only drug that effectively kills adult parasites, and no licenced vaccine is currently available. To survive within the vasculature, schistosomes must interact with their host and extracellular parasite antigens are likely to be involved in these interactions. Micro-exon genes (MEGs) encode for secreted and cell surface protein families, where symmetrical micro-exons (from 6bp up to 81bp) are present in the coding sequence and can give rise to multiple protein isoforms through exon skipping. MEGs also exhibit high non-synonymous/synonymous substitution rates and possibly play a role in immune evasion through alternative splicing and antigenic variation. *S. mansoni* lacking the oesophageal gland (a MEG upregulation hotspot) cannot establish infection in immunosuppressed mice, so it is strongly theorised that the MEGs expressed at the oesophagus play a role in immune evasion or suppression. Our aim is to characterise the role of *S. mansoni* MEG proteins during mammalian infection. In a first instance, we will aim to identify the MEG transcripts variants expressed at different life stages of the parasite's life cycle through long read RNA sequencing. These MEGs will then be expressed recombinantly in mammalian cells and tested against a library of human immune receptors using SAVEXIS to identify new host:parasite interactions. The function of these interactions will then be tested in cellular assays and possibly in a murine model of *S. mansoni* infection.

Poster 77 : Deconvoluting the mode-of-action of novel antileishmanial compounds

Dr Nathaniel Jones, *University of York*

NG Jones[1]; EA Alpizar-Sosa[2]; L Filipe[2]; MP Barrett[3]; J Mottram[1]; PW Denny[2];
[1] York Biomedical Research Institute, Department of Biology, University of York, UK; [2] Department of Biosciences, University of Durham, UK; [3] School of Infection & Immunity, University of Glasgow, UK

Compounds from the GSK Leishbox are active anti-leishmanials identified by phenotypic screening, but many of them have an unknown mode-of-action. This study aimed to identify the targets of a subset of nine LeishBox compounds, reduced to four following initial triage. To achieve this, drug-resistant strains of *L. major* and *L. mexicana* promastigotes were generated by *in vitro* evolution and then subjected to whole-genome sequencing. Following SNP calling and identification of coding mutations, four potential targets were selected for genetic validation, including two hypothetical proteins, one encoding a predicted amino acid transporter (AATP11), and one encoding a folate biopterin transporter (FBT).

Initially, null mutants were generated in *L.mx T7/Cas9* to separate likely essential targets from non-essential genes. Null mutants could be generated for all four genes, suggesting that these are not the sole targets of the compounds. Furthermore, since two of the proteins were predicted to be transporters, mutations in their genes were hypothesized to confer resistance to the compounds. When the Δ*aatp11* strain was treated with compound 3, it resulted in a 2-fold increase in the EC_{50} of the compound. Addback and over-expressor strains have been generated to confirm that this phenotype is gene-specific. The Δ*fbt* strain is currently being assayed. This target is part of a tandem gene array that is often mutated in response to anti-folates such as methotrexate.

Poster 78 : A First report of *Pseudosuccinea columella* (Say, 1817), an Alien Intermediate Host of Liver Fluke in Malawi

Sam Jones, *Liverpool School of Tropical Medicine*

S Jones[1]; P Makaula[2]; A Juhasz[1]; L Cunningham[1]; J Archer[1]; J Musaya[2]; S Kayuni[1]; JR Stothard[1];
[1] Liverpool School of Tropical Medicine, UK; [2] Malawi-Liverpool-Wellcome Trust Clinical Research Programme, Malawi

Commencing in October 2021, quarterly malacological surveys have been carried out at 12 locations in Mangochi, Chikwawa and Nsanje Districts in Malawi. These studies have been aimed at determining the spatial distributions of intermediate snail hosts of parasites of medical and veterinary importance. In March 2023 the lymnaeid snail *Pseudosuccinea columella* was first noted in Nsanje District and then again identified in Mangochi and Chikwawa Districts in June 2023, as well as an additional site in Nsanje. Of note one of the sites in Mangochi where the snail was found is directly connected to Lake Malawi, expanding the list of potential intermediate hosts of parasites in the lake. Specimens of *P. columella* were initially identified through the snail's characteristic shell micro-sculpture, differentiating it from the more common *Radix natalensis.* Molecular typing was then performed on snails transported back to the Liverpool School of Tropical Medicine and analysis of the mitochondrial ribosomal 16S region confirmed that the samples were *P. columella.* While *P. columella* is a well-known intermediate host for human and animal fascioliasis, through both *Fasciola hepatica* and *Fasciola gigantica*, no evidence of infection was found through either observed cercarial shedding or molecular xenomonitoring. However, the presence of this snail in Malawi, especially in the lower Shire valley and Lake Malawi raises concerns for altered local transmission for human and animal fascioliasis.

Poster 79 : Untargeted Metabolomics links alterations of host tyrosine metabolism with susceptibility to *Schistosoma mansoni* infection

Dr Mireille Kameni, *University of Bamenda*

M Kameni[6]; E Kamguia Meyo[4]; G Blackburn[7]; P Whitfield[7]; GJ van Dam[2]; PL Corstjens[2]; K Lennard[8]; C Demarta-Gatsi[5]; T Spangenberg[5]; P Lamberton[1]; J Nono Komguep[3];
[1] University of Glasgow , UK; [2] Leiden University Medical Centre, Netherlands; [3] Unit of Immunobiology and Helminth Infections/IMPM/MINRESI, Cameroon; [4] Institute of Medical Research and Medicinal Plant Studies(IMPM);, Cameroon; [5] Global Health Institute of Merck, a subsidiary of Merck KGaA, Darmstadt, Germany, Ares Trading S.A., Switzerland; [6] Unit of Immunobiology and Helminth Infections, Institute of Medical Research and Medicinal Plant Studies (IMPM), Ministry of Scientific Research and Innovation, Yaoundé, Cameroon; [7] Glasgow Polyomics, Wolfson Wohl Cancer Research Centre, UK; [8] Division of Chemical and Systems Biology, Faculty of Health Sciences, University of Cape Town, Cape Town, South Africa

Background: Hepatosplenic schistosomiasis caused by *Schistosoma mansoni* (Sm) remains a significant public health concern, particularly in endemic regions like Cameroon. Understanding the metabolic changes induced by Sm infection is crucial for uncovering unprecedented host signatures of the infection and markers of disease pathogenesis.

Methods: In this study, we employed LC-MS untargeted metabolomic profiling on plasma samples obtained from school-aged children in areas of low and moderate endemicity for Sm in Cameroon, strong holds of parasite persistent transmission that withstand elimination. Diagnosis of Sm infection was conducted using the Kato Katz (KK) method and complemented by an up-converting phosphor-lateral flow circulating anodic antigen (UCP-LF CAA) assay. Liver morbidity was assessed by ultrasonography (US). Children were stratified into four groups based on infection and liver fibrosis status: Group 1 - Infected without liver fibrosis (KK+US-); Group 2 - Infected with liver fibrosis (KK+US+); Group 3 - Not infected with liver fibrosis (KK-US+); and Group 4 - Not infected without liver fibrosis (KK-US-). From each group, three successive batches of patients were screened by untargeted metabolomics probing of isolated plasma to uncover differentially abundant metabolites and associated pathways during Sm infection and/or associated liver fibrosis. Every hit was searched for robust occurrence in the list of differential metabolites through a first 'discovery' metabolomics run, a 'validation' run and then a 'stability in front of polyparasitism' run (for the latter, studied groups included individuals coinfected with malaria or hepatitis). Comparative analyses were performed in each run using various statistical methods, including fold change analysis, t-tests, sPLSDA, VIP score, heatmap visualization, and ROC curve analysis.

Results: Our metabolomic profiling identified significant alterations in multiple metabolic pathways associated with Sm infection and liver fibrosis susceptibility. Strikingly, alteration of Tyrosine metabolism unprecedentedly emerged as a robustly perturbed pathway across the discovery and the validation runs during Sm infection, suggesting its potential as a candidate biomarker for Sm infection. Moreover, in the polyparasitism run, which examined the host plasma metabolome under conditions of concurrent infections, the persistence of alterations in tyrosine metabolism in Sm infected hosts highlighted the resilience of this pathway as a biomarker of Sm infection in the field (where coinfected sites are usually the norm).

Conclusion: This study highlights the consistent perturbation of tyrosine metabolism in the plasma of Sm infected hosts and its potential as a pathognomonic characteristic of Schistosomiasis to inform adjunct diagnostic, monitoring or therapeutic tools.

Keywords: Metabolomics, *Schistosoma mansoni*, live

Poster 80 : Reduced plasma levels of GM-CSF as biomarker of *Schistosoma mansoni* infection in school aged children

Elvis Leonel Kamguia Meyo, *Laboratory of Molecular Biology and Biotechnology, CRSPP/IMPM/MINRESI*

S Kamdem[2]; E Kamguia Meyo[4]; A Oumarou[5]; B Bitye Zambo[1]; F Brombacher[6]; C Demarta-Gatsi[3]; T Spangenberg[3]; J Nono Komguep[1];

[1] Unit of Immunobiology and Helminth Infections/IMPM/MINRESI, Cameroon; [2] Department of Pathology, University of Utah, Salt Lake City, UT,USA, United States; [3] Global Health Institute of Merck, a subsidiary of Merck KGaA, Darmstadt, Germany, Ares Trading S.A., Route de Crassier 1, 1262 Eysins, Switzerland; [4] Unit of Immunobiology and Helminth Infections, Laboratory of Molecular Biology and Biotechnology/CRSPP/IMPM/MINRESI, Cameroon; [5] Ministry of Public health, Yaoundé, Cameroon, Cameroon; [6] Cape Town Component, International Centre for Genetic Engineering and Biotechnology, Cape Town, South Africa

Schistosomiasis is a neglected tropical disease (NTD) that persists despite decades of intensive global control efforts. Diagnosis in low burden settings and logistically easy to use morbidity monitoring tools are lacking and much needed to facilitate the elimination of the disease as a public health problem by 2030. Since, the infection associates with a highly dynamic host cytokine response, we comprehensively evaluated the potential of host plasma cytokines as biomarkers of intestinal Schistosomiasis and/or associated liver fibrosis. We performed a cross-sectional study on school children from a *Schistosoma mansoni* endemic area in rural Cameroon. Participants were screened for schistosomiasis and liver fibrosis using Kato Katz and ultrasonography, respectively. Plasma cytokines were screened and compared between phenotypical distinct groups (harbouring or not infection or morbidity) by Luminex in a discovery set of samples; identified candidate cytokines of dissimilar plasmatic levels were further confirmed by ELISA on a validation set of samples. Data were compiled in Excel software, analysed with RStudio and graphs plotted with GraphPad Prism. From a preliminary screening of 27 plasma cytokines by Luminex on the discovery set of participants, only three candidate cytokines were altered by the host infectious or pathology profile i.e. GM-CSF, IL-2 and VEGF. Cytokine-specific ELISA assays on a separate set of validation participants confirmed children with *S. mansoni* infection to present with significant lower plasma levels of GM-CSF and IL-2 when compared to *S. mansoni*-negative children. Further assessment of the biomarking potential of these cytokines revealed a positive correlation between plasma levels of GM CSF, but not those of IL-2, and *S. mansoni* egg burdens in infected individuals. Moreover, when applying a threshold of plasma GMCSF levels for the screening of *S. mansoni* at-risk children, we could achieve an augmentation of the sensitivity of a single Kato-katz by at least 20%. Finally, a Receiver operating Characteristic Curve of GM-CSF performance as a predictive marker of *S. mansoni* infection in our study population yielded an Area Under the Curve of 75%, confirming the possible use of plasmatic levels of GM-CSF as good predictive marker of *S. mansoni* infection. In conclusion, our work revealed the potential of biomarking *S. mansoni* infection by comparative measurements of plasma GM-CSF. A basis for the refined use of plasma GM-CSF as a Schistosomiasis adjunct diagnostic tool is herein suggested.

Poster 81* : Measuring the impact of treatment regimens on the evolution of anthelmintic resistance

Benedict Karani, *University of Glasgow*

BE Karani[3]; J McIntyre[3]; F Kenyon[1]; R Laing[3]; SR Doyle[2]; JA Cotton[3];

[1] Moredun Research Institute, UK; [2] Wellcome Trust Sanger Institute, UK; [3] School of Biodiversity, One Health and Veterinary Medicine, University of Glasgow, UK

Parasitic worms are widespread pathogens that cause enormous negative impacts on the health of humans and animals. These worms are largely controlled by use of anthelminthic drugs such as ivermectin. The large-scale use of anthelmintics in livestock especially sheep has led to worm populations developing resistance. Despite the spread of anthelmintic resistance (AR), there's limited knowledge on the genetic basis of the resistance to some drug classes. This project aims to use modern genomic approaches to study the evolutionary dynamics of AR genes by comparing different approaches to treatment with ivermectin, a major anthelminthic drug in livestock. This is key in identifying the genetic markers driving AR in *Teladorsagia circumcincta*, a common parasitic worm in the UK sheep; and how different ivermectin treatment approaches impact the rate at which AR evolves.

Poster 82* : Using deep-amplicon sequencing to understand the dynamics of gastrointestinal nematodes present in co-grazed host species kept in a Scottish zoological collection

Dr Rob Kelly, *Royal (Dick) School of Veterinary Studies and The Roslin Institute, University of Edinburgh*

RF Kelly[1]; E Galbraith[1]; O Zahid[1]; ND Sargison[1];
[1] Royal (Dick) School of Veterinary Studies and The Roslin Institute, University of Edinburgh, UK

Aims: Grazing animal species kept in zoological collections can be at risk of significant gastrointestinal nematodes (GIN) infections, due to life-cycle maintenance in confined grazing areas and proximity to other susceptible hosts. Yet little work has been undertaken to understand the GIN species dynamic within co-grazed host-species. This pilot study aimed to describe the species diversity of nematode species found in faeces of grazing animals in a Scottish zoological collection.

Methods: At a single time-point, fresh faecal samples were collected randomly from the ground of the studied animals' enclosures including horses, sheep, alpacas, deer and tapirs. Faecal worm egg counts (FWECs) were conducted using a saturated saline centrifugal flotation cuvette technique. The nematode species profile was estimated through deep-amplicon sequencing of ITS-2 and mitochondrial markers to understand infection dynamics between host-species.

Results: There was a 95% prevalence of gastrointestinal nematode infection. Magnitude in faecal worm egg counts varied between individual host-species ($p<0.01$). Apart from goats with a FWEC 460epg, all other animal groups had egg counts <200epg. There were various gastrointestinal parasite species found across host-species, including the potentially pathogenic species *Haemonchus contortus* and *Teladorsagia circumcinta.* Potential transmission dynamics, between host species, were explored by phylogenetic trees. Mitochondrial markers were also compared using other animal holdings in the UK to explore the population structuring.

Conclusion: The variation in egg counts and parasite species suggest how different host-species and management factors could influence transmission. A high prevalence of GINs was found across all host-species. In particular, the increased egg count found in goats and the presence of potential pathogenic species (*H. contortus*) indicates the importance of regularly monitoring levels of parasitic infections in mixed-host-species zoological collections to develop sustainable control programmes.

Poster 83* : The Origin and Evolution of *Plasmodium falciparum*

Martha Kivecu, *University of Edinburgh*

M Kivecu[2]; B Omondi[2]; RW Nowell[1]; P Sharp[2];
[1] University of Stirling, UK; [2] The University of Edinburgh, UK

The malignant human malaria parasite *Plasmodium falciparum* evolved from the zoonotic transmission of a gorilla parasite, *Plasmodium praefalciparum*. However, the exact mechanism and timing of this event remain a matter of debate. Some have suggested that *P. falciparum* arose around 60,000 years ago, while others have suggested that it occurred after the origin of agriculture, less than 10,000 years ago.
In both humans and a bacterial pathogen, *Helicobacter pylori*, it has been reported that levels of genetic diversity decrease with distance from the area of origin of modern humans in East Africa. In an analysis of the genetic diversity using two housekeeping genes from 519 samples, Tanabe *et al.* (2010), found that the genetic diversity of *P. falciparum* also decreased with distance from the eastern part of Central Africa. They interpreted this as evidence that *P. falciparum* emerged from Africa at the same time as humans, around 60,000 years ago.

Now, thousands of *P. falciparum* whole genome sequences are available. This study aims to determine the geographical distribution of genome-wide genetic diversity and its implications for the origin of *P. falciparum*. Genome-wide genetic diversity was calculated for a total of 10, 333 sequences from 88 study sites in 33 countries. As expected, it was found that the genome-wide nucleotide diversity was higher in African sites (0.050%) as compared to other regions of the world (0.038% in Oceania, 0.035% in Asia, 0.031% in S America).

For the 46 study sites across Africa, the 'least-cost' geographical distance from each of 2,000 uniformly spaced potential sites of origin was calculated, and the Spearman correlation between genome-wide genetic diversity and least-cost geographical distance was calculated. The highest negative correlation was obtained for locations in the Western part of central Africa, close to the range of Western lowland gorillas (*Gorilla gorilla*) that harbour *P. praefalciparum*, i.e., the genetic diversity of *P. falciparum* declines with distance from where western gorillas are found. Since this correlation is expected to be disrupted by demographic factors over time, this finding is consistent with a very recent origin of *P. falciparum.*

Poster 84* : Sphingosine kinase (SPHK), a new drug target for treating Schistosomiasis

Ziada Kiwanuka, *Aberystwyth University*

Z Kiwanuka[1]; J Forde-Thomas[1]; K Hoffmann[1];
[1] IBERS, Aberystwyth University, UK

Schistosomiasis is a neglected tropical disease (NTD) that currently affects over 230 million people in subtropical and tropical regions with a further 779 million people at risk of becoming infected globally. *Schistosoma mansoni* is one of the three major parasitic trematodes that causes schistosomiasis, with 54 million people infected annually with this species. Praziquantel is known to be the only drug recommended by the (WHO) for the treatment and control of all *Schistosoma* species worldwide, however, there is evidence suggesting that resistance is indeed a possibility.

In the absence of a vaccine, and to prepare for praziquantel resistant, new chemical matter needs to be brought into the drug discovery pipeline to sustain schistosomiasis control.

SPHK, is an enzyme that phosphorylate sphingolipid sphingosine to produced sphingosine 1 phosphate (S1P). S1P regulates various cellular processes and survival of the parasite when in the host. Dysregulation of SPHK and S1P signalling has been implicated in several diseases, including cancer, inflammation, and autoimmune disorders. Understanding how *Schistosoma mansoni* parasites are affected by inhibiting Sphk1 provides insights into potential drug targets for treatment against schistosomiasis

SPHK1 Inhibitors were screened against adult worm pairs (50 μM) followed by dose response titration (25 μM, 12.5 μM, and 6.25 μM) with phenotypic scoring conducted at 24, 48, and 72 hours using the WHO-TDR scoring matrix. Worm death, motility effect and egg production effects were observed.

Determined from BLASTp interrogations, the likely target of these SPHK1 inhibitors is Smp_157100, a *S. mansoni* protein that contains conserved amino acid residues to those found in critical positions within the human functional homologue.

Moving forward, additional phenotypic screens using chemical analogues of the most potent SPHK inhibitor will be performed as well as SPHK activity in *S. mansoni* will be measured in adult worms treated with the SPHK inhibitors compared to controls. Confirmation of these results in adults will be performed using an antibody (S1P antibody) that detects phosphorylated sphingosine (S1P). Additionally, Long-term culture of the worms treated with compounds will be performed to observe the occurring effects upon worm development. Finally, RNAi will be used to knock-down s*mp_157100* to provide further support that SPHK is essential to *S. mansoni*. The results of these experiments will provide complementary evidence for progressing SPHK as an urgently needed novel drug target for schistosomiasis.

Poster 85 : To process or not to process: the role of the PfRh5 pro-domain in red blood cell invasion.

Dr EIlen Knuepfer, *Royal Veterinary College*

M Hart[2]; M Higgins[1]; E Knuepfer[2];
[1] University of Oxford, Department of Biochemistry, UK; [2] Royal Veterinary College, UK

Invasion of red blood cells (RBCs) by *Plasmodium* merozoites is a critical event in the life cycle of these parasites that ensures their continued survival within the host. Invasion progresses through several morphological steps, including gliding motility, deformation of the host RBC, a calcium 'flux' at the merozoite-RBC interface, and finally, parasite entry and resealing of the host cell. Throughout invasion, merozoites secrete numerous proteins from specialised secretory organelles, called micronemes and rhoptries, that facilitate each step.

One such ligand, *Plasmodium falciparum* Reticulocyte binding protein homolog 5 (PfRh5), is part of an essential pentameric complex hypothesised to mediate the calcium flux via an unknown mechanism. Importantly, just before, or during invasion itself, PfRh5 is proteolytically cleaved by the aspartic protease, Plasmepsin X (PMX), to release a short (~14 kDa) N-terminal pro-domain from the remainder of the protein. However, precisely when and why PMX processing occurs is not known.

Recently, we developed a Dimerisable Cre-Recombinase (DiCre) 'conditional swap' system to replace wild type PfRh5 with mutant copies upon rapamycin treatment[1], thus enabling us to investigate the functional importance of the PfRh5 pro-domain and PMX processing. We, as others have recently shown[2], demonstrate that the PfRh5 pro-domain is important for successful invasion, and that providing parasites with a pre-processed PfRh5

copy lacking a pro-domain does not complement the loss of full-length PfRh5. Finally, we show that parasites expressing a non PMX-cleavable version of PfRh5 also cannot invade, demonstrating that PMX processing is essential but needs to occur in a tightly ordered sequence of molecular events for invasion to proceed.

1 Farrell B *et al.* Structure of the PfRCR complex which bridges the malaria parasite and erythrocyte during invasion. *Nature* 2024; 625: 7995.

2 Triglia T *et al*. Plasmepsin X activates the PCRCR complex of *Plasmodium falciparum* by processing PfRh5 for erythrocyte invasion. *Nat Commun* 2023; 14: 2219.

Poster 86 : Intricate balance of dually-localized catalase modulates infectivity of *Leptomonas seymouri*

Natalia Kraeva, *Life Science Research Centre, University of Ostrava*

N Kraeva[1]; L Chmelová[1]; A Saura[1]; D Feder[3]; J Lukeš[2]; A Kostygov[1]; V Yurchenko[1];
[1] Life Science Research Centre, Faculty of Science, University of Ostrava, Ostrava, Czechia; [2] Institute of Parasitology, Biology Centre, Czechia; [3] Universidade Federal Fluminense, Instituto de Biologia, Programa de Pós-Graduação em Ciências e Biotecnologia, Niterói, Brazil

In this work, we analyzed subcellular localization and function of catalase in trypanosomatids. We demonstrated that catalase in *Leptomonas seymouri* is present in the cytoplasm and a subset of glycosomes, and that its cytoplasmic retention is H_2O_2-dependent. The ablation of catalase in this parasite is not detrimental *in vivo*, while its overexpression resulted in a substantially higher parasite prevalence in the experimental infection of *Dysdercus peruvianus*. We propose that the capacity of studied flagellates to modulate the catalase activity in the midgut of its insect host facilitates their development and protects them from the oxidative damage at elevated temperatures.

Poster 87 : Novel components of the expression-site body and surrounding splicing bodies discovered by TurboID proximity labelling in African Trypanosomes

Dr Lianne Lansink, *University of York*

L Lansink[2]; M Jones[2]; AA Dowle[1]; J Correia Faria[3];
[1] University of York, UK; [2] Department of Biology, University of York, UK; [3] Biology Department & York Biomedical Research Institute, University of York, UK

African trypanosomes use antigenic variation to evade the host immune response. They hide by obscuring invariant membrane-bound antigens under a dense coat of one out of >2000 Variant Surface Glycoprotein (VSG) isoforms. Their ability to express a single *VSG* at any given time, *monogenic expression*, is essential for successful antigenic variation. However, the molecular mechanisms underpinning this complex process are not fully understood.
In *Trypanosoma brucei* bloodstream-form, the single active-*VSG* is transcribed by RNA-Polymerase I within the expression-site body (ESB). In spatial proximity to the ESB are other subnuclear bodies including the *Spliced-Leader*-(*SL*)-associated body (SLAB), essential for *trans*-splicing, and the NUFIP 'body', also thought to play a role in splicing. This spatial integration of transcription and splicing facilitates mRNA maturation and combined with other mechanisms enables the production of vast amounts of the active-*VSG* (10% of the total proteome).

VSG-Exclusion Protein 2 (VEX2) specifically localises to the ESB and establishes an inter-chromosomal bridge via VEX1 to the *SL*-locus; other *VSGs* are excluded from this sub-nuclear 'transcription and splicing factory'.

To define the protein network within the ESB and spatially proximal bodies and better understand the sub-nuclear context in which VEX1 and VEX2 operate, we fused VEX1-C, VEX2-N and VEX2-C with TurboID and performed proximity labelling. Proteomic analysis of proximally enriched proteins captured known interactors (VEX2, VEX1 and CAF-1 subunits), as well as components of the ESB and neighbouring bodies that have been previously identified, therefore validating our approach. Notably, we identified novel components of the 'transcription and splicing factory', and their localisation has been subsequently validated using super resolution microscopy. Among these were three novel ESB-specific components, a range of ESB-enriched proteins and one new SLAB component as well as four NUFIP enriched proteins. We are currently functionally characterising the novel ESB-specific components, two of which are stage-specific, and defining their very own spatial interactome.

Following the discovery of the VEX proteins and ESB1, the identification of novel ESB-specific factors using proximity labelling constitutes a significant advance in the understanding of the molecular mechanisms governing the biology of antigenic variation regulation in African trypanosomes.

Poster 88 : Screening a protein kinase library for new chemical starting points against *Schistosoma mansoni*

Dr Kristin Lees, *Aberystwyth University*

K Lees[1]; JE Forde-Thomas[1]; MG Taylor[2]; E Holmes[2]; R Street-Jeakings[2]; BJ Hulme[1]; M Evans[1]; B Dankwa[1]; N Caldwell[2]; B Baragaña[2]; IH Gilbert[2]; KF Hoffmann[1];
[1] Aberystwyth University, UK; [2] Wellcome Centre for Anti-Infectives Research, University of Dundee, Dundee, UK

Schistosomiasis a neglected tropical disease caused by blood flukes of the genus *Schistosoma.* This parasitic trematode currently infects over 200 million people worldwide, resulting in around 12,000 deaths annually. At present, Praziquantel is the only drug available to treat schistosomiasis. However, it is ineffective against the juvenile stages of the parasite, necessitating multiple treatments. With no anti-*Schistosoma* vaccine on the horizon, mass drug administration programs form the predominant line of defence in controlling this disease. Therefore, new drugs are desperately needed.

To identify novel chemicals with anti-schistosomal action, we collaborate closely with our partners at the Drug Discovery Unit in Dundee. One approach we employ to identify prospective therapeutic leads is to screen pre-existing compound libraries. The KCGS Protein Kinase Set (v1) is one such library that contains 187 inhibitors of 215 human kinases. These 187 compounds were initially tested at a single point concentration (10 μM) on our automated high-throughput platform (Roboworm) against *Schistosoma mansoni* schistosomula. There were 26 hits from this initial screen. Compounds with pan-kinase effects (e.g. targeting 5 or more human kinases) or with published cell toxicity data were discarded. The remaining 15 compounds were purchased (where possible), resynthesized by Dundee or re-supplied by SGC for validation and titration studies in schistosomula and adult worms. To investigate the wider chemical series of the 15 hits, commercial analogues were acquired for further phenotypic screening and DMPK analysis. Hit-to-lead studies are now being undertaken for multiple chemical series.

Poster 89 : Novel environmental biomarkers for liver fluke control.

Mia Ley, *Aberystwyth University*

M Ley[1];
[1] Aberystwyth University, UK

Fasciola hepatica is a species of parasitic flatworm of global concern, due to its ability to cause fascioliasis in both humans and animals with a significant impact on livestock species. An estimated 90 million humans and 700 million ruminants are at risk of liver fluke infection, with economic losses a serious issue for farming communities. This study intends to uncover novel environmental biomarkers for liver fluke control to improve field diagnostics and subsequently highlight risk areas for *F. hepatica* infection. To begin, uninfected *Galba truncatula* snails, an invertebrate host of *F. hepatica*, as well as infected snails at day 10 and day 42 post infection will be harvested to generate the transcriptome for *G. truncatula* and to determine key genetic differences between uninfected and infected snails. The study will then focus on the collection of environmental DNA (eDNA) from water inhabited by *G. truncatula* to identify potential biomarkers that could be used for the development of a field-based diagnostic test. Current diagnostic tests are laboratory-based and include faecal egg counts (FEC) and enzyme-linked immunosorbent assays (ELISA), but new diagnostic methods are warranted to achieve quick and accessible diagnosis of liver fluke presence onsite to minimise transmission risk.

Poster 90* : Repurposing drugs to tackle amoebic gill disease in Atlantic Salmon

Yee Wan Liu, *School of Biodiversity, One Health and Veterinary Medicine, University of Glasgow*

Y Liu[6]; B Cheaib[1]; P McGinnity[2]; N Ruane[2]; R Williams[5]; FL Henriquez-Mui[4]; J Archibald[3]; M Barret[7]; MS Llewellyn[1];
[1] University of Glasgow , UK; [2] Marine Institute, Newport, Ireland; [3] Dalhousie University, Halifax, Canada; [4] University of the West of Scotland, UK; [5] Institute of Biomedical and Environmental Health Research, School of Health and Life Science, University of West Scotland, UK; [6] School of Infection and Immunity, University of Glasgow,UK; [7] School of Infection & Immunity, University of Glasgow, UK

Amoebic gill disease (AGD) is a devastating disease that causes multi-million-dollar losses annually in salmonid fish farming. The causative agent of AGD in Atlantic Salmon is *Neoparamoeba perurans* which belongs to the *Paramoebidae* family. An interesting feature of most of the *Paramoebidae* is the endosymbiotic relationship they have with the *Perkinsela*-like organism (PLO). The PLO is a *kinetoplastid* distantly related to the Tritryps parasites. As there appears to be a high level of metabolic interdependence between host and symbiont, eliminating the PLO, which resides adjacent to the nucleus of its symbiotic host, will hypothetically eliminate *N. perurans*. To this end, we have chosen to co-opt frontline and experimental trypanocidal drugs to target the PLO.

N. perurans cannot be grown axenically, so traditional drug screening methods are impeded by bacterial activity within the amoebic culture. To assay *in vitro* efficacy, holographic microscopy was used to assay cell motility and viability under drug pressure. Drugs which showed good *in vitro* efficacy were then deployed during two rounds of *in vivo* trials on the West Coast of Ireland, with promising signs of efficacy. We hope that providing a market for trypanocidal drugs in aquaculture may not only improve fish welfare but also lower their cost of production for deployment in a medical context in the tropics.

Keywords: endosymbiosis, Kinetoplastid, amoeba, amoebic gill disease, anti-leishmanial, anti-trypanosomal, holographic microscopy, drug screening method

Poster 91* : Targeted genetic knockouts in *Leishmania mexicana* reveal roles for lipid metabolism in drug sensitivity

Lizzie Marriott, *Durham University*

L Marriott[1]; PW Denny[1];
[1] Durham University, UK

Leishmaniasis and invasive fungal diseases, both caused by eukaryotic pathogens, are diseases for which historical neglect has left us with a limited armoury of therapeutics. Emerging issues with the few drugs we do have for the treatment of these diseases present an increasing risk to global health, especially within vulnerable populations. As such there is a pressing need for novel antimicrobials and a comprehensive understanding of their mode of action. Previous study has implicated the central role of lipids and their synthesis in the modes of action of both amphotericin B and miltefosine, two anti-microbials currently used in the clinical treatment of leishmaniasis and fungal infections. To further the goal of building a comprehensive understanding of the modes of action of these drugs in the context of lipid metabolism, a curated library of CRISPR/Cas9 modified *Leishmania mexicana*, engineered with genetic knockout of key genes within lipid metabolism pathways were subjected to drug sensitivity and phenotypic screening. This library screening approach was able to uncover genes for which knockout contributes to decreased membrane integrity and resultant sensitivity to drug pressure. Of note however are several genes for which the knockout increases resistance to one or both drugs, implicating the gene product or it's associated metabolites in the drug mode of action. The most significant changes in drug sensitivity were found after knockout of inositol phosphoryl ceramide synthase (IPCS), a key enzyme in *Leishmania spp.* sphingolipid synthesis, where a 4-fold increase in resistance to miltefosine accompanied by a 2-fold increase in sensitivity to amphotericin B were observed. Initial screening of drug sensitivity in genetically modified *L. mexicana* has provided a candidate list of genes that will be further studied in parallel investigations in fungi to further elucidate key areas within the mode of action of these drugs. Additionally, the library screening approach in both organisms will be applied to assess the mode of action of novel compounds with anti-leishmanial activity.

Poster 92 : *Amphilina bipunctata* (Riser 1948) a monozoic cestode from North American Sturgeon in the Dawes Collection, Museum of Life Sciences, King's College London: a comment on zoogeography and validity

Dr Andrew McCarthy, *Higher Education Animal Sciences, Canterbury College*

AM McCarthyY[1];
[1] Museum of Life Sciences, King's College London, UK

Members of the cestode genus *Amphilina* are monozoic cestodes parasitic in the body cavity (coelom) of sturgeons. It has been suggested that they are neotenic forms of strobilate tapeworms that once developed as adults in a now extinct reptile definitive host, possibly a fish-eating dinosaur. During examination of the Dawes Collection of helminth microscope slides at the Museum of Life Sciences, King's College London, a single specimen, (D901), of a monozoic cestode was discovered labelled "*Amphilina bipunctata*, *Ex. White & Green Surgeon, Oregon. U.S.A*." Subsequent research revealed that this species had first been described by Riser (1948) from specimens found at the Hopkins Marine Station of Stanford University in a jar mis-labelled

"Trematoda", the label with the specimens having the following information, *"Dodson, Oregon. From coelom of sturgeon. Aug 1923. Carl D. Duncan".*

Since its description *A. bipunctata* has been relegated to a junior synonym of *Amphilina japonica* Goto & Ishii,1936. Though this status is currently accepted, we venture to pose the question as to whether the situation might be re-examined, citing the following. *Amphilina japonica* of Goto and Ishii (1936) was described from the Sakhalin Sturgeon *(Acipenser mikadoi)* a species known to inhabit the Amur and Tumin Rivers in Russia, Sakhalin Island and the Sea of Japan, locations geographically distant from Oregon on the West Coast of North America (most probably the Columbia River at Dodson) where the sturgeon species from which *A. bipunctata* was described were obtained; the White Surgeon *(Acipenser transmontanus)* and the Green Sturgeon *(Acipenser medirostris)*. In his description of *A. bipunctata* Riser (1948) mentioned several morphological features that he felt differentiated the North American species from *A. japonica,* these including the size and shape of the testes and ova, the extent of the testes with respect to the vitellaria, and the configuration of the Mehlis gland. In a footnote to their Table 1, Margolis & McDonald (1986) mention that Malmberg (University of Stockholm) concluded that the two species could actually be distinguished on the basis of the shape of the larval (lycophore) hooks.

It is envisaged that detailed morphological studies, perhaps using new technologies such as Micro-CT Scanning, and also the use of molecular genetic analyses might shed light on the true systematic relationship between *A. bipunctata* and *A. japonica*.

Acknowledgements: The author gratefully acknowledges Dr Gillian Sales Curator of the Museum of Life Sciences at Kings College London for access to the Dawes Collection and provision of research facilities.

References: Goto, S. and Ishii, N. (1936) On a new cestode species*, Amphilina japonica*. *Japanese Journal of Experimental Medicine*. 14, pp. 81-83.

Margolis, L. and McDonald, T.E. (1986) Parasites of the White Surgeon, *Acipenser transmontanus*, from the Fraser River, British Columbia. *Journal of Parasitology* 72 (5) pp.794-796.

Riser, N.W. (1948) *Amphilina bipunctata* n. sp. A North American Cestodarian. *Journal of Parasitology* 34 (6), pp.479-485.

Poster 93 : *Trypanosoma cruzi* PUF3 RNA-binding protein modulates genes linked to mitochondrial morphology and function

Dr Ana Maria Mejia-Jaramillo , *Universidad de Antioquia*

AM Mejía-Jaramillo[1]; GF Fernandez[1]; H Ospina-Zapata[1]; AM Murillo[1]; DE Jimenez[1]; LA Gómez[2]; C Lowenberger[3]; O Triana-Chávez[1];
[1] Universidad de Antioquia, Colombia; [2] Universidad Eafit, Colombia; [3] Simon Fraser University, Canada

The RNA-binding PUF proteins are post-transcriptional regulators found throughout the eukaryotic domain that control the stability and translation of transcripts through the binding to specific recognition sequences in the 3´untranslated regions (3´-UTRs) of mRNAs. Few PUF proteins have been characterized in *Trypanosoma cruzi.* Considering that the control of gene expression in this parasite is mainly at the posttranscriptional level, further studies are needed to determine the functional depiction of the PUF family. Here, we characterized the PUF3 protein by knocking out and overexpressing the gene in *T. cruzi* epimastigotes and studied different genetic and biological features. The RNA-seq analysis in both genotypes showed significant changes in the number of regulated transcripts compared with wild-type parasites. Thus, the differentially expressed genes analysis in the knockout (Δ*TcPuf3)* and the overexpressor (pTEX*TcPuf3)* were 238 and 187, respectively. However, the crucial distinction lies in the direction of the change in expression. In the knockout, a more

significant proportion of genes was negatively regulated (166 out of 238). In contrast, in the overexpressor, positively regulated genes were predominant (166 out of 187). Additionally, when we predicted the subcellular location of the differentially expressed genes, the results revealed a notable overrepresentation of nuclear genes encoding mitochondrial proteins. To ascertain the direct targets of PUF3 among the identified genes, we searched for the PUF3 UGUAYAUW binding motif in the 3'-UTR. Our analysis reveals that 25% of differentially expressed genes in the knockout parasites possess at least one binding site for PUF3 in their 3'-UTR. Interestingly, a parallel proportion of 18.82% was observed in the overexpressor. Since our transcriptomic result suggests an overrepresentation of mitochondrial genes, we wanted to determine whether overexpression or knock-out of *TcPuf3* could lead to changes in both mitochondrial structure and cellular respiration. When mitochondria from Δ*TcPuf3* and pTEX*TcPuf3* parasites were analyzed by transmission electron microscopy (TEM), it was observed that the overexpressor had an abnormal mitochondrial morphology, evidenced by swelling. The results associated with cellular respiration showed that both the Δ*TcPuf3* and pTEX*TcPuf3* had lower ATP-linked respiration and a diminished bioenergetic reserve, which suggests an impaired electron transport system capacity. Likewise, the mitochondria from overexpressing parasites showed hyperpolarization. Additionally, several biological features were altered, such as growth, cell cycle, cell infection, metacyclogenesis, ROS production, and response to benznidazole, depending on energy obtained from mitochondria. In conclusion, our results suggest that although PUF3 is not an essential protein in *T. cruzi*, it strongly influences mitochondrial transcripts, affecting mitochondrial morphology and function.

Poster 94 : Functional analysis of *Trypanosoma cruzi* spliceosome proteins

Arthur Menezes, *University of São Paulo*

AT Menezes[1]; AM Silber[1]; JF Nascimento[2]; PP Coltri[1];
[1] Biomedical Science Institute - USP, Brazil; [2] Instituto de Ciências Biomédicas, Brazil

Trypanosoma cruzi currently infects 6 to 7 million people worldwide, causing Chagas disease, which, when symptomatic, is characterized by cardiomyopathy, digestive system failure, or both. Trypanosomatids' genes are transcribed as polycistronic pre-mRNAs, which are individualized by SL trans-splicing, by the addition of a spliced leader sequence (SL-RNA) to each ORF in these pre-mRNAs. SL trans-splicing is carried out by the spliceosome, which is composed of a core of 5 ribonucleoproteins (RNPs) and several regulatory proteins conserved among trypanosomes and other eukaryotes. However, details on the function and activity of specific proteins in trypanosomatids' spliceosomes remain largely unexplored. Previously, we analyzed the *T. cruzi* spliceosome by mass spectrometry. The present work intends to characterize two of the detected proteins, TcRRM (TcCLB.510143.80) and TcHEL67 (TcCLB.506213.120), which are orthologous to the human SF3b4 and DDX3X proteins, respectively. In humans, these proteins participate in several cellular processes, including pre-mRNA splicing regulation. We overexpressed the TcRRM and TcHEL67 proteins in *T. cruzi* epimastigotes, and used CRISPR-Cas9 to edit their coding sequences. We analyzed the variations in splicing efficiency of endogenous α-tubulin, GAPDH and Poly-A polymerase transcripts by RT-qPCR. Using immunofluorescence, we confirmed that the TcRRM and TcHEL67 proteins localize within the parasite's nucleus. Besides, immunoprecipitation assays showed that TcRRM seems to be associated to U2, U5 and U6 spliceosome snRNAs, while TcHEL67 do not show any strong association. Our findings suggest that the TcRRM and TcHEL67 proteins may have a role in regulating splicing in *T. cruzi* epimastigotes. We intend to further explore the results by *in vitro* splicing assays and use CLIP-seq to check for RNA association for these proteins.

Poster 95 : *Trypanosoma carassii*, a model for whole host interaction studies

Sarah Monic, *University of Cambridge*

S Monic[1]; 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% in densely populated 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, RNA pol I and T7 polymerase. These cell lines have been used to infect transparent zebrafish larvae, facilitating the tracking of all trypanosomes infecting a host. The infection in zebrafish larvae has been followed by fluorescence quantification and the distribution by fluorescence microscopy over the course of an infection.

Poster 96 : Development of a LAMP detection assay for *Dictyocaulus viviparus* lungworm

Sirapat Nak-On, *University of Glasgow*

S Nak-On[1]; P Campbell[1]; J McIntyre[1]; A Antonopoulus[1]; T Chontananarth[2]; R Laing[1];
[1] University of Glasgow, Glasgow, UK; [2] Srinakharinwirot University, Bangkok, Thailand

The bovine lungworm, *Dictyocaulus viviparus,* is highly pathogenic and disease outbreaks can be difficult to predict and manage. High performance molecular detection tools could improve diagnosis of this parasite and help with the implementation of strategic control measures. Loop-mediated isothermal amplification (LAMP) is a rapid and isothermal DNA amplification assay, which could be developed for field-based detection. First, genomic DNA was extracted from single *D. viviparus* L3 larvae to amplify and clone the ITS2 DNA region into the recombinant plasmid (DviITS2). A novel DviLAMP primer set was designed to specifically target DviITS2 with the flanking 5.8S ribosomal DNA region. Genomic DNA and the DviITS2 plasmids from different individual larvae were tested as template in the LAMP protocol. Successful amplification with the LAMP primers was detected by conventional and colorimetric LAMP assays, with gel electrophoresis, real-time analysis and colour change observation. The colorimetric DviLAMP assay can specifically detect the *D. viviparus* ITS2 locus with naked eye observation after 45 and 90 minutes of incubation at 64 °C for 1 ng and 1 pg of the plasmid DNA, respectively. Future work will detect the dual-labelled (biotin and FAM) LAMP amplicon in a lateral flow system and investigate the utility of the assay as a point of care test to detect *D. viviparus* larvae in nasal swabs and/or faeces. Therefore, the development of DviLAMP could significantly improve the sensitivity of lungworm diagnosis in the field.

Poster 97* : A hybrid whole genome sequencing approach to studying the population structure of *Eimeria* parasites

Conor Noonan, *Royal Veterinary College*

C Noonan[2]; J Pachebat[1]; M Hay[2]; S Hill[2]; DP Blake[2]; D Xia[2];
[1] Aberystwyth University, UK; [2] Royal Veterinary College, UK

Eimeria are a genus of Apicomplexan parasites which can infect all major livestock. Ingestion of this parasite leads to coccidiosis, an intestinal disease whose clinical signs include haemorrhagic diarrhoea, diminished weight gain, and mortality in severe infection. The genus is comprised of more than 1,500 species, each of which are monoxenous and mostly host-specific in their lifecycle. Of particular economic importance are species which infect chickens, as coccidiosis incurs costs upwards of £10.4 billion to annual global poultry production. Despite their impact in the agriculture sector, little is known about the genetic diversity of these parasites, and how this variation contributes to the rising level of resistance to current treatment strategies. Population genetic studies have typically utilised a select number of markers to infer evolutionary relationships between these parasites, and existing reference sequences remain incomplete due to both technical limitations and the inherent repetitive structure of *Eimeria* genomes.

This project combines the use of whole genome short-read sequencing and the most recent advancements in long-read sequencing technologies to overcome both technical and biological bottlenecks. We developed a novel Nanopore sequencing workflow to improve upon the quality of *Eimeria* reference genomes by generating long reads capable of spanning expansive regions of low complexity. Acting as scaffolds, these large sequences augment the *de novo* assembly of *Eimeria* genomes by properly orienting shorter sequences and bridging gaps between them. In doing so, we reduced the number of contigs in existing *Eimeria* genomes from 4,664 down to 250 whilst maintaining an equivalent level of completeness. Furthermore, these sequences were able to capture information within repeat-rich regions and sources of structural variation exceeding 1,000 base pairs in length, including insertions, deletions, inversions, repeat contractions, and repeat expansions.

Moreover, the availability of contiguous and complete reference sequences for *Eimeria* spp. was exploited in our whole genome short-read sequencing analysis of 18 *Eimeria tenella* clones selected for drug resistance and precocious development. Loci attributed to these phenotypes were identified for further investigation, including missense mutations, insertions, and deletions in genes coding for proteins involved in nucleotide binding, protein modification, and redox catalysis.

Our findings show that current *Eimeria* assemblies can be substantially improved upon with the use of Nanopore long reads. By resolving gaps and capturing low-complexity regions, more information is retained which benefits downstream analyses of whole genome sequencing data. Furthermore, the ability of long reads to retain large structural variants allows for more complete genotyping. The application of these methods to field samples may be used to enhance our understanding of the dynamics of these organisms, including the global distributions of different species, regional differences within and between species, as well as the genetic determinants of drug resistance.

Poster 98 : Direct demonstration that histone modification impacts gene expression in trypanosomes

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. We 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.

Poster 99 : *Cryptosporidium* spp. in cattle in a Jamaican watershed

Dr Corinne Ong, *Toronto Metropolitan University*

A Morris[1]; S Noble[2]; J Lindo[2]; C Ong[1];
[1] Toronto Metropolitan University, Canada; [2] University of the West Indies, Jamaica

Cryptosporidium is a protozoan parasite associated with gastrointestinal illness in humans and a wide range of vertebrate animals. Cattle, particularly calves, are widely recognized as major reservoir hosts of zoonotic *Cryptosporidium* spp. Despite this, and cattle being one of the most significant economic mainstays in St. Elizabeth, Jamaica, there is no published record to date on the presence of *Cryptosporidium* in Jamaican cattle nor their potential role as zoonotic reservoirs. In the present study, 119 faecal samples were collected from beef and dairy cattle (ages 1 month to 11 years) from 10 farms in St. Elizabeth parish. A modified acid-fast (MAF) staining microscopy technique was performed to detect *Cryptosporidium* oocysts from 29 (24%) of samples. Molecular screening by polymerase chain reaction (PCR), amplifying the 18S rRNA gene, confirmed *Cryptosporidium* presence in 10 (8%) of samples. PCR positive isolates were subjected to nested PCR and sequence analysis of the 18S rRNA gene, which identified a *C. parvum* genotype of bovine origin in faecal specimens from 2 calves. Further sequence analysis of the 60 kDa glycoprotein (gp60) gene, identified the C. hominis IbA9G2 subtype, previously reported in humans and a calf. This reports the first molecular characterization of *Cryptosporidium* spp. from cattle in Jamaica, and their potential role as zoonotic reservoirs of the protozoan parasite. Further molecular investigation is warranted to better understand direct and indirect transmission dynamics of cryptosporidiosis between livestock and humans in the region.

Poster 100 : A novel 'target enrichment' based approach for genomically characterising *Giardia duodenalis* in human and animal directly from clinical samples

Dr Rossella Panarese, *University of Glasgow*

R Panarese[1]; P Capewell[1]; C Alexander[2]; W Weir[1];
[1] University of Glasgow , UK; [2] Scottish Microbiology Reference Laboratories (Glasgow), UK

Giardia duodenalis(*Giardia*)is an intestinal protozoan parasite which causes disease in both animals and humans across the globe. It is becoming increasingly apparent that in developed countries there exist major knowledge gaps on *Giardia* ecology and epidemiology. For example, in Scotland, despite being considered an

"exotic" disease, recent studies have suggested the presence of an endemic cycle of disease within the country which is not associated with travel-related infections. However, endemic transmission routes are poorly understood and, in particular, the level of zoonotic risk is unknown. Current molecular markers for parasite genotyping are low-resolution and are only effective in a minority of field samples. Moreover, only a low proportion of *Giardia* isolates can be adapted to culture, which presents a major obstacle to generating good-quality DNA preps for genomic sequencing. Therefore, the present study aims: (i) to establish a laboratory workflow to allow sequencing of *Giardia* directly from faecal samples collected from human and animal cases in Scotland; (ii) to generate high-quality exomes to establish a robust minimum set of genotyping markers for analysing relationships between parasite isolates and (iii) to compare the genomes of endemic and foreign travel-associated isolates to begin to investigate transmission routes in Scotland. The laboratory workflow fundamentally consists of four steps: library preparation performed on *Giardia* DNA extracted directly from faecal samples, validation of a hybridisation capture protocol targeting the parasite exome, enrichment amplification conducted on the captured libraries and, lastly, short read sequencing. For the genome capture hybridisation, we designed an array of overlapping biotinylated RNA capture probes evenly spaced across the *Giardia* exome. An additional set of capture probes was designed to specifically cover the loci most commonly used for typing, including the *bg*, *gdh* and *tpi* genes located on chromosomes 4 and 5 of the parasite. A process of iterative testing and refinement of the library preparation and target enrichment methodology has allowed us to develop an optimised protocol. We have successfully applied this to a *Giardia*-positive faecal sample from a Scottish wildcat, achieving a high level of parasite DNA enrichment. Reads mapped to the genome corresponding to the exome probe design. The loci which were over-represented on the probe design had a higher depth than the other regions, including the three key loci used for typing. The gene sequences at these loci identified the isolate as belonging to 'assemblage F' typically found in felines. Having demonstrated 'proof of principle' for this approach, we now intend to apply it to a number of human and animal samples. Ultimately, it is envisioned that the genome capture methodology may provide a rapid and accurate typing method for this parasite, including samples that do not readily PCR, allowing a better understanding of the epidemiology of *Giardia* in Scotland and further afield.

Poster 101* : A drug discovery journey: N6-Methyltubercidin cures a cutaneous *Leishmania amazonensis* mouse model

Cassandra Present, *University of Antwerp: Laboratory of Microbiology, Parasitology and Hygiene*

C Present[1]; R Donola Girão[2]; C Lin[3]; G Caljon[1]; S Van Calenbergh[3]; O Moreira[2]; L Alexandre de Souza Ruivo[2]; MM Batista[2]; R Azevedo[2]; DG Batista[2]; MN Soeiro[2];
[1] University of Antwerp, Belgium; [2] Instituto Oswaldo Cruz, Rio de Janeiro, Brazil; [3] Ghent University, Belgium

Cutaneous leishmaniasis is a neglected tropical disease caused by the intracellular protozoan parasite *Leishmania*. It is transmitted when female sand flies bite mammalian hosts, such as humans, to obtain a bloodmeal. The disease mainly occurs in tropical and subtropical areas but is spreading to previously unaffected areas. Currently, there are a few antileishmanial drug treatments available, such as antimonials, amphotericin B, miltefosine and paromomycin. However, these drugs have multiple practical shortcomings, their mode of action is unknown or poorly understood, or they face the development of drug resistance. Therefore, it is essential to develop new drugs for the treatment of leishmaniasis. Nucleoside analogues are considered promising alternative drug treatments. Since *Leishmania* parasites are unable to synthesise their own purines *de novo*, they rely completely on salvaging purines from the host. This process provides interesting targets for drug discovery of new antileishmanial compounds. In this project, we explored the *in vitro* and *in vivo* activity of N^6-methyltubercidin, also known as CL5564, against *L. amazonensis*, after it demonstrated

potent activity against *Trypanosoma cruzi* and *L. infantum*. With selectivity indices of 278 and 43, respectively, CL5564 was 6.5 -fold (p = 0.0002) more potent dan miltefosine, a reference antileishmanial drug, against intracellular amastigote forms in peritoneal mouse macrophages. Combination treatment of CL5564 and miltefosine on *ex vivo* amastigotes resulted in an additive effect. These results stimulated us to study the activity of CL5564 in a mouse model of cutaneous *Leishmania* infection: BALB/c female and male mice infected by *L. amazonensis* and treated with CL5564 (10 mg/kg, intralesional route for five days) presented a >93% reduction of paw lesion size, similar to Milteforan™ given orally at 40 mg/kg, while the combination (10 + 40 mg/kg of CL5564 and Milteforan™, respectively) caused >96% reduction. The qPCR data confirmed the suppression of parasite load after treatment, but only the combination approach reached 66% of parasitological cure. These results warrant additional studies with nucleoside derivatives.

Poster 102 : It's about time! Do rhythmic interactions between mosquitoes and their microbiota influence malaria transmission?

Naomi Riithi, *University of Edinburgh*

N Riithi[2]; JP Mooney[1]; SE Reece[2];
[1] Institute for Immunology and Infection Research, University of Edinburgh., UK; [2] Institute of Evolution Ecology, University of Edinburgh, UK

Mosquitoes are the most important vectors of infectious disease, transmitting not only malaria parasites (*Plasmodium spp*.) but also a variety of lethal pathogens such as Dengue Fever, West Nile virus, Yellow Fever, Zika virus and Chikungunya virus, among others. Mosquito symbionts (microbes) are increasingly being recognised as crucial and influential players in vectorial capacity – a measure of transmission potential. Mosquito gut-microbiota modulate the vector immune system and produce antiparasitic proteins to block *Plasmodium* transmission. Therefore, the microbial community in the mosquito conveys critical roles in vector development and influences the survival and fecundity of adults. Mosquitoes exhibit a daily rhythm when they forage for blood or sugar, which imposes a rhythm on the diversity and abundance of the microbial community. This, in turn, may generate rhythmicity in the impacts of mosquito immune responses and microbial-produced antiparasitic proteins on *Plasmodium* transmission. Nevertheless, the potential for microbe-related rhythms to influence parasite development and affect mosquito vectorial capacity remains unknown. Here, I will discuss the experimental approaches needed to address this gap. Since mosquito-biting times of day are changing, understanding how rhythms impact on host-parasite-vector interactions is critical. Demonstrating the impact of microbial rhythms in transmission could inform malaria control and drive novel interventions.

Poster 103* : Genomic analysis of Amoebic Gill Disease causing *Neoparamoeba perurans* in Atlantic salmon aquaculture

Brendan Robertson, *University of Glasgow*

BA Robertson[1]; L Covington[1]; B Cheaib[2]; MS Llewellyn[1];
[1] University of Glasgow , UK; [2] Centre for Infectious Diseases, Universitätsklinikum Heidelberg, Germany

Amoebic Gill Disease (AGD) is a severe infection of farmed Atlantic salmon caused by *Neoparamoeba perurans* and has continued to spread globally. We still know little about how *N. perurans* spread between farms, production cycles, regions, and countries. Such an understanding could inform fallowing and control in

salmonid aquaculture. Genomic analyses required to achieve *N. perurans* molecular epidemiology, however, are frustrated by characteristically high levels of intracellular bacterial contamination. A handful of *N. perurans* genes have been successfully sequenced (18S rRNA, cytochrome oxidase I, ITS, etc) but with an insufficient genetic resolution to be useful to the farmer or regulator. A draft genome of *N. perurans* was generated at the Llewellyn lab to enable hundreds of new sequencing markers to be developed. These markers can be sequenced directly from gill swabs at low cost to provide epidemiologically relevant information. A highly multiplexed testing strategy is under development with the intent to sequence a series of AGD samples from Ireland and Scotland in 2019-2023 for molecular epidemiological analysis. Gill swab samples from Atlantic salmon affected by AGD were analysed. DNA extractions were conducted on all samples and amplified through qPCR to determine the presence of the 18S rRNA gene of *N. perurans.* A multiplex PCR was developed using 96 primer pairs across two 48 primer pair reactions to amplify genes across the nuclear, mitochondrial, and kinetoplast genome from qPCR data*.* PCR products were barcoded and cleaned. Library preparation was conducted as per Nanopore specifications. PCR products were sequenced using the Nanopore MinION system. Basecalling was completed per MinKNOW 23.04.3 protocol. Sequences were aligned with phylogeny estimated and analysed in R, MEGA, and Figtree. Extracted DNA of 164 gill swabs from Atlantic salmon in Scotland and Ireland revealed 120 (73.5%) to be positive for *N. perurans*) presence at or before 30 amplification cycles of the 18S rRNA gene and quantified (ng/µl) with Ct scores greater than 30 considered negative. The best candidates for DNA sequencing based on DNA quantity, and epidemiological considerations (location & time) were chosen to maximise the success of genome sequencing and allow for phylogenetic investigation inclusive of spatiotemporal changes. Analysis of phylogenetic trees indicated a high level of genetic mixing and reinfection year over year in the Scottish and Irish farmed salmon populations signifying that treatments should remain focused on aquaculture cage hygiene and therapeutic freshwater fallowing as prophylactic measures are unlikely to prevent reintroduction of AGD. To date, there is no effective means of attributing AGD infections to a source. Although incomplete, this work has taken steps towards achieving this by demonstrating that high-resolution genetic information may be readily derived directly from gill swab samples to inform AGD treatment and control.

Poster 104 : Stem cell proliferation driven by opposite sex excretory-secretory products (ESPs) in *Schistosoma mansoni*

Dr Eman Shakir*, Kingston University*

E Shakir[2]; R Kirk[2]; G Rinaldi[1]; AJ Walker[2];
[1] Aberystwyth University, UK; [2] Kingston University, UK

The human parasitic flatworm *Schistosoma mansoni* genome encodes more than 260 protein kinases, important regulatory proteins that are highly conserved across eukaryotes. Our previous studies demonstrated that the exchange of male or female excretory-secretory products (ESPs) between groups of opposite-sex adult worms rapidly activated the p38 mitogen-activated protein kinase (p38 MAPK) and extracellular signal-regulated kinase (ERK) pathways within the worms. Moreover, treatment with SB203580 and U0126 significantly attenuated the ESP-mediated phosphorylation (activation) of p38 MAPK and ERK, respectively. Next, to investigate stem cell proliferation, Click-it EdU and Alexa Fluor staining were performed in *in vitro* cultured worms. In homosexual culture, adult males and females exhibited a significant cell proliferation reduction over six days, compared to day zero controls. Remarkably, however, exposure to opposite sex ESPs significantly increased cell proliferation in the testicular lobes of males, ovaries and vitellaria of females, and the surface tegument layer of both sexes. This effect was attenuated by either SB203580 or U0126, highlighting the involvement of p38MAPK and ERK pathways in the ESP-mediated responses. We next conducted proteomic

analysis of the male/female ESPs, revealing 705 proteins in total, with 583 common to males and females, and 74 and 48 exclusively present in ESPs of the male and female, respectively. Analysis of extracellular vesicle (EV)-depleted fractions revealed 362 proteins in total, 286 in common, and 62 and 14 exclusive to males and females respectively. Although the molecules responsible for stimulating responses in opposite sex worms have not yet been definitively identified, future research aims to elucidate the nature of the critical ESPs and further characterise their effects on opposite sex worms. These findings provide valuable insights into male-female schistosome interactions that might open potential avenues for the development of novel strategies to control schistosomiasis, a disease affecting over 240 million people.

Poster 105 : Exploring the *Trichuris peptidome* as a source of novel antimicrobials

Dr Rebecca Shears, *Manchester Metropolitan University*

R Shears[2]; A Irvine[1]; R Thomas[2]; A Lewis[2]; T Britten[2]; L Atkinson[1]; A Mousley[1];
[1] Queen's Univeristy Belfast, UK; [2] Manchester Metropolitan University

Trichuris (whipworm) is a genus of parasitic worms that live in the gastrointestinal tract of mammals. These parasites come into direct contact with the host colonic microbiota and are known to modulate this microbial community to promote their own survival. One way that these worms may perturb the host gut microbiota is through the secretion of antimicrobial peptides (AMPs), which are key, yet somewhat underappreciated, components of the invertebrate immune system Data from our research team shows that at least four newly discovered whipworm AMPs have efficacy against *Pseudomonas aeruginosa, Klebsiella pneumoniae, Escherichia coli* and *Acitenobacter baumannii* (all of which are WHO priority pathogens, posing the biggest threat to human health due to high rates of antimicrobial resistance), while an additional peptide shows broad spectrum efficacy against clinically relevant Gram-negative and positive bacterial pathogens. In addition, all five peptides demonstrate low toxicity against host cells and four of the five are highly stable against host serum proteases. This combination of potent antimicrobial activity, low host toxicity and high serum stability is rare for AMPs, with many drug discovery programs undergoing great efforts to improve the pharmacological profiles of AMP candidates. Given that many parasitic helminths, including *Trichuris* species, have evolved to live in close contact with their host and associated microbial communities, this combination may be a pre-requisite for such AMPs, which must be stable enough to withstand host conditions (e.g. body temperature and proteolysis) whilst causing minimal toxicity. This work may pave the way for the discovery and development of novel antimicrobials from other helminths, which with a few exceptions, are largely underexplored.

Poster 106* : Novel RELM-β binding molecule identified from *Heligmosomoides polygyrus bakeri*

Vivien Shek, *University of Dundee*

V Shek[1]; DJ Smyth[1]; HJ McSorley[1];
[1] University of Dundee, UK

Helminth infections persist by influencing host immunity through their molecular products, thereby preventing immune ejection. In addition, the immunomodulatory properties of these molecules can prove useful in suppressing immune-mediated diseases. The intestinal nematode *Heligmosomoides polygyrus bakeri (Hpb*) secretes many immunomodulatory molecules, notably: HpARI and HpBARI, which antagonise the IL-33 pathway, and HpTGM which mediates a regulatory response by inducing Treg cells. All three molecules are structurally similar, consisting of a string of consecutive atypical Complement Control Protein (CCP) domains.

This led to the hypothesis that CCP domain-containing proteins may represent a family of immunomodulatory molecules used by *Hpb* to evade the host immune response.
HpARI and HpTGM are not classified as CCP domain-containing proteins by current protein domain prediction tools, prompting the development of a new atypical CCP motif. Through sequence alignment analyses of HpARI, HpBARI, HpTGM and all *Hpb* genes classified as conventional CCP domains defined by InterPro IPR000436, a novel atypical CCP motif was generated. To identify CCP domain-containing proteins like the *Hpb* immunomodulators, this motif was screened against an in-house *Hbp* transcriptome and genomic data from WormBase ParasSite. Selected candidates were produced as recombinant proteins using the Expi293 expression system and assessed for interactions with host immune proteins using the Avidity-based Extracellular Interaction Screening (AVEXIS) assay.

We confirmed known parasite-host interactions using this technique, including HpARI-IL-33, HpBARI-ST2 and HpTGM-TGFβR. Additionally, we have identified a novel interaction between the *Hpb*-secreted CCP-domain protein "61365" with host RELM-β, a cysteine-rich secretory cytokine predominantly expressed in the gastrointestinal tract. RELM-β is involved in regulating intestinal homeostasis, promoting airway inflammation, and driving antiparasitic activity, making RELM-β an interesting target. Current work involves elucidating the mechanism in which 61365 modulates RELM-β.

Taken together, this approach has identified a novel parasite-host interaction pair which will be further explored. The methods and analyses described here may assist the development of a larger pipeline in identifying, producing, and characterising new helminth immunomodulatory molecules which may prove beneficial for the development of novel anthelminthics or therapeutics for immune-mediated diseases.

Poster 107 : Identification of schistosome-derived immunomodulatory proteins.

Vivien Shek, *University of Dundee*

V Shek[1]; HH Smits[2]; HJ McSorley[1];
[1] University of Dundee, UK; [2] Leiden University Medical Centre, Netherlands

Type 2 immune responses are commonly associated with host defence against helminth infections and the development of allergic inflammation. Helminths are highly effective at initiating immunoregulatory mechanisms; there is increasing interest in dissecting out the regulatory pathways they induce and identifying the specific molecular mediators from parasites involved in these pathways.
IL-4 inducing Principle from Schistosome Eggs (IPSE) is a glycoprotein secreted from the eggs of *Schistosoma mansoni* and has been shown to modulate the host immune response by inducing IL-4 production in basophils and IL-10 production in regulatory B cells. In addition to its immunomodulatory capacity, IPSE is an interesting molecule due to its unusual mode of action: IPSE contains a secretory signal peptide at the N-terminus, enabling trafficking of the protein to the secretory pathway, and a nuclear localisation sequence (NLS) that directs the protein to the nucleus. Typically, proteins possess only one of these signals, as they are either secreted or localised to the nucleus. In addition to IPSE, *S. japonicum*-derived molecule Sj16 serves as another example of a NLS-containing immunomodulatory molecule. Sj16 has been shown to induce IL-10 production and inhibit the maturation of dendritic cells upon entering the host nucleus. Thus, we hypothesised that the presence of a NLS on helminth molecules could be a mechanism used by parasites to modulate the host immune response by altering host cellular activity.

Here, we treated human monocyte derived dendritic cells (MoDC) with *S. mansoni* soluble egg antigen (SEA) for 24 hrs and carried out subcellular fractionation and DIA proteomics to identify other S*chistosome*-derived proteins like IPSE that migrate into host cell nuclei.

The mass spectrometry results identified 7700 human and 550 *S. mansoni* proteins, and IPSE was significantly enriched in the nuclear fraction of SEA-treated MoDCs alongside 14 other *S. mansoni* proteins. The proteomics data set will provide new insights into how the host immune response is altered during *S. mansoni* infection, which may prove beneficial for developing novel anthelmintics.

Poster 108 : *Leishmania (Leishmania) infantum* infection alters the lipidome of human macrophage

Cínthia Siess-Portugal, *University of São Paulo School of Medicine*

C Siess-Portugal[1]; CY Ozaki[1]; EM Ramos-Sanchez[3]; AB Chaves-Filho[2]; MY Yoshinaga[2]; S Miyamoto[2]; H Goto[1];

[1] Departamento De Medicina Preventiva, Faculdade de Medicina da Universidade de São Paulo (USP), Brazil; [2] Instituto de Química, Universidade de São Paulo, Brazil; [3] Universidad Nacional Toribio Rodríguez de Mendoza de Amazonas.Facultad de Medicina, Escuela Profesional de Medicina Humana, Peru

Leishmaniases represent neglected diseases caused by parasites of the genus *Leishmania*. *Leishmania (Leishmania) infantum*, in the Americas, is responsible for - Visceral Leishmaniasis (VL). VL can result in death if not adequately treated. In addition to the typical systemic symptoms of VL, changes in the plasma lipid profile are noted in humans as well as in dogs and mice. These lipid changes are characterized by increased levels of triglycerides (TG) and very low-density lipoproteins (VLDL) and a reduction of high-density lipoproteins (HDL). In human infections, we previously identified a correlation between high levels of TG and VLDL and a greater risk toward active disease development. In our recent study, a change in the expression of genes related to lipid metabolism was demonstrated in *L. infantum* infected-THP-1 macrophages. The aim of the present study was to identify the alterations in the lipidome of human macrophages during infection by *L. infantum*. The monocytic THP-1 cell line was differentiated using 20 ng/mL of PMA for 24 hours and maintained for 48 hours in RPMI 1640 medium with 5% heat-inactivated foetal bovine serum, at 37˚C and 5% CO2. The THP-1 macrophage was infected with stationary phase *L. infantum* promastigotes for 6 hours at the beginning of the experiment, and then evaluated at time zero and 24 hours post-infection. The untargeted lipidomic analysis revealed an increase in lipid species of lysophosphatidylcholine (2), lysophosphatidylglycerol (1), phosphatidylinositol (1), phosphatidylcholine (9), phosphatidylethanolamine (3), fatty acids (3), sphingolipids (3), and cholesterol ester (1) in infected macrophages compared with control or uninfected macrophages. Thus, we may conclude that the interaction between *Leishmania* and macrophages leads to alterations in the lipid profile of host cells during the first 24 hours of infection. These findings suggest a crucial role of lipid changes in the pathogenesis of VL. Understanding these pathophysiological mechanisms may contribute for the development of new therapeutic strategies. Additionally, the identification of specific lipid biomarkers may be useful for early diagnosis and monitoring the progression of VL.

Poster 109 : Lipidomic analysis of intracellular *Leishmania (Leishmania) infantum* amastigotes

Cínthia Siess-Portugal, *University of São Paulo School of Medicine*

C Siess-Portugal[1]; CY Ozaki[1]; EM Ramos-Sanchez[3]; AB Chaves-Filho[2]; MY Yoshinaga[2]; S Miyamoto[2]; H Goto[1];

[1] Departamento De Medicina Preventiva, Faculdade de Medicina da Universidade de São Paulo (USP), Brazil; [2] Instituto de Química, Universidade de São Paulo, Brazil; [3] Universidad Nacional Toribio Rodríguez de Mendoza de Amazonas.Facultad de Medicina, Escuela Profesional de Medicina Humana, Peru

Visceral Leishmaniasis, caused by *Leishmania infantum* in Brazil, is caused by a digenetic parasite presenting as promastigote (in the vector insect) and amastigote (intracellular). The amastigotes reside in the cell's phagolysosome. While the promastigote develops inside the phlebotomines gut and relies more on glycolysis, the intracellular amastigote uses β-oxidation as energy sources. Thus, lipids constitute one of the sources of energy for the intracellular parasite. In addition, the amastigote can incorporate lipids from the host into its lipid composition, playing a significant role in the intracellular growth of *Leishmania*. The main aim of the present study is to characterize the lipidome of *L. infantum* amastigotes isolated from infected THP-1 macrophages. Human macrophages derived from THP-1 monocytes were infected with stationary phase *L. infantum* promastigotes, at 10:1 parasite:cell ratio. The intracellular parasites were recovered and isolated through centrifugation in a Ficoll400 column at time zero (after 6 hours of parasite:macrophage co-culture) and 24 hours after infection. A comprehensive analysis of the lipid composition of the amastigotes was conducted through lipidomics at both time points. We analyzed the lipidome of *L. infantum* amastigotes in comparison with the lipid composition of the infected macrophage and identified 265 differentially expressed lipid molecular species, demonstrating significant differences between the lipidome of infected human macrophages and intracellular *L. infantum*. In contrast to the macrophages that exhibited a greater enrichment in phospholipids associated with polyunsaturated fatty acids, sphingomyelins, and triglycerides with long and unsaturated chains, *L. infantum* intracellular amastigotes showed a higher concentration of cholesterol esters, triglycerides containing short and saturated fatty acids, and ceramides. Characterizing the lipids in *L. infantum* amastigotes can contribute to a better understanding of the parasite's lipid metabolism. These data could also reveal new targets for the development of therapeutic strategies and allow the identification of specific lipid biomarkers for the prognosis and monitoring of the progression of Visceral Leishmaniasis.

Poster 110 : Peptide microarray IgM and IgG screening of pre-SARS-CoV-2 human serum samples from Zimbabwe for reactivity with peptides from all seven human coronaviruses: a cross-sectional study

Yasmine Socratoff, *University of Edinburgh*

Y Socratoff[1];
[1] University of Edinburgh, UK

Background: The emergence of the SARS-CoV-2 virus as a global pandemic has led to millions of deaths worldwide, yet Africa has shown notably lower infection and mortality rates compared to other continents. Various factors have been proposed to explain this difference, including demographic, lifestyle, and climatic variations. However, the role of the immune system, which plays a significant role in SARS-CoV-2 infection and COVID-19 outcomes, has been overlooked. Recent research suggests that pre-existing cross-reactive immune responses in African populations, potentially induced by previous exposure to other Human Coronaviruses (HCoVs) or endemic pathogens like *Plasmodium falciparum*, could contribute to this epidemiological pattern.

Methods: To investigate pre-existing cross-reactive immunity to SARS-CoV-2 in an African population, we conducted a cross-sectional study using pre-pandemic serum samples collected from adults living in Zimbabwe. Serum samples from four villages were analyzed for SARS-CoV-2 serological cross-reactivity using rapid diagnostic tests and screened for cross-reactivity with peptides from the proteomes of seven human coronaviruses. Peptide analysis revealed complex IgM and IgG response profiles against peptides from various coronaviruses, including the spike, nucleocapsid, and polyprotein 1AB proteins. Additionally, we compared identified peptides to the Human Immune Epitope Database (HIED) to assess potential cross-reactivity with antigens from endemic pathogens and food immunogens.

Findings: The overall prevalence of cross-reactivity with SARS-CoV-2 among pre-pandemic serum samples from two villages was 31.9%. Peptide analysis highlighted the presence of IgM and IgG responses against peptides across coronaviruses, with some peptides sharing motifs with antigens from pathogens endemic to Zimbabwe, including *Trypanosoma* spp and *Plasmodium* spp, as well as plant and food immunogens, and human autoantigens.

Interpretation: The implications of these cross-reactive antibodies on SARS-CoV-2 infection and COVID-19 outcomes remain uncertain. However, their presence underscores the importance of considering pre-existing immunity in interpreting SARS-CoV-2 seroepidemiology studies and evaluating COVID-19 vaccine efficacy in Africa. Further characterization of SARS-CoV-2 immune phenotypes and responses in African populations is warranted. This study sheds light on the potential role of pre-existing immunity in shaping the epidemiological landscape of COVID-19 in Africa and underscores the need for continued research in this area.

Poster 111 : Ruminating over host-parasite interaction models for fluke driven immune responses

Corey Steele, *Aberystwyth University*

C Steele[1]; RE Wonfor[1]; RM Morphew[1]; M Robinson[2];
[1] Aberystwyth University, UK; [2] Queen's University Belfast, UK

Rumen fluke, *Calicophoron daubneyi*, is a gastro-intestinal parasite which infects grazing livestock worldwide, particularly cattle, resulting in paramphistomosis. Recently, rumen fluke prevalence is rapidly increasing across the UK and Ireland, creating a need for further research to understand how this parasite infects its host, and how it affects the hosts immune response to promote its survival. Helminth extracellular vesicles (EVs) are recognised to contain predicted and confirmed immune modulators, allowing them to manipulate their hosts in order to facilitate survival. Rumen fluke have also been demonstrated to produce EVs yet their actions on the host immune system is yet to be investigated. Historically, *in vitro* models, such as cell cultures and more recently organoids, have been utilised to investigate the effect of infectious diseases on their hosts. However, such approaches cannot account for the multiple cell types present within tissues and thus do not fully represent natural infection interactions. As a result, there is increasing interest in the development of tissue explant models, whereby animal tissues are maintained *in vitro*. This research project is developing an *in vitro* explant model of the bovine rumen to allow exploration of the rumen fluke-host interaction. Currently, the rumen explant model is being assessed for its response to liposaccharide (LPS). With an optimised bovine rumen explant model, the role of rumen fluke EVs can be assessed following *in vitro* stimulation. This work will lead to the establishment of a more representable model of natural infections and will investigate how rumen fluke EVs affect host immune responses.

Poster 112 : Morphological and phylogenetic analysis of *Bothridium pithonis* (Cestoda: Diphyllobothriidea) in Python Snakes (*Malayopython reticulatus*) in Thailand

Assoc.Prof Dr Sivapong Sungpradit,*Faculty of Veterinary Science, Mahidol University*

S Sungpradit[1]; T Rawangchue[2]; T Chamsai[3]; C Pabutta[3]; K Tonchiangsai[3]; N Tanpradit[4]; N Sangkachai[3]; P Aramsirirujiwet[5]; P Wongluechai[3]; P Sedwisai[3];
[1] Department of Pre-clinic and Applied Animal Science, Faculty of Veterinary Science, Mahidol University, Thailand; [2] Parasitology Unit, Center for Veterinary Diagnosis, Faculty of Veterinary Science, Mahidol University, Thailand; [3] Monitoring and Surveillance Center for Zoonotic Diseases in Wildlife and Exotic

Animals, Faculty of Veterinary Science, Mahidol University, Thailand; [4] Department of Clinical Sciences and Public Health, Faculty of Veterinary Science, Mahidol University, Thailand; [5] Department of National Park, Wildlife and Plant Conservation, Ministry of Natural Resources and Environment, Thailand

Limited information exists on the morphology and molecular identification of snake tapeworm parasites in Thailand. This study, conducted by the Center for Veterinary Diagnosis, Faculty of Veterinary Science, Mahidol University, Thailand, analyzed five tapeworm samples from three python snake alimentary tracts preserved in 70% alcohol. The scolices and segments of the tapeworms underwent morphological characterisation using permanent slide preparation and scanning electron microscopy. Genomic DNA extracted from these samples was amplified and sequenced using four molecular markers: nuclear (1*8S rRNA*) and mitochondrial (cytochrome c oxidase subunit I, *12S rRNA* and *16S rRNA*) genes. The tapeworms were identified as *Bothridium pithonis* (Cestoda: Diphyllobothriidea), with the sequences showing 99%-100% similarity to the reference sequences of *B. pithonis* in the GenBank database. Phylogenetic analyses of nuclear and mitochondrial markers were conducted, and intra- and inter-species genetic distances were determined. The results revealed that all mitochondrial markers are suitable for genetic study of *Bothridium* in snakes.

Poster 114 : L-threonine 3-dehydrogenase protects *Trypanosoma cruzi* from genetic damage and oxidative stress

Prof Omar Triana-Chavez, *Universidad de Antioquia*

P Garcia-Huertas[2]; A Mejia-Jaramillo[2]; CR Machado[1]; O Triana-Chavez[2];
[1] Universidade Federal de Minas Gerais, Brazil; [2] Universidad de Antioquia, Colombia

Benznidazole and nifurtimox are the front-line drugs used to treat *Trypanosoma cruzi* infections. The resistance of *T. cruzi* to these drugs has been reported as one of the leading causes of treatment failure against Chagas disease. However, most of the mechanisms of such resistance remain unknown. Several studies have shown mutagenic and genotoxic effects of Bz, double-stranded breaks in treated parasites possibly caused by nucleotide oxidation and cell cycle arrest, mainly in G0/G1. Other studies have proposed that the mechanism of action of Bz is related to the induction of oxidative stress caused by reactive oxygen species (ROS).

L-threonine 3-dehydrogenase (TDH) plays an essential role in L-threonine catabolism. It catalyzes the NAD(P)+-dependent oxidation of L-threonine to 2-amino-3-oxobutyrate, which is a precursor in the production of glycine and acetyl-coenzyme A. Interestingly, this enzyme was found overexpressed in Bz resistant *T. cruzi* parasites. However, more is needed to know about its role in this process. For this, TDH was overexpressed in Bz-sensitive parasites, and their response to Bz, oxidative stress, and genetic damage was evaluated. Moreover, some biological features, such as *in vitro* growth, mitochondrial membrane potential, and cell infectivity, were also estimated. Our results showed that TDH-overexpressing parasites did not have changes in their growth, but they were more resistant to Bz and H_2O_2, and their cellular infectivity increased compared to the control. We found no alterations in the mitochondrial membrane potential or cell cycle in these parasites after Bz treatment. Finally, we submitted the overexpressing TDH parasites to the effect of the MMS compound, an alkylating agent, and gamma radiation that causes the breaking of double-stranded DNA; we found that these parasites were also resistant to genetic damage. Although no previous information relates to TDH with Bz resistance, we propose that TDH has a protective effect on oxidative stress, genetic damage caused by Bz, and the impact of compounds such as H_2O_2, MMS, and gamma radiation in *T. cruzi*.

Poster 115* : Structural and functional dissection of VSG-Exclusion Protein 2 in African trypanosomes

Leon Walther, *University of York*

L Walther[1]; H Hashimoto[3]; J Van Der Merwe[1]; M Tinti[2]; JC Mottram[1]; JN Blaza[1]; E Debler[3]; J Faria[4];
[1] University of York, UK; [2] University of Dundee, UK; [3] Thomas Jefferson University, Philadelphia, United States; [4] York Biomedical Research Institute, Department of Biology, University of York, UK

African Trypanosomes are extracellular parasites that evade the host immune system by periodic switching their homogenous coat made of a variant-surface-glycoprotein (VSG). Trypanosomes have a vast repertoire of >2600 *VSG* genes and pseudogenes, but *VSG* genes can only be expressed from a limited subset of transcription-units referred to as 'expression-sites', however, only one is active at a time.

VEX2, a large (224 kDa) member of the Superfamily 1 (SF1) helicases has been shown to be critical to sustain singular *VSG* expression. Indeed, the loss of VEX2 results in multiple *VSG* expression at the single cell level. VEX2 colocalizes with the ESB and interacts with VEX1, which is associated with the *Spliced-Leader*-(*SL*)-array thereby forming an inter-chromosomal bridge between those nuclear compartments. So far there is little molecular understanding of those interactions and how they are controlling monoallelic expression.

Firstly, we aim to understand VEX2 substrate specificity and the potential role of the N-terminal domain in the regulation of the helicase activity. To that end, we are currently recombinantly expressing VEX2 full-length and AlphaFold guided helicase-domain constructs in different expression systems including *Leishmania tarentolae* (LEXSY), to pursue a biochemical and structural characterization, the latter using single-particle cryoEM. So far, a recombinant helicase core has been produced in baculovirus-infected insect cells showing that VEX2 is an ATP-dependent RNA:DNA helicase. Notably, precision editing of key residues using CRISPR/Cas9 indicated that the helicase activity is required for parasite survival and *VSG* expression control.

Secondly, VEX2 is an orthologue of human Senataxin, which is an RNA:DNA helicase involved in R-Loop metabolism. Whether VEX2 shows similar activity in context of R-Loop resolution at the active-*VSG* expression-site remains elusive, therefore, we performed DRIP-Seq before and after VEX2 depletion and found interesting and unexpected patterns.

Thirdly, we are currently investigating key post-translational modifications with a focus on SUMOylation and phosphorylation via LC-MS/MS analysis in both bloodstream and procyclic cells to understand their impact on the regulation of VEX2 function and localisation during different cell cycle stages.

Poster 116 : Association of current intestinal schistosome infection status with periportal fibrosis: a systematic review and meta-analysis

Lauren Wilburn, *University of Oxford, Big Data Institute*

LE Wilburn[2]; A Esuzie[3]; D Thakrar[1]; N Roberts[1]; R Malouf[1]; G Chami[2];
[1] University of Oxford, UK; [2] University of Oxford, Big Data Institute, UK; [3] Queen's University Belfast, UK

Background: Periportal fibrosis (PPF) is a severe morbidity caused by exposure to intestinal schistosome infections. In the context of repeated mass drug administration, it is unknown whether PPF correlates with schistosome infection. We aimed to assess the association between current infection status and intensity of *S. mansoni*, *S. japonicum*, or *S. mekongi* and periportal fibrosis (PPF).

Methods: In this systematic review and meta-analysis, we searched the Cochrane Central Register of Controlled Trials, Embase, Global Health, Global Index Medicus and Medline on May 18, 2022, for studies of

original research. Only studies that actively diagnosed current schistosome infection at the time of PPF measurement were included and underwent a risk of bias assessment. A meta-analysis of data extracted from published reports was conducted when the findings of more than three studies could be combined. Pooled effect sizes for binary PPF outcomes against current schistosome infection status were calculated using inverse-variance weighted random effects meta-analysis. The protocol was prospectively registered on 19th May 2022 with PROSPERO (CRD42022333919).

Findings: We identified 2646 references; 37 were included in the systematic review and 28 were used to calculate pooled effect sizes across 16777 participants. PPF was heterogeneously defined; with the Niamey protocol most often used to guide ultrasound assessments. Individuals with any current schistosome infection were 2·42 (95% CI:1·65-3·55) times more likely to have PPF but heterogeneity was high (I2 statistic 94·35%). This association was not observed in studies with a low risk of bias. Subgroup analyses showed significant differences between study design, PPF outcome classifications, and studies that followed an ultrasound protocol and those that modified it. No significant association was found between a secondary outcome of schistosome infection intensity and PPF status.

Interpretation: Guidelines use current schistosome infection as a proxy for PPF morbidity. This study supports that current infection status but not intensity is associated with an increased likelihood of having PPF. Further work is needed to identify associations of current infection status with different severity stages of PPF to develop effective guidelines for morbidity.

Poster 117* : The protective effect of soap against the attachment or penetration of schistosome cercariae to mouse tails

Jiaodi Zhang, *Imperial College London*

J Zhang[3]; AK Pitol[1]; L Braun[2]; MR Templeton[3];
[1] Liverpool School of Tropical Medicine, UK; [2] London School of Hygiene and Tropical Medicine, UK; [3] Imperial College London, UK

Introduction: Schistosomiasis is a neglected tropical disease which is spread through skin contact with water containing *Schistosoma* cercariae. Drug treatment with praziquantel has been the major control method, but it cannot prevent reinfection. Water, sanitation and hygiene interventions can be a sustainable measure to reduce transmission and are recommended as one of the core strategic interventions for schistosomiasis control by the World Health Organization. The use of soap is central to many hygiene practices; however, there is limited knowledge about whether and how the use of soap can protect individuals against schistosomiasis infection. Our previous systematic review demonstrated that soap on skin can act as a barrier to prevent cercariae from penetrating skin. In this experimental study, mouse tails were used to better understand the potential protective mechanism of soap against schistosome cercariae.

Methods: Laboratory experiments were carried out to study the efficacy of soap at preventing the attachment or penetration of *Schistosoma mansoni* cercariae into mouse tails. Mouse tails were stored at -20°C and thawed prior to use. For non-soap samples, ~2 cm of mouse tail tips were exposed to water with *S. mansoni* cercariae for 15, 30 and 60 minutes, and then removed. Tails were washed with water to remove cercariae that did not attach irreversibly to nor penetrated the tails. The experiment could not distinguish between attachment and penetration. The attachment or penetration of cercariae was determined by quantifying the number of cercariae in the liquid before and after exposure. For soap-treated samples, the same procedure was adopted to determine cercarial attachment or penetration at 60 minutes, but tails were treated

with soap before being exposed to cercariae. One powder soap Kleesoft and one bar soap B29 which are commonly used in a schistosomiasis-endemic region of Tanzania were tested. 2 cm of mouse tail tips were treated with water mixed with powder soap Kleesoft at 10, 100 and 1000 mg/L or with B29 at 1000 mg/L by immersing the tail tips in the soap solution, and then rinsed in water to wash soap off the tails.

Results: *S. mansoni* cercariae were able to attach to or penetrate thawed mouse tails that had been previously frozen. The percentage of attachment or penetration to mouse tails was significantly related to exposure time, achieving a maximum of 54.5±11.0% at 60 minutes. Powder soap Kleesoft provided partial protection against the attachment or penetration of *S. mansoni* cercariae to mouse tails, and this protection was significantly associated with the soap concentration of tail treatment, reaching 87% of reduction under 1000 mg/L tail treatment. However, bar soap B29 was not able to provide protection even under the highest concentration of 1000 mg/L tail treatment.

Conclusions: While neither soap type provided complete protection against attachment or penetration, powder soap was found to be more protective than bar soap, which may be especially important for long duration water contact activities such as washing clothes by hand.

Poster 118 : Environmental influences on the distribution and ecology of the fluke intermediate host *Galba truncatula*: A systematic review

Christopher Smith, *Aberystwyth University*

C Smith[1]; ER Morgan[3]; R Jones[2];
[1] Aberystwyth University, UK; [2] IBERS, UK; [3] Queen\'s University Belfast, UK

Galba truncatula is the main host of the liver fluke *Fasciola hepatica* across Europe, North Africa, and South America. As such, understanding the environmental preferences of this species is vital for creating strategies to control this and other trematode infections such as rumen flukes. This systematic literature review evaluated the current understanding of the snail's environmental preferences to identify factors that might aid control and areas where further research is needed. Searches were conducted across Web of Science and Google Scholar between March and August 2023, with results screened to find papers which explicitly focused on *G. truncatula*, with a total of 198 papers evaluated. These studies were assessed for risk of bias using the Mixed Methods Appraisal Tool, and the country of origin, habitat type and habitat pH were noted, along with any other information regarding the snail's environmental preference noted by the authors. The results indicate that the snail is most commonly found in farmland open drainage systems, on river and stream banks, and in pools and ponds, and is capable of surviving across a wide range of climates. However, the impacts of many environmental factors are unclear due to limited data and inconsistent results and terminology between studies. The pH tolerance of the snail is around 5-9, but within this range there are a roughly equal number of studies which state that *G. truncatula* prefers acidic, basic, or neutral habitats. Overall, the results show that many aspects of the snail's environmental preferences require further research to clarify abiotic and biotic impacts on populations. This information will be vital in creating robust risk assessments of fluke infection, predicting how the snail and hence trematode transmission may be impacted by climate change, and the opportunities for environmental control strategies.

Poster 119 : Multi-host parasites in heterogenous landscapes: Do we see between-species transmission and host specificity of *Bartonella grahamii* strains infecting fragmented populations of water voles (*Arvicola amphibius*) and field voles (*Microtus agrestis*)?

Dr Laura MacKenzie, *University of Livepool*

L MacKenzie[3]; S Telfer[2]; R Hassall[4]; R Birtles[1]; S Brierley[1]; I Goodhead[1]; X Lambin[2];
[1] University of Salford, UK; [2] University of Aberdeen, UK; [3] University of Livepool, ; [4] UK Center for Ecology and Hydrology, UK

Many parasites infect multiple host species and transmission between sympatric hosts is assumed to be commonplace. Between-species transmission could be key to parasite persistence and spread, especially where host populations exist in highly heterogeneous landscapes, are small and prone to stochastic extinction. However, previous genetic studies of the multihost parasite *Bartonella*, found evidence of cryptic host specificity of bacterial strains, challenging the assumption of frequent between-species transmission. We investigate whether this finding of cryptic host specificity is universal or whether dynamics of between-species transmission may be context dependent. Using a whole-genome sequencing approach, we compare patterns of host specificity and between-species transmission of *Bartonella grahamii* strains in the context of two contrasting populations: a highly fragmented population of water voles (*Arvicola amphibius*) and sympatric field voles (*Microtus agrestis*) and a community of field and bank voles (*Myodes glareolus*) inhabiting more continuous habitat. Statistical analysis of single nucleotide polymorphism (SNP) patterns showed that *B. grahamii* strains were host generalists in the context of the fragmented population, commonly infecting both hosts. In contrast, in the continuous population *B. grahamii* showed indications of cryptic host specificity. We suggest that patterns of between-species transmission may be context dependent. In highly heterogenous landscapes and fragmented populations, host generalist strains may have a competitive advantage over host specialists, as the ability increasing their persistence and spread.

Poster 120 : Parasitic profile of five species of terrestrial achatina snail in Cross River State, Nigeria: public health implications

Jenavine Onyinye Mbah, *University of Calabar*

J Onyinye Mbah[1];
[1] University of Calabar, Nigeria

A total of 760 snails of the genera *Achatina*, belonging to five species (*Achatina achatina, Achatina belteata, Achatina degneri, Achatina fulica* and *Achatina marginata*) were sampled between January and August 2021, from six communities located in six Local Government Areas (LGAs) in the Central Senatorial District, Cross River State. A. achatina was the most abundant species collected (32.89%) while *A. degneri* was the least collected (11.19%). A greater number of *Achatina* snails were collected in the wet season than in the dry season with no observed dominance of any of the species. Overall, 319 (42%) snails were infected with parasites. *A. fulica* had the highest prevalence of parasitic infection (50.50%) while *A. marginata* had the least parasitic infection (28%). Snail species sampled in Boki LGA had the highest prevalence of parasitic infection (56.25%), while Obubra LGA recorded the least prevalence (21.28%). Mean intensity of *Angiostrongylus* spp. in *A. achatina* was 4.780 (4.56 – 5.00; 95% CI), while *Strongyloides* spp. was 4.667 (4.11-5.22; 95%CI). Testing parasite species diversity in the snail species assessed using diversity indices, *A. balteata* recorded the highest values for Shannon-Wiener (1.653) and Margalef's indices (1.995), and also for species dominance using the Simpson index (0.22). Public health education and provision of adequate toilet facilities are recommended for control of snail-borne parasites.

Poster 122 : Development of a qPCR Duplex Assay for simultaneous detection *of Fascioloides magna* and *Galba truncatula* in eDNA samples: Monitoring beyond boundaries

Amir Reza Varzandi, *University of Turin*

AR Varzandi[1]; S Zanet[1]; E Rubele[1]; F Occhibove[1]; R Vada[1]; F Benatti[1]; E Ferroglio[1];
[1] University of Turin, Italy

Parasites constitute a significant economic burden and highly impact environmental, public, and animal health. The emergence of many parasitic diseases is environmentally mediated, and they share the same biogeography with humans and both domestic and wild animals. American liver fluke, *Fascioloides magna* – a trematode parasite of domestic and wild ungulates – is an example of the anthropogenic introduction of an "invasive alien species" in Italy and Europe. Multiple introductions to Europe have led to the biogeographical expansion of the parasite across the Danube region mainly provided by the presence of suitable habitats for all hosts involved in the parasite's life cycle, human-assisted transport, and drastic environmental events such as flooding. In Italy, it was introduced and established in La Mandria Regional Park (LMRP) near Turin in 1865 along with imported wapitis (*Cervus elaphus canadensis*) from North America (Bassi, 1875), but with no reported expansion to the surrounding areas. LMRP isolated *F. magna* focus, poses an important threat of possible expansion since the enclosed area is vulnerable to occasional bidirectional passage of roe deer. Additionally, tributary rivers to the Po river system, traversing the enclosed area, could further bolster the possibility of such spread. In this study, we developed a duplex qPCR assay for *F. magna* and its principal intermediate host *Galba truncatula* optimized for testing eDNA samples to meet the needs for surveillance of the parasite. Moreover, we validated the developed assay *in natura* by testing samples derived from filtered water and sediments collected inside and outside LMRP's fenced-off area. Our findings for the first time demonstrate the presence of *F. magna's* eDNA outside the park's internal fenced-off area.

Poster 123 : The increasing concern surrounding the presence of *Angiostrongylus cantonensis* in Mediterranean Europe

Dr Claudia Paredes-Esquivel, *Liverpool School of Tropical Medicine*

S Jaume Ramis[2]; A Martínez-Ortí[4]; N Pons García[2]; S Delgado-Serra[2]; M Arango-Colonna[2]; MD Bargues[4]; S Mas-Coma[1]; LR Haines[3]; C Paredes-Esquivel[2];
[1] Unidad de Parasitología Sanitaria, Facultad de Farmacia, Universidad de Valencia, Spain; [2] Universitat de les Illes Balears, Spain; [3] Department of Biological Sciences, University of Notre Dame, United States; [4] Universitat de Valencia, Spain

Neuroangiostrongyliasis, an emerging zoonotic disease caused by the gastropod-borne nematode *Angiostrongylus cantonensis*, poses a growing concern. The arrival of the rat lungworm in Mediterranean Europe was first reported in Mallorca in 2018, by conducting parasitological surveillance in hedgehogs. More recently the parasite was reported in Valencia, mainland Spain. In humans, this infection can lead to painful and complex clinical manifestations associated with eosinophilic meningitis, often due to the accidental or deliberate ingestion of raw or undercooked snails. In Mallorca, we have also detected *A. cantonensis in R. rattus* and *R. norvegicus*, the primary definitive hosts, while 11% of gastropods from endemic hotspots carry the parasite. Knowledge gaps persist regarding competent intermediate and paratenic hosts and their impact on food industries, wildlife and human populations. Given frequent ship traffic between Mallorca

and other European countries, proactive surveillance is vital for early diagnosis in the region. Following a One Health approach, we present recent updates on the epidemiological scenario of this important pathogenic invader in a tourism-based area where snails are historically consumed.

Poster 124 : LeishGEM: A genome-scale database for knockout mutant life cycle fitness phenotyping and subcellular protein localisation in *Leishmania mexicana*

Dr Ulrich Dobramysl, *University of Oxford*

U Dobramysl[3]; E Ferreira[4]; RP Neish[1]; LD Davidson[8]; R Pereira[2]; R Etzensperger[2]; S Aellig[2]; M Young[5]; J Smith[5]; J Damasceno[7]; JD Sunter[6]; J Mottram[1]; E Gluenz[2]; R Wheeler[9];
[1] University of York, UK; [2] Institute of Cell Biology, University of Bern, Switzerland; [3] University of Oxford, UK; [4] University of York, Centre for Immunology and Infection, UK; [5] Institute of Infection, Immunity and Inflammation, University of Glasgow, UK; [6] Department of Biological and Medical Sciences, Oxford Brookes University, Oxford, UK; [7] Wellcome Centre for Integrative Parasitology, Institute of Infection, Immunity and Inflammation, University of Glasgow, UK; [8] Oxford Brookes University, UK; [9] Peter Medawar Building for Pathogen Research, Nuffield Department of Medicine, University of Oxford, UK

The success of *Leishmania* parasites as pathogens is encoded in their genome. However, as eukaryotes, their genome is large and relatively complex. Despite extensive efforts for the functional characterisation of protein-coding genes in *Leishmania*, the role and localisation of most is still unclear. Indeed, according to TriTrypDB only 14% of the 8,267 *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 systematically address this by:

1) Determining the fitness of deletion mutants of protein-coding genes (genome-wide, 8,267 genes) by generating uniquely genetically barcoded deletion cell lines and assessing growth fitness as promastigotes, axenic amastigotes, amastigotes in macrophages, and in a mouse footpad infection.
2) Visualising the sub-cellular localisation proteins in promastigotes and axenic amastigotes by tagging at both the N and C termini (if lacking an ortholog in or divergent from *T. brucei*, 2,700 target genes) in the LeishTag sub-project.
3) Analysing the subcellular localisation via LOPIT-DC fractionation.

We are now making the fitness phenotyping and localisation data generated by the LeishGEM project so far available at http://leishgem.org/. As of now, this database contains fitness data for 2,305 gene deletion mutants, subcellular localisation for 1,209 tagged cell lines where we have completed localisation annotation, and 3,782 proteins for which LOPIT-DC fractionation and mass spectroscopy yielded information on the subcellular localisation. This is the first data release of a transformative resource for the function of thousands of genes in a family of important human pathogens.

Poster 125* : Epidemiology of apicomplexan parasites in livestock-infesting ticks in northwest Nigeria

Haruna Adamu Mamman, *University of Salford*

HM Adamu[1];
[1] *University of Salford Tick Infections (USALTI) Group, School of Environment and Life Sciences, University of Salford, UK*

Background: Northwestern Nigeria is an important hub within a vast regional transboundary livestock trade network through which thousands of animals raised in the semi-arid Sahel must pass each year to fulfil the demand for meat in the densely populated coastal regions to the south. This network can facilitate the rapid spread of pathogens across large distances, especially in the absence of any form of importation control at borders. Ticks and tick-borne pathogens present a well-established threat to livestock health in the region but almost no work has been done to assess the impact of transboundary livestock trading on their diversity, distribution and epidemiology. This study aimed to establish fundamental information about the diversity of ticks infesting cattle and camels.

This study aims at documenting the fauna of ticks at different time of the year in Northwestern Nigeria and molecularly assessing the occurrence of Apicomplexan tick-borne pathogens from cattle and camel tick species in Northwestern Nigeria.

Method: Three surveys of ticks infesting cattle in Zamfara and Sokoto state of Northwestern Nigeria were carried out in 2017, 2019 and 2021. The ticks were identified morphologically to the species level and were screened molecularly, first using Apicomplexan-specific nested Polymerase Chain Reaction and in comparison, the positive results were screened again using Next Generation Sequencing (NGS) targeting a broad range of apicomplexan species.

Results: 600 adult tick species were collected from a total of 169 livestock across the 3 sampling years. Between the years, 5 livestock markets were surveyed for tick on both cattle and camels. Overall 9 tick species were encountered in both states - *Amblyomma variegatum, Hyalomma dromedarii, Hyalomma truncatum, Hyalomma rufipes, Hyalomma impressum, Hyalomma impeltatum, Hyalomma marginatum, Rhipicephalus boophilus decoloratus* and *Rhipicephalus sanguineus*. Across the year, the camel tick *Hyalomma dromedarii* showed consistency in dominating other less common tick species. Notably is the absence of *Amblyomma variegatum* in 2017 but accounted for 17% and 30% in 2019 and 2021 respectively revealing an increasing prevalence in the region while *Hyalomma impeltatum* showed a declining trend from 37% in 2017 to 13% in 2019 and 7% in 2021. The molecular results revealed the presence of apicomplexan species as - *Theileria annulata, Theileria mutans, Theileria velifera, Babesia cabali, Babesia occultans,* and free-living apicomplexan species.

Conclusion: The study affirms the presence of a wide variety of tick species in Northern Nigeria, with a particularly notable prevalence of the camel tick, *Hyalomma dromedarii*, among cattle. This suggests the encroachment of desert-adapted tick species into regions experiencing discernible effects of climate change. Shedding light on the

Poster 126* : Exploring cryptic novel Thraustochytrida (Labyrinthulomycetes, Stramenopiles) diversity in *P. olseni* infected clam populations along the French Atlantic Coast

Elisa Chailler, *Station Biologique de Roscoff*

E Chailler[1]; M Perennou[2]; S Itoïz[2]; H Le Bayon[1]; M Smits[2]; E Derelle[2]; A Bidault[2]; N Le Goïc[2]; P Soudant[2]; A Chambouvet[1];
[1] *CNRS, Sorbonne University, France;* [2] *Univ Brest, CNRS, IRD, Ifremer, LEMAR, Plouzané, France, France*

Thraustochytrids (Labyrinthulomycetes, Stramenopiles) are enigmatic heterotrophic unicellular protists which are ubiquitous and abundant in a wide variety of environments. Their ecological role remains largely unknown

and underestimated, yet these osmo-heterotrophic protists play a fundamental role in the cycling of organic material as they have the ability to degrade numerous organic substrates including refractory organic matter. Even if, for now, the majority of described Labyrinthulomycetes are saprotrophic, some members have been described as parasites or pathogens and implicated in mortality events of numerous benthic invertebrates or algae. One hypothesis is that some of these organisms can be opportunistic parasites that become pathogenic in host stress conditions. Global biodiversity is currently threatened by anthropogenic eutrophication of environments, increasing temperatures, and biotic factors; these factors can weaken organisms and make them vulnerable to pathogenic agents such as opportunistic parasites like thraustochytrids. This is the case of Manila clam populations (*Ruditapes philippinarum*) which are threatened by another unicellular exotic parasite, *Perkinsus olseni* (Perkinsea, Alveolata). Accidentally introduced into Europe from Asia with its host, *P. olseni* spread along the European Atlantic coastline following a latitudinal gradient with recurrent mortality events in Spain and Portugal. The aim of this poster is to evaluate the genetic diversity and the distribution of novel Thraustochytrids associated with *P. olseni*-infected clams along the French Atlantic coastline.

Poster 127 : Insights into the exoerythrocytic development of *Haemoproteus attenuatus* in the Thrush Nightingale *Luscinia luscinia*

Dr Melanie Tchoumbou, *Nature Research Center*

M Tchoumbou[1]; M Duc[1]; M Ilgūnas[1]; T Iezhova[1]; G Valkiūnas[1];
[1] Nature Research Centre, Lithuania

Haemoproteus parasites (Haemosporida, Haemoproteidae) are cosmopolitan blood pathogens. Despite their importance for avian host fitness and health, little is known about the exo-erythrocytic development of most described species. Recent discoveries based on molecular markers showed that tissue stages of haemoproteids damage various internal organs, sometimes resulting in severe and even lethal avian haemoproteosis, including cerebral pathologies. This study aimed to identify and describe the exo-erythrocytic (tissue) stages of *Haemoproteus* parasites in *Luscinia luscinia* (Muscicapidae). Blood and tissue samples of 8 infected individuals were examined under a microscope and using PCR-based methods. Organs were examined for exo-erythrocytic stages by histology methods and application of in situ hybridization tools using genus-specific and lineage-specific oligonucleotide probes targeting the 18S ribosomal RNA of the parasites. Exo-erythrocytic meronts of *H. attenuatus* (lineage ROBIN01) were found and described for the first time in 6 bird individuals. Most meronts were located in the lungs; a few also were found in the liver, heart and pectoral muscle. Mature meronts contained numerous roundish merozoites. Megalomeronts were observed in the gizzard and the heart of two individuals, which also contained meronts. Based on the morphology and site of infection, these megalomeronts likely belong to parasites of *Haemoproteus majoris* group, which present in co-infection with *H. attenuatus*. This study reports new pathological aspects of haemoproteosis during infection of *H. attenuatus* and probable co-infection with *H. majoris*, opening new directions for better understanding pathology during haemoproteosis. This study was funded by the Research Council of Lithuania (S-PD-22-71).
Key words: Avian haemosporidians, *Haemoproteus*, Phylogeny, In situ hybridization, Meront, Megalomeront

Poster 128 : Schiff base complexes: A glimpse of hope for anti-onchocercal drug leads

Dr Evans Mainsah Ngandung, *University of Buea*

EM Ngandung[2]; JC Shirri[2]; F Cho-Ngwa[2]; PT Ndifon[3]; JN Yong[2]; G Parkinson[1]; MH Todd[1];
[1] University College London, UK; [2] University of Buea, Cameroon; [3] University of Yaounde I, Cameroon

The devastating effects of onchocerciasis have continued to be more and more visible, ranging from human pain and suffering to huge economic losses. It is one of the neglected tropical diseases with over 37 million persons affected and a risk population of over 120 million, with over 95% of this population in Africa. Ivermectin, the only drug of choice for mass drug administration programs is only microfilaricidal, requiring long treatment duration and has severe side effects in cases of *Loa loa* co-infection. Also, many endemic zones of Africa are hard hit by wars and insecurity, thereby frustrating the WHO MDA programs. The onchocerciasis burden is made worse by there being no drug effective against macrofilariae thereby necessitating the search for safe and more effective drug leads. There is growing interest in emodepside as DNDi receives USD 20 million to accelerate the development of innovative new drugs for onchocerciasis elimination. Our previous work demonstrated that Schiff base complexes derived from isoniazid were potential macrofilaricides, active against adult female worms at IC_{50} values less than 10 µg/mL. In continuation of our investigation on this class of compounds, we are currently studying the anti-onchocercal activities of an expanded library of compounds and their metal complexes designed for superior solubility.
Keywords: Onchocerciasis, Schiff bases, metal complexes, macrofilaricides, *Loa loa*.

Poster 129* : Hide and Caecum: Searching for antimicrobials in the equine tapeworm *Anoplocephala perfoliata*

Holly Northcote, *Aberystwyth University*

H Northcote; P Wititkornkul[1]; PM Brophy[1]; RE Wonfor[1]; RM Morphew[1];
[1] Aberystwyth University, UK;

Gastrointestinal (GI) helminths have been observed to create significant changes to the GI microbiome they share their environment with. Helminths, largely nematodes, have also been shown to create a plethora of antimicrobial peptides (AMPs) secreted through secretory products (ESP, which contain extracellular vesicles (EVs) which has been hypothesised as a mechanism for host-microbiome changes. *Anoplocephala perfoliata* (Aper), an equine tapeworm, has often been neglected in molecular research. However, the recent generation of a transcriptome and secretory (ESP and EV) proteomes provides a foundation for a greater understanding of its host-parasite relationships. Utilising a comparative bioinformatic investigation the current work has identified AMPs within *A. perfoliata* and two additional flatworms and localised them in EVs. AperEV-free ESP (AperESP) and the cytosolic proteome (AperCY) were also further investigated for AMPs. Furthermore, Aper peptidomes (Somatic, ESP, EVs) were extracted, using an acidic methanol treatment, and identified via MSMS. Finally, AperEVs and AperESP antimicrobial activity was assessed following incubation with *Escherichia coli* and *Bacillus megaterium* and the optical density (OD) monitored over 24 hours. In total, 34 unique IDs were identified in *A. perfoliata* as AMPs in contrast to 130, and 13 from a rumen fluke and a liver fluke respectively. Notably, 6, 8, and 4 of these retrieved IDs were localised in the parasite's EV proteomes. Within *A. perfoliata*, a further four and 25 potential AMP IDs were resolved in the AperCY and AperESP, respectively. Preliminary peptidome analysis of the same fractions within *A. perfoliate* demonstrates the potential to identify a plethora of novel peptides and possible AMP agents and for the first time the identification of peptides within the EVs. Despite promise of AMP activity through bioinformatic, proteomic and peptidomic analysis, initial optical density assays of *A. perfoliata* EVs demonstrated no antimicrobial activity. The current data suggests that *A. perfoliata* can produce a range of different potential AMP proteins and/or peptides and these can be localised within their EVs. Whilst initial assays have not demonstrated antimicrobial activity, bioinformatic data suggests *A. perfoliata* may have the ability to affect/alter the microbial environment around

them through AMPs. Alternatively, proteins resembling potential AMPs may not possess antimicrobial activity, similar to helminth defence proteins. previous studies. Thus, further microbial investigation is needed.

Poster 130 : Interrogating the role and regulatory functions of autophagy-related pathways during *Trypanosoma brucei* differentiation and host adaptation

Aro Nugawela, *York University*

A Nugawela[2]; N Thompson[2]; M Cayla[1];
[1] Department of Biology, University of York, UK; [2] YBRI and Department of Biology, University of York, UK

Trypanosoma brucei (*T. brucei*) is the causative agent of human African trypanosomiasis and the cattle wasting disease, Nagana. The spread of trypanosomiasis relies on successful transmission of parasites between the mammalian host and their *Tsetse* fly vector; a process which requires stage-specific pre-adaptation to the new host. In the mammalian bloodstream (BSF) parasites differentiate from the replicative "slender" to the quiescent "stumpy" forms, a differentiation event which is characterised by extensive morphological remodelling, which pre-adapts these parasites for survival in the insect host. Our work centres on understanding the regulatory role of autophagy-related pathways and differentiation.
The lysosome-dependent autophagy pathway in eukaryotic models is widely understood to facilitate the degradation and recycling of cellular components. Through the visualization autophagosomes and autolysosomes, we present preliminary data which links autophagy activation to a key point in the differentiation process. We also investigate the role of lysosome exocytosis in the release of parasite peptidases/proteases in initiating quorum-sensing dependent differentiation and their function in tissue invasion using *in vivo* and *in vitro* methodologies.

We outline our initial steps in the development of a novel high throughput (HTP) bioimaging system, optimised for prolonged visualisation of the highly motile BSF form *T. brucei*. This system aims to provide a resource for routine and prolonged live-cell visualisation and particle tracking across the time-course of differentiation *in vitro*. This HTP system will then be used in combination with a kinome-wide RNAi screen to identify the protein kinases regulating the autophagy and differentiation pathways in this pathogen.

Poster 131* : How is VEX2 recruited to the expression-site body in *Trypanosoma brucei*?

Elizabeth Spink, *University of York*

E Spink[1]; K Hayton[1]; S Stevens[1]; J Faria[1];
[1] University of York, UK

Antigenic variation allows *Trypanosoma brucei* to evade the immune system, survive, and proliferate in its mammalian host. This process is dependent on monoallelic expression of variant surface glycoproteins (VSGs). While the parasites possess an extensive repertoire of VSG genes, only one expression-site (VSG-ES) is active at one time. Stochastic antigenic switching allows the cells to evade the immune response, whereas cells expressing multiple VSGs are easily cleared by the immune system.

The expression-site body (ESB) is a subnuclear structure where the single active VSG-ES is transcribed by RNA polymerase I (Pol-I). VSG-Exclusion-2 (VEX2) is a large RNA:DNA helicase found at the ESB which has been shown to be essential for VSG monoallelic expression. Previous analysis using Hi-C and scRNA-Seq

showed that VEX2 depletion leads to dramatic changes in genome organisation that result in multiple antigen expression, however the mechanism is not fully understood.

We sought to dissect VEX2 function and recruitment to the ESB by using a tet-inducible system to overexpress wild-type, mutated, and truncated versions of VEX2.These results were analysed using fluorescence microscopy and transcriptomics.

Super resolution microscopy showed that VEX2 overexpression leads to its accumulation at the active-VSG-ES and at the periphery of the nucleolus, revealing intrinsic affinity for sites of Pol-I transcription. We also found that such specificity is mediated by sequences within its N-terminus and that the helicase activity is essential for VSG expression control.

In addition, by specifically blocking transcription at the active-VSG-ES, we found that active transcription is crucial for VEX2 localization to the ESB, indicating the role of RNA in its compartmentalisation within this structure. Future research will aim to identify the RNA sequences that VEX2 interacts with.

Poster 132* : Whipworm host defence peptides – novel opportunities for parasite control?

Richard Thomas, *Manchester Metropolitan University*

R Thomas[2]; A Irvine[1]; D Mckenzie[1]; C McCoy[1]; MS Asif[2]; J Ashworth[2]; N Pionnier[2]; L Atkinson[1]; A Mousley[1]; R Shears[2];
[1] Queen's University Belfast, UK; [2] Manchester Metropolitan University, UK

Host defence peptides (HDPs) are key components of the invertebrate innate immune system where they provide protection against microbial threat. In the context of parasitic helminth infection, worm-derived HDPs may play a critical role in modulating host-parasite-microbiome interactions, particularly in microbe-rich environments such as the gastrointestinal tract. HDPs have been identified in *Ascaris* and *Anisakis* species, *Toxocara canis, Heligosomoides polygyrus, Onchocerca ochengi* and the free-living nematode *C. elegans*. Using a computational approach, we have identified HDPs within the peptidomes of *T. muris, T. trichiura* and *T. suis*, some of which have antimicrobial and/or immunomodulatory activity in vitro. We have also identified several HDPs within *T. muris* and *T. trichiura* excretory/secretory content. Given that *Trichuris* infections alter the composition of the host's gut microbiota to enable chronic infection, targeting *Trichuris* derived AMPs therapeutically, perhaps through vaccination, could be a novel opportunity for parasite control.

Poster 133* : Temporal trends and spatial patterns of Cutaneous Leishmaniasis in the most densely populated Moroccan region.

Imane El Idrissi Saik, *Hassan II University of Casablanca*

I EL Idrissi Saik[2]; H Talimi[3]; B Badri[4]; S Bouhout[4]; M Riyad[1]; M Lemrani[5];
[1] Hassan II University of Casablanca, Morocco; [2] Hassan II University of Casablanca and Institut Pasteur du Maroc, Morocco; [3] Institut Pasteur du Maroc and University Abdelmalek Essaadi, Morocco; [4] Ministry of Health of Morocco, Morocco; [5] Institut Pasteur du Maroc, Morocco

Introduction: Cutaneous leishmaniasis (CL) caused by protozoa of the *Leishmania* genus and transmitted through infected sand fly bites, poses a significant public health burden in Morocco. Our study focuses on the Casablanca-Settat region, the most densely populated region of the country, and aims to analyze the spatio-

temporal trends of CL in this area retrospectively for over 14 years (2009-2022). The study explores epidemiological patterns, seasonal variations, and the impact of climate variables on CL incidence.

Methodology: Data, including CL cases, demographic information, and climatic factors, were collected and analyzed using Geographic Information Systems (GIS) and R statistical packages.

Results: Results revealed a steady increase in CL cases until 2019, followed by a significant rise. All the reported CL cases were caused by *Leishmania tropica*. Incidence rates vary among groups, with higher rates in females and children aged 5-14. Seasonal analysis indicates a notable increase in cases during spring and winter. Spatial distribution analysis identifies the highest hotspot in El Brouj (Settat province), the only recognized endemic CL focus in the region, emphasizing the need for targeted interventions. Pearson correlation analysis shows a significant positive association between CL cases and minimum temperature and precipitation.

Conclusion: This retrospective study provides crucial insights into CL epidemiology in the Casablanca-Settat region, emphasizing the role of climate variables in disease incidence. The findings underscore the importance of tailored prevention strategies, increased disease awareness, and local control programs to improve case reporting and curb CL transmission.

Keywords: Cutaneous leishmaniasis, *Leishmania tropica*, spatio-temporal distribution, climate, Morocco

Poster 134* : Exploring the mode of action of anti-leishmanial natural product analogues

Hannah Asiki, *University of Oxford*

H Asiki[2]; Y Biddick[3]; A Sozanschi[3]; M Amaral[1]; E Levatti[1]; R Wheeler[2]; EA Anderson[3]; A Tempone[1];
[1] Butantan Institute, Brazil; [2] Peter Medawar Building for Pathogen Research, Nuffield Department of Medicine, University of Oxford, UK; [3] Chemistry Research Laboratory, University of Oxford, UK

The dehydrodieugenol family of natural products has specific activity against all *Leishmania* species tested.[1, 2] We have developed an active analogue with both cross-linking and click capabilities which allows access to various techniques for target identification including whole cell localisation and photo-affinity protein pull-downs. Using *L. mexicana* for our investigations, we demonstrate mitochondrial localisation using both fluorescence light microscopy and transmission electron microscopy.
The ascididemin family of natural products also shows promise as an antiparasitic, having activity against *P. falciparum* and various *Trypanosomes*,[3] although little is known about its mode of action. We have developed a range of analogues which are highly potent against *L. infantum*, *L. mexicana*, and *T. cruzi*. SAR knowledge from these analogues is a first step towards introducing a handle that can be used for bioorthogonal labelling. Additionally, this family shows metal ion binding capabilities which could contribute to compound mode of action.

Finally, we have explored the benzyltetrahydroisoquinoline alkaloids as antiparasitics. By synthesising a range of natural products and related analogues, we show good antiparasitic activity against *L. infantum*, *L. mexicana*, and *T. cruzi*.[4] We combine cell biology techniques such as monitoring cell cycle interruption, changed morphology and dsDNA damage to reveal that kinetoplast disruption likely plays a key role in the mode of action for this compound family.

These natural product families each have unique and varied chemical structures, allowing us to discover interesting and potentially novel modes of action against *Leishmania*.

References

1. Grecco, S. S.; Costa-Silva, T. A.; Sousa, F. S.; Cargnelutti, S. B.; Umehara, E.; Mendonça, P. S.; Tempone, A. G.; Lago, J. H. G. *Journal of Venomous Animals and Toxins including Tropical Diseases* 2018, *24* (1), 27.

2. Amaral, M.; Asiki, H.; Sear, C. E.; Singh, S.; Pieper, P.; Haugland, M. M.; Anderson, E. A.; Tempone, A. G. *RSC Medicinal Chemistry* 2023, *14* (7), 1344-1350.

3. Copp, B. R.; Kayser, O.; Brun, R.; Kiderlen, A. F. *Planta Med* 2003, *69* (6), 527-531

4. Sozanschi, A.; Asiki, H.; Amaral, M.; de Castro Levatti, E. V.; Tempone, A. G.; Wheeler, R. J.; Anderson, E. A. *JACS Au* 2024, *4* (2), 847-854.

Poster 135 : Endoparasites of captive snake collections, in the UK

Shea Murray, Niamh Lysaght, *Liverpool School of Tropical Medicine*

S Murray[1]; N Lysaght[1]; E Crittenden[1]; N Casewell[1]; JR Stothard[1]; J LaCourse[1]; A Juhasz[1]
[1] Liverpool School of Tropical Medicine, UK

Many snakes are asymptomatic carriers of parasites throughout their life. However, even if they are asymptomatic, captive and free-ranging reptiles can harbour and excrete a wide range of diseases that can also cause infections in humans. This zoonotic aspect makes this topic significant not only in veterinary medicine but also in human medicine. Endoparasites are very common in both wild and captive snakes. Reptiles harbour a broad spectrum of internal parasites, including diverse species of protozoans, nematodes, cestodes, pentastomids, acanthocephalans and trematodes. The exotic pet trade, illegal wildlife trade, snake bite research and private collections are a source of risk in zoonotic transmission between snakes and humans. In the present project, snake faeces were collected, and investigated for intestinal parasites, from several sources, including the LSTM's venom research centre, exotic pet shops, and zoos from UK. Faecal samples were obtained from these animals in artificial enclosures, with emphasis on avoiding soil contamination. Samples were processed and screened for parasites via several methods, including a *Giardia/Cryptosporidium* Rapid Diagnostic 'Quick Chek®', PCR, and microscopical examination following faecal staining, and flotation techniques. These combined approaches and techniques expand knowledge of the range of intestinal parasites in these reptiles and enhance understanding of effective management of captive snakes in improving animal welfare and reducing risk of zoonotic transmission.

Poster 136 : Assessing effects of landscape and woodland patch attributes on the density of *Ixodes ricinus* within woodland creation sites in Scotland

Benjamin Miller, *University of Liverpool*

BE Miller[1]; A Hacket Pain[2]; B Purse[3]; S Burthe[3]; J Medlock[4]; K Park[5]; E Fuentes-Montemayor[5]; S Venkatesan[1]; C Millins[1];
[1] Institute of Infection, Veterinary and Ecological Sciences (IVES), University of Liverpool, UK; [2] Department of Geography and Planning, School of Environmental Sciences, University of Liverpool, UK; [3] UK Centre for Ecology and Hydrology (UKCEH), UK; [4] Medical Entomology and Zoonoses Ecology, UK Health Security Agency, Porton Down, UK; [5] Biological and Environmental Sciences, Faculty of Natural Sciences, University of Stirling, Stirling, UK

Tick-borne zoonoses are an increasing threat to the health of people and livestock in northern latitudes, and this could be exacerbated by policy driven increases in woodland cover. Diseases such as Lyme borreliosis (LB), a tick-borne bacterial disease caused by infection with *Borrelia burgdorferi* sensu lato, cause tens of thousands of infections in Europe annually, with increased infections recorded year-on-year. The primary vector of LB

is *Ixodes ricinus* L., a tick species common in woodland habitats. However, little is known about how tick-borne disease risks will change in the future as planted woodland patches increase in size, complexity, and connectivity. Additionally, how host species assemblages establish over time in UK woodlands remains poorly understood. We predicted that increasing woodland connectivity and reducing the distance between patches will favour patch utilisation by host species such as deer which are important for presence and density of *I. ricinus*. More complex woodland structures found in older woodlands could favour small mammal populations, which are transmission hosts for *B. burgdorferi* s.l. and more suitable ground vegetation conditions could increase off-host tick survival through increasing humidity. To investigate this, a snap-shot survey of 60 broadleaf woodlands in Scotland selected along gradients of patch size, connectivity and woodland age was conducted between June and July 2023. *Ixodes ricinus* nymphal density, ground vegetation, temperature and humidity were measured along twenty 10m transects in each woodland, and an hour of constant dragging was carried out to collect additional nymphs. Out of 60 woodlands, 32 were found to have populations of *I. ricinus* where the tick population appeared to be established (>6 nymphs collected from site), while the remaining 28 sites were found to have very low or absent tick densities (<6 nymphs). In sites where *I. ricinus* was present, mean nymph densities ranged between 0.26 (±0.55) and 4.15 (± 2.90) nymphs/10m^2. Generalised linear mixed models were used to determined how woodland age, size, connectivity, and vegetation could influence i) the presence or apparent absence of *I. ricinus* populations at a site and ii) the density of nymphs recorded on individual transects nested within sites. Results from this study could be used to inform woodland managers and users about the risk from tick-borne pathogens and how these risks could change over time.

Poster 137 : Characterisation of the chronic infection-driving trypanosomes in cattle through single cell transcriptomics

Dr Emma Briggs, *University of Edinburgh*

E Briggs[1]; S Larcombe[1]; E Paxton[2]; KR Matthews[1]; L Morrison[2];
[1] Institute of Immunology & Infection Research, University of Edinburgh, UK; [2] The Roslin Institute, University of Edinburgh, UK

African trypanosomes cause livestock and human disease across sub-Saharan Africa. Although Human African Trypanosomiasis is on track for elimination, Animal African Trypanosomiases (AAT) remains a significant economic burden that causes ~US$4.5 billion losses in annual GDP due to the associated wasting disease, as well as significant animal suffering. AAT in cattle, Nagana, results in 3 million livestock deaths alone each year.

Most trypanosome research has focused on laboratory-adapted *Trypanosoma brucei* 'monomorphic' lines that proliferate indefinitely in culture and generate unrealistic acute infections. Recently there has been increased focus on 'pleomorphic' parasites more relevant to field infections, which exhibit quorum sensing whereby 'slender forms' develop to arrested 'stumpy forms' as parasite density increases. Stumpy forms are pre-adapted for tsetse fly vector transmission. Recently, we have found that in *Trypanosoma brucei* chronic stage infections of mice (several weeks) when parasitaemia in the blood remains high, the overwhelming majority of parasites in the bloodstream are non-proliferating forms pre-adapted for transmission to the tsetse. Additionally, 'intermediate' forms that have undergone cell cycle arrest and express stumpy-associated transcripts but had not undergone full morphological change associated with stumpy forms, were evident in chronic infection. These forms may be key to understanding how trypanosomes establish and maintain chronic infections.

In cattle chronic infections are the norm, but at tens of magnitude lower blood parasitaemia than that observed in mouse models. Thus, cattle infections are an essential foundation for exploring the roles of slender, stumpy

and ‘indeterminate’ forms in chronicity and providing insight into the infection status dominant in the field. Two calves were infected with pleomorphic *T. brucei* and parasitaemia was followed over 60 days. Single cell transcriptomics was performed at four discrete time points from early, mid and late infections, together capturing the transcriptomes of 37,602 individual parasites. Coupled with microscopy, we find populations of parasites that are highly similar to both slender and stumpy forms to be prevalent in the blood throughout infection. Although stumpy-like forms isolated from cattle do not have the classical stumpy morphology frequently observed in mouse models, we find these forms have transcriptomic profiles most similar to mature stumpy forms. Dividing parasites were infrequently observed through-out infection, consistent with lower blood parasitaemia. Finally, comparison of *in vitro* generated slender and stumpy forms with both mouse and cattle derived *T. brucei* revealed host-specific transcriptomic changes.

Poster 138* : Sexual reproduction maintains mitochondrial genome fitness in Trypanosomatid parasites

Zihao Chen, *University of Edinburgh*

Z Chen[2]; E Wadsworth *[2]; S Cooper[4]; M Geerts[3]; P Buscher[1]; F Van den Broeck[3]; N Savill[2]; A Schnaufer[2]; *[1] Institute Tropical Medicine, Antwerp, Belgium; [2] University of Edinburgh, UK; [3] Institute of Tropical Medicine, Antwerp, Belgium; [4] Institute of Immunology & Infection Research, University of Edinburgh, UK*

Trypanosomatids are unicellular, flagellated eukaryotes that cause a number of diseases in humans and livestock. These parasites are all transmitted by different insect vectors and also renowned for their extraordinarily massive and complex mitochondrial DNA, the kinetoplast. The kinetoplast DNA (kDNA) in trypanosomatids forms a chainmail-like network that contains two types of interlinked DNA molecules: 20 to 50 copies of identical maxicircles and thousands of highly heterogeneous minicircles. Maxicircle genes encode subunits of the mito-ribosome, the electron transport chain and the F_OF_1-ATP synthase. The pre-mRNAs of several maxicircle genes (twelve in *Trypanosoma brucei*) require post-transcriptional editing directed by short “guide RNAs” (gRNAs) encoded on minicircles. The abundance of editing sites entails that a diverse population of minicircles is necessary for editing all maxicircle-encoded mRNAs.

The lifecycle of several trypanosomatid parasites involves developments in insect vectors and mammalian hosts. As typically not all maxicircle genes are needed in the mammalian stage, the selective pressure on kDNA relaxes temporarily and allows minicircle populations to change. During cell division, imperfect replication and segregation of kDNA result in fluctuation in the minicircle populations and copy number of insect-stage specific gRNA genes may randomly drift towards a dangerous low level approaching elimination. Loss of essential gRNAs may render the parasites incapable of establishing themselves in the insect vector and deprived of the opportunity of transmission.

We propose that sexual reproduction is key in countering random genetic drift in kDNA. For trypanosomatids such as *T. brucei*, *T. cruzi* and *Leishmania* spp, it has been shown that sexual reproduction happens exclusively in the insect vector and results in completing mixing of the mitochondrial genome in the progeny. Hence, sexual reproduction reshuffles minicircles among insect-transmissible isolates. The circulation potentially rescues underrepresented gRNA genes by replenishing it with copies from another parental cell line in which the gRNA gene is more abundant. Hence, sexual reproduction lowers the risk of losing the ability to express insect stage-specific genes after generations of clonal reproduction in mammalian host.

In support of this concept, we demonstrate that absence of sexual reproduction has profound impacts on kDNA of tsetse-transmissible and tsetse-independent subspecies (or ecotypes) of *T. brucei*. We sequenced, assembled and compared kDNA genomes from 262 *T. brucei* isolates of diverse geographical origin. Compared to the highly complex kDNA in *T. brucei* subspecies capable of sexual recombination (i.e. *T. b. brucei*, *T. b. rhodesiense* and *T. b. gambiense* type 2), we observed different degrees of reduction in kDNA complexity in the asexual subspecies. We confirmed that in three groups of kDNA independent *T. b. evansi* and *T. b .equiperdum*, the minicircle genomes consist of thousands of a single minicircle class specific and therefore diagnostic of each group. Unexpected for a putatively kDNA independent subspecies, *T. b. equiperdum* group OVI retains a minicircle population with moderate complexity and is potentially capable of generating fully edited mRNAs of A6 and RPS12, the only edited maxicircle genes required in the mammalian host. Further, we report a highly streamlined and conserved minicircle population characteristic of *T. b. gambiense* type 1 isolates. The significantly lower gRNA coverage in *T. b. gambiense* type I suggests that only a minor fraction of cells within each population still retain the ability to survive in the tsetse vector, which may help

Poster 139 : Anti-*Wolbachia* drugs have no effect on the viability of extracellular *Wolbachia*

EIlen Masters, *Liverpool School of Tropical Medicine*

EK Masters[1]; A Yousef[2]; Y Wu[1]; JD Turner[1]; JM Foster[3]; SA Ward[1]; MJ Taylor[1];
[1] Liverpool School of Tropical Medicine, UK; [2] Kuwait University, Kuwait; [3] New England Biolabs Inc., United States

Wolbachia is an obligate symbiont of medically important filarial nematodes and is required for normal worm development, growth, embryogenesis, transmission of microfilariae and adult worm longevity. *Wolbachia* depletion with doxycycline leads to immediate and permanent sterility of adult female worms and a highly reduced adult lifespan. Other compounds in the tetracycline and rifamycin classes of antibiotics have also been shown to be active against *Wolbachia*. Interestingly, within the fluoroquinolone class some antibiotics are active whereas others have no effect on *Wolbachia* infection. More recently, candidates from the AWOL consortium were shown to have a much more rapid kill profile. The mechanism of action by which these drugs clear *Wolbachia*, and the reasons behind the differences in speed and efficacy are not yet understood. We show that anti-*Wolbachia* drugs from each class consistently increase autophagic flux in two insect cell lines and *Brugia malayi*, but not in mammalian cells. Drugs ineffective against *Wolbachia*, and sub-optimal concentrations of the anti-*Wolbachia* antibiotics, did not induce autophagy. Inhibition of autophagy, at early and late stage, reduced the effect of the drugs in C6/36 cells and *B. malayi*. Interestingly, the activation of autophagy by anti-*Wolbachia* drugs was also seen in uninfected cells, indicating its effect is independent of *Wolbachia* infection. To further investigate the role of the host cell, *Wolbachia* were purified from C6/36 cells and treated with anti-*Wolbachia* drugs. No change in bacteria viability was seen after a 7-day exposure to anti-wolbachial drugs, measured by BacLight membrane staining. This assay is based on membrane integrity, and since it is possible for the bacteria to be dead with an intact membrane, the ability of the drug exposed *Wolbachia* to re-infect was assessed. All drug exposed *Wolbachia* were able to re-infect C6/36 cells. Ultrastructural morphology of purified *Wolbachia*, naturally release *Wolbachia* and drug exposed bacteria were indistinguishable. Since *Wolbachia* are obligately intracellular, with a degenerate genome, and unable to replicate extracellularly, it could be that they are metabolically quiescent and therefore unaffected by anti-*Wolbachia* drugs, many of which are known to target protein synthesis. In order to test this, we plan on performing RNAseq on *Wolbachia* at different time points since exiting the host cell. Together, this data shows that induction of autophagic flux is required for anti-*Wolbachia* activity, that this induction is independent of *Wolbachia* infection, and that anti-*Wolbachia* drugs have no direct effect on extracellular *Wolbachia*.

Poster 140* : Determining the optimum timing for treatment of gastrointestinal nematodes with tree fodder through mathematical modelling

Anna Ciezarek, *Queen's University Belfast*

A Ciezarek[1]; E Morgan[1]; A Aubry[2]; K Theodoridou[1];
[1] Queen's University Belfast, UK; [2] Agri-Food Biosciences Institute, Hillsborough., UK

Increasing levels of anthelmintic resistance has led to amplified interest in alternative control methods for gastrointestinal nematode infections of livestock. One method is using alternative feed stuffs such as tree leaves. These can induce a displacement effect by reducing consumption of contaminated pasture, and may also contain secondary metabolites, such as condensed tannins, with anthelmintic properties. However, these are limited resources and cannot be fed year-round, so optimisation of the timing of use is critical. This study uses mathematical modelling to predict the effect of these treatments at different times of year on GIN epidemiology. The GLOWORM-FL model framework was extended to include parasitic stages of the lifecycle to facilitate modelling the effects of tannins on different lifecycle parameters. Parameter values were calculated based on effect sizes reported in published *in vivo* and *in vitro* studies of tree leaf tannins on GINs. The model was run across four different locations along a north-south gradient across western Europe, and for 52 individual years (1970 – 2022) in each location to determine spatial and temporal variations. The effects of tannin-rich and no tannin forages are compared. Across all locations, the effect of displacement on adult worm burden is highest at the start of the year and gradually decreases throughout. The effect of tannin-rich fodder has a different seasonal pattern with the greatest level of effect occurring during the rise in adult parasite numbers, but before the peak. This therefore differs between species, location, and year to different degrees. These findings support the use of model optimisation of treatments to gain maximum benefit and the framework developed here can also be applied to the use of many different treatment strategies.

Poster 141 : Native microbiota-mediated changes of host immunity and body condition correlate to enhanced virulence when challenged by a novel pathogen invader

Ian Will, *University of Oxford*

I Will[2]; EJ Stevens[1]; K King[2];
[1] Keele University, UK; [2] University of Oxford, UK

The frequency of emerging disease is expected to grow as ongoing climate change and other anthropogenic effects drive new host and pathogen interactions. Shaping the outcomes of those interactions are the resident host microbiota. The microbiota has been shown to produce both reduced and enhanced virulence in hosts challenged by pathogens, depending on the context and organisms involved. In the context of emerging disease interactions, how the microbiota affects infection outcomes may be especially difficult to predict. As such, we investigated host gene expression to understand the molecular causes of microbiota-mediated enhanced virulence during novel pathogen invasion. We conducted transcriptomic analyses of *Caenorhabditis elegans* nematodes colonized by an ecologically relevant model microbiota community infected by the invasive bacterial pathogen, *Staphylococcus aureus*. We found microbiota and pathogen co-colonized hosts may become more susceptible to severe infection by changes in collagen biology mediated by the microbiota. Furthermore, microbiota colonized hosts increased expression of immunity genes and both increased (heat-shock) and decreased (unfolded protein) stress responses during infection, which could encompass both causes and effects of enhanced virulence. Taken together, our results indicate that molecular changes in the

hosts mediated by a typically beneficial microbiota may incur significant costs when challenged by a novel invading pathogen.

Poster 143* : Population genomics of praziquantel treatment response by *Schistosoma mansoni* from Uganda

Shannan Summers, *London School of Hygiene and Tropical Medicine*

S Summers[3]; F Allan[1]; T Bhattacharyya[3]; MA Miles[3]; A Bustinduy[3]; SR Doyle[2]; BL Webster[1];
[1] Natural History Museum, UK; [2] Wellcome Trust Sanger Institute, UK; [3] London School of Hygiene and Tropical Medicine, UK

Human populations at risk of schistosomiasis, particularly in sub-Saharan Africa, typically receive treatment with Praziquantel (PZQ) via annual mass drug administration programmes to reduce morbidity and to control transmission. These programmes have successfully decreased the prevalence and intensity of infections in many endemic countries; however, the impact of the drug pressure on the *Schistosoma* (S.) populations, particularly at the genetic level, is poorly understood and understudied. This project aims to characterise the genomic diversity of Ugandan *Schistosoma mansoni* populations (pre- and post-PZQ treatment) to determine the impact of PZQ treatment from a clinical trial that aimed to find the appropriate treatment regimen for preschool-aged children with intestinal schistosomiasis in Lake Albert, Uganda. Eggs were collected from participant stool, hatched, and individually stored on QIAcard FTA cards. Low-input DNA recovery followed by library preparation and multiplexing was undertaken before whole genome sequencing of 96 individual miracidia sequencing libraries using 150 bp paired-end reads on an Illumina Novaseq S4 lane. Mean coverage was calculated in 25kb sliding windows and ranged from 0.1x-16.5x coverage of the *S. mansoni* genome (version 10). We characterised the population structure and genomic diversity of *S. mansoni* populations within the context of published genomic data from different regions of Uganda and five other countries. Principal component analysis revealed that parasites from Lake Albert form a panmictic population and show a lack of population structuring. However, clear population structuring was shown at the regional and country level where parasites from Lake Albert could be distinguished from Lake Victoria and Koome Islands. We detected no high-frequency functionally impactful variants in the candidate PZQ target, the transient receptor potential melastatin praziquantel channel (SmTRPMPZQ). In conclusion, our findings suggest that PZQ treatment has not had a significant impact on the population structuring of parasites in Lake Albert. As control efforts to eliminate schistosomiasis intensify to reach the WHO neglected tropical disease roadmap targets, there is a need to monitor how *Schistosoma* populations are evolving in response to treatment. The lack of high frequency functionally impactful variants in SmTRPMPZQ found makes PZQ resistance difficult to monitor currently.

Poster 144 : Investigating gene expression regulation during early ookinete development using *Plasmodium chabaudi chabaudi* genetic crosses

Dr Joanne Power, *University of Glasgow*

BJ Power[1]; KK Modrzynska[1];
[1] School of Infection and Immunity, University of Glasgow, UK

Genetic linkage analysis of the cloned progeny of *Plasmodium chabaudi chabaudi* genetic crosses has historically been used to determine loci responsible for drug resistance and drug sensitivity, most notably in pyrimethamine and chloroquine resistance phenotypes. The usefulness of *P. c. chabaudi* strains for such

experimental evolution studies stems from their genetic diversity. For example, pyrimethamine-resistant *P. c. chabaudi* strain 47AS and pyrimethamine-sensitive *P. c. chabaudi* strain 10AJ differ by >144k single nucleotide polymorphisms (SNPs), equating to approximately 1 SNP every 100 bp. Coupled with the ability to genetically cross and transmit these rodent malaria strains through *Anopheles* mosquitoes, genetic analyses such as quantitative trait loci (QTL) analysis and linkage group selection (LGS) have been possible in this *Plasmodium* species, while such experiments are not possible in experimental strains of *P. berghei* or *P. falciparum* .

Using genetic crosses of two divergent *P. c. chabaudi* (AS and AJ) strains, we aim to investigate gene expression regulation during zygote-to-ookinete development *in vivo* in the *Anopheles stephensi* mosquito midgut, paying particular attention to maternal and paternal allele inheritance. In addition, should either of these strains fail to transmit or cross-fertilise, we have acquired, and transmitted through *An. stephensi* mosquitoes for the first time, the *P. chabaudi* subspecies isolate *P. c. esekanensis* EF.

These data represent the establishment of the full *P. c. chabaudi* life cycle and the first generation of *P. c. chabaudi* genetic crosses at the University of Glasgow. This study also demonstrates the first transmission of *P. c. esekanensis* EF in an experimental setting. Using female Theiler's Original (TO) mice housed under normal light conditions, we have measured the synchronicity of morphological stages across the intraerythrocytic developmental cycle (IDC), quantified gametocytaemia over 24 hours, and demonstrated transmission of three *P. c. chabaudi* strains (*Pcc*AS, *Pcc*AJ, and *Pc*EF) through *An. stephensi* mosquitoes. Further optimisation of ookinete generation and purification is underway, with finalised protocols providing new avenues by which the *P. c. chabaudi* malaria model can be used to investigate the evolution and pathogenesis of *Plasmodium* parasites.

Poster 145* : Community and stakeholder's preferences for alternative schistosomiasis prevention strategies beyond mass drug administration in fishing communities of Mayuge District, Eastern Uganda

Sande Slivrsteri, *MRC/UVRI and LSHTM Uganda Research Unit*

S Slivestri[1]; M Moses Arinaitwe[4]; L Lazaaro Mujumbusi[3]; L Lucy Pickering[5]; E Edith Nalwadda[3]; A Agnes Ssali[3]; K Keila Meginnis[2]; J Janet Seeley[6]; P Lamberton[2];
[1] MRC/UVRI and LSHTM Uganda Research Unit; London School of Hygiene and Tropical Medicine, Uganda; [2] School of Biodiversity, One Health and Veterinary Medicine, University of Glasgow, UK; [3] Medical Research Council/Uganda Virus Research Institute and London School of Hygiene & Tropical Medicine Uganda Research Unit, Uganda; [4] Vector Borne and Neglected Tropical Diseases division, Ministry of Health, Uganda; [5] School of Social & Political Sciences, University of Glasgow, UK; [6] Department of Global Health and Development, London School of Hygiene and Tropical Medicine, UK

Background: Schistosomiasis remains a significant public-health concern in Uganda, with over 4 million estimated infections and 55% of the population at risk. Mass drug administration (MDA) with praziquantel is the current main control strategy. To reduce schistosomiasis transmission and morbidity, additional interventions are needed. Understanding community preferences for prevention strategies beyond MDA is vital for sustainable control. Previous work conducted by our group between 2017 and 2019 using focus group discussions, in-depth interviews, observations and participations, aimed to understand how people perceive and manage their risk of *Schistosoma mansoni* infection, identifying potential interventions to reduce infections. These data then informed discrete choice experiments which aimed to understand individual's willingness to pay and/or work for different interventions.

Objectives: The objective of this study, was to disseminate research findings with stakeholders, gather feedback, and elicit stakeholders' preferences in comparison with individual level results. Our overarching aim is to inform tailored interventions aligning with community needs and individual preferences, for different control interventions against schistosomiasis beyond MDA.

Methods: Between January and March 2021, we held 15 stakeholder workshops in Bugoto (5), Musubi (5), and Bwondha (5), communities on the shores of Lake Victoria in Uganda. Stakeholders included fisher folk (predominantly man), community opinion leaders, and school-age children. Discussions focused on interventions for reducing Risk to Self (RTS) and Risk to Others (RTO) which had been previously identified in 2017-2019. RTS interventions included sensitization by community healthcare workers, murals, public radio, water filtration, and construction of water taps. RTO interventions comprised constructing latrines at the lake, market, or within a 5-minute walk from homes, enforcing fines for open defecation, and maintaining latrines. Stakeholder groups ranked interventions based on preference and justifications. The order of the rankings where then compared to the rankings from the individual level surveys in 2019. Findings were validated through three larger meetings (one in each community) and four district workshops with the district health team, water engineer, district executive council, and district WASH partners. Facilitators and barriers to each of the top three RTS and RTO interventions where then discussed in more detail.

Results: Constructing public latrines near the lake and/or market, were the most preferred intervention for RTO, in comparison to the discrete choice experiment results when individuals reported preferring latrines within a 5-minute walk from home. Community sensitization and access to safe tap water were favoured for prevention of RTS by

Poster 146 : Leishmaniasis in Nigeria: Unravelling the past, navigating the present, and charting the future

Dr Olayinka Osuolale, *Elizade University*

O Osuolale[1];
[1] Elizade University, Nigeria

Background: Leishmaniasis, a parasitic disease transmitted by sandflies, poses significant public health challenges in Nigeria. This study undertakes a comprehensive differential analysis of leishmaniasis research in the country to unravel trends, identify gaps, and address challenges encountered in existing research. Given the neglected tropical nature of the disease, understanding the current state of leishmaniasis research becomes crucial for exploring future investigations and public health strategies.

Methods: Bibliometrix, a R-based software tool, and Research Rabbit, a curation tool, we systematically gathered and analyzed a corpus of 62 articles on leishmaniasis in Nigeria. The articles, including preprints and published works spanning decades, utilized diverse methodologies and thematic areas. This thorough review allowed for examination of the multifaceted aspects of leishmaniasis research in the country.

Results: Geographical distribution analyses revealed a patchwork of leishmaniasis cases across Nigeria, extending from the arid plains of the Northern states to the lush vegetation of the South-South. Identified hotspots in Plateau, Kwara, and Oyo states underscored the pervasive impact of the parasite on both humans and dogs. Recent studies hinted at a potential increase in cases, particularly in previously less-affected regions like the South-South, indicating a shifting transmission dynamic. Trend analysis demonstrates, emphasizing leishmaniasis' longstanding presence in Nigeria dating back to 1924. Current research highlighted the potential for further spread, calling for vigilance and proactive measures to curb its spread. The multifaceted nature of

leishmaniasis in Nigeria was evident through 4 epidemiological, 9 clinical, 6 entomological, and 4 immunological studies.

Conclusions: However, gaps remain. Data inconsistencies, limited surveillance in certain regions, and the need for robust and standardized diagnostic tools hinder our grasp of the true burden of leishmaniasis. Addressing these challenges demands intersectoral collaboration, encompassing diverse stakeholders – researchers, clinicians, public health officials, and communities affected by the disease.

Poster 147* : Transmission-relevant heterogeneities in *Tribolium castaneum* infected with a eugregarine gut parasite

Jacob Cohen, *University of Liverpool*

J Cohen[1]; M Viney[1]; A Fenton[1];
[1] *University of Liverpool, UK*

Theoretical models have repeatedly demonstrated that host heterogeneities in susceptibility and infectiousness can affect the dynamics of parasite spread. However, these predictions have rarely been tested experimentally. Here I present results from a series of experiments using the red flour beetle (*Tribolium castaneum*) as the host, infected with Eugregarines, an apicomplexan gut parasite. We quantified heterogeneities in susceptibility and infectiousness in this system using six different colonies of beetles established from field-collected individuals trapped in distinct locations and at different times. We challenged larvae of each of the colonies with the same dose of parasite infective particles to assay host susceptibility, and assayed host infectiousness by quantifying the parasite infective particles produced by infected larvae. We show that the colonies differ in their susceptibility to the Eugregarines, but not in their infectiousness. We can then use different combinations of these colonies to vary population-level heterogeneity in free-running mesocosm experiments, allowing us to directly test model predictions about the consequences of host heterogeneities on parasite transmission.

Index

A

B

C

D

E

F

G

H

I

J

K

L

M

N

O

P

Q

R

S

T

U

V

W

Y

Z

JOURNAL FACTSHEET

PeerJ
Life & Environment

The Journal of Life and Environmental Sciences

The peer-reviewed & Open Access journal publishing primary research and reviews in biology, life sciences, environmental sciences, and medicine.

Years publishing	10
Subjects	125
Articles published	15,694
Monthly views	500,000*
Publication speed	30 days†
Impact Factor	3.06
Scimago Ranking	0.695
SNIP	0.894
Citescore	5.1

* All journals
† Median days to 1st decision

Reputation

Publishing high-quality research
From the world's top institutions

Professional & experienced team
More than 125 years publishing experience

Industry-leading service
95% of authors recommend us

Institutional publishing
148 institutions signed up

Editors & Advisors

1,933 Editors and Advisors


Mina J. Bissell
Lawrence Berkeley Nat. Lab


John Gurdon
University of Cambridge


Dorothy Bishop
University of Oxford


Mario R. Capecchi
University of Utah

Audience

Widely read and cited
500,000 monthly views*,
56,000 content alert subscribers

Regular press coverage
In top outlets across US, EU & world

Comprehensively indexed
Web of Science, MEDLINE, JCR, PubMed, Scopus, Google Scholar, ++

Publishing Made Easy

Relaxed reference formatting
Clarity is the only requirement

Press release & sharing tools
Maximize readership of your work

Great looking articles
Fully typeset article proofs

Straightforward submission
Easy to use, fast and loved by authors

Peer review

Robust, Developmental review
1 editor, 2+ reviewers

Excellent review quality
Above average quality and depth

Signed reviews (optional)
Opt-in to fair and transparent science

peerj.com/life-environment

calibre SCIENTIFIC

Empowering Scientific Discovery Globally

agar scientific · ELKay · medline scientific · Labtech Serving scientists · SciQuip

Learn more about Calibre Scientific

Return to Contents page 207

Return to Contents page 210

TIME	START	END	DAY 1	DAY 2			DAY 3			DAY 4		
				CE	DC	FFE	CE	DC	FFE	CE	DC	FFE
MORNING 1	09:00	10:30	Registration	Plenary 1			10	11	12	19	20	21
BREAK	10:30	11:00										
MORNING 2	11:00	12:30		1	2	3	13	14	15	22	23	24
LUNCH	12:30	13:00								Close		
WORKSHOP	13:00	14:00	Database Workshops	ECR Conversation			EDI Conversation					
AFTERNOON 1	14:00	15:30		4	5	6	16	17	18			
BREAK	15:30	16:00										
AFTERNOON 2	16:00	17:30	Database Workshops	7	8	9	Plenary 2: Medalists					
	17:30	18:00					BSP AGM					
EVENING 1	18:00	19:30	Drinks	Posters 1			Posters 2					
EVENING 2	19:30			Young Parasitologists Party			Meeting dinner					

CE: Control & elimination (LT1) **DC**: Disease complexity (LT2) **FFE**: Form, function and evolution (LT3)

Return to Contents page 213