British Society for Parasitology

Spring Meeting 2012 Abstract Book.

Welcome

We are delighted to welcome you all to the city of Glasgow and the 50th Spring Meeting of the British Society for Parasitology. This is a first in several ways. Clearly it is the first Golden Jubilee for the BSP, but it is also the first Spring Meeting at the University of Strathclyde and also the first in the city of Glasgow since before almost anyone can remember. ICOPA was of course held here a few years ago, and I think all will agree that it was an outstanding conference in many respects.
We at the University of Strathclyde are particularly pleased that we are able to host this celebratory meeting at our University. We have a very long record of supporting research in parasitology, with particularly seminal contributions being made in the understanding of toxoplasmosis. In recent years we have reorganised our internal structures (which university has not?) with one outcome being our outstanding new building to house the Strathclyde Institute of Pharmacy and Biomedical Sciences. This large department was set up in 2006 to facilitate close research interactions across the life sciences and pharmaceutical sciences.
We hope that you will find this meeting very memorable in many ways. Firstly for the science, which promises to be outstanding. Secondly for the networking opportunities, with more than 550 participants the chances should be great. Thirdly for the city itself. We are holding two events in iconic buildings of the city. The Reception on Monday evening is in the City Chambers and the Conference Dinner will be in the Kelvingrove Art Gallery and Museum. Do make sure that you take the opportunity to see the excellent collection of paintings by the Glasgow Boys.
Thank you for joining this anniversary meeting of the BSP. We hope that you find it extremely stimulating and enjoyable.

Catherine Lawrence
Owain Millington
Graham Coombs

University of Strathclyde

BSP2012 Local Organising Committee

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Venue

Reception and Registration

Registration for the meeting will be from 2.00 pm to 6.00 pm on Monday 2nd April the Foyer of the Arbuthnott Building (Map page 9).

The reception desk will re-open at 8.30am on Tuesday 3rd April in the John Anderson Building (Map page 9)

Other facilities that will be made available at the reception desk will include:

- Urgent message pickup
- Conference Organising Committee contact details
- Internet access codes
- An information board
- Tourist Information

Safety

On the continuous sounding of the fire alarm, evacuate by the nearest safe route to the assembly area on the concourse opposite the main entrance. Emergency exits are clearly identified with illuminated green signs.

Please note that the fire alarms will be tested briefly between 9.00am and 10.00am on Wednesday morning.

In the event of an emergency, dial x2222 from any internal phone to contact Security Services.

Identification

We would like to remind all delegates that it is important to wear name badges at all times in order to identify themselves to the organizers, volunteer helpers and university staff. The conference committee can be identified by name badges which display the University of Strathclyde logo and student volunteers will be wearing BSP polo shirts.

Internet Facilities

Delegates can access the internet via several different wireless networks. Strathclyde University has guest Wi-Fi passes valid for the duration of the conference. Desktop computers with internet access will also be available in the Lord Todd Dining Room.

Access codes to sign onto desktops and SU-Guest-Wi-Fi can be picked up from the Conference Reception desk. To configure your Wi-Fi, set internet proxy to:

http://www-config.strath.ac.uk/proxy.config

Upon opening your internet browsed, you will be prompted to enter your unique username/password. If there are any problems, please contact the Conference Registration desk who can provide details of IT Services.

Information for Oral Presentations
Oral presentations will be given in rooms K314, K317, K325, K326, K327 and K412 in the John Anderson Building (see map page 9)

Loading of oral presentations will take place in the lecture theatre appropriate to the session.

Please ensure that your presentation is loaded by 8:45 am at the latest if you are presenting in the morning sessions or by 1:45 pm if you are presenting in the afternoon sessions.

There will be a student volunteer in each room who will help you load your presentation and help you if any problems arise with the equipment.

The scientific programme is very full and speakers are respectfully requested to keep to their timeslots so that delegates who wish to move between sessions can do so.

Information for Poster Presentations
Posters may be put up from 4:00 pm on Monday 2nd April and must be removed by 1:00 pm on Thursday 5th April. You will be provided with a number for your poster that will correspond to a particular poster board. All poster boards are located in the rooms 511/512 in the Colville building (see map page 9). Please note that the use of blue tack or drawing pins is prohibited and only the Velcro tabs provided may be used to secure your poster to the boards. This will be made available at the Conference Reception desk. All posters must be displayed in time for the poster session on Tuesday afternoon.

Student Prizes
Prizes for the best oral and poster presentations by student delegates have been generously provided by Biomed Central on behalf of the *Malaria Journal* and *Parasites and Vectors*, and by Cambridge University Press on behalf of *Parasitology*.

Entrants for the student competitions are marked with and asterisk (*) throughout the programme and abstracts. Please use the tear out voting slip at the back of the abstract book to register your vote, these should be handed in to the Conference Reception desk at the end of the session. You may only vote once for each category!

Food and Refreshments
All coffee breaks will be provided in room 511 in the Colville meeting (Map Page 9).

Lunch will be served in the Lord Todd Dining Room (Map page 9).

Delegates are reminded to ensure that their name badges are easily seen by university staff serving food and refreshments.

In addition to the refreshments and meals provided by the conference, the Orbit Café and Business School Café will be open from 9.30 am to 2.30 pm throughout the conference for purchase of refreshments and light snacks.

The Todd's Bar in the Lord Todd will be open for the purchase of drinks and light refreshments

Social Programme

Monday 2nd April: A welcome drinks reception, hosted by Glasgow City Council, will be held in the magnificent Glasgow City Chambers after the Public Understanding of Science lecture from 6.30 pm. A grand and imposing edifice overlooking George Square, the City Chambers is an impressive symbol of Glasgow's political strength and historical wealth. Completed in 1888, the City Chambers has for over a hundred years been the headquarters of successive councils serving the City of Glasgow. For a map see page XX

Tuesday 3rd April: Poster event with a wine reception, from 6.00pm. The poster session will be held in the exhibition area in rooms 511/512 of the Colville Building (see map)

A Young Parasitologists' Pub Quiz has been organised in the Lord Todd Bar from 8:00 pm after the Poster viewing. All students will have tickets for this in their delegate packs and will include snacks and drink vouchers.

Wednesday 13th April: Conference Dinner in the Kelvingrove Museum. Enjoy a wonderful evening among one of the finest civic collections in Europe housed within this Glasgow landmark. Here you can explore collections that include everything from fine and decorative arts to archaeology and the natural world while enjoying your drinks reception. The evening commences with a welcome drinks reception at 7pm accompanied by a recital on the Museums 100 year old organ. Dinner will be served at 8.00 pm. After this the room will be cleared for the Ceilidh by Teannaich. Dancing fimnishes at 11.30 pm. Don't forget your kilts!

Getting There: Kelvingrove Art Gallery and Museum is located in picturesque Kelvin Park in the popular West End of the city.
By Subway: 10 minutes walk from Kelvin Hall Subway station. On exiting the subway station, turn left and follow Dumbarton Road to the Museum.
By Bus: First Bus services 9, 16, 18, 42, 62 and 64 all stop outside Kelvingrove.

Eating and drinking
For other places to eat and drink and catch up with friends and colleagues, your conference delegate packs will include a City Guide to help you find the many excellent pubs, bars, restaurants and shopping to keep you entertained. If you are extending your stay then additional tourist information will also be available at the Conference Reception desk.

Left Baggage Facilities
On Thursday, delegates wishing to do so may leave their luggage in 'left luggage' in the Arbuthnott building up to 2:00 pm. Signs will indicate the room but please ask at Arbuthnott reception if in doubt.

Public Understanding of Science Lecture
SIBS 101, Arbuthnott Building

The Scottish Encounter with Tropical Disease

Mike Barrett
Wellcome Trust Centre for Molecular Parasitology, Institute of Infection, Immunology and Inflammation, CMVLS, University of Glasgow, G12 8TA

Many of those diseases specifically associated with the tropics are caused by parasites (a term derived from the Greek "parasitos" meaning "one who feeds at the table of another"). An extraordinary number of the parasites that affect mankind today were discovered by Scottish investigators in the nineteenth and early twentieth Centuries. Leishmaniasis, for example, a disease that afflicts millions of people around the world today, is named after Glaswegian William Leishman. *Trypanosoma brucei* the causative agent of sleeping sickness was named after another Scottish doctor, David Bruce, who is credited with identifying the parasites responsible for this devastating disease. Ronald Ross's discovery that Anopheles mosquitoes transmit malaria parasites won him Briton's first Nobel prize. These pioneering Scottish doctors and scientists were all influenced by Sir Patrick Manson, from Oldmeldrum in Aberdeenshire, who is considered as the founding father of the discipline of tropical medicine. Manson was a relative of David Livingstone whose accounts of his explorations in Africa first exposed many Europeans to the impact of tropical disease on mankind. The ethos of the Scottish Enlightenment on education had created this remarkable generation of medical men and women intent on discovering the causes of disease, with a view to their cure. The legacy of these early breakthroughs in medical research lives on in Scotland today where active programmes of research into Parasitology continue to bring forward new discoveries and progress in combating tropical disease.

Keynote Talks
K325, John Anderson Building

In the long list of parasitic disease that infect man, foremost perhaps are malaria and schistosomiasis. During the last half century there have been many research and control activities waged against both diseases, often separately or in conjunction, across many parts of the globe. To celebrate 50 years of the BSP two Past President's will give a personal account, flavoured by their experiences, in each disease and review progress made – highlighting both successes and failures. Looking to the future they will outline their hopes as to where research and control should be best placed in forthcoming years.

50 years of Parasitology: Schistosomiasis

Prof David Rollinson, Natural History Museum, London

50 years of Parasitology: Malaria
Prof Geoff Targett, London School of Hygiene and Tropical Medicine, London,

BSP AGM and Forum

The BSP council, has an exciting new format for the AGM with various additional features of a scientific nature around this. The council would encourage you to attend and make a contribution, it is only through having your opinions heard that the Society can adapt and change in the manner you wish it to. Much has happened in the first 50 years of the Society, help take us into the next 50 years by becoming an active part of our Society.

Plenary Lecture
K325, John Anderson Building

Parasites, people and tomorrow's world

Robert May, Lord May of Oxford, Oxford University

This talk will consider the past interplay between human populations and parasites – broadly defined – and will speculate on what the future might be. Beginning with an outline of humanity's demographic history, I will contrast recent past patterns in developed and developing countries. The part played by advances in medical and epidemiological understanding will be discussed, with emphasis on some of the peculiarities in what has tended to receive attention and what has not. I shall conclude with some tentative thoughts about likely future trends.

Wright Medal Lecture
K325, John Anderson Building

The British Society for Parasitology (BSP) awards an annual medal to commemorate the life of Dr Chris Wright, Natural History Museum, by formal recognition of an individual's research excellence and expertise in parasitology.

The BSP 2012 Wright Medalist is Professor Mark Taylor, Liverpool School of Tropical Medicine, for his outstanding work on filarial nematode diseases, in particular lymphatic filariasis and onchocerciasis.

***Wolbachia* and filarial nematodes: mutual friends and dangerous enemies**

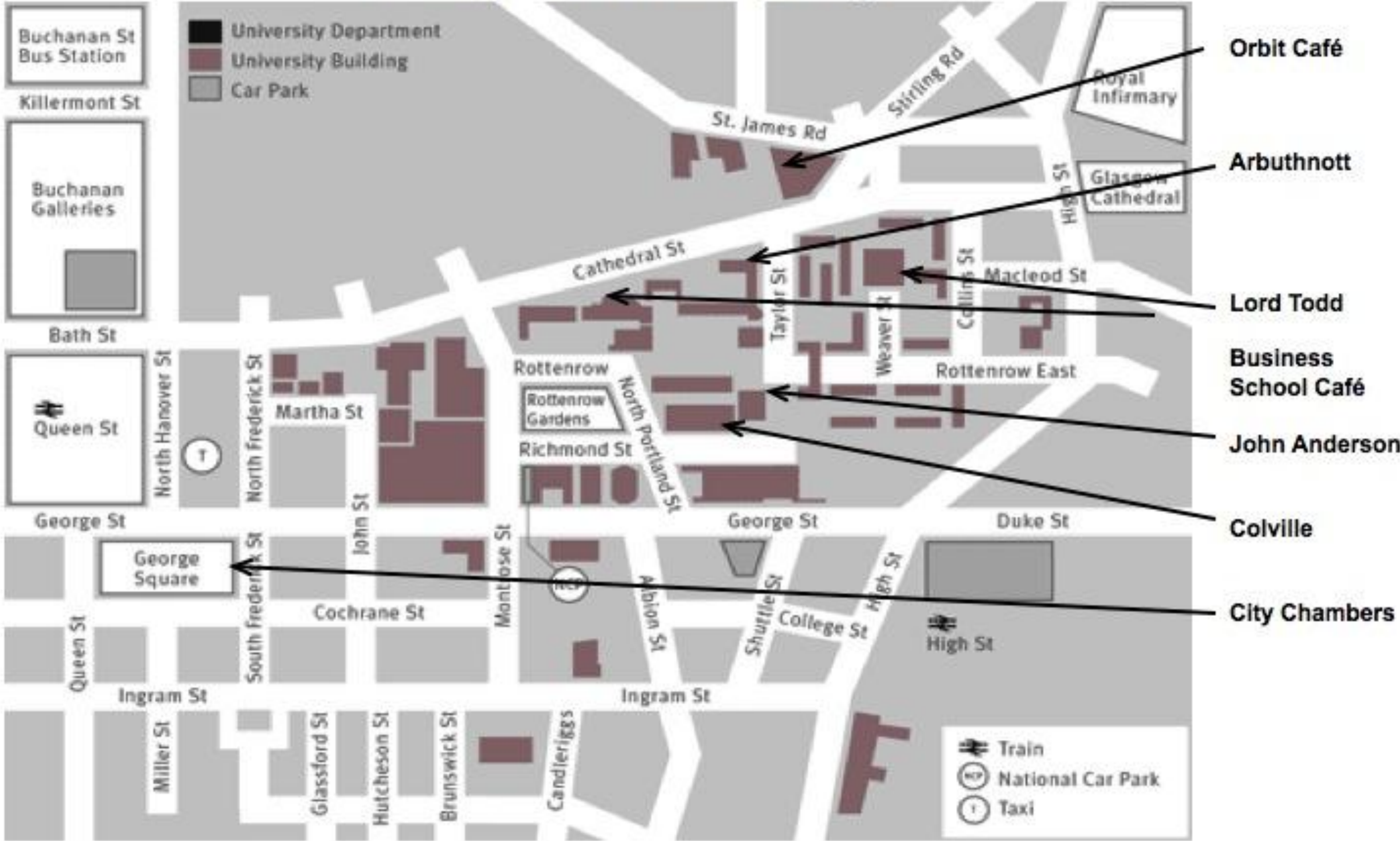
Wolbachia bacterial endosymbionts have evolved a mutualistic symbiosis with filarial nematodes that is essential for parasite development, fertility and survival. Whilst the bacteria are beneficial for the nematode, their release into the host contributes to adverse events following anti-filarial treatment and the inflammatory pathogenesis of river blindness and elephantiasis. Their mutualistic symbiosis has been exploited in a new approach to the treatment of lymphatic filariasis and onchocerciasis with antibiotics, which deplete the nematodes of their bacterial symbiont leading to an initial sterilisation of adult worms followed later by their death. Anti-*Wolbachia* therapy, therefore delivers a safe macrofilaricidal treatment with superior therapeutic outcomes compared to all standard anti-filarial treatments, with the added benefit of substantial improvements in clinical pathology. A-WOL, an international consortium of academic and industrial partners funded by the Bill & Melinda Gates Foundation was formed to discover and develop new anti-*Wolbachia* drugs and regimes against onchocerciasis and lymphatic filariasis, with the goal of delivering an alternative and complimentary strategy for the treatment, control and elimination of this public health burden.

BSP 50 Debate
K325, John Anderson Building

50 years of control of parasitic diseases: Is the end game now in sight?

Please join us for an open floor debate to assess how the future control of parasitic diseases will be shaped in the next decade and beyond. The debate will feature the views of a select panel of Past President's of the BSP and will be Chaired by Professor Peter Winstanley (President of the Royal Society of Tropical Medicine and Hygiene) and joined by Dr Lorenzo Savioli (Director of WHO NTD Control) and Dr Andy Forbes (Merial). All questions from the floor will be formally recorded in an 'earmarked' article for publication within Parasites and Vectors, so please come prepared.

Strathclyde University Map



OUTLINE TIMETABLE

MONDAY 2 APRIL 2012						
12:00 -18:00	REGISTRATION					
17:00-18:00	**PUBLIC UNDERSTANDING OF SCIENCE** **Prof Mike Barrett,** **University of Glasgow** ***SIBS101***					
18:00-20:00	**CIVIC RECEPTION GLASGOW CITY CHAMBERS**					
TUESDAY 3 APRIL 2012						
09.00-10.15	Prof David Rollinson Prof Geoff Targett ***K325***					
10:15-11:00	**BSP AGM and FORUM** ***K325***					
11:00-11:30	**Coffee break**					
	SESSION A	SESSION B	SESSION C	SESSION D	SESSION E	SESSION F
11:30 -13:00	Tryp/leish 1	Malaria 1	Vet 1	Helminth Molecular 1	Mapping parasitic diseases across the globe	Imaging
13:00- 14:00	**LUNCH**					
14:00-15.30	Tryp/leish 2	Tryp/leish 3	Helminth Immunology 1	Helminth Molecular 2	Eco 1	
15:30-16:00	**Coffee break**					
16:00-17:30	Tryp/leish 4	Tryp/leish 5	Vet 2	Helminth Immunology 2	Eco 2	
18:00-20:00	**POSTER SESSION + DRINKS**					
20:00-??	**YOUNG PARASITOLOGIST PARTY TODD BAR**					

WEDNESDAY 4 APRIL 2012					
09.00-10.00	**PLENARY LECTURE** ***Lord May of Oxford*** **Parasites, people and tomorrow's world** ***K325***				
10.00-10.30	**WRIGHT MEDAL LECTURE** **Professor Mark Taylor,** **Liverpool School of Tropical Medicine,** ***Wolbachia* and filarial nematodes: mutual friends and dangerous enemies** ***K325***				
	SESSION A	SESSION B	SESSION C	SESSION D	SESSION E
10:30-11:00	**Coffee break**				
11.00-12.30	Tryp/leish 6	Malaria 2	Vet 3	Helminth molecular 3	Helminth Immunology 4
12:30- 14:00	**LUNCH**				
14.00-15.30	Tryp/leish 7	Malaria 3	Helminth Immunology 3	Helminth molecular 4	Eco 3
15:30-16:00	**Coffee break**				
16.00 -17:30	Tryp/leish 8	Tryp/leish 9	Vet 4	Helminths – Treatment and control	Eco 4
PAST PRESIDENTS **CONFERENCE DINNER KELVINGROVE MUSEUM**					

THURSDAY 5 APRIL 2012					
09.00-10.30	**BSP 50 Debate** **50 years of parasite control: is the end game now in sight?** ***K325***				
10:30-11:00	**Coffee break**				
11:00 -12:30	Tryp/leish 10	Tryp/leish 11	Helminth Immunomodulation	Sex hormones, immunity and protozoan parasites	Eco 5
12:30- 14:00	**LUNCH**				
MEETING CLOSE					

DETAILED TIMETABLE

TUESDAY 3 APRIL 2012					
09.00-09.40	**KEYNOTE 1** **Prof David Rollinson, Natural History Museum, London UK** **50 years of Parasitology: Schistosomiasis**				
09.40-10.20	**KEYNOTE 2** **Prof Geoff Targett, London School of Hygiene and Tropical Medicine, London, UK** **50 years of Parasitology: Malaria**				
10.20-11.00	**BSP AGM AND FORUM**				
	Session A **K325**		**Session B** **K314**		**Session C** **K317**
11.30-13.00	**Trypanosomiasis/Leishmaniasis Seminar 1** *Chair*: Mark Field Cell biology; surfaces and immune evasion	11.30-13.00	**Malaria 1** *Chair*: Paul Horrocks Control of Malaria	11.30-13.00	**Veterinary Parasitology 1** *Chair*: Mike Stear
11.30-12.00	**OR1** *Invited Speaker:* Etienne Pays, Université Libre de Bruxelles, Belgium Adaptation of African trypanosomes to man	11.30-12.00	**OR36** *Invited Speaker*: Janet Hemingway, Liverpool School of Tropical Medicine, UK Health Impacts of Product Development Partnerships	11.30-12.00	**OR70** *Invited Speaker:* Andy Forbes, Merial, Lyon, France Helminth Control in Ruminants – taking the line of least resistance
12.00-12.15	**OR2** Lucy Glover A telomere-specific DNA damage response in African trypanosomes	12.00-12.15	**OR37** Colin Sutherland Evaluation of a novel molecular marker for monitoring artemisinin resistance in *Plasmodium falciparum* malaria	12.00-12.15	**OR71** Valerie Relf Helminth egg output on UK Thoroughbred studs
12.15-12.30	**OR3** Mark Carrington The trypanosome haptoglobin haemoglobin receptor and human infectivity	12.15-12.45	**OR38** *Invited Speaker:* Sanjeev Krishna, St Georges, London, UK Antimalarials – trials and triumphs	12.15-12.30	**OR72** Fiona Kenyon Effect of anthelmintic treatment approach on the number and species of ovine gastrointestinal nematode parasites present.
12.30-12.45	**OR4*** Paul Manna Regulators of endosomal membrane trafficking in *Trypanosoma brucei*			12.30-12.45	**OR73** Michael Stear The relationship between parasitism and production in Scottish sheep.
12.45-13.00	**OR5*** James Hall Mosaic VSGs in African trypanosome antigenic variation	12.45-13.00	**OR39*** Simon Hemelaar Stability of hotspots after implementation of community wide vector control with indoor residual spraying	12.45-13.00	**OR74*** Stewart Blair A Questionnaire Based Survey of Current Endoparasite Control Practices on Sheep Farms in Northern Ireland
13.00-14.00	**LUNCH Lord Todd**				

	Session A K325		Session B K314		Session C K317
14.00-15.30	**Trypanosomiasis/Leishmaniasis Seminar 2** Cell biology; cell cycle *Chair*: Tansy Hammarton	14.00-15.30	**Trypanosomiasis/Leishmaniasis Seminar 3** In the field *Chair*: Jeremy Sternberg	14.00-15.30	**Helminth Immunology 1** *Chair*: Richard Grencis & Andrew MacDonald
14.00-14.30	**OR6** *Invited Speaker:* Sergio Schenkman, Universidade Federal de São Paulo, Brazil Dephosphorylation of eIF5A is required for survival at stationary growth phase in *Trypanosoma cruzi*	14.00-14.30	**OR40** *Invited Speaker:* Bruno Bucheton, CIRDES, Burkina Fasso New evidences of human trypanotolerance in West Africa: perspectives to better understand host-parasite interactions and improve control strategies.	14.00-14.30	**OR75** *Invited Speaker:* Phil Cooper, Liverpool School of Tropical Medicine, UK The relevance of the hygiene hypothesis as an explanation for the allergy epidemic in Latin America
14.30-14.45	**OR7** Brice Rotureau Continuous production of infective trypanosomes in the tsetse fly	14.30-14.45	**OR41** Jeremy Sternberg Disease progression in Human *Trypanosoma brucei rhodesiense* infection: CNS humoral and cellular responses	14.30-14.45	**OR76*** Dries Masure The gastro-intestinal immune response during the expulsion of *Ascaris suum*
14.45-15.00	**OR8** Jane Andre A diversity of TBCC domain-containing proteins in trypanosomatid parasites	14.45-15.00	**OR42*** Tapan Bhattacharyya Towards Trypanosoma cruzi lineage-specific serology for Chagas disease	14.45-15.15	**OR77** *Invited Speaker:* Rick Maizels, University of Edinburgh, UK Immune regulation and regulators in nematode infections
15.00-15.15	**OR9** Cristina Costa Identification and functional characterisation of CRK12:CYC9, a novel CDK-cyclin complex in *Trypanosoma brucei*	15.00-15.15	**OR43*** Lauren Sullivan Hope on the Horizon: development of a new prototype lateral flow diagnostic test for Human African Trypanosomiasis.	15.00-15.15	
15.15-15.30	**OR10** Lori Peacock Meiosis is an inherent feature of the life cycle of *Trypanosoma brucei* subspecies	15.15-15.30	**OR44** Emily Adams Molecular amplification tools for the diagnosis of Human African Trypanosomiasis – A systematic review.	15.15-15.30	**OR78** Peter Cook Epigenetic Control of Th2 Induction by Dendritic Cells
15.30-16.00	**COFFEE BREAK**				

	Session A K325		Session B K314		Session C K317
16.00-17.30	**Trypanosomiasis/Leishmaniasis Seminar 4** Drugs: Mechanism of action and resistance *Chair*: David Horn	16.00-17.30	**Trypanosomiasis/Leishmaniasis Seminar 5** Genomics *Chair*: Fred Bringaud	16.00-17.30	**Veterinary Parasitology 2** *Chair*: Mike Stear
16.00-16.30	**OR11** *Invited Speaker:* Mike Barrett, University of Glasgow, UK Assessing drug modes of action and resistance using metabolomics	16.00-16.30	**OR45** *Invited Speaker:* Steve Beverley, Washington University, USA Genomic studies of Leishmania and RNA Viruses in South America	16.00-16.30	**OR79** *Invited Speaker:* Nicholas Jonsson, University of Glasgow, Glasgow Evolution of host resistance to cattle tick infestation – implications for vaccine design
16.30-16.45	**OR12** Jean-Claude Dujardin Genetic markers for SSG-resistance in *Leishmania donovani* and SSG-treatment failure in visceral leishmaniasis patients of the Indian subcontinent	16.30-16.45	**OR46*** Louisa Messenger Multiple mitochondrial introgression events and heteroplasmy in *Trypanosoma cruzi*	16.30-16.45	**OR80** Stewart Burgess Transcriptomic analysis of the host response to infestation with the ectoparastic mite *Psoroptes ovis*
16.45-17.00	**OR13** Susan Wyllie The anti-trypanosome drug fexinidazole shows potential for treating visceral leishmaniasis	16.45-17.00	**OR47** Richard McCulloch Coordinated modularity of DNA replication and transcription in *Trypanosoma brucei*	16.45-17.00	**OR81*** Beth Wells Development of a diagnostic test for sheep scab using biomarkers
17.00-17.15	**OR14** Jane Munday Aquaporin 2: A Resistance Marker for Pentamidine and Melarsoprol in *Trypanosoma brucei*	17.00-17.15	**OR48** Andrew Jackson Comparative genomics of African trypanosomes	17.00-17.15	**OR82** James Campbell Human and ruminant fascioliasis in central Vietnam, 2007-2010
17.15-17.30	**OR15** Jean Rodgers Melarsoprol cyclodextrin inclusion complexes; an oral treatment for human African trypanosomiasis	17.15-17.30	**OR49** Tim Downing Population structure and adapative evolution of a recent *Leishmania* outbreak	17.15-17.30	**OR83*** Heather McDougall The search for "hidden antigens" in the liver fluke, *Fasciola hepatica*
18.00-20.00	**POSTER SESSION**				

<table>
<tr><th colspan="6">Wednesday 5th April</th></tr>
<tr><td>09.00-
09.05</td><td colspan="5">Welcome Address
Prof Kenny Miller, Pro-Vice Principal, University of Strathclyde</td></tr>
<tr><td>09.05-
10.00</td><td colspan="5">PLENARY LECTURE
Lord May of Oxford
Parasites, people and tomorrow’s world</td></tr>
<tr><td>10.00-
10.30</td><td colspan="5">WRIGHT MEDAL LECTURE
Prof Mark Taylor
Wolbachia and filarial nematodes: mutual friends and dangerous enemies</td></tr>
<tr><td></td><td>Session A
K325</td><td></td><td>Session B
K314</td><td></td><td>Session C
K317</td></tr>
<tr><td>11.00-
12.30</td><td>Trypanosomiasis/Leishmaniasis Seminar 6
Metabolism
Chair: Michael Ginger</td><td>11.00-
12.30</td><td>Malaria 2
Malaria genomics and molecular biology
Chair: Andy Waters</td><td>11.00-
12.30</td><td>Veterinary Parasitology 3
Chair: Lee Innes</td></tr>
<tr><td>11.00-
11.30</td><td>OR16 Invited Speaker: Malcolm Walkinshaw, University of Edinburgh, UK
Targeting the glycolytic pathway of trypanosomes by structure-based and screening approaches</td><td>11.00-
11.30</td><td>OR50 Invited Speaker: Elizabeth Winzeler, Novartis Research Foundation
Short term evolution of malaria parasites</td><td>11.00-
11.30</td><td>OR84 Invited Speaker: Alasdair Nisbet, Moredun Research Institute, Scotland
Control of a parasitic nematode in sheep by vaccination with a recombinant antigen cocktail</td></tr>
<tr><td>11.30-
11.45</td><td>OR17 Darren Creek
Global metabolomics of bloodstream-form Trypanosoma brucei guides rational improvements to cell culture medium and drug discovery</td><td>11.30-
11.45</td><td>OR51* Murad Ali Mubaraki
Metabolic Fingerprinting of Plasmodium falciparum</td><td>11.30-
11.45</td><td>OR85 Tom McNeilly
Desensitisation as a method of mitigating production losses associated with parasitic nematode infections of livestock</td></tr>
<tr><td>11.45-
12.00</td><td>OR18 Julius Lukes
Eukaryotic life without haem: the aerobic kinetoplastid flagellate Phytomonas does not require haem for viability</td><td>11.45-
12.00</td><td>OR52* Jakob Jesperson
Analyzing Plasmodium falciparum erythrocyte membrane protein 1 gene expression by a next generation sequencing method</td><td>11.45-
12.00</td><td>OR86 Thierry Monney
Chimeric antigens for the vaccination against Neospora caninum: study in the pregnant and in the non pregnant mouse model</td></tr>
<tr><td>12.00-
12.15</td><td>OR19 Paul Michels
Channel-forming activities of glycosomal membrane proteins from Trypanosoma brucei</td><td>12.00-
12.15</td><td>OR53* Larissa Laine
Structural, functional and biochemical characterisation of Plasmodium falciparum pyruvate dehydrogenase complex</td><td>12.00-
12.15</td><td>OR87* Joaquín Prada Jimenez de Cisneros
IgA better than FEC to indicate resistance in naturally infected lambs</td></tr>
</table>

12.15- 12.30	**OR20*** Ana Marta Franco da Silva How thiol dependent reductase 1 regulates metabolism in *Leishmania*	12.15- 12.30	**OR54*** Aline Freville *Plasmodium falciparum* Inhibitor-3 Homolog Increases Protein Phosphatase Type 1 Activity and Is Essential for Parasitic Survival.	12.15- 12.30	**OR88*** Johnny Vlaminck Detection and characterisation of an immunodominant antigen present on the surface of *Ascaris suum* L3 larvae
12.30- 14.00	**LUNCH**				
14.00- 15.30	**Trypanosomiasis/Leishmaniasis Seminar 7** Gene expression *Chair*: Gloria Rudenko	14.00- 15.30	**Malaria 3** Malaria Pathogenesis and vaccines *Chair*: Colin Sutherland	14.00- 15.30	**Helminth Immunology 3** *Chair*: Richard Grencis & Andrew MacDonald
14.00- 14.30	**OR21** *Invited Speaker:* Chris Tschudi, Yale University, USA Insights into the Biology of African trypanosomes by Next-generation Sequencing	14.00- 14.30	**OR55** *Invited Speaker:* Lars Hviid, Centre For Medical Parasitology, Copenhagen Protective immunity to malaria, and how the *Plasmodium falciparum* parasites try to evade it	14.00- 14.30	**OR89** *Invited Speaker*: Nicola Harris, Swiss Vaccine Research Institute, Switzerland A novel role for interleukin-1 beta in promoting chronicitiy of intestinal helminths.
14.30- 14.45	**OR22** Sam Alsford VSG gene sequences control monotelomeric VSG expression in African trypanosomes	14.30- 14.45	**OR56** Alexandra Rowe Induction of Strain-Transcending Antibodies Against Group A PfEMP1 Surface Antigens from Virulent Malaria Parasites	14.30- 14.45	**OR90** Matt Taylor PD-1 mediated Th2 cell hypo-responsiveness determines susceptibility to helminth infection
14.45- 15.00	**OR23** Megan Povelones Histone H1 regulates antigenic variation in *Trypanosoma brucei*	14.45- 15.00	**OR57** Alan Brown Molecular basis for evasion of the malaria parasite by cytoadhesion to human brain tissue	14.45- 15.15	**OR91** *Invited Speaker:* Adrian Mountford, University of York, UK Th2-type and wound healing responses after repeated exposure to schistosome larvae.
15.00- 15.15	**OR24*** Valentin Faerber The role of CNOT10 in the process of mRNA turnover in *Trypanosoma brucei*	15.00- 15.15	**OR58** Sandy Douglas *Plasmodium falciparum* neutralisation by anti-RH5 antibodies which block the RH5-basigin interaction		
15.15- 15.30	**OR25** Pegine Walrad The post-transcriptional trans-acting regulator, TbZFP3, coordinates transmission-stage enriched mRNAs in *Trypanosoma brucei.*	15.15- 15.30	**OR59** Arturo Reyes-Sandoval CD8+ T Effector Memory Cells Protect against pre-erythrocytic Malaria	15.15- 15.30	**OR92** Kelly Hayes Simvastatin as an Anti-Helmintic
15.30- 16.00	**COFFEE BREAK**				

	Session A K325		Session B K314		Session C K317
16.00- 17.30	**Trypanosomiasis/Leishmaniasis Seminar 8** Signaling/differentiation *Chair*: Keith Matthews	16.00- 17.30	**Trypanosomiasis/Leishmaniasis Seminar 9** Immunology *Chair*: James Alexander	16.00- 17.30	**Veterinary Parasitology 4** *Chair*: Lee Innes
16.00- 16.30	**OR26** *Invited Speaker:* Gerald Spaeth, Institut Pasteur, France A touch of Zen: Genetic analysis of *Leishmania* stress signaling	16.00- 16.30	**OR60** *Invited Speaker:* Paul Kaye, University of York, UK Immunopathology in leishmaniasis: friend or foe	16.00- 16.30	**OR93** *Invited Speaker:* Daland Herrmann, Friedrich-Loeffler-Institut, Wusterhausen, Germany *Toxoplasma gondii*: genetic diversity around the world and insight into genotypes and virulence of *T. gondii* in Germany
16.30- 16.45	**OR27** Helen Price Effects of BBS1 deletion on parasite morphology and infectivity in *Leishmania major*	16.30- 16.45	**OR61** Marc Karam In *Leishmania major*-induced inflammation, IL-13 down-regulates IL-1β and up-regulates IL-6 in an IL-4 independent mechanism.	16.30- 16.45	**OR94** Emily Clark Strategies for assessing genetic diversity in *Eimeria* species parasites of poultry.
16.45- 17.00	**OR28** Balazs Szoor Dissecting differentiation signalling pathways in *Trypanosoma brucei*	16.45- 17.00	**OR62** Juliane Schroeder A protective role for MAP kinase phosphatase 2 in the control of parasite infection	16.45- 17.00	**OR95** Frank Katzer Increased *Toxoplasma gondii* positivity relative to age in 125 Scottish sheep flocks; evidence of frequent acquired infection
17.00- 17.15	**OR29*** Laura Munro Functional analysis of LmxMPK2, a MAP kinase essential for *Leishmania mexicana* amastigotes	17.00- 17.15	**OR63*** Debanjan Mukhopadhyay Suppression of host immunity by polarization of monocytes is a hallmark of Indian Post Kala-azar Dermal Leishmaniasis	17.00- 17.15	**OR96*** Alison Burrells Evidence of the three main clonal *Toxoplasma gondii* lineages in British wild carnivores.
17.15- 17.30	**OR30*** Nathaniel Jones Validating protein kinases of *Trypanosoma brucei* as drug targets: a kinome-wide RNAi screen	17.15- 17.30	**OR64*** Alice Halliday Toll-like receptors in cutaneous Leishmaniasis and as targets for vaccine adjuvants	17.15- 17.30	**OR97** Adam Reid Genome sequences of four *Eimeria* species reveal that most gene sequences are interrupted by homopolymeric amino acid repeats

THURSDAY 5 APRIL 2012					
09.00-10.30	**BSP 50 DEBATE** **50 years of parasite control: is the end game now in sight?**				
	Session A **K325**		**Session B** **K314**		**Session C** **K317**
11.00-12.30	**Trypanosomiasis/Leishmaniasis Seminar 10** Mitochondrion *Chair*: Achim Schnaufer	11.00-12.30	**Trypanosomiasis/Leishmaniasis Seminar 11** Vector biology *Chair*: Paul Bates	11.00-12.30	**Helminth Immune Modulation** *Chair*: Billy Harnett
11.00-11.30	**OR31** *Invited Speaker:* Ken Stuart, Seattle Biomed, USA The functional proteome of the *Trypanosoma brucei* mitochondrion	11.00-11.30	**OR65** *Invited Speaker:* Marcos Pereira, Universidade Federal de Minas Gerais, Brazil The host skin microcirculation analysis during blood feeding by *Rhodnius prolixus*	11.00-11.30	**OR98** *Invited Speaker:* Padraic Fallon, Trinity College, Dublin, Ireland Schistosome modulation of inflammatory diseases: the good and bad of a two-sided coin.
11.30-11.45	**OR32** Lucio Freitas-Junior Targeting the kDNA replication machinery for drug discovery in *Leishmania*	11.30-11.45	**OR66** Matthew Rogers Introducing the molecular sieve theory of *Leishmania* transmission	11.30-11.45	**OR99** Laura Webb Steady state dendritic cells depend on Type I IFN responsiveness for optimal induction of T cell responses against *Schistosoma mansoni*
11.45-12.00	**OR33** Frédéric Bringaud The procyclic trypanosomes express two mitochondrial enzymes for acetate production from acetyl-CoA	11.45-12.00	**OR67** Rod Dillon Maintenance of gut-microbial homeostasis in *Lu. longipalpis* and implications for *Leishmania* transmission.	11.45-12.15	**OR100** *Invited Speaker:* Francisca Mutapi, University of Edinburgh. Immunomodulation during natural and experimental human helminth infection.
12.00-12.15	**OR34** Roderick Williams ATG5-deletion mutants reveal interplay between macroautophagy and mitochondrial homeostasis in *Leishmania major*	12.00-12.15	**OR68** Mauricio Viana Sant'Anna Colonisation resistance in the bloodsucking sand fly *Lutzomyia longipalpis: Leishmania* protects its host from bacterial pathogenesis.		
12.15-12.30	**OR35** Hassan Hashimi Functional characterization of a putative mitochondrial cation/proton antiporter in both life stages of *Trypanosoma brucei brucei* and the akinetoplastic *Trypanonsoma brucei evansi*	12.15-12.30	**OR69*** Johannes Doehl Investigating the roles of *Leishmania major* HASPB and SHERP proteins during metacyclogenesis in *Phlebotomus papatasi*		
MEETING CLOSE					

TUESDAY 3 APRIL 2012					
09.00- 09.35	**KEYNOTE 1**				
09.35- 10.10	**KEYNOTE 2**				
10.20- 11.00	**BSP AGM AND FORUM**				
	Session D **K326**		**Session E** **K327**		**Session F** **K412**
11.30- 13.00	**Molecular Helminthology 1** Helminth Neurobiology Chair: Paul McVeigh	11.30- 13.00	**Mapping parasitic diseases across the globe** *Chair*: Thomas K. Kristensen & Robert Bergquist	11.30- 13.00	**Imaging Parasite Infections** *Chair*: Jim Brewer
11.30- 12.00	**OR102** *Invited Speaker:* Steven Husson, K.U.Leuven, Belgium Neuropeptidergic elminthe in *Caenorhabditis elegans*	11.30- 11.45	**OR136** *Invited Speaker:* Laura Rinaldi, Swiss TPH, Switzerland Geospatial tools in veterinary parasitology: from sampling to modeling	11.30- 12.00	**OR170** *Invited Speaker:* Rogerio Amino, Institut Pasteur, France Role of *Plasmodium* host cell traversal in the evasion of liver innate immunity
12.00- 12.15	**OR103** Johnathan Dalzell RNA interference as a receptor deorphanisation tool in plant pathogenic nematodes	11.45- 12.00	**OR137** *Invited Speaker:* John Malone, Louisiana State University, USA Ecological niche models and the distribution and abundance of hookworms in Bolivia	12.00- 12.15	**OR171** Michael Lewis Real-time in vivo imaging of mice infected with transgenic *Trypanosoma cruzi* expressing 'red-shifted' firefly luciferase
12.15- 12.30	**OR104** Louise Atkinson *FMRFamide like peptide*-11 function and elminthes n in *Globodera pallida*	12.00- 12.15	**OR138** *Invited Speaker:* Penelope Vounatsou, Swiss TPH, Switzerland Mapping the geographical distribution of schistosomiasis in Nigeria from compiled survey data	12.15- 12.45	**OR172** *Invited Speaker:* Claire Forestier, Clermont Université, France Imaging host cell infection by *Leishmania donovani* provides a new view of the early infection process
12.30- 12.45	**OR105*** Glenn Horan *Macrostomum lignano*: a platform for studying flatworm biology and validating targets for parasite control				
12.45- 13.00	**OR106*** Michael Stevenson Acetylcholinesterase biology of plant parasitic nematodes	12.15- 12.30	**OR139** *Invited Speaker:* Uwem Ekpo, University of Agriculture, Abeokuta, Nigeria Mapping the geographical distribution of schistosomiasis in Nigeria from compiled survey data	12.45- 13.00	**OR173** Elmarie Myburgh In vivo imaging models of African trypanosomiasis to support drug discovery programs

		12.30- 12.45	**OR140** *Invited Speaker:* Russell Stothard, Liverpool School of Tropical Medicine Use of personal GPS-dataloggers to infer water contact patterns and social networks that influence transmission of intestinal schistosomiasis among mothers and young children		
		12.45- 13.00	**Discussion**		
13.00- 14.00	**LUNCH Lord Todd**				

	Session D **K326**		**Session E** **K327**
14.00- 15.30	**Molecular Helminthology 2** RNAi in Helminths and *C. elegans* as a model. *Chair*. Aaron Maule	14.00- 15.30	**Ecoparasitology** 1 Ecology of microbial infection *Chair*. Sam Brown
14.00- 14.30	**OR107** *Invited Speaker*. Tim Day, Iowa State University, USA RNAi approaches to G protein coupled receptor deorphanisation and characterization.	14.00- 14.30	**OR141** *Invited Speaker:* Brit Koskella, Oxford University, UK Adaptation of bacteriophages to natural plant pathogen populations
14.30- 14.45	**OR108** Eileen Devaney The microRNAs of *Caenorhabditis elegans* – could they play a role in drug resistance?	14.30- 14.45	**OR142** Olivier Restif Unravelling the within-host dynamics of an acute bacterial infection.
14.45- 15.00	**OR109** Paul McVeigh A reverse-genetics approach to control target discovery in liver fluke	14.45- 15.00	**OR143** Rachel Norman Manipulating wild, managed population densities to control disease
15.00- 15.15	**OR110** Gillian Stepek Identification of essential astacin metalloproteases in parasitic nematodes of veterinary importance	15.00- 15.15	**OR144*** Tim Dale Squirrelpox: An Epidemic on Merseyside and its Aftermath
15.15- 15.30	**OR111*** Cassandra Longhi Generation of a Recombinant *Teladorsagia circumcincta* Antigen Using *Caenorhabditis elegans*	15.15- 15.30	**OR145** Joanna Randall Protozoan infection alters the regulation of host population dynamics – a cockroach-gregarine story
15.30- 16.00	**COFFEE BREAK**		

	Session D **K326**		**Session E** **K327**
16.00- 17.30	**Helminth Immunology 2** *Chair*: Richard Grencis & Andrew MacDonald	16.00- 17.30	**Ecoparasitology 2** BES SESSION: Ecology meets medicine *Chair*: Mike Begon
16.00- 16.30	**OR112** *Invited Speaker:* Maria Yazdanbakhsh, Leiden University, Netherlands Modulation of the immune system by parasitic elminthes: data from human studies	16.00- 16.30	**OR146** *Invited Speaker:* Les Real, Emory University, USA Evolutionary and ecological dynamics of epidemic rabies
16.30- 16.45	**OR113** Alex Phythian-Adams CD11c positive cells are critical for maintenance of Th2 responses and survival during chronic *Schistosoma mansoni* infection	16.30- 16.45	**OR147** Jo Lello Is Co-infection a Key Driver of Inter-Individual Infection Heterogeneity in School Aged Children?
16.45- 17.15	**OR114** *Invited Speaker:* Judi Allen, University of Edinburgh, UK Macrophages in helminth infection: Where inflammation is anti-inflammatory	16.45- 17.00	**OR148** Sarah Knowles An experimental test for interactions among co-infecting parasites in a wild mammal system
		17.00- 17.15	**OR149** A Laudisoit Microdiversity inside macrobiodiversity : zoonotic risk along the Congo river
17.15- 17.30	**OR115*** Laura Appleby Analysis of phenotype and function of monocyte subsets in human schistosomiasis	17.15- 17.30	**OR150** Poppy Lamberton On-going onchocerciasis transmission under long-term ivermectin control
18.00- 20.00	**POSTER SESSION**		

	WEDNESDAY 4TH APRIL 2012		
09.00-09.05	**Welcome Address** **Prof Kenny Miller, Pro-Vice Principal, University of Strathclyde**		
09.05-10.00	**PLENARY LECTURE** ***Lord May of Oxford***		
10.00-10.30	**WRIGHT MEDAL LECTURE**		
	Session D **K326**		**Session E** **K327**
11.00-12.30	**Molecular Helminthology 3** Drug resistance in helminth parasites. *Chair*: Jacqui Matthews	11.00-12.30	**Helminth Immunology 4** **Parasite Immunology Sponsored Session** *Chair*: Richard Grencis & Andrew MacDonald
11.00-11.30	**OR116** *Invited Speaker:* John Gilleard, University of Calgary, Canada Population genetics of anthelmintic resistance in the small ruminant parasitic nematodes *Haemonchus contortus* and *Teladorsagia circumcincta*	11.00-11.30	**OR151** *Invited Speaker:* David Artis, University of Pennsylvania, USA Mechanisms of immune regulation at barrier surfaces
11.30-11.45	**OR117** Lindy Holden-Dye Emodepside is an activator of the calcium-activated potassium channel, SLO-1	11.30-11.45	**OR152*** Lucy Jones Alternatively activated dendritic cells regulate CD4+ T-cell elminthes n *in vitro* and *in vivo.*
11.45-12.00	**OR118*** Erin McCammick Multidrug resistance protein transcriptional responses to triclabendazole / triclabendazole metabolites in *Fasciola hepatica* newly excysted juveniles	11.45-12.15	**OR153** Invited Speaker: David Dunne, University of Cambridge, UK Metazoan parasites, IgE, immunity and allergy.
12.00-12.15	**OR119*** Roz Laing A population genetics approach to ivermectin resistance in *Haemonchus contortus* and *Teladorsagia circumcincta*		
12.15-12.30	**OR120** Claire McArthur Updated findings from an ongoing cattle parasite survey	12.15-12.30	**OR154** Matthew Little Phenotypic analysis of colonic macrophages in $CX3CR1^{+/eGFP}$ mice infected with the parasitic nematode *Trichuris muris.*
12.30-14.00	**LUNCH**		

	Session D K326		Session E K327
14.00- 15.30	**Molecular Helminthology 4** Molecular and cell biology of elminthes. *Chair*: Eileen Devaney	14.00- 15.30	**Ecoparasitology 3** Molecular ecology of infection *Chair*: Lina Bayer-Wilfert
14.00- 14.30	**OR121** *Invited Speaker:* Frederic Landmann, University of California Santa Cruz, USA Wolbachia in Filarial Nematodes: Mechanisms of Symbiosis	14.00- 14.30	**OR155** *Invited Speaker:* Steve Patterson, University of Liverpool, UK Genetic diversity, immunity and resistance to multiple pathogens in a natural rodent population
14.30- 14.45	**OR122** Denis Voronin Nematode autophagy regulates *Wolbachia* populations and identifies a novel mode-of-action for anti-filarial treatment	14.30- 14.45	**OR156*** Jewelna Osei-Poku Gut microbial diversity in field-caught mosquitoes
14.45- 15.00	**OR123** Sabrina Munshi *Schistosoma mansoni* methyl-CpG binding domain protein (SmMBD2/3): a novel component of the schistosome epigenetic machinery	14.45- 15.00	**OR157** Barbara Tschirren Toll-Like Receptor 2 (*TLR2*) mediates the resistance to *Borrelia afzelii* in a natural reservoir host
15.00- 15.15	**OR124** Collette Britton microRNAs of parasitic nematodes – a role in larval arrest?	15.00- 15.15	**OR158** Martha Betson Molecular epidemiology of ascariasis
15.15- 15.30	**OR125*** Anna Protasio Skin- *vs.* Mechanically transformed schistosomula – a transcriptional comparison.	15.15- 15.30	**OR159*** Ricardo Ramiro Sex and species recognition in *Plasmodium*
15.30- 16.00	**COFFEE BREAK**		
16.00- 17.30	**Helminths – Treatment & Control** *Chair*: Mark Taylor Moses Bockarie	16.00- 17.30	**Ecoparasitology 4** The role of host and parasite behaviours in infection *Chair*: Heather Ferguson
16.00- 16.30	**OR126** *Invited Speaker:* María-Gloria Basáñez, Imperial College, London Modelling the impact of MDA programmes on helminth infections: what do we know about drug effects	16.00- 16.30	**OR160** *Invited Speaker:* Jaap de Roode, Emory University, USA Monarch butterflies practice herbal medicine: consequences for infectious disease and host-parasite coevolution
16.30- 16.45	**OR127** Francesca Tamarozzi Long-term impact of large scale community-directed delivery of doxycycline for the treatment of onchocerciasis	16.30- 16.45	**OR161*** Ellie Sherrard-Smith Weather effects on tick burdens of otters, *Lutra lutra*
16.45- 17.00	**OR128** Loiuse Ford A·WOL drug discovery – screening of focused anti-infective libraries for novel compounds with efficacy against *Wolbachia* endosymbionts of filarial nematodes	16.45- 17.00	**OR162** Issa Lyimo Reshaping the fitness landscape of host species choice in African malaria vectors using interventions: a strategy for evolutionary sustainable control?

17.00-17.15	**OR129** Darren Cook A·WOL drug discovery: Screening diversity libraries to discover novel areas of chemical space with anti-*Wolbachia* properties.	17.00-17.15	**OR163** Stéphane Cornet Malaria infection increases bird attractiveness to uninfected mosquitoes
17.15-17.30	**OR130** Hugo Turner Uncertainty surrounding the projections of the long term impact of ivermectin treatment on human onchocerciasis.	17.15-17.30	**OR164*** Maya Kaushik The impact of *Toxoplasma gondii* on host behaviour: studies on mechanism of action

THURSDAY 5 APRIL 2012			
09.00-10.30	**BSP 50 DEBATE** **50 years of parasite control: is the end game now in sight?**		
	Session D **K326**		**Session E** **K327**
11.00-12.30	**Sex Hormonses, Immunity and Protozoan Parasites** *Chair*: Craig Roberts	11.00-12.30	**Ecoparasitology 5** Integrating evolution and ecology into epidemiology *Chair*: Mark Woolhouse
11.00-11.30	**OR131** *Invited Speaker*: Sabra Klein, Johns Hopkins, USA Placental hormones alter the outcome of influenza virus infection in female mice	11.00-11.30	**OR165** *Invited Speaker:* Sebastian Bonhoeffer, ETH, Switzerland Population biology of drug resistance: Comparing viral, bacterial and microparasitic infections
11.30-11.45	**OR132** Lilach Sheiner Identification of new apicoplast proteins, and functional characterization via single step conditional mutants	11.30-11.45	**OR166** Dan Nussey Immune ageing in a wild mammal population
11.45-12.00	**OR133** Audrey Dubourg Enterocyte function is compromised by a *Giardia*-secreted mediator.	11.45-12.00	**OR167** Andy Fenton Comparing approaches for inferring the occurrence of interspecific parasite interactions
12.00-12.15	**OR134*** Katrin Kremer Functionally different subsets of micronemes in *Toxoplasma gondii*	12.00-12.15	**OR168** Lina Bayer-Wilfert Dynamics of reciprocal selective sweeps in an insect-virus system
12.15-12.30	**OR135** Craig Roberts The ability of progesterone to modulate dendritic cell and macrophage function provides a potential mechanism for the ability of sex and pregnancy to modulate the outcome of *T. gondii* infection.	12.15-12.30	**OR169*** Thibaud Boutin Quantifying cross-species transmission from pathogen sequence data: classical swine fever virus in Europe as a case example
MEETING CLOSE			

Oral Presentations

OR1 Adaptation of African trypanosomes to Man

Etienne Pays
Laboratory of Molecular Parasitology, IBMM, Université Libre de Bruxelles
12, rue des Professeurs Jeener et Brachet, B6041 Gosselies, Belgium

The evolutionary origin of Man in the African continent has imposed the requirement to resist endemic parasites, in particular African trypanosomes (prototype: *Trypanosoma brucei*). Therefore, human serum is provided with an efficient system of innate immunity against these parasites, as discovered by A. Laveran in 1902. However, two *T. brucei* clones, termed *T. b. rhodesiense* and *T. b. gambiense*, managed to escape this immunity system, enabling them to grow in humans where they cause sleeping sickness. We have identified the gene allowing *T. b. rhodesiense* to resist trypanolysis by human serum, which led us to discover that the trypanolytic factor is apolipoprotein L1 (apoL1). ApoL1 is a human-specific serum protein bound to HDL particles that also contain another human-specific protein termed « haptoglobin-related protein » (Hpr). Following the binding of hemoglobin (Hb) to Hpr, the apoL1-bearing HDL particles are avidly taken up by the trypanosome through their binding to a parasite surface receptor for the Hp-Hb complex. After endocytosis apoL1 kills the parasite by generating anionic pores in the lysosomal membrane. In our laboratory, mutant versions of apoL1 have been constructed, which are no longer neutralized by the resistance protein of *T. b. rhodesiense* and are therefore able to kill this human pathogen. Unexpectedly, we have recently discovered that similar mutants do actually exist in nature: in Africans and Americans of recent African origin, even a single allele of these mutants allows protection against infection by *T. b. rhodesiense*, but the price to pay is a high frequency of end-stage renal disease when doubly allelic. The evidence of natural selection of these apoL1 mutations despite their deleterious potential for kidneys highlights the importance of the resistance to trypanosomes in the evolution of Man. The mechanism by which mutant apoL1 triggers end-stage renal disease is currently studied.

OR2 A telomere-specific DNA damage response in African trypanosomes

Lucy Glover, Sam Alsford, David Horn
London School of Hygiene and Tropical Medicine, WC1E 7HT

In *Trypanosoma brucei*, antigenic variation is triggered by DNA double strand breaks (DSBs) at the active telomeric Expression Site (ES). Subsequent repair by homologous recombination allows for variant surface glycoprotein (*VSG*) exchange, but relatively little is known about the DNA damage response at these loci. We have shown that natural breaks are detected at both an active and silent ES and appear clustered towards the telomere. By exploiting an I-*Sce*I meganuclease-based system, single DSBs can be generated at different chromosomal loci. A *VSG*-adjacent DSB triggers a massive increase in RAD51 dependent *VSG* exchange, typically involving recombination within local 70-bp repeats. The DSB response (DSBR) at telomeric and non-telomeric loci revealed DNA resection (ssDNA formation), the focal phosphorylation of histone H2A and accumulation of the ssDNA-binding factor, Replication Protein A (RPA). RPA, RAD51 and γH2A foci all colocalize. Importantly, clear differences in the DSB response were revealed; the γH2A response was stronger at the non-telomeric locus while the RPA response was stronger at the telomere. Genetic dissection revealed that a histone acetyltransferase (HAT3) is specifically required for an effective DSB response at a chromosomal internal locus. We conclude that *T. brucei* chromosomes are segregated into distinct domains in terms of the DSB response and that this has important implications for the control of telomeric *VSG* exchange.

OR3 The trypanosome haptoglobin haemoglobin receptor and human infectivity

Matthew Higgins, Olga Tkakchenko, Alan Brown, Jenny Reed & Mark Carrington
Department of Biochemistry, University of Cambridge

The uptake of host haptoglobin haemoglobin (HpHb) by a specific receptor provides some of the haem required by bloodstream form trypanosomes. The HpHb receptor (HpHbR) is exploited by the primate-specific innate immunity factor TLF1, a high density lipoprotein particle that contains both a ligand for the HpHbR and the trypanolytic apolipoprotein L1. Like any other receptor on the bloodstream form trypanosome cell surface, the HpHbR has to be able to bind its ligand in the context of the variant surface glycoprotein coat and this imposes two opposing constraints on the structure of a receptor, the requirements to: (i) be shielded by the VSG monolayer and (ii) access a large ligand, such as TLF1, that cannot penetrate the VSG coat.
Here, the structure of the HpHb receptor has been determined. The receptor is an elongated three helical bundle with an axis longer than the VSG. The three helical bundle is a conserved motif for trypanosme cell surface proteins and may be the evolutionary precursors of the VSG. The binding site for HpHb is above the top of the VSG coat and can thus bind ligand. An amino acid polymorphism unique to human infective *T. b. gambiense* causes the affinity for HpHb to be reduced 20-fold and if binding of TLF1 is monovalent this may sufficient to greatly reduce uptake and contribute to TLF1 resistance.

OR4* Regulators of endosomal membrane trafficking in *Trypanosoma brucei*

Paul T. Manna, Vincent Adung'a, Catarina Gadelha, Amy Puttick and Mark C. Field*
Department of Pathology, University of Cambridge, Tennis Court Road, Cambridge, CB2 1QT, UK

Clathrin mediated endocytic trafficking is crucially important for viability in bloodstream stage *Trypanosoma brucei.* Comparative genomic and functional analysis indicates that the mechanism(s) for clathrin recruitment are divergent from higher eukaryotes, with multiple factors, including the clathrin recruiting AP2 complex, absent. By proteomic analysis of clathrin complexes, we identified a cohort of clathrin-associated proteins, TbCAPs, several of which are restricted to the trypanosomatids. By colocalization, knockdown and reverse IP we demonstrate that these proteins are bona fide clathrin interaction partners, and interestingly several possess highly novel architectures, implicating very distinct mechanisms within trypanosomes compared to host cells. Further, a search of the *T. brucei* genome for additional clathrin adaptor proteins revealed a potential member of the AP180/CALM family of clathrin adaptors (TbCALM). TbCALM localises to the endocytic system and knockdown in bloodstream form parasites demonstrates the protein to be essential. Unexpectedly no detectable defect in clathrin distribution or early endocytosis is seen in the knockdown cells, suggesting that TbCALM is dispensable for early stages of clathrin-mediated endocytosis. Further analysis revealed gross morphological changes to the endocytic network in TbCALM depleted cells, including the appearance of enlarged vacuolar structures positive for both endocytosed ConA and the lysosomal marker protein p67. These data underscore the very distinct pathways that subtend clathrin functions in trypanosomes when compared with higher eukaryotes.

OR5* Mosaic VSGs in African trypanosome antigenic variation

James P. J. Hall & J. David Barry
Wellcome Trust Centre for Molecular Parasitology, University of Glasgow, 120 University Place, Glasgow G12 8TA

African trypanosome antigenic variation is mediated by a dense, uniform coat of variable surface glycoprotein (VSG). The coat covers the entire parasite surface, shielding invariant antigens, but is itself highly immunogenic. By switching to express different *VSG* genes, parasites escape the immune responses targeting their predecessors, allowing the population to persist in the face of the immune system. The *Trypanosoma brucei* genome reveals the capacity for antigenic variation: ~2000 silent *VSG*s. Yet the majority are damaged by fragmentation, frameshifts and stop codons. Segmental gene conversion allows expressed *VSG*s to be constructed from fragments of silent *VSG* donors, allowing *VSG* repair and giving access to a greater extent of the archive. Furthermore, forming mosaic VSGs potentially gives trypanosomes the ability to generate completely new antigens.
Is sequential mosaic *VSG* formation a mechanism of antigenic variation? That is, do differences between related mosaics change confer sufficient epitope change to escape antibodies? To address this, *T. brucei* infections were sampled and expressed *VSG*s sequenced. Patterns of *VSG* expression were explored with reference to the sequenced genome, and mosaic *VSG*s were identified. These antigens were expressed in a non-switching line of *T. brucei*, and their antigenic properties experimentally examined. The results show related mosaic VSGs that are antigenically distinct, suggesting that mosaic VSG formation directly contributes to *T. brucei* antigenic variation.

OR6 Dephosphorylation of eIF5A is required for survival at stationary growth phase in *Trypanosoma cruzi*

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We have found that eIF5A, a protein known to be involved in translation elongation, is one of the major phosphorylated proteins in exponentially growing epimastigotes of *Trypanosoma cruzi,* which undergoes dephosphorylation when cells reach the stationary phase. This protein is essential in several eukaryotes and undertakes a unique post-translational modification, called hypusination, which consists in the addition of spermidine followed by hydroxylation to a conserved lysine residue. By using mass spectrometry analysis, we found that *T. cruzi* eIF5A is also hypusinated at lysine 53 and mainly phosphorylated at serine 2 (a phosphorylation conserved in yeast), in addition to two other unique, phosphorylations and several methylations. In exponentially growing *T. cruzi* part of the eIF5A sediments with dense fractions in sucrose gradients as do polysomes. When the cells reach stationary phase, polysomes are largely decreased, while relatively more eiF5A remains present in dense fractions. These eIF5A complexes are sensitive to RNAse, EDTA and disappear after treatment of cells with puromycin, suggesting that they represent structures related to the translation machinery, enriched under stationary stress. To answer why eiF5A is dephosphorylated at the stationary phase, wild type forms of eiF5A (WT), or mutants replacing the Ser by Ala (S2A) or Asp (S2D) were overexpressed in the parasites. Both WT and S2D overexpressors increase cellular growth, while S2A has no effect. When the cells reach stationary phase the S2D overexpressor stop moving and become fragile, in contrast with the other cell lines. In parallel, while WT and S2A eiF5A accumulates normally in more dense fractions at the stationary phase, the S2D overexpressor remains in the light fractions. Taken together, this results indicate first that phosphorylation of eIF5A promotes cell growth at the exponential phase, and second that the accumulation of non-phosphorylated eIF5A in dense structures is required for survival at stationary phase.

OR7 Continuous production of infective trypanosomes in the tsetse fly

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African trypanosomes are flagellated protozoan parasites causing sleeping sickness and transmitted by the bite of the tsetse fly. To complete their life cycle in the insect, trypanosomes reach the salivary glands and transform into metacyclic infective forms. The latter are expelled with the saliva at each blood meal during the whole life of the insect. Here, we reveal the existence of two distinct modes of trypanosome proliferation occurring simultaneously in the salivary glands. The first cycle produces two equivalent epimastigote cells that are not competent for infection and attached to the epithelium. It is predominant at the early steps of infection, ensuring a rapid colonization of the glands. The second mode of proliferation is more frequent at later stages of infection and involves an asymmetric division. It produces a trypomastigote daughter cell that matures into the metacyclic form released in the saliva, as demonstrated by the expression of specific molecular markers, the calflagins. The amount of these calcium-binding proteins increases exclusively in the new flagellum during the asymmetric division, showing the commitment of the future daughter cell to differentiation. The coordination of these two alternative cell cycles contributes to the continuous production of infective parasites, turning the tsetse fly into an efficient and long-lasting vector for African trypanosomes.

OR8 A diversity of TBCC domain-containing proteins in trypanosomatid parasites

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Cell shape in *Trypanosoma brucei* is maintained by a subpellicular corset of microtubules and attachment of the flagellum. Regarding microtubule formation a/b-tubulin heterodimerization is the result of a well-defined tubulin folding pathway involving a number of intermediates that sequentially interact with several cofactor proteins, including TBCC. We have previously shown one such TBCC-containing protein, TbRP2, localises to transitional fibres radiating from the mature basal body and is essential for axoneme construction. Here, we show localisation of two further TBCC domain-containing proteins, a novel, large trypanosomatid-specific protein that contains three TBCC-like domains and is also found at basal bodies, plus a *T. brucei* homologue of TBCCD1 that localises to basal bodies, the anterior end of the trypanosome cell and the bi-lobe structure implicated in Golgi biogenesis. This suggests a diversity of cellular functions for the TBCCD1 protein. We are also intrigued by how these TBCC-containing proteins achieve a discrete sub-cellular localisation. Focusing on TbRP2, we have characterised TOF and LisH motifs that are responsible for basal body localisation; similar motifs are also found in other trypanosome basal body proteins. Collectively, and when taken in the context of data available for other eukaryotes, our characterisation of a diverse trypanosome TBCC domain-containing protein family points towards the co-option of LisH/TOF motifs for basal body localization of TbRP2 and the possibility of a conserved association between TBCCD1 and Golgi positioning/function.

OR9 Identification and functional characterisation of CRK12:CYC9, a novel CDK-cyclin complex in *Trypanosoma brucei*

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Cell cycle progression in eukaryotes is regulated by cyclin dependent kinases (CDKs) and their corresponding activating cyclin partners. Several CDC-2 related kinases (CRK1-4 and CRK6-12) and cyclins (CYC2-11) have been described in *Trypanosoma brucei*, although *in vivo* interaction has only been demonstrated for CRK3 with CYC2 and CYC6, which regulate the G1/S and G2/M transitions, respectively. Here we show that CRK12 and CYC9 form a complex *in vivo* in both bloodstream and procyclic stages and that CRK12 is an active protein kinase. CRK12 and CYC9 were functionally characterised using RNA interference, showing them both to be essential for bloodstream form cell growth. However, while depletion of CRK12 had little effect on cell cycle progression, but generated cells with enlarged flagellar pockets and defects in endocytosis, CYC9 depletion specifically inhibited furrow ingression during cytokinesis, indicating its involvement in cell cycle regulation. These results may suggest that CRK12 and/or CYC9 interact with other proteins to carry out some of their functions.

OR10 Meiosis is an inherent feature of the life cycle of *Trypanosoma brucei* subspecies

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Trypanosoma brucei undergoes genetic exchange in the salivary glands of the tsetse vector and the process is thought to involve meiosis and fusion. Genetic exchange has been assumed to be a rare event in the trypanosome life cycle and to occur only in mixed infections. Recently, we identified the probable meiotic division stage of *T. b. brucei* by following expression of key meiosis genes tagged with yellow fluorescent protein. We have now extended these observations to other *T. brucei* subspecies, including the human pathogens *T. b. rhodesiense* and Group 1 *T. b. gambiense*. To date, a total of four different *T. brucei* subspecies strains have been shown to express meiosis-specific genes exclusively in the nucleus of dividing epimastigotes during the early phase of colonisation of the fly salivary glands. As expression of meiosis-specific genes occurred during transmission of clonal trypanosome populations, meiosis appears to be an inherent part of the *T. brucei* life cycle. These results demonstrate the ubiquity of meiosis across trypanosome subspecies.

OR11 Assessing drug modes of action and resistance using metabolomics

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A surprising number of currently used drugs act via unknown mechanisms. Since many pharmaceuticals inhibit enzymes, which in turn cause perturbations to the metabolic network, the use of emerging metabolomic technologies offers the potential to reveal how drugs work in a hypothesis free manner. LC-MS based metabolomics approaches have been implemented in the study of African trypanosomes and we have developed techniques that allow the identification and relative quantification of hundreds of metabolites simultaneously. Using the trypanocidal drug eflornithine, a known inhibitor of ornithine decarboxylase, for example, we have shown it to induce expected changes in abundance of both the target enzyme's substrate ornithine and its product putrescine with further impacts on other polyamines in the parasites. Perturbation to other aspects of metabolism was minimal until the cells begin to die. Moreover, in assessing resistance mechanisms we showed that parasites resistant to eflornithine show no significant changes to their metabolic network, although a loss of eflornithine uptake was demonstrable using mass spectrometry, and confirmed by molecular analysis which revealed loss of a transporter necessary for eflornithine uptake. Other drugs too have been shown to impact on cellular metabolism pointing to a key role for metabolomics in determining drug modes of action and resistance.

OR12 Genetic markers for SSG-resistance in *Leishmania donovani* and SSG-treatment failure in visceral leishmaniasis patients of the Indian subcontinent

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Antimony-resistant (SSG-R) *L. donovani* is widespread in the visceral leishmaniasis endemic regions in India and Nepal. The current standard to identify SSG-R *Leishmania* is a laborious *in vitro* assay of which the result has little clinical value since SSG-R parasites are also found in SSG-cured patients. In this study, candidate genetic markers for clinically relevant SSG-resistant parasites identified by full genome sequencing were validated on a large set of clinical strains. This showed that 3 genomic locations suffice to specifically detect the SSG-resistant parasites found only in patients experiencing SSG-treatment failure (sensitivity: 77.8%, specificity: 100.0%, positive predictive value: 100.0%, negative predictive value 92.0%). These parasite genetic markers show not only a better performance to detect and predict SSG-treatment failure of the patient, they are also much easier to apply compared to the current laborious *in vitro* SSG-susceptibility test. These findings allow the development of rapid assays to monitor the emergence and spread of clinically relevant SSG-resistant *Leishmania* parasites.

OR13 The anti-trypanosome drug fexinidazole shows potential for treating visceral leishmaniasis

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Safer and more effective oral drugs are required to treat visceral leishmaniasis, a parasitic disease that kills 50-60,000 people each year. Here we report that fexinidazole, a drug currently in phase I clinical trials for treating African trypanosomiasis, shows promise for treating visceral leishmaniasis. This 2-substituted 5-nitroimidazole drug is rapidly oxidized *in vivo* in mice, dogs and humans to sulfoxide and sulfone metabolites. Both metabolites of fexinidazole were active against *Leishmania donovani* amastigotes grown in macrophages, whereas the parent compound was inactive. Pharmacokinetic studies with fexinidazole (200 mg kg^{-1}) showed that fexinidazole sulfone achieves blood concentrations in mice above the EC_{99} value for at least 24h following a single oral dose. A once daily regimen for 5 days at this dose resulted in a 98.4% suppression of infection in a mouse model of visceral leishmaniasis, equivalent to that seen with the drugs miltefosine and Pentostam, which are currently used clinically to treat visceral leishmaniasis. In African trypanosomes, the mode of action of nitro-drugs involves reductive activation via an NADH-dependent bacterial-like nitroreductase. Overexpression of the leishmanial homologue of this nitroreductase in *L. donovani* increased sensitivity to fexinidazole by 19-fold indicating that a similar mechanism is involved in both parasites. These findings illustrate the potential of fexinidazole as an oral drug therapy for treating visceral leishmaniasis.

OR14 Aquaporin 2: A Resistance Marker for Pentamidine and Melarsoprol in *Trypanosoma brucei*

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Aquaporins (AQPs) are solute channels allowing the entry of water, glycerol and other small molecules into cells. There are 3 aquaporins in *T. brucei*: AQP1, AQP2 and AQP3. AQP2 was recently identified as a possible factor in resistance to pentamidine and melarsoprol in *T. brucei*[1]. We have investigated AQP2 in several pentamidine and melarsoprol resistant lines, and examined drug resistance and uptake of pentamidine in knock-out and knock-in mutants. AQP2 has been lost or chimerised with AQP3 in our *in vitro*-selected *T. b. brucei* pentamidine resistant line B48, and in two *in vivo*-selected melarsoprol resistant lines, one in a *T. b. brucei* background and the second a *T. b. gambiense* line. Deletion of *AQP2* produced pentamidine- and melarsoprol-resistant trypanosomes in which high affinity uptake of pentamidine was lost. AQP2 re-expression rescued these phenotypes. Conversely, expression of a wild-type copy of AQP2 in the B48 line re-sensitised this line to pentamidine and melarsoprol, and restored uptake of pentamidine. Expression of the chimeric AQP2/3 found in B48 in *AQP2*$^{-/-}$ had no effect on pentamidine sensitivity. We are currently exploring whether AQP2 mediates uptake of pentamidine directly or through regulation of other transporters.

OR15 Melarsoprol cyclodextrin inclusion complexes; an oral treatment for human African trypanosomiasis

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Melarsoprol, is currently the only drug available for the treatment of CNS-stage human African trypanosomiasis (HAT) caused by *T.b.rhodesiense* infection. The solubility characteristics of melarsoprol necessitate its production as a 3.6% solution in propylene glycol. This limits administration of the drug to a strictly intravenous route and demands hospitalisation throughout the course of the protracted treatment regimen.
Cyclodextrins are naturally occurring oligosaccharide molecules. They take the form of a truncated cone or torus with a hydrophilic exterior and a hydrophobic interior cavity that can be occupied by guest molecules. We have used melarsoprol cyclodextrin inclusion complexes, delivered as a series of 7-daily oral doses, to cure CNS-stage trypanosome infections in the well established *T.b.brucei* GVR35 mouse model of HAT. These complexes cleared the parasites from the CNS compartment quickly after commencing the regimen. In addition, BBB integrity was restored to normal levels 24 hours after completion of the drug regimen, as measured by contrast enhanced magnetic resonance imaging. The treatment also produced a significant resolution of the neuroinflammatory reaction associated with CNS-stage trypanosome infections. No overt signs of toxicity were present.
These studies indicate that melarsoprol cyclodextrin inclusion complexes should be of great value in delivering an orally available treatment for CNS-stage *T.b.rhodesiense* infections offering both increased safety and decreased costs.

OR16 Targeting the Glycolytic Pathway of Trypanosomes by Structure-Based and Screening Approaches

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The ten enzymatic steps required to convert glucose to pyruvate are conserved among mammals, protozoa and bacteria. The trypanosomatid parasites *Trypanosoma brucei* (Tb), *Trypanosoma cruzi* (Tc) and *Leishmania mexicana* (Lm) all have peroxisome-like organelles called glycosomes that sequester the first seven enzymes in the pathway. A number of differences in allosteric control mechanisms for some of the enzymes in the pathway have also evolved. The aim of our work is to capitalize on the differences between the parasite and host enzymes and develop specific small molecule inhibitors as potential drug leads.
We are focusing on phosphofructokinase (PFK), phosphoglycerate kinase, phosphoglycerate mutase and pyruvate kinase (PYK), and high-throughput screens (HTS) have been run on most of these proteins at the NIH Chemical Genomics Center, under the Pathways to Discovery programme. In parallel to the HTS approach we are using structure-based and virtual screening to identify potential allosteric and active-site inhibitors. A detailed structural study of pyruvate kinases from the three parasites Lm, Tc and Tb has uncovered some unexpected differences in their allosteric behaviour especially compared with the mammalian host enzymes. X-ray structures of PYK complexed with the original anti-trypanosomatid compounds including suramin show they bind as competitive inhibitors of ATP.

OR17 Global metabolomics of bloodstream-form *Trypanosoma brucei* guides rational improvements to cell culture medium and drug discovery

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Novel metabolomics technology enables simultaneous detection of many small molecule metabolites, providing phenotypic information about intracellular metabolic activity in an untargeted manner. Accurate mass LC-MS based metabolomics has been applied to cell extracts and spent medium from long slender bloodstream-form *T. brucei* under typical *in vitro* culture conditions. Quantitative targeted, semi-quantitative untargeted and stable-isotope labelled precursor metabolite profiling approaches revealed the active metabolic pathways in *T brucei*, which included many well-studied pathways, and some novel metabolites and pathways that were not predicted by genome annotation or biochemical literature. This knowledge of the active metabolic capability of the system allowed rational development of a reduced-component culture medium by removing the non-physiological and unnecessary high concentrations of 35 nutrients present in standard culture media. In addition to cost benefits, the removal of unnecessary medium components allows clearer detection of metabolic changes induced by cellular perturbations such as drug treatment or gene knockout. The minimal medium also increased the sensitivity of drug screening assays for compounds that have a mechanism of action or uptake susceptible to inhibition by excess nutrient concentrations, as demonstrated by the 400-fold decreased IC_{50} for the anti-folate drug methotrexate.

OR18 Eukaryotic life without haem: the aerobic kinetoplastid flagellate *Phytomonas* does not require haem for viability

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Heme is an iron-coordinated porphyrin that is universally essential as a protein cofactor for fundamental cellular processes, such as electron transport in the respiratory chain, oxidative stress response, or redox reactions in numerous metabolic pathways. Kinetoplastids represent a rare example of organisms that depend on oxidative metabolism but are heme auxotrophs. Seeking to understand the metabolism of *Phytomonas serpens* , for which heme is fully dispensable, we searched for heme-containing proteins in its *de novo* sequenced genome and examined several cellular processes for which heme has been so far considered indispensable. We found that *P. serpens* lacks most of the known hemoproteins and does not require heme for electron transport in the respiratory chain, protection against oxidative stress, or desaturation of fatty acids. Although heme is still required for the synthesis of ergosterol, its precursor lanosterol is instead incorporated into the membranes of *P. serpens* grown in the absence of heme. In conclusion, *P. serpens* is a flagellate with unique metabolic adaptations that allow it to bypass all requirements for heme. To our knowledge, this is the first example of a eukaryote totally lacking heme.

OR19 Channel-forming activities of glycosomal membrane proteins from *Trypanosoma brucei* Melisa Gualdrón-López[1], Vasily Antonenkov[2] & Paul Michels[1]
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Glycosomes are specialized peroxisomes found in all kinetoplastid organisms, such as the parasites of the *Trypanosoma* and *Leishmania* species. The organelles harbour most enzymes of the glycolytic pathway. How glycolytic metabolites are transported across the boundary membrane is unclear. As for peroxisomes, the membrane is impermeable for bulky solutes (ATP, NAD(P), acyl-CoAs) which seem to be translocated by specific transporter molecules. We hypothesized that the glycosomal membrane, similarly to those of yeast, plant and mammalian peroxisomes, contain channels allowing the selective permeation of the smaller glycolytic metabolites. To verify this prediction, we isolated a highly purified glycosomal fraction from bloodstream-form *Trypanosoma brucei* and transferred their solubilized membrane proteins to planar lipid bilayers, resulting in the reconstitution of channels as evidenced by electrophysiological methods. Three main channel-forming activities were detected with current amplitudes 70-80 pA, 20-25 pA, and 8-11 pA, respectively (holding potential +10 mV and 3.0 M KCl as an electrolyte). The 20-25 pA channel is anion-selective (P_{K+}/P_{Cl-} ~0.31), while the other two types of channels are slightly selective for cations (P_{K+}/P_{Cl-} ratios ~1.15 and ~1.27 for the high- and low-conductance channels, respectively). The anion-selective channel showed an intrinsic current rectification that suggests a functional asymmetry. These results indicate that the membrane of glycosomes contains several types of pore-forming channels connecting the glycosomal lumen and the cytosol.

OR20* How thiol dependent reductase 1 regulates metabolism in *Leishmania*
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Thiol Dependent Reductase 1 (TDR1) is a parasite-specific, glutathione-S-transferase (GST)-like enzyme, with an unusual two-domain structure, that has thiol transferase and dehydroascorbate reductase activities. To dissect the protein's function in the parasite, we generated a *TDR1* null mutant. Promastigote virulence was little affected by *TDR1* deletion, but metabolomics analyses of the mutant revealed changes in the levels of metabolites participating in *Leishmania* energy and amino acid metabolism (glycerol-3-phosphate, proline, argininosuccinic acid and S-adenosylhomocysteine). This is consistent with TDR1 having a role in enzyme redox regulation via glutathionylation, a reversible post-translational modification. Recombinant TDR1 was able to catalyse the deglutathionylation of protein and peptide substrates, showing that TDR1 can function as a glutaredoxin-like deglutathionylating enzyme. The 2.3 Å structure of the TDR1 revealed a unique trimeric structure in which the subunits contain two homologous domains, each adopting a GST-fold with distinctive features. Our data suggest that TDR1 functions as a deglutathionylating enzyme key for redox regulation of enzymes in *Leishmania*.

OR21 Insights into the Biology of African trypanosomes by Next-generation Sequencing

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Identifying genes essential for survival in the host is fundamental toward unraveling the biology of human pathogens and understanding mechanisms of pathogenesis. The protozoan parasite *Trypanosoma brucei* causes devastating diseases in humans and animals in sub-Saharan Africa and the publication in 2005 of the genome sequence provided the first glance at the coding potential of this organism. Although at that time there was a catalogue of predicted protein coding genes, the challenge remained to identify all authentic genes, including their boundaries, and to monitor gene expression profiles during different developmental stages. We used next generation RNA sequencing (RNA-Seq) to map transcribed regions and the single-nucleotide resolution genomic map of the *T. brucei* transcriptome revealed 1,114 novel transcripts. Many of the new transcripts potentially encode small proteins (30 to 100 amino acids) with a considerable number having a predicted signal peptide or a single trans-membrane domain. RNAi-induced down-regulation of 42 transcripts encoding small proteins with matching mass spectrometry data linked 8 to essential functions, validating an in-depth analysis of this small proteome.
Relatively little is known about the transcriptome during trypanosome development in the tsetse fly vector. We applied the power of RNA-Seq to determine mRNA abundance profiles in trypanosomes isolated from several insect tissues. Midgut, proventriculus and salivary gland datasets will be discussed with special emphasis on RNA-binding proteins and their possible role as important regulators of gene expression in trypanosomes.

OR22 *VSG* gene sequences control monotelomeric *VSG* expression in African trypanosomes

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African trypanosomes achieve host immune evasion through antigenic variation. This requires monotelomeric, RNA polymerase I (RNAP-I) mediated transcription of a variant surface glycoprotein (*VSG*) gene expression site (*ES*) and reversible silencing of other telomeric *VSG-ESs*. Studies on cis-acting elements have revealed that monotelomeric expression continues when the *VSG-ES* promoter is replaced with an *rDNA* promoter or when a telomere is deleted. Chromatin structure is important for monotelomeric *VSG* expression, and we have demonstrated that knockdown of replication-dependent histone chaperones, or a histone, derepresses silent *VSG ES* promoters. However, derepression is incomplete. We have also explored the role of the *VSG*. To determine whether *VSG* cross-talk or silencing is dependent upon telomere-proximity, we inserted a 'mini-*VSG* reporter cassette' at a telomere-distal locus. The *VSG* was strikingly and homogeneously silenced at this locus and silencing was relaxed when the 3'-untranslated region (UTR) was replaced with an unrelated sequence. Using telomere-mediated fragmentation, we fused similar reporter cassettes to *Trypanosoma brucei* chromosome ends. These cassettes exhibited cross-talk, whereby expression was variable, and active transcription had a negative impact on the original *VSG*. Our results indicate that the *VSG* gene makes a major contribution to cross-talk and silencing in the context of monotelomeric *VSG* expression.

OR23 Histone H1 regulates antigenic variation in *Trypanosoma brucei*

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Trypanosoma brucei evades the mammalian immune system through antigenic variation of its major surface coat protein, variant surface glycoprotein (VSG). Although the genome of *T. brucei* contains ~1000 antigenically distinct VSGs, only one is expressed at a time from one of ~14 subtelomeric VSG expression sites (ESs). Chromatin structure plays an important role in silencing inactive ESs, thereby ensuring monoallelic exclusion. The chromatin of trypanosomes displays several unusual properties, which may be adaptations to its parasitic lifestyle and unique genome organization. We have investigated the role of the linker histone H1 in chromatin organization and ES regulation in *T. brucei*. *T. brucei* histone H1 proteins have a domain structure distinct from that of higher eukaryotes. However, despite these differences, we find that *T. brucei* histone H1 proteins are associated with chromatin and play an important role in maintenance of higher-order chromatin structure. Depletion of histone H1 causes global changes in chromatin accessibility. This effect is particularly striking at silent ES promoters, leading to transcriptional derepression. Knockdown of histone H1 also results in an increase in VSG switching. We propose that histone H1 functions to suppress VSG switching through mechanisms involving both transcriptional control and homologous recombination.

OR24* The role of CNOT10 in the process of mRNA turnover in *Trypanosoma brucei*

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In the protozoan parasite *T. brucei,* gene expression is controlled mainly at the level of mRNA degradation. This process starts with deadenylation by the CAF1-NOT complex and is followed by either 5'->3' or 3'->5' digestion. The trypanosome CAF1-NOT complex is built on the scaffold protein NOT1, to which the remaining subunits CAF1, NOT2, NOT5, DHH1 and a putative homologue of CNOT10 are attached. CAF1 is the catalytic subunit. The functions of the other subunits are unclear.
We investigated the role of the putative CNOT10. It is around 20 kDa smaller than its counter part in humans and has only poor sequence similarity. We showed that the putative CNOT10 is part of the complex and interacts directly with CAF1 and NOT1. Depletion of CNOT10 led to a proliferation defect and, more interestingly, to a halt of mRNA degradation. We investigated the transcriptome of CNOT10- and CAF1-depleted cells by RNA-Sequencing and found that the effect for CNOT10 was even stronger than for CAF1. When we further investigated the effect of CNOT10 RNAi, we saw that its depletion led to NOT1 instability and detachment of CAF1 from the complex. We speculate that the attachment of CAF1 to the complex is required for its recruitment to mRNAs.

OR25 The post-transcriptional *trans*-acting regulator, *Tb*ZFP3, coordinates transmission-stage enriched mRNAs in *Trypanosoma brucei*.

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Post-transcriptional gene regulation is essential to eukaryotic development. This is particularly emphasized in trypanosome parasites where genes are co-transcribed in polycistronic arrays but not necessarily co-regulated. The small CCCH protein, *Tb*ZFP3, has been identified as a *trans*-acting post-transcriptional regulator of Procyclin surface antigen expression in *T. brucei*. To investigate the wider role of *Tb*ZFP3 in parasite transmission, a global analysis of associating transcripts was carried out. Examination of selected transcripts revealed their increased abundance through mRNA stabilization upon *Tb*ZFP3 ectopic overexpression, dependent upon the integrity of the CCCH zinc finger domain. Reporter assays demonstrated that this regulation was mediated through 3'-*UTR* sequences for two target transcripts. Global developmental expression profiling of the cohort of *Tb*ZFP3-selected transcripts revealed their significant enrichment in transmissible stumpy forms of the parasite. Immunofluorescent assays demonstrate that *Tb*ZFP3 colocalises with a P body marker in starvation granules, and with a subset of *procyclin* transcripts in stumpy stage parasites. This analysis of the specific mRNAs selected by the *Tb*ZFP3mRNP provides evidence for a developmental regulon with the potential to stabilize and coordinate genes important in parasite transmission.

OR26 A touch of Zen: Genetic analysis of *Leishmania* stress signalling

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Our laboratory studies the molecular basis of virulence of *Leishmania*, an important human pathogen that produces serious diseases world wide. Our research program is focused on the analysis of *Leishmania* signal transduction pathways that are relevant for intracellular parasite development and survival. Through development and application of novel methods of molecular parasitology, quantitative phosphoproteomics, and kinase activity determination, we recently revealed that the *Leishmania* stress response is regulated mainly at post-translational levels by stress-regulated protein kinases that phosphorylate parasite-specific residues in otherwise conserved heat shock proteins and chaperones. We currently elucidate these signalling mechanisms by mapping specific protein kinase – phosphoprotein relationships and by genetic assessment of selected phosphorylation sites using conditional null mutant analysis. The talk will focus on the genetic analysis of two chaperone proteins, STI1 and cyclophilin 40, and the functional analysis of their phosphorylation sites by plasmid shuffle approach and complementation assay.

OR27 Effects of *BBS1* deletion on parasite morphology and infectivity in *Leishmania major*

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Bardet-Biedl syndrome (BBS) is a human genetic disorder with a spectrum of symptoms caused by primary cilium dysfunction. The disease is caused by mutations in one of at least 16 identified genes, of which 7 encode subunits of the BBSome, a protein complex required for specific trafficking events to and from the primary cilium. The molecular mechanisms associated with BBSome function remain unknown. We have generated null mutant lines of the BBSome subunit *BBS1* in *Leishmania major*. The parasites have no apparent defects in growth, motility or differentiation *in vitro* but accumulate vesicles at the flagellar pocket. Trafficking of the lipophilic marker FM4-64 occurs as wild type in *BBS1* null procyclic promastigotes but is defective in the metacyclic stage of the mutant parasites. Further, infectivity of these parasites for macrophages in vitro is reduced compared to the wild type control. Mouse infectivity studies are currently in progress. We hypothesise that specific trafficking events are defective in *Leishmania* in the absence of *BBS1*, which leads to inhibition of differentiation from promastigote to amastigote stages and therefore reduced virulence. This is the first report of an association between the BBSome complex and pathogen infectivity.

OR28 Dissecting differentiation signalling pathways in *Trypanosoma brucei*

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Despite the detailed *in silico* analysis of the TriTryp kinome and phosphatome, the identification of major signalling events in Trypanosomes is almost entirely missing.
One exception is a protein phosphatase cascade, regulating differentiation events from bloodstream stumpy forms to vector adapted procyclic forms. In transmissible stumpy forms a tyrosine phosphatase (*Tb*PTP1) preventing cells from differentiation until it is inactivated by the differentiation trigger citrate/cis-aconitate (CCA), which is controlled by the carboxylate transporter PAD proteins, expressed on the stumpy cells' surface at ambient temperature of the tsetse-fly blood meal. Recently, we identified *Tb*PIP39, a DxDxT phosphatase as a downstream regulator of this pathway and showed it is activated upon tyrosine-phosphorylation and negatively regulated by *Tb*PTP1. Beside the CCA, other differentiation triggers as mild acid, pronase and glucose depletion were identified. To establish if these triggers operate through the CCA/ PAD/PTP1/PIP39 pathway, we used pleomorphic *Tb*PIP39 RNAi line and antibody against the tyrosine-phosphorylated *Tb*PIP39. We monitored *Tb*PIP39 ablated cells' ability to differentiate upon different external stimuli and found that only pronase treated cells differentiated as well as the parental cells. Also, in pronase treated pleomorph cells the phospho-*Tb*PIP39 level was lower than in CCA/mild acid treated cells, suggesting the *Tb*PTP1 was unaffected in this case. Taken these together, we suggest that the mild acid acts through the *Tb*PTP1 regulated pathway, but pronase operates via an independent pathway.

OR29* Functional analysis of LmxMPK2, a MAP kinase essential for *Leishmania mexicana* amastigotes.

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LmxMPK2 is a mitogen-activated protein (MAP) kinase homologue in *Leishmania mexicana* that is expressed in both the amastigote and promastigote stages. Generation of homozygous gene knock out mutants revealed a reduction in cell proliferation and a range of morphological alterations with cells showing multiple flagella, kinetoplasts and nuclei, lobed cell bodies, spiked posterior ends and division furrow ingression from the posterior end. Localisation studies are underway through the use of GFP-tagged LmxMPK2. In infection studies, the homozygous gene knock out mutants were unable to cause lesions in infected Balb/c mice whereas genomic add-backs caused the disease making LmxMPK2 a promising drug target. Recombinant expression of LmxMPK2 resulted in an active enzyme already phosphorylated on tyrosine and threonine which is able to phosphorylate myelin basic protein (MBP) despite the lack of activation by a MAPK kinase. Co-expression with the human phosphotyrosine phosphatase PTP1B led to LmxMPK2 being dephosphorylated on tyrosine but not threonine residues retaining the ability of tyrosine autophosphorylation maintaining equal levels of MBP phosphorylation. This suggests that LmxMPK2 is an unusual MAP kinase in which threonine phosphorylation alone leads to a fully activated.

OR30* Validating protein kinases of *Trypanosoma brucei* as drug targets: a kinome-wide RNAi screen.

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[2] Institute of Structural and Molecular Biology, University of Edinburgh

Inhibitors of human protein kinases have been developed into drugs for clinical use, demonstrating that PKs are druggable targets. Parasite PKs may represent novel drug targets. To identify essential PKs we have performed a large-scale, targeted RNAi screen of the *T. brucei* kinome. We developed a high-throughput method to generate stem-loop RNAi constructs by modifying the pRPa^{isl}/*T.b.brucei* 2T1 system for Gateway cloning. This system was validated with CRK3, a known essential gene, and used to generate a library of plasmids targeting the 183 PKs of *T. brucei.* Two independent BSF RNAi clones were generated for each PK and were tested for loss-of-fitness phenotypes *in vitro* using an alamar blue cell viability assay. Selected lines were assessed *in vivo* using a mouse model. We identified 59 PKs that are essential or important for growth in BSF *T. brucei,* of which 30 have not previously been investigated. The loss-of-fitness cohort included known drug targets such as GSK3 and CK1.2, further validating the RNAi system. No phenotypes were observed in RNAi lines targeting PK genes known to be redundant in BSF parasites, suggesting the system is not generating false positives. We are characterising novel essential PKs and developing cellular assays to identify PKs regulating signalling pathways involved in autophagy and differentiation.

OR31 The functional proteome of the *Trypanosoma brucei* mitochondrion

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Trypanosoma brucei has a single large mitochondrion that contains the hallmark of the Kinetoplastida, a single network of unusual mitochondrial DNA (kinetoplast DNA, kDNA) adjacent to the flagellar basal body. These features are shared by the related pathogens *Trypanosoma cruzi* and leishmania. Proteomic and bioinformatic analyses reveal that the *T. brucei* mitochondrion contains about 1,200 proteins. These proteins are products of the nuclear genome and imported into the organelle, with the notable exception of 17 that are encoded in kDNA. Many mitochondrial proteins are in complexes including those of the oxidation/phosphorylation system, of which some are kDNA encoded. Another complex, the mitochondrial ribosome, translates the proteins encoded in kDNA many from mRNAs that undergo the post-transcriptional maturation process of RNA editing. Editing employs several complexes including the editosomes that perform the central catalytic processes of mRNA cleavage, uridylate addition and removal and ligation. Editing is regulated of during the life cycle and affects energy production. Genetic studies of gene function in bloodstream form *T. brucei* show that components of the editosomes and mitochondrial tRNA synthetases are essential for parasite viability. Overall, the mitochondrial proteome is the product of two genetic systems that function in an integrated fashion to produce a highly specialized organelle with unique characteristics that provide multiple potential therapeutic targets.

OR32 Targeting the kDNA replication machinery for drug discovery in *Leishmania*

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The kDNA is the unique mitochondrial genome of kinetoplastid parasites. It encodes several distinct proteins involved in kDNA replication, a process that has many particularities and that has been proposed as a drug target for kinetoplastids.
A list of 2,152 “hits” was selected from a screening campaign of 200,000 compounds against intracellular *Leishmania donovani*. To identify which of these hits were targeting the kDNA replication machinery, the compounds were tested in dose response in a viability assay for promastigotes of *L. donovani,* epimastigotes of *Trypanosoma cruzi,* bloodstream forms of *T. brucei* and *T. evansi*, which lacks a normal kDNA replication machinery and was used as a control. Using 70% activity cutoff, 12 compounds were identified with potential to be interfering with the kDNA replication process.
A replication assay using the thymidine analog EdU was performed to phenotypically confirm if compounds interfere with kDNA replication. We found many unusual phenotypes that are suggestive of defective kDNA replication. Currently we are testing these compounds on *T. brucei* replication assay. Compounds targeting kDNA replication are promising for drug development for trypanosomiasis and leishmaniasis, and can also aid on the better understanding of kinetoplast biology.

OR33 The procyclic trypanosomes express two mitochondrial enzymes for acetate production from acetyl-CoA:
ASCT is involved in ATP production, but not ACH

Yoann Millerioux, Pauline Morand, Marc Biran, Muriel Mazet, Marion Wargnies, Charles Ebikeme, Kamel Deramchia, Jean-Michel Franconi & Frédéric Bringaud
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The procyclic form of *Trypanosoma brucei* needs to transfer acetyl-CoA produced in the mitochondrion to feed the essential cytosolic fatty acid biosynthesis, using the so called "acetate shuttle". This pathway requires the mitochondrial conversion of acetyl-CoA into acetate by acetate:succinate CoA-transferase (ASCT) and an unknown enzymatic activity. We have identified an acetyl-CoA thioesterase (ACH) gene encoding a mitochondrial enzyme involved in this process, since repression of ASCT by inducible RNAi in the ACH null background abolishes acetate production, as opposed to both single ASCT and ACH mutants. ASCT is involved in ATP production while ACH is not, since the ASCT null mutant is ~1000-time more sensitive to oligomycin, a specific inhibitor of the mitochondrial F_0/F_1-ATP synthase, than the wild-type cells and the ACH null mutant. This was confirmed by RNAi repression of the F_0/F_1-ATP synthase F_1ß subunit, which is lethal when performed in the ASCT null background, but not in the wild-type cells or ACH null background. We concluded that acetate is produced from both ASCT and ACH, however only ASCT is important, together with the F_0/F_1-ATP synthase, for ATP production in the mitochondrion of the procyclic trypanosomes.

OR34 *ATG5*-deletion mutants reveal interplay between macroautophagy and mitochondrial homeostasis in *Leishmania major*

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This study has revealed, by using a variety of approaches, that ATG5 is part of a functional ATG12-ATG5 conjugation system, previously thought to be absent from *Leishmania*, and consequently crucial for ATG8-dependent autophagosome formation. Gene-deletion studies have also shown, however, that ATG5 is required for mitochondrial integrity and phospholipid balance in this organelle. *L. major* mutants lacking *ATG5* (Δ*atg5*) had abnormal morphology with increased mitochondrial mass, reduced mitochondrial membrane potential, higher levels of reactive oxygen species and elevated phosphatidylethanolamine content. Δ*atg5* mutants were less able to differentiate and had greatly reduced virulence to macrophages and mice, demonstrating macroautophagy to be important for the host-parasite relationship.

OR35 Functional characterization of a putative mitochondrial cation/proton antiporter in both life stages of *Trypanosoma brucei brucei* and the akinetoplastic *Trypanonsoma brucei evansi*

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The leucine zipper EF-hand-containing transmembrane protein (Letm1) is a ubiquitous mitochondrial protein that serves as a cation/proton antiporter across the inner membrane. It remains controversial whether the cation in question is K^+ or Ca^{2+}, as there are data supporting both scenarios. Furthermore, Letm1 is believed to anchor mitoribosomes to facilitate translation of mitochondrial genes in yeast. RNAi-silencing of Letm1 in PS and BS *Trypanosoma brucei brucei*, plus *Trypanosoma brucei evansi*, indicate this protein is essential in all cell types, since its ablation results in mitochondrial swelling. This phenotype is consistent with a role in cation efflux from the matrix. Complementation by expression of the human ortholog of Letm1, which has been demonstrated to be able to transport Ca^{2+}, rescues cell growth when the endogenous protein is downregulated. Furthermore, mitochondrial translation is indeed compromised in PS. However the results from *T.b. evansi*, where translation is non-existent, suggest the primary role of this protein is cation/proton exchange. More importantly, these results indicate that among the possible reasons explaining the energy expenditure needed to maintain an active mitochondrion in the BS, which does not produce energy as in the PS, is cellular ion homeostasis.

OR36 Health Impacts of Product Development Partnerships

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Over 40 PDPs have been established in the last 20 years to stimulate industry to accelerate the development of new drugs, vaccines, insecticides and diagnostics for a range of tropical diseases. The products supported by these PDPs are now being used to impact on disease transmission and prevalence.

The Innovative Vector Control Consortium (IVCC) is the only vector control PDP supporting the development of public health pesticides and diagnostics for malaria, dengue and other insect borne disease prevention. The impact on policy and practice of IVCC supported products 7 years after the PDP's inception will be discussed.

OR37 Evaluation of a novel molecular marker for monitoring artemisinin resistance in *Plasmodium falciparum* malaria

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There is evidence of reduced susceptibility of the malaria parasite *Plasmodium falciparum* to artemisinin derivatives, manifesting as delayed parasite clearance times *in vivo*. If artemisinin resistance spreads, it would threaten global malaria control. We lack validated molecular markers for monitoring these resistance phenotypes. Using genome-wide strategies in the rodent malaria parasite *Plasmodium chabaudi* we have identified a mutation in the *mu* chain of the AP2 adaptor protein complex (*pcap2-mu*) that arose along with increased artemisinin resistance. We have screened for genetic polymorphisms in the *P. falciparum* orthologue, *pfap2-mu* in field isolates, from an ACT clinical trial in Burkina Faso, that were tested *in vitro* for their response to artemisinin derivatives and other antimalarial drugs, and in pre- and post- treatment samples from an *in vivo* ACT trial carried out in Kenya. Genetic polymorphisms in *pfap2-mu* were analysed for association with several endpoints in both trials that might indicate a drug resistant parasite phenotype. Preliminary results indicate that polymorphisms in this adaptor protein subunit may be associated with *in vitro* and *in vivo* responses to artemisinin derivatives, quinine and lumefantrine. Further evaluation of *pfap2-mu* as a potential molecular marker of artemisinin resistance is now needed.

OR38 Antimalarials – trials and triumphs

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The history of discovery of antimalarials provides fascinating insights for the discovery and development of new classes that are urgently needed. Quinine was discovered well before the parasites that it is still used to treat, and artemisinins were identified at around the time the British Society of Parasitology was convened. The history of these antimalarials will be presented with emphasis on aspects that may be of interest to modern drug developers.
A brief overview of the how artemisinins might work in the context of emerging resistance will also be presented as this class of antimalarial is important for treating most patients with malaria. Some discussion of what we mean by artemisinin resistance, and how we can monitor it, will also be attempted.

OR39* Stability of hotspots after implementation of community wide vector control with indoor residual spraying

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Malaria tends to be clustered in hotspots of high transmission intensity. The heterogeneity of malaria creates opportunities for targeted interventions, but it is unclear if hotspots remain stable after implementation. We aimed to establish the stability of hotspots after intervention with Indoor Residual Spraying (IRS). Two surveys were conducted in the Mwanza region, Tanzania, before and after implementation of IRS. In total, 3031 people were included in both surveys. Parasite carriage was determined using sensitive nested PCR. Responses against malaria specific antibodies, AMA-1 and MSP-1, were used to determine the stable spatial patterns in transmission intensity. In the first survey, before implementation of IRS, parasite prevalence was 31.1% but varied between villages, from 24.9% to 60%. Two hotspots ($p \leq 0.01$) and two coldspots ($p \leq 0.01$) were detected. The current ongoing work aims to determine the stability of these hotspots and coldspots over time, 12 months after the initial survey and IRS implementation. We describe the persistence of hotspots after community-wide implementation of vector control. The success of any intervention greatly depends on its ability to reduce malaria transmission in hotspots.

OR40 New evidences of human trypanotolerance in West Africa: perspectives to better understand host-parasite interactions and improve control strategies.

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Since first identified, human African trypanosomiasis (HAT) or sleeping sickness has long been described as invariably fatal. Increasing data however argue that infection by *Trypanosoma brucei gambiense* (*Tbg*), the causative agent of HAT, results in a wide range of outcomes in its human host and importantly that a number of subjects in endemic areas are apparently able to control infection to low levels. We will review here results obtained during the long term follow-up of patients refusing treatment and serological suspects from Côte d'Ivoire and Guinea. Alternative natural progressions of HAT were observed in addition to the "lethal" classical one: (i) a progression to an apparently spontaneous resolution of infection (with negative parasitology and PCR) associated with a progressive drop in antibody titres and (ii) a progression to an apparently aparasitemic and asymptomatic latent infection associated with strong long lasting serological responses (>10 years) as this is also observed in serological suspects testing positive to the highly specific trypanolysis test for *Tbg* (SERO TL+). Comparing cytokine responses in HAT patients and SERO TL+ evidenced contrasting immunological responses in these two categories of subjects. The SERO TL+ status is associated with high plasma levels of IL-8, IL-6 and TNF-□ and low IL-12 levels suggesting that the inflammatory response in these individuals is mainly triggered by innate immunity. Furthermore in SERO TL+, high IL-10 and low TNF-□ levels were predictive of subsequent disease development whereas high IL-8 levels were associated with individuals becoming negative in serology. These data thus provide further evidences that trypanotolerance exists in humans as described in cattle and mice. The consequences/impacts on HAT epidemiology will also be discussed in regard of implementing sustainable HAT control strategies.

OR41 Disease progression in Human *Trypanosoma brucei rhodesiense* infection: CNS humoral and cellular responses.

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Analysis of cerebrospinal fluid (CSF) is crucial to stage diagnosis in Human African trypanosomiasis (HAT). Presently the diagnostic criteria are based on the observation of trypanosomes in the CSF and/or raised white cell counts. It would be desirable to obtain more specific CSF or preferably serum diagnostics, but this will require a better understanding of the pathophysiological evolution of trypanosomiasis in the brain. We have investigated the development of inflammatory cytokine and CNS immunoglobulin responses to infection in *T.b.rhodesiense*-infected subjects from Uganda. Our results reveal that while overall disease progression is accompanied by increases in cellular and humoral responses in the CNS, intriguingly neurological signs of infection and alterations of inflammatory cytokine profile in the CSF are also observed in early stage cases, suggesting an early CNS involvement in *rhodesiense* HAT. Our results will be discussed in the context of the biology of CNS invasion by trypanosomes and the development of new diagnostics for staging of HAT.

OR42* Towards *Trypanosoma cruzi* lineage-specific serology for Chagas disease

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Chagas disease, caused by the protozoan *Trypanosoma cruzi*, remains an important parasitic disease in the Americas. It can be fatal in the acute phase, but life-long chronic infection may be asymptomatic, or lead to debilitation and death by cardiac and/or intestinal complications. Genetically diverse, *T. cruzi* is classified into the intra-species lineages TcI-TcVI, displaying disparate geographical distributions and ecologies. The varying disease outcomes may be linked to parasite lineage, and complicated by mixed infections. The work presented here addresses the development of lineage-specific serology to identify an individual's history of exposure to *T. cruzi* lineages. The molecular diversity of the parasite surface antigen TSSA was analysed across a panel of reference biological clones encompassing *T. cruzi* genetic and ecological diversity, revealing lineage-specific B-cell epitopes. We demonstrate here the capacity of synthetic peptides based on the TcII/V/VI common epitope to be recognised by antibodies in human sera from Brazil, Chile, and reported for the first time, Ecuador. Further, we report the first TcIII- and TcIV-specific serology, from experimental murine models. A genomic approach to identify *T. cruzi* lineage-specific epitopes can be used successfully in developing a differential serology to investigate an individual's history of *T. cruzi* lineage exposure, and lead to a greater insight into the link with Chagas disease outcome. Overall, this approach represents a potential new tool in Chagas disease epidemiology.

OR43* Hope on the Horizon: development of a new prototype lateral flow diagnostic test for Human African Trypanosomiasis.

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Currently the diagnosis of Human African Trypanosomiasis (HAT) mainly relies on the Card Agglutination Test for Trypanosomiasis (CATT), which has severe limitations. Our aim was to develop a lateral flow test based on trypanosome antigens. We used sera from *T. b. gambiense* infected and non-infected patients to identify infection specific diagnostic trypanosome proteins. The trypanosome proteins identified were then cloned into *E. coli* for recombinant expression and purification. The recombinant proteins were then screened by ELISA against 145 patients' sera from the WHO HAT specimen bank. Invariant Surface Glycoprotein (ISG) 65 was selected for development into a lateral flow format test and 80 randomised patients' sera were used to evaluate this prototype. Here we describe the results showing that an un-optimised ISG65 lateral flow test matches the reported CATT sensitivity and specificity scores. We intend to collaborate with BBInternational to further develop this test by optimising conditions used in the ISG65-lateral flow test and to evaluate the use of other antigens for lateral flow format.

OR44 Molecular amplification tools for the diagnosis of Human African Trypanosomiasis – A systematic review.

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Recently we investigated the accuracy of molecular diagnostics for Human African Trypanosomiasis. We wanted to know if the accuracy of these tests warranted their implementation in endemic settings; and whether limited resources should be re-directed towards this goal. Here, we found that PCR tests seem to have an acceptably high specificity and sensitivity for diagnosis of stage I HAT. However, this conclusion is based on multiple-microscopy based techniques as reference standards, which may have low sensitivity, and a patient population that was not always representative. Accuracy was not sufficient for diagnosis of stage II disease.
Data from studies assessing diagnostic molecular amplification tests for HAT were extracted and pooled to calculate accuracy. 16 articles evaluating molecular amplification tests fulfilled the inclusion criteria: PCR (n = 12), NASBA (n = 2), LAMP (n = 1) and a study comparing PCR and NASBA (n = 1). 14 articles, including 19 different studies were included in the meta-analysis. Summary sensitivity for PCR on blood was 99.0% (95% CI 92.8 to 99.9) and the specificity was 97.7% (95% CI 93.0 to 99.3). Differences in study design and readout method did not significantly change estimates although use of satellite DNA as a target significantly lowers specificity. Sensitivity and specificity of PCR on CSF for staging varied from 87.6% to 100%, and 55.6% to 82.9% respectively.

OR45 Genomic studies of *Leishmania* and RNA Viruses in South America

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One manifestation of leishmaniasis is a disfiguring mucocutaneous form (MCL) involving dissemination to nasopharyngeal areas and tissue destruction, caused primarily by species from the South American *Leishmania* subgenus *Viannia*. We reported previously that metastatic *L. guyanensis* bear high levels of a novel dsRNA totivirus (LRV1), while non-metastatic lines have low levels or lack LRV1. LRV1-infected *Leishmania* were associated with a hyper-inflammatory response dependent on the host Toll-like receptor 3 (TLR3) signaling. Thus, LRV in metastatic parasites subverts the host immune response and promotes dissemination. This is the first parasite factor implicated causally in phenotypes relevant to metastatic mucocutaneous disease, with LRV acting as a 'parasite within a parasite'. The closely related species *L. braziliensis* is responsible for the great majority of human MCL. Thus we are surveying *L. braziliensis* strains to establish the distribution and potential for clinical association(s) of LRVs in parasites taken from humans. Several novel LRVs have been identified; these and the host parasite are being characterized by virus and genome sequencing.

OR46* Multiple mitochondrial introgression events and heteroplasmy in *Trypanosoma cruzi*

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Mitochondrial DNA is a valuable taxonomic marker due to its relatively fast rate of evolution. In *Trypanosoma cruzi*, the causative agent of Chagas disease, the mitochondrial genome has a unique structure consisting of 20-50 maxicircles (~20kb) and thousands of minicircles (0.5-10kb). *T. cruzi* displays remarkable genetic heterogeneity and is recognized as a complex of six discrete typing units (DTUs) each broadly associated with disparate ecologies and geographical distributions. The availability of whole genome sequences has advanced high resolution genotyping techniques and re-invigorated interest in exploring cryptic sub-DTU diversity. We developed a maxicircle multilocus sequence typing (mtMLST) scheme and evaluated it against current nuclear typing tools using a panel of isolates belonging to the oldest and most widely occurring lineage TcI. Gross nuclear-mitochondrial phylogenetic incongruence was observed at multiple levels, including among different populations as well as major DTUs. These observations indicate that genetic recombination is geographically widespread and continues to influence the natural population structure of TcI, challenging the traditional paradigm of clonality in *T. cruzi*. In parallel, we exploited read depth data, generated by 454 sequencing of the TcI reference maxicircle genome, to provide the first evidence of mitochondrial heteroplasmy (multiple mitochondrial genomes in an individual cell) in *T. cruzi*.

OR47 Coordinated modularity of DNA replication and transcription in *Trypanosoma brucei*

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The nuclear genome of the parasitic protist *Trypanosoma brucei* has an unusual organisation, with each chromosome comprising just a few discrete transcription units. To address how DNA replication occurs in the context of such modular organization, we have mapped binding sites for the initiator protein ORC1/CDC6 by chromatin immunoprecipitation and have identified replication origins genome-wide by Marker Frequency Analysis. ORC1/CDC6 binding sites and replication origins display remarkably precise co-localisation with the boundaries of the transcription units. We observe a strikingly small number of active origins, the spacing of which is greater than seen in any other eukaryote. Finally, we show that reducing levels of ORC1/CDC6, in addition to perturbing DNA replication, impacts upon transcription, leading to increased levels of mRNA for genes at the boundaries of the transcription units and to derepression of silent, telomere-proximal *Variant Surface Glycoprotein (VSG)* genes, which are critical in the evasion of host immunity by this parasite.

OR48 Comparative genomics of African trypanosomes

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A comparative analysis of genome sequences for *Trypanosoma congolense* IL3000 and *T. vivax* Y486 with the reference sequence for *T. brucei* 927 shows that the principal disparities in repertoire concern genes expressed at the cell surface. I present a cell surface phylome for African trypanosomes* that uncovers structural diversity in familiar families and reveals uncharacterized families that could encode important effectors in the host-parasite interaction. Evolutionary changes affecting the variant surface glycoprotein (*VSG*) and its expression are foremost among the species differences. The canonical, bloodstream-stage *VSG* expression site has evolved only in *T. brucei.* The phylome demonstrates that Expression Site-Associated Genes (*ESAGs*) often belong to widespread gene families, but are lineages unique to *T. brucei*, (except for *ESAG6*). Through phylogenetic analysis of the *VSG*, I show that *T. congolense* employs variant antigens derived from multiple ancestral *VSG* lineages, while in *T. brucei VSG* lineages are less diverse and more recently derived, and ancestral gene lineages have been repeatedly co-opted to novel functions. These histories are reflected in fundamental differences between species in the scale and mechanism of recombination among *VSG*. These results demonstrate how past *VSG* evolution indirectly determines the ability of contemporary parasites to generate novel variant antigens, and suggest that the current model for antigenic variation in *T. brucei* is only one means by which these parasites maintain infections.
* www.genedb.org/Page/trypanosoma_surface_phylome

OR49 Population structure and adapative evolution of a recent *Leishmania* outbreak

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Leishmaniasis is a potentially fatal disease caused by *Leishmania* parasites endemic to tropical and sub-tropical regions. Over 51,000 people die annually from the visceral form and consequently biological characterisation of ongoing epidemics is needed to infer their origin, evolution and transmission. We used the genome of a Nepalese clinical strain as a high-quality reference to detect whole-genome variation in global and clinical strains. We sequenced clinical strains that represent a genetically monomorphic but phenotypically variable outbreak – all were isolated from patients with documented treatment outcomes. Placing clinical diversity in an evolutionary context showed that the majority of epidemic strains originated after a population crash during the Indian anti-parasite spraying campaigns in the 1960s. However, a minority from the same small region was related to African isolates. By comparing patient treatment backgrounds with the phylogenetic distribution of the epidemic sample and genome-wide scans for mutations related to experimentally induced drug resistance, new drug-susceptibility variants were discovered. We combined analyses of clinical parasites to provide a foundation for continuous monitoring of novel and drug-resistant outbreaks that act as a threat to public health.

OR50 Short term evolution of malaria parasites

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The ability of the *P. falciparum* parasites to acquire drug resistance through single nucleotide polymorphisms and copy number amplifications as well as to evade the host immune response makes it difficult to control the global malaria burden. My group has been using long term in vitro evolution of cloned parasites, grown in the presence and absence of drugs and full genome sequencing of patient isolates to determine how quickly the parasite will acquire evasive genetic changes that may allow parasites to escape drugs and vaccines. Sequencing and microarray analysis shows that single nucleotide polymorphisms arising in core chromosomal regions are relatively common and that the core genome is very stable. In contrast, large-scale deletions and rearrangements of subtelomeric regions containing members of gene families involved in immune evasion were more frequent during mitotic growth. Our data show copy number amplifications arise frequently and apparently, exclusively, in response to drug pressure. Our findings predict rapid evolution and diversification in a clonal parasite population and predict the rapid appearance of multiple haplotypes even in a single human malaria infection.

OR51* Metabolic Fingerprinting of *Plasmodium falciparum*

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Despite intesive research there remains major knowledge gaps in our understanding of the metabolic network of the malaria parasite *Plasmodium falciparum.* This deficiency exacerbates our ability to identify new targets for drug discovery at a time when new targets are urgently required. In order to address this problem we have adopted a chemical biology strategy whereby biologically selective inhibitors have been used to perturb concentration and fluxes in intermediary metabolic pathways generating "fingerprints" of metabolites in a time-related manner. In this initial study, a number of mitochondrial inhibitors selective for specific electron transport chain complexes and mitochondrial transporters were used to assess mitochondrial function in asexually growing parasites. Metabolite identification was conducted using a targeted LC-MS/MS metabolomics approach. Despite the differing modes of action of the inhibitors, the metabolic fingerprint from these experiments was consistent with the parasite mitochondrion playing a key role in pyrimidine biosynthesis. This metabolic fingerprint leading to parasite death was quite distinct from fingerprints obtained from biologically distinct inhibitors e.g. heme-binding antimalarials. In contrast to genomic and proteomics approaches, metabolomics therefore appears to better represent the parasites' phenotype in response to drug perturbation. Metabolic fingerprinting will therefore have significant utility in understanding the mode of action, efficacy and toxicity of pharmaceutical drugs.

OR52* Analyzing *Plasmodium falciparum* erythrocyte membrane protein 1 gene expression by a next generation sequencing method

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Plasmodium falciparum infections are the cause of the vast majority of severe malaria cases and are responsible for >1 million deaths every year. The virulence is highly associated with the expression of certain members of the *Plasmodium falciparum* erythrocyte membrane protein 1 (PfEMP1) family, encoded by ~60 highly variable *var* genes per haploid genome. This variable nature has constituted a roadblock in *var* expression studies aimed at identifying semi-conserved domains responsible for high virulence. Here we present the first effective method for sequence analysis of the extracellular domain of *var* genes expressed in field samples: a sequential next generation sequencing technique applied on *var* expression tags and subsequently on long range PCRs of expressed *vars*. The results obtained with this method supports quantitative PCR data showing group A and domain cassette 8 PfEMP1s being expressed at particularly high levels in severe childhood malaria.

OR53* Structural, functional and biochemical characterisation of *Plasmodium falciparum* pyruvate dehydrogenase complex

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Pyruvate dehydrogenase complex (PDC) comprises three enzymes; pyruvate decarboxylase (E1), dihydrolipoamide acyltrasferase (E2) and dihydrolipoamide dehydrogenase (E3). E2 forms the central structure of the large complex to which E1 and E3 bind. PDC converts pyruvate to acetyl-CoA, which in humans feeds into the citric acid cycle in the mitochondrion. Intriguingly, in the malaria parasite, *P. falciparum* (*Pf*), the sole PDC is found in the apicoplast and produces acetyl-CoA for fatty acid biosynthesis. Recently, PDC has been shown to be essential for parasite progression from the liver stage to the symptomatic intraerythrocytic stage. Thus, inhibiting PDC could prevent development of malaria. This work focuses on identifying and characterising structural and biochemical differences between human and parasite PDC that may be exploitable for the development of urgently required new anti-malarial drugs.
Codon optimised mature *Pf*E2 and *Pf*E3 have been recombinantly expressed in *E. coli*. r*Pf*E2, purified to near homogeneity with a final yield of 1–1.5 mg/L of bacteria is lipoylated by its expression host and is enzymatically active. Preliminary models for the solution structure of r*Pf*E2 determined using analytical ultracentrifugation (AUC) and small–angle x-ray scattering (SAXS) will be presented. r*Pf*E3 is insoluble; optimisation of the expression protocol will be described.

OR54* Plasmodium falciparum Inhibitor-3 Homolog Increases Protein Phosphatase Type 1 Activity and Is Essential for Parasitic Survival.

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Protein Phosphatase type 1 (PP1) is one of the major phosphates involved in protein dephosphorylation process. Many studies evidenced that PP1 is tightly controlled by several regulators as essentials as PP1 itself. Here we report the identification and characterization of a PP1 regulator in *Plasmodium falciparum*, designated PfI3 (inhibitor 3), which shared significant identity with Human I3. Nuclear Magnetic Resonance analysis showed that PfI3 belongs to the disordered protein family. High affinity interaction of PfI3 and PfPP1 was demonstrated *in vitro* using ELISA and Pull Down assay. We further showed that the conserved (41)KVVRW(45) motif was crucial for this interaction as the replacement of the Trp(45) by an Ala(45) severely decreases the binding to PfPP1. Surprisingly, PfI3 was unable to rescue a yeast strain deficient in I3 (Ypi1). This lack of functional orthology was supported as functional assays *in vitro* have revealed that PfI3, unlike yeast I3 and human I3, increases PfPP1 activity. Reverse genetic approaches suggest an essential role of PfI3 in the growth and/or survival of blood stage parasites. Finally, the main localization of a GFP-tagged PfI3 in the nucleus is compatible with a regulatory role of PfI3 on the activity of nuclear PfPP1.

OR55: Protective immunity to malaria, and how the *Plasmodium falciparum* parasites try to evade it

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Most people living in areas with stable transmission of *P. falciparum* parasites eventually acquire substantial immunity to malaria. However, the protection afforded is incomplete, fragile, and takes years to develop. Undoubtedly, a major reason for this is the ways the parasites have evolved to circumvent the host immune responses to infection. Antigenic polymorphism (allelic variation) and clonal antigenic variation are well-established major evasive strategies used by *P. falciparum*, but recent studies indicate that additional components are of importance. In this talk, I will review the available evidence, and discuss its implications for our understanding of immunity to malaria and for development of vaccines to combat this scourge.

OR56 Induction of Strain-Transcending Antibodies Against Group A PfEMP1 Surface Antigens from Virulent Malaria Parasites

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The PfEMP1 family of variant surface antigens encoded by *var* genes are adhesion molecules that play a pivotal role in malaria pathogenesis and clinical disease. PfEMP1 is a major target of protective immunity, however, development of drugs or vaccines based on PfEMP1 is problematic due to extensive sequence diversity within the PfEMP1 family. Here we identified the PfEMP1 variants transcribed by *P. falciparum* strains selected for a virulence-associated adhesion phenotype (IgM-positive rosetting). The parasites transcribed a subset of Group A PfEMP1 variants characterised by an unusual PfEMP1 architecture and a distinct N-terminal domain (DBLα1.5 or DBLα1.8). Antibodies raised in rabbits against the N-terminal domains showed functional activity (surface reactivity with live infected erythrocytes, rosette inhibition and induction of phagocytosis) down to low concentrations (<10 μg/ml of total IgG) against homologous parasites. Furthermore, the antibodies showed strain-transcending activity against heterologous rosetting parasites, including clinical isolates from four sub-Saharan African countries. The existence of shared surface epitopes that can be targeted by strain-transcending antibodies suggests that development of anti-rosetting therapies to prevent severe malaria is a realistic goal.

OR57 Molecular basis for evasion of the malaria parasite by cytoadhesion to human brain tissue

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The malaria parasite, *Plasmodium falciparum,* evades the human immune system by cytoadherence of parasitized erythrocytes to cellular receptors. Sequestration leading to accumulation of erythrocytes within the brain is characteristic of cerebral malaria, an often-fatal complication in infected individuals. Binding to host receptors is mediated by *P. falciparum* erythrocyte membrane protein-1 (PfEMP1), a large family of diverse, modular proteins. Here we present, for the first time, the molecular architecture of an interaction between a host ligand (intercellular-adhesion molecule-1 [ICAM-1]) and a PfEMP1. The structure, and supporting biophysical data, demonstrates that the PfEMP1-ICAM-1 interaction is mediated fully by a single Duffy binding-like (DBL) domain binding to the tip of ICAM-1. As ICAM-1 is strongly implicated in cerebral malaria this work is important in guiding the choice of vaccine components and helping us understand better PfEMP1's role in antigenic variation and evasion from the immune system.

OR58 *Plasmodium falciparum* neutralisation by anti-RH5 antibodies which block the RH5-basigin interaction

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Vaccines against *P. falciparum's* asexual blood-stage have not achieved clear efficacy in clinical trials. Challenges include antigenic polymorphism, recombinant antigen production, and achievement of high antibody titres without excessive reactogenicity. We have previously shown that vaccines based upon the full-length reticulocyte-binding protein homologue 5 (RH5) induce antibodies which neutralise all tested laboratory-adapted parasite strains. More recently, we have found that neutralisation of recently-isolated parasites by anti-RH5 is more potent than with anti-AMA1 antibodies, and have identified synergistic effects of mixtures of anti-RH5 IgG with other polyclonal antisera. We hypothesised that blockade of the interaction of RH5 with its receptor basigin was likely to be a mechanism of action of anti-RH5 antibodies. We have found that vaccine-induced polyclonal anti-RH5 serum is capable of blocking this interaction, as well as merozoite attachment to erythrocytes. We have also raised a panel of RH5-specific monoclonal antibodies: those which block the RH5-receptor interaction are capable of neutralising parasites. Minimal linear epitopes recognised by these antibodies were mapped, and are likely to be within or close to RH5's receptor binding site. These data support prompt clinical testing of RH5-based vaccines, and shed light upon the mechanism of action of anti-RH5 antibodies.

OR59 CD8$^+$ T Effector Memory Cells Protect against pre-erythrocytic Malaria

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Identification of correlates of protection for infectious diseases including malaria is a major challenge and has become one of the major obstacles to develop effective vaccines. We investigated the protection conferred by combinations of adenoviral and Modified Vaccinia Ankara (MVA) vectors expressing pre-erythrocytic malaria antigens. By classifying CD8$^+$ T cells into effector (T_E), effector/memory (T_{EM}) and central memory (T_{CM}) subsets using CD62L$^+$ and CD127$^+$ markers, we found striking differences in T-cell memory generation. While MVA induces accelerated T_{CM}, which could be efficiently boosted by subsequent adenoviral administration, it failed to protect against malaria. In contrast, adenoviral vectors, which permit persistent antigen delivery, elicit a prolonged T_E and T_{EM} response that requires long intervals for an efficient boost. A preferential T_{EM} phenotype was maintained in liver, blood and spleen upon Ad/MVA prime-boost regimens and animals were protected against malaria sporozoite challenge. Blood CD8$^+$ T_{EM} cells correlated with protection against malaria liver-stage infection, assessed by estimation of number of parasites emerging from the liver into the blood. The protective ability of antigen-specific T_{EM} cells was further confirmed by transfer experiments into naive recipient mice. Thus, we identify persistent CD8 T_{EM} populations as essential for vaccine-induced pre-erythrocytic protection against malaria, a finding that has important implications for logical vaccine design.

OR60 Immunopathology in leishmaniasis: friend or foe

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Chronic infectious diseases are often associated with varying degrees of tissue pathology. In the case of the infection with the protozoan parasite *Leishmania donovani*, the causative agent of human visceral leishmaniasis, pathology is markedly organ-specific, both in terms of its histopathologic basis and in its relationship to host protection vs. parasite persistence. This presentation will describe recent advances in our understanding of the cellular basis of host-protective granulomatous inflammation and of the lymphoid tissue remodeling that is characteristic of parasite persistence in the spleen. I will discuss our progress at using intravital imaging data to develop computational models of inflammation that may prove useful for pre-clinical drug evaluation and illustrate how an understanding of pathology can be used to identify new therapeutic approaches.

OR61 In *Leishmania major*-induced inflammation, Interleukin-13 down-regulates IL-1β and up-regulates IL-6 in an IL-4 independent mechanism.

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Infection with high dose of *Leishmania major* induces a sustained hyperalgesia in BALB/c mice. Although the cascade of cytokines involved in provoking the low pain thresholds, some anti-inflammatory cytokines, especially Interleukin-13 (IL-13), was shown to reduce this hyperalgesia during the treatment period (6 days) and for two extra days. This effect was accompanied by a significant decrease in the levels of IL-1β and a significant increase in the levels of IL-6 in the paws of mice throughout the whole experiment (3 weeks) showing that the hypoalgesic effect of exogenous IL-13 is IL-6 and IL-1β independent. Those results suggest that exogenous IL-13 reduces hyperalgesia, up-regulates IL-6 and down regulates IL-1β through different mechanisms. The ability of IL-13 to down-regulate TNF-α is suspected to mediate its hypoalgesic effect. On the other hand, since the production, secretion and effects of IL-4 are deeply affected by IL-13, it was hypothesized that IL-13 down-regulates IL-1β and up-regulates IL-6 by upregulating the levels of IL-4 during the whole experimental period. In this study, we investigated the effect of IL-13 treatment on the levels of IL-4 in the paws of mice during and after the treatment period. Our results showed that there is no correlation between the levels of IL-4 and neither the decreased IL-1β levels nor the increased levels of IL-6. Therefore, we conclude that IL-13 down-regulates IL-1β and up-regulates IL-6 in an IL-4 independent manner.

OR62 A protective role for MAP kinase phosphatase 2 in the control of parasite infection

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MAP kinase phosphatase 2 (MKP-2) is a nuclear phosphatase which negatively regulates the MAP kinase signalling pathway by dephosphorylating MAP kinases ERK, JNK and p38. In order to investigate a potential role of MKP-2 in immune responses against different pathogens we recently created MKP-2 deficient mice that were bred onto a C57BL/6 genetic background. We have shown that these mice, unlike their wild-type counterparts, were unable to control footpad infections with *Leishmania mexicana* and developed progressively growing lesions with high parasite burdens. Analysis of the parasite specific immune response showed a diminished Th1 and an enhanced Th2 response. Interestingly, MKP-2 was found to negatively control the expression of Arginase-I in macrophages and consequently MKP-2 deficiency resulted in increased macrophage Arginase-I production, decreased nitric oxide production and enhanced parasite growth. Nevertheless, infection of MKP-$2^{-/-}$ mice with another species, *L. major,* did not result in increased disease severity compared with wild-type animals despite there being a diminished Th1 response in MKP-$2^{-/-}$ mice and their macrophages being more permissive to infection than those of their wild-type counterparts. The immunological and cell biological mechanisms underlying these apparently paradoxical observations are being investigated.

OR63* Suppression of host immunity by polarization of monocytes is a hallmark of Indian Post Kala-azar Dermal Leishmaniasis

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Post Kala-azar Dermal Leishmaniasis (PKDL), caused by persistence of the parasite *Leishmania donovani,* occurs following cure from Visceral Leishmaniasis. As the immune status of monocytes-macrophages, essential host cells for the *Leishmania* parasite is a grey area in the context of PKDL, this study evaluated the functional status of circulating monocytes in Indian PKDL.
The study population included 15 patients with PKDL whose intracellular concentrations of proinflammatory and antiinflammatory cytokines, levels of nitric oxide (NO), reactive oxygen species (ROS) and non protein thiols in monocytes were measured along with expression of TLR-2, TLR-4, CD14, CD16, CD80 and CD86. Furthermore, monocyte polarization was measured in terms of intracellular levels of iron and gene expression related to iron signaling, arginase-I and Vitamin D signaling pathway.
In PKDL patients, circulating monocytes showed decreased expression of CD16, CD86, TLR-2 and TLR-4 as also decreased levels of NO, ROS and proinflammatory cytokines. Conversely, expression of arginase-I, levels of thiols and IL-10 within monocytes was raised, concomitant with a high intracellular iron load and altered vitamin D signaling.
Conclusions: In PKDL, altered immune functions causes monocyte polarization towards alternative activation. Therefore, chemotherapy should aim to repolarize monocytes into the classically activated form.

OR64* Toll-like receptors in cutaneous Leishmaniasis and as targets for vaccine adjuvants

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Despite our wide understanding of the immunopathology of cutaneous Leishmaniasis, an effective vaccine has not yet been developed for the disease. An area which requires attention in the development of an effective vaccine is adjuvant discovery and design. Toll-like receptors (TLRs) are major targets for adjuvants and have been shown to be crucial for defence against a number of infections. TLR2 recognises lipopeptides and has been linked to BCG efficacy, and has additionally been shown to recognise surface glycolipids of *Leishmania in vitro*. In this study, *in vivo* experimental infections show that TLR2 has a role in protection to cutaneous Leishmaniasis, as shown by increased lesion sizes, parasite burdens and regulatory immune responses in TLR2$^{-/-}$ mice infected with *L. major* and *L. mexicana*. Mice lacking TLR2 co-receptors TLR1 and TLR6 do not show increased susceptibility to infection, suggesting either mono-TLR2 function or alternative co-receptor involvement. These results suggest that TLR2 is involved in the protective immune responses to *Leishmania* and is a potential target for vaccine adjuvants. However, an experimental vaccine model showed that TLR2-targeting lipopeptides are ineffective adjuvants which can result in exacerbated disease upon challenge. Further research aims to uncover the mechanisms involved in TLR2 activation by *Leishmania*, and immune responses to vaccines containing lipopeptide adjuvants.

OR65 The host skin microcirculation analysis during blood feeding by *Rhodnius prolixus*

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Triatomine bugs are hematophagous insects and the vectors for *Trypanosoma cruzi* in the Americas Triatomines are vessel feeders, obtaining blood directly from the vessels of their vertebrate hosts.The objective of this study was to use the cibarial pump (CP) electromyogram and the analysis of venule wall movements during the engorgement phase to better understand the feeding process of *R. prolixus* on the mouse ear skin. A good synchrony between the contractions of the CP and vessel wall movements, as well as, the reduction in the blood pumping frequency was observed in all experiments. The CP frequency was inversely proportional to the average of the vessel area (functional diameter). At the beginning of the feeding process, the CP was at its higher frequency, resulting in a low functional diameter of the vessel. Platelet deposition and leukocyte recruitment were observed on the venular endothelium after removal of the mouthparts. This microenvironment in the feeding site triggers blood coagulation increasing blood viscosity. Probably, the increase of blood viscosity is responsible for the difficulties in maintaining the CP frequency by the insects.

OR66 Introducing the molecular sieve theory of *Leishmania* transmission

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A highly enriched population of metacyclic promastigotes is delivered by sand fly bite. Metacyclogenesis is accompanied by the accumulation of a viscous glycan gel secreted by the parasites. The promastigote secretory gel (PSG) blocks the sand fly gut and embeds the parasites in the gut lumen. PSG is formed of a dense 3D matrix of filamentous proteophosphoglycan which impairs the bloodfeeding behaviour of the sand fly and promotes transmission by regurgitation.
Here we extend the ‘blocked fly hypothesis’ of *Leishmania* transmission by showing that the non-infective promastigote forms of *Leishmania mexicana* bind to PSG in vitro and are immobilised within the gel-like plug. In contrast, the infective metacyclic forms cannot bind to PSG and is freely motile with it. This feature of PSG allows metacyclics to accumulate in high proportions in the anterior midgut, foregut and proboscis of the sand fly and ‘sieves’ non-infective forms from the infectious bite. These results identify a new example of stage-specific binding during the life cycle of *Leishmania* which influences vectorial capacity.

OR67 Maintenance of gut-microbial homeostasis in *Lu. longipalpis* and implications for *Leishmania* transmission.

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Lutzomyia longipalpis naturally harbour populations of *Leishmania infantum* parasite in the gut but the extent to which the parasite activates or suppresses the immune system of the insect vector is unknown. *Leishmania* do not elicit a strong reactive oxygen species (ROS) response although our gene silencing experiments suggest that an activated ROS response would kill the parasite.
To further investigate the sand fly immune response a Caspar homologue for a negative regulator of Imd immune pathway was identified. Caspar expression was significantly reduced in females between 3 and 6 days after a blood feed containing *Leishmania mexicana*. RNA interference was used to reduce Caspar expression in female *Lu. longipalpis* which were subsequently fed with *Leishmania* in a blood meal. Sand fly gut populations of both *Le. mexicana* and *Le. infantum* were significantly reduced in Caspar depleted females. The results support the hypothesis that Imd pathway effector molecules have anti-leishmanial activity that reduce the population of this parasite. The activation of the sand fly immune system, via depletion of this single gene, leads to the abortion of *Leishmania* development and the disruption of transmission by the phlebotomine sand fly.

OR68 Colonisation resistance in the bloodsucking sand fly *Lutzomyia longipalpis*: *Leishmania* protects its host from bacterial pathogenesis.

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Lu. longipalpis males and females are plant feeders but only females feed on blood for egg maturation. During their feeding visits, *Lu.longipalpis* have ample opportunity to ingest bacteria, yeasts, viruses and protozoans from plants and animals but little is known about the impact of these microbes on *Leishmania* growth and development within the sand fly gut. Gram-positive and gram-negative bacteria, as well as yeasts were isolated from the midguts of urban *Lu. longipalpis* in Teresina, a region endemic for visceral leishmaniasis in Northeast Brazil. Prior midgut colonization with different indigenous bacteria and yeast species significantly reduced numbers and infection rates of *Leishmania mexicana* promastigotes within the sand fly midgut. The antileishmanial effect increased proportionally with increase in bacterial load. Conversely, previous *Lu.longipalpis* infections with *Leishmania mexicana* extended the longevity of the sand fly when insects were challenged with the insect pathogen *Serratia marcescens*, when compared to bloodfed uninfected sand flies. One of the bacteria able to promote CR towards *Leishmania* in the females is an acetic acid bacterium of the genus *Asaia* isolated for the first time from wild caught *Lu. longipalpis* in Teresina. *Asaia* stably colonizes *Lu. longipalpis* male and female midguts; transformed *Asaia* expressing GFP were observed within the sand fly gut up to 10 days after inoculation. *Asaia* is an insect symbiont that is easy to transform, its ability to be venereally and vertically transmitted in sand flies is currently being assessed. This bacterial species is a potential candidate for studying sand fly paratransgenesis.

OR69* Investigating the roles of *Leishmania major* HASPB and SHERP proteins during metacyclogenesis in *Phlebotomus papatasi*

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During metacyclogenesis in the *Leishmania* life cycle, procyclic promastigotes differentiate into mammalian-infective metacyclic parasites within the sand fly vector. We have shown previously that parasites deleted for the *L. major* LmcDNA16 locus, (a region of chromosome 23 coding for the stage-regulated HASP and SHERP proteins), do not complete metacyclogenesis in the sand fly midgut, although metacyclic-like stages can be generated in *in vitro* culture (Sádlová *et al.,* 2010, Cellular Microbiology 12:1765). To determine the contribution of individual genes in the locus to this phenotype, we have generated a range of mutants in which target HASPB and SHERP genes are reintroduced into their original genomic locations within the *L. major* cDNA16 double deletion mutant. Replacement strains have been characterized with respect to gene copy number and stage-regulated protein expression, passaged through susceptible mice and then used to infect the *L. major* specific sand fly vector, *P. papatasi*. Following infection, the progress of parasite metacyclogenesis was monitored over twelve days by midgut dissection, parasite measurements and microscopy. Surprisingly, HASPB protein expression could not be detected in the replacement mutants within the sand fly midgut, although it was readily detected when the same parasite lines were cultured *in vitro.* These observations suggest a requirement for an as-yet-un-identified regulatory component for HASPB expression within the midgut, which is not required in culture.

OR70 Helminth Control in Ruminants – taking the line of least resistance

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Anthelmintic resistances has become a dominant force in parasitology research and in turn in approaches to parasite control, yet many of the current recommendations are not evidence-based, nor do they take into account their impact on the animals themselves, the interests of the farmer or the practicalities of livestock farming. Although there are intrinsic conflicts between anthelmintic-based control and the avoidance of anthelmintic resistance, they do not have to be mutually incompatible; nevertheless, it may require some paradigm shifts. One of the keys to this is an understanding of host-parasite interactions and their variability, and in changing the emphasis in diagnostics and monitoring from the parasite to the host. What actually matters, particularly in agricultural settings, is not just how many parasites there are, but what impact they are having on their domestic animal hosts. This is particularly relevant to infections with many of the common, endemic helminth parasites of ruminants, in which frank, clinical parasitic disease may be uncommon, yet sub-clinical infections frequently result in economically important, sub-optimal performance. The question that veterinarians should pose therefore is not simply which animals need treating, but, which animals would most benefit from treatment? It is incumbent on veterinarians and parasitologists to take into consideration some of the broader issues, such as green house gas emissions, in their advice to farmers, and, at the same time, not to forget that it is efficiency of production at the individual animal level that may have the most telling impact biologically, environmentally and economically.

OR71 Helminth egg output on UK Thoroughbred studs

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As Thoroughbred (TB) breeding studs in the UK rely heavily on anthelmintics to control helminth infections and relatively few establishments implement regular faecal sample analysis, it is important that knowledge of parasite distributions in managed horse populations be made available to those implementing parasite control programmes. Faecal samples from 1221 TBs, residing at 22 UK studs, were screened to determine the prevalence of infection in relation to equine age, gender and management practices. Strongyle egg distribution between individuals was highly over-dispersed (arithmetic mean = 95 epg, k = 0.111). The mean strongyle faecal egg count and degree of aggregation between individuals varied significantly between age and sex categories. Although, samples with ≥200 strongyle eggs per gram (epg) were received from a large proportion (16/22) of studs, those samples accounted for only 12% of all equines screened. Strongyle infections of ≥200 epg were associated with a number of management factors including the number of animals per establishment, last anthelmintic administered, co-grazing resident and visiting equines, rotational group grazing, resting pastures from grazing and moving equines to 'clean' pasture post-treatment. The findings from this study will pave the way for more efficient evidence-based strategies for targeted selective treatment (TST) in TBs.

OR72 Effect of anthelmintic treatment approach on the number and species of ovine gastrointestinal nematode parasites present.

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One concern with part flock treatment strategies such as targeted selective treatment (TST) strategies is that they may, over time, lead to increased pasture contamination. A five year experimental field study at Moredun has compared the TST approach with whole-flock treatment strategies in which anthelmintic was administered monthly, strategically (3 times per season) or on the appearance of clinical signs in some lambs. The replicated experiments were conducted each summer with 176 grazing lambs and also incorporated 4 worm-free tracer lambs that grazed for one month in May and September. As expected, total mean (min, max) worm recovery was lowest for monthly treatment (12538 (1150, 47300)) with similar total mean worm recoveries from the other treatment groups (26222 (1500, 88300); 28519 (100, 82200) and 26553 (1150, 80950) from TST, strategic and clinical groups, respectively). A range of species were identified, with *Teladorsagia circumcincta*, *Nematodirus battus* and *Trichostrongylus vitrinus* being most common. The interaction effect of treatment and year on the mean number of nematodes (total and species-wise) was not statistically significant (p = 0.45-0.89), suggesting that TST did not lead to an increased pasture contamination across years, compared with other strategies.

OR73 The relationship between parasitism and production in Scottish sheep.

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Understanding the relationship between the intensity of gastrointestinal nematode infection and lamb growth is essential for efficient sheep husbandry but different studies have given conflicting results. Following infection, sheep produce a type 2 response with increased parasite specific IgA and IgE activity, eosinophilia and mastocytosis. For natural and deliberate infection with *Teladorsagia circumcincta,* IgA and possibly eosinophils are associated with the control of parasite size and fecundity while IgE and mast cells are associated with the regulation of worm number. Mast cell degranulation is associated with destruction of tight junctions between epithelial cells, loss of appetite, reduced digestive efficiency, leakage of protein into the gut lumen and relative protein deficiency while IgA appears non-pathogenic. We therefore hypothesised that reduced bodyweight was associated with IgE rather than IgA activity. Analysis of 200 Texel lambs indicated that increased IgE activity was associated with reduced faecal egg counts and increased body weight ($p<0.05$) while increased IgA activity was associated with reduced egg counts but had no effect on growth. The absence of a growth effect for IgA was confirmed in a subsequent analysis of 1000 Scottish Blackface sheep. These results indicate that there is no simple trade-off between immunity and growth. The relative importance of IgA and IgE changes over time as the IgE response matures. Therefore the relationship between parasitism and production is also likely to change over time

OR74 A Questionnaire Based Survey of Current Endoparasite Control Practices on Sheep Farms in Northern Ireland

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For 40 years or so helminths have been controlled by the use of a small number of broad spectrum anthelmintics. However, over-reliance on a small portfolio of chemotherapeutic agents, coupled with anthelmintic abuse (under dosing, lack of rotation and overuse) has undermined the utility of many anthelmintics as long-term parasite control options. Of particular concern is the fact that reports of anthelmintic resistance are common-place, making effective parasite control increasingly challenging. Eighty Northern Ireland farmers’ attitudes to parasite control were surveyed, with particular attention on factors that may contribute to the development of resistance. The majority of respondents treated lambs most frequently, often following a set programme rather than providing treatment at the first signs of disease. There were a variety of obvious deviations from best practice in helminth control (SCOPS) that could encourage the development of anthelmintic resistance. For example, the majority of farmers (>60%) calculate anthelmintic dosage based on animal weight estimates, ~20% do not rotate anthelmintics and ~25% had relied on a single anthelmintic for a year or more. To ensure sustainable control there must be a change from historic routine blanket treatments to a more considered approach that encompasses education, anthelmintic rotation, the targeted use of anthelmintics and resistance testing alongside management and husbandry changes.

OR75 The relevance of the hygiene hypothesis as an explanation for the allergy epidemic in Latin America

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A high prevalence of asthma has been reported from Latin America and prevalence appears to be increasing in some countries. Asthma is caused by complex interactions between environmental exposures and genetic susceptibility but recent increases in asthma prevalence are likely to be caused by changes in environmental exposures. Such exposures are likely to include poverty, and alterations in lifestyle and other factors associated with the process of urbanization. It has been suggested that the hygiene hypothesis is unlikely to be relevant in Latin America because the asthma epidemic largely affects the urban poor whose living environment can hardly be considered hygienic. Recent studies from Brazil and Ecuador, however, indicate that most asthma in children is not associated with classical allergy and may be mediated by non-allergic inflammatory mechanisms. However, chronic childhood infections, such as helminth infections, may have an important role in the attenuation of atopy even though this may have little impact on asthma prevalence. Chronic infectious diseases of childhood, particularly helminth infections, may reduce the severity and persistence of allergic symptoms during childhood through their effects on atopy. If this is true, then a reduction in the prevalence of these infections that is an inevitable consequence of improvements in sanitation and living environments, a process that is occurring throughout Latin America, might be predicted to be associated with an increase in atopy, a shift in asthma from non-atopic to atopic, and an increase in the persistence and severity of childhood asthma.

OR76* The gastro-intestinal immune response during the expulsion of Ascaris suum

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Ascaris infections remain the most prevalent helminth infection in man and a major cause of abdominal disorders in tropical climate, despite mass drug treatments. Because of their high homology, *A. suum* infections in pigs can be used as a model for human infections with *A. lumbricoides*. During an infection with Ascaris suum in pigs, 4th stage larvae (L4) are expelled from the small intestine between 10 and 28 days post infection. The objective of this study was to identify effector mechanisms that play a key role in the expulsion phase. To investigate the contribution of the hepato-tracheal migration to the expulsion of *A. suum*, we infected pigs orally with either *A. suum* eggs or lung stage larvae. Animals that bypassed the initial migration were still able to expel the parasite 7 days after arrival in the small intestine. Whereas in natural infections we found a bias towards a Th1 type response, animals bypassing the hepato-tracheal migration showed no increase in Th1 or Th2 cytokine expression. In both experiments there was an upregulation of genes involved in innate immunity and a marked influx of eosinophils in the small intestine coinciding with the expulsion. We are currently investigating the cross-talk between eosinophils and L4 larvae. Taken together, our findings indicate that the expulsion of *A. suum* is a locally triggered phenomenon with eosinophils playing a prominent role.

OR77 Immune regulation and regulators in nematode infections

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Immunity to intestinal and tissue nematode infections involves innate and adaptive type-2 cell populations, cytokines and antibodies. In infection with the intestinal nematode parasite *Heligmosomoides polygyrus bakeri,* type 2 immunity is inhibited by a suite of immunomodulatory effects including expanded Foxp3+ regulatory T cell populations, skewed dendritic cell and macrophage phenotypes, B cell hyperstimulation and multiple innate cell responses within the intestinal environment. Infected mice adopt an immunoregulated phenotype, with abated allergic and autoimmune reactions, reflecting systemic changes resulting from localised parasitism. The ability of the helminth to influence this spectrum of host immunological responses is likely to be mediated by products released by live parasites, represented by *H. polygyrus* excretory-secretory material (HES) collected from adult worms cultivated in vitro. HES directly induces Foxp3+ regulatory T cells, and recapitulates the suppressive effect of infection on airway allergy. High-throughput transcriptomic and proteomic analysis to identify all major components of HES now offers a range of novel immunomodulators as well as candidate vaccines for the induction of protective immunity, with potential for translation into major human and animal diseases.

OR78 Epigenetic Control of Th2 Induction by Dendritic Cells

Cook, P.C.[1], Deaton, A. [2], Owen, H. [2], Thomas, G., Borger J.G. [1], Jones, L.H. [1], Phythian-Adams, A.T.[1], Lundie, R.J.[1], Webb, L.M.[1], Grainger J.R. [1], Maizels, R.M.[1], Ivens, A.[1], Bird, A.[2], MacDonald, A.S.[1]
[1]IIIR, University of Edinburgh, Edinburgh, EH9 3JT, UK. [2]Wellcome Trust Centre for Cell Biology, University of Edinburgh, Edinburgh EH9 3JR, UK

Dendritic cells (DCs) play a critical role in Th2 priming during helminth infection, yet the mechanism by which they direct Th2 polarisation is poorly understood. Since Th2 inducing DCs display minimal transcriptional activation, we investigated whether Th2 priming by DCs is dependent on epigenetic regulation of gene transcription via methyl-CpG binding domain protein-2 (MBD2), which links CpG methylation to repressive chromatin structure. We generated mice with conditional deletion of MBD2 in CD11c+ cells ($CD11c^{cre}MBD2^{fl/fl}$) and found these animals displayed significantly impaired Th2 development following injection of eggs from the parasitic helminth *Schistosoma mansoni*. This shows that MBD2 is important in controlling Th2 priming by DCs. To further address this, we found bone marrow derived DCs from global $MBD2^{-/-}$ mice pulsed with soluble egg antigen (SEA) from *S. mansoni*, were less able than WT DCs to promote Th2 responses *in vitro* and *in vivo*. This demonstrates epigenetic regulation of DCs, via MBD2, can be critical for Th2 induction and development. Ongoing work is investigating which genes are targeted by MBD2, with the aim being identification of specific mechanisms employed by DCs that are fundamental for Th2 promotion.

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OR80 Transcriptomic analysis of the host response to infestation with the ectoparastic mite *Psoroptes ovis* (*P. ovis*)

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Moredun Research Institute, Pentlands Science Park, Edinburgh. EH26 0PZ.

Sheep scab is a highly contagious ectoparasitic disease of sheep caused by the mite *Psoroptes ovis* (*P. ovis*). Host response to infestation is directed against mite secretory/excretory products and is typical of an immediate hypersensitivity reaction. Vaccine candidates have been identified by fractionating mite protein extracts. However, efficacy is low and the isolation of the individual protective proteins has proven difficult. To further improve development of a sheep scab vaccine we must first gain a better understanding of the host-parasite relationship. To further characterise the early events (first 24 hours) in the host response to infestation we utilised an ovine transcriptome microarray to interrogate the transcriptional events associated with the local skin response to infestation. Clustering and network analyses of this dataset have enabled identification of the key signalling events involved in the host response to infestation with *P. ovis*. This has provided unique insights into the pro-inflammatory response instigated by *P. ovis*, with a key role identified for the transcription factor NF-kB. It has also provided vital information regarding the nature of the mite factors that may trigger this response. This study has increased our knowledge and understanding of the host response to *P. ovis*, identifying potential strategies for novel methods of disease control, including vaccine development.

OR81* Development of a diagnostic test for sheep scab using biomarkers

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Sheep scab is a highly contagious ectoparasitic disease caused by the mite, *Psoroptes ovis.* The disease causes intensely pruritic lesions with severe dermatitis and is a major welfare and production issue in the national flock. Sheep scab was recently made notifiable in Scotland indicating that diagnostic tests will be crucial tools in future disease control. Serological diagnostic tests indicate exposure to the parasite but do not indicate current infestation or disease. Biomarkers in the serum may be useful in overcoming this limitation. Recent microarray studies identified over 600 host genes differentially expressed in circulating leukocytes following *P. ovis* infestation. They were initially filtered to identify the most promising biomarker candidates, resulting in a final list of 178 potential biomarkers, 13 of which were assessed using a range of sera from sheep scab infested and naïve sheep. Promising results have been obtained for 2 selected proteins showing they are up-regulated following infestation, correlate with disease progression and fall rapidly post-treatment. Further work includes combining the biomarker diagnostic with a *P. ovis* specific antibody test currently being developed at the Moredun Research Institute, to give a full profile of disease status of individual animals in a flock. This would provide the sheep industry with a powerful diagnostic test crucial for any control or eradication programme.

OR82 Human and ruminant fascioliasis in central Vietnam, 2007-2010

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Fascioliasis has been documented in 45 of 64 provinces in Vietnam and is a significant problem in the centre of the country, with seropositivity rates of up to 8% in humans in some regions. Over 3 million head of cattle are kept in Vietnam, mainly in the coastal and plain areas and high rates (15-79%) of *Fasciola* infection have been documented.
As effective prevention and control measures are hindered by a lack of data on the current burden of disease, this study was conducted to examine the epidemiology of human and veterinary fascioliasis in Quang Ngai in central Vietnam. Additionally, efforts were made to identify possible reservoirs and risk factors of infection in both the human and veterinary communities.
Our study has confirmed that fascioliasis represents a significant veterinary problem in central Vietnam and action should be taken to prevent and control this infection not only to avoid poor reproductivity and/or weight loss but to prevent transmission to humans. Ruminants represent the major reservoir of infection for human populations in these rural areas. Less than 10% of our surveyed population owned a buffalo, whereas 50% owned at least one cow, it may be worth focusing particularly on control of bovine fascioliasis. From our survey, antihelmintic use in cattle was less than 5%. Administration of triclabendazole would have a positive impact. Additional efforts should be also made to educate and inform at-risk populations of the known risk factors to avoid morbidity from *Fasciola.*

OR83* The search for "hidden antigens" in the liver fluke, *Fasciola hepatica*

Heather M^c^Dougall, Alasdair Nisbet, Dave Knox, & Philip Skuce
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With the increasing incidence of Fasciolosis and emergence of flukicide resistant parasite populations, a vaccine to control *Fasciola hepatica* is an attractive alternative control strategy. Hidden gut antigens have provided protection against infection with blood-feeding parasites, including the gastrointestinal nematode, *Haemonchus contortus* and the cattle tick, *Rhipicephalus (Boophilus) microplus*. Here, we attempt to identify hidden gut antigens in *F. hepatica.*
A screen with a panel of lectins identified 7 with an affinity for gut glycoproteins. Two lectins, PNA and JAC, showed a preference for glycoproteins on either the gastodermal cells or gut lamellae, respectively. These were used to enrich a crude somatic extract by affinity chromatography. Subsequent proteomic analysis of the resultant fractions revealed that, in addition to a number of other proteases, the PNA lectin enriched for a Cathepsin D-like enzyme. Further investigation localised this protease to the gastrodermal cells in the gut and some reproductive organs of the parasite. Other studies of the somatic extract identified the presence of an aspartyl protease which is optimal at low pHs and potentially has a role in digestion of haemoglobin (a potential food source). Vaccination with aspartyl proteases has elicited protection against infection with Schistosomes and hookworms, this enzyme may also prove to be a promising vaccine candidate for *F.hepatica*.

OR84 Control of a parasitic nematode in sheep by vaccination with a recombinant antigen cocktail

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Teladorsagia circumcincta is the major cause of parasitic gastroenteritis in small ruminants in temperate regions. As sheep can acquire a protective immune response against *T. circumcincta* in natural and experimental circumstances, vaccination is a possible alternative for control. We have developed a strategy to identify putative host protective antigens by studying local antibody responses directed at proteins specific to post-infective larvae. Antigens were also selected on the basis of their potential immunomodulatory role at the host/parasite interface. Recombinant versions of five immunogenic molecules were combined with recombinant versions of three proteins that have potential immunoregulatory activities and were administered to sheep as a single vaccine formulation. The animals were subsequently subjected to a trickle challenge infection regime with *T. circumcincta* infective larvae. The trial was performed twice. In both trials, vaccinated sheep had significantly lower mean faecal egg counts (FEC) over the period of the experiment, with an overall mean FEC reduction of 72% (Trial 1) and 58% (Trial 2). During the peak egg shedding periods vaccinated sheep shed 92% and 73% fewer eggs than control sheep in Trials 1 and 2 respectively. At post mortem, vaccinated sheep had 75% (Trial 1) and 57% (Trial 2) lower nematode burdens in the abomasum than those in the control group. These results will be presented in full and discussed in terms of their potential impact compared to previous recombinant and native vaccines against helminths.

OR85 Desensitisation as a method of mitigating production losses associated with parasitic nematode infections of livestock.

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Gastrointestinal parasites are widely considered to be the major cause of poor growth rates in grazing ruminants worldwide. The majority of production losses associated with temperate parasite species of sheep such as *Trichostrongylus colubriformis* have been attributed to the anti-parasite immune response. Methods to down-regulate the immune response may reduce production losses associated with these parasites. In this study, helminth-free lambs (n=9) were desensitised to *T. colubriformis* by three weekly injections of somatic antigens into the rectal submucosa. A control group (n=9) was immunized with PBS alone. One week after the final injection animals were trickle infected with 2000 *T. colubriformis* L3 larvae/day for nine weeks. Throughout the infection desensitized lambs exhibited significantly increased voluntary feed intakes ($P < 0.001$) and live-weights ($P=0.01$), resulting in a 4kg advantage in live-weight and a 2kg advantage in carcass-weight at the end of the study. Desensitised animals exhibited less fecal scouring and had significantly fewer intestinal globule leukocytes ($P<0.05$) but greater numbers of intestinal mast cells, suggesting that desensitization resulted in impaired mast cell degranulation. Antibody responses and parasitological data were unaffected. These results indicate that desensitization represents a useful and novel approach to mitigate production losses associated with ruminant nematode infections.

OR86 Chimeric antigens for the vaccination against *Neospora caninum*: study in the pregnant and in the non pregnant mouse model

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The overall goal of our investigations on *N. caninum* is to develop a vaccine that limits both the cerebral infection and the transplacental transmission. We created four different chimeric proteins composed of the predicted putative antigenic domains of three *N. caninum* proteins (NcMIC1, NcMIC3 and NcROP2) placed in different order. Balb/C mice were vaccinated with the different antigens and challenged with *N. caninum* tachyzoites. One of the chimeric proteins (recNcMIC3-1-R) conferred a significant protection against cerebral infection and was further tested in a pregnant mouse model. However, any significant protection against transplacental transmission and against cerebral infection in the pregnant dams was observed. In both vaccination trials, the vaccine induced a Th2 biased immune response with high IgG1 and high IL-4 titers in sera. The high IgG1/IgG2a ratio remained true after vaccination in both models. However, while the non pregnant mice showed a higher IFN-y/IL-4 ratio after challenge, the pregnant mice showed an overall lower cytokine production with a higher IL-4/IFN-y ratio. The lack of protection observed in the pregnant mouse model is thought be due to a modulation of the immune response toward a Th2 type immune response during pregnancy, thus hampering the mixed Th1/Th2- type immune response associated with protection in the non pregnant mouse model.

OR87* IgA better than FEC to indicate resistance in naturally infected lambs

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Essentially all grazing sheep are infected with gastrointestinal nematodes and faecal egg counts are widely used to assess the intensity and severity of infection. However, their relationship with worm number is non-linear for *Teladorsagia* circumcincta; due to density-dependent constraints on fecundity sheep with high worm burdens produce few eggs. We propose IgA activity against fourth-stage larvae as an alternative marker of the severity of infection with *T. circumcincta.* IgA activity is closely related to worm fecundity. A dynamic data-driven model was developed to predict the results of selection after 10 generations when selecting for reduced FEC or selecting for high plasma IgA responses. The response to selection was 50% faster when selecting on plasma IgA. These results are compatible with independent field observations. Plasma IgA is also cheaper and easier to sample and analyse therefore it offers a good alternative to FEC for farmers and breeders.

OR88 Detection and characterisation of an immunodominant antigen present on the surface of *Ascaris suum* L3 larvae.

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Roundworms are universal and important parasites of the small intestine of humans (*Ascaris lumbricoides*) and pigs (*Ascaris suum*). A continued exposure to *Ascaris* induces immunity at the level of the gut in pigs and possibly also humans, protecting the host against the migrating larvae**.** The aim of this project was to identify & characterise parasite antigens against which this immune response is directed. Pigs were immunized by trickle infection for 30 weeks and subsequently challenged and euthanized two weeks after challenge. At necropsy, there was a 100% reduction in L4's recovered from the intestine and a 97,2% reduction in white spots on the liver in comparison with challenge controls. Antibodies purified from the mucus were subsequently used to probe larval extracts resulting in the specific recognition of a 12 kDa antigen (As12) by antibodies of immune pigs. As12 is present on the surface of infective L3 larvae of *A. suum* and *A. lumbricoides* and appears to be shed off actively by the larvae. Furthermore, the As12 is highly resistant to different enzymatic and chemical treatments and is most likely a glycolipid. Since this molecule could be of significant importance to the survival of the parasite during the initial stages of infection, further studies are currently being undertaken to unravel its composition and structure.

OR89 A novel role for interleukin-1 beta in promoting chronicitiy of intestinal helminths.

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Intestinal helminthes often form long-lived infections, living for 1 to 2 years as adult worms within the intestinal lumen of their human hosts. Such chronicity no doubt results from the long co-evolution between helminths and their mammalian hosts, however the molecular mechanisms by which these organisms avert immune rejection are not clear. In the search for factors regulating the chronicity of infection we compared cytokine production within the intestine of animals infected with the nematodes *Heligmosomoides polygyrus* (Hp) or *Nippostrongylus brasiliensis* (Nb) which repectively form chronic or acute infections in wildtype C57BL/6 mice. This analysis revealed that production of interkeukin-1 beta (IL-1β) within the intestine is increased in Hp infected mice compared to Nb infected mice. We further determined that IL-1β normally functions to attenuate protective immunity and promote parasite chronicity following Hp infection, as demonstrated by a more rapid expulsion of adult parasites and enhanced type 2 adaptive immune responses in IL-1$\beta^{-/-}$ mice. Enhanced Th2 cell expansion in the absence of IL-1β was driven by an increased production of intestinal IL-25 at early timepoints following infection and a subsequent expansion of type 2 innate lymphoid cells. Although IL-1β levels correlated with increased dissemination of intestinal bacteria in Hp infected mice, IL-1β production did not require the presence of commensal bacteria and Hp products were observed to activate caspase-1 *in vitro* . These data indicate that Hp has the capacity to mediate inflammasome activation directly and reveal a novel and unexpected role for IL-1β in promoting helminth chronicity.

OR90 PD-1 mediated Th2 cell hypo-responsiveness determines susceptibility to helminth infection

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Susceptibility to helminths is synonymous with immune suppression, which prevents the development of protective memory and results in impaired Th2 immunity. Although Th2 cell anergy has been proposed as a regulatory outcome, the intrinsic fate of Th2 cells during chronic helminth infection is largely unknown. Using a permissive model of filariasis, *Litomosoides sigmodontis* infection of BALB/c IL-4gfp reporter mice, we demonstrate that Th2 cells are conditioned towards an anergic phenotype during infection resulting in susceptibility. Despite increasing in number during infection, parasite-induced IL-4gfp$^+$ Th2 cells became functionally hypo-responsive denoted by an impaired intrinsic ability to produce IL-4, IL-5 and IL-2 proteins. Th2 cell hypo-responsiveness correlated with PD-1 expression, and mAb-mediated blockade of PD-1 during infection led to a long-term recovery in Th2 cell functional quality and resistance to infection. Antibody-mediated blockade of PD-L1 and PD-L2 demonstrated that whilst both ligands down-regulated Th2 responses during infection, only PD-L2 determined susceptibility. Thus, Th2 cell intrinsic hypo-responsiveness and the PD-1 pathway are key immune regulatory elements determining the outcome of filarial nematode infection.

OR91 Th2-type and wound healing responses after repeated exposure to schistosome larvae.

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Invasive larvae of *Schistosoma mansoni* infect the mammalian host via a percutaneous route and cross the epidermis and dermis before exiting via the vasculature and/or lymphatics. The larvae elicit a rapid but transient inflammatory response in the skin and prime an antigen–specific $CD4^+$ response in the draining lymph nodes. However, in a murine model of repeated infection, it was discovered that local and systemic $CD4^+$ cell responses were significantly down-regulated, in advance of the known down-regulation observed after chronic exposure to eggs. Responsiveness was heavily influenced by the Th2-type immune environment at the skin site of repeated infection. The skin of multiply- infected mice contain an abundance of eosinophils, mast cells, arginase-1^+ $Ym1^+$ (alternatively-activated) macrophages, and $MHCII^{hi}$ antigen presenting cells which were functionally compromised. This dermal environment is reminiscent of a wound healing response and is accompanied by angiogenesis as evidenced by significant increases in angiopoietin 2, matrix metalloproteinases, and other pro-angiogenic factors. This response may be due to tissue damage caused by migrating larvae. Nonetheless, excretory/secretions released by invasive larvae induced similar effects in endothelial cells *in vitro*, and caused the growth of new vessel into Matrigel plugs administered *in vivo.* Therefore, it appears that wound healing is promoted by both host- and parasite-derived factors which act together to aid host and parasite survival.

OR92 Simvastatin as an Anti-Helmintic

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Statins are 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors that are typically used to lower cholesterol. Animal models have also shown that they are beneficial in treating a variety of autoimmune diseases by skewing an immune response to a Th2 phenotype. The efficacy of statin treatment in helminth infections, where Th2 responses are protective, has never been assessed.
Trichurs muris is a caecal-dwelling nematode that elicits a resistant Th2 or susceptible Th1 phenotype in inbred strains of mouse. Typically susceptible mice are the AKR strain that produces large amounts of IFN-gamma.
Simvastatin treatment of AKR mice significantly decreased worm burdens though did not overtly affect Th1 or Th2 cytokine production. Simvastatin treatment of *Trichinella* infected animals however had no significant effects on worm burden or cytokine production suggesting that simvastatin might be active against the egg stage of *T. muris*. Indeed, treatment of animals at the point of infection reduced worm burden whereas treatment after hatching (d5 post-infection) did not. As hatching is known to be triggered by intestinal bacteria, numbers of bacteria at the time of hatching was ascertained and were found to be unaffected by treatment. Recent advances in genomic sequencing of *T. muris* have now highlighted a potential HMG coA Reductase gene in this parasite that would explain the effects of simvastatin and could provide a putative target for future anti-helmintics.

OR93 *Toxoplasma gondii*: genetic diversity around the world and insight into genotypes and virulence of *T. gondii* in Germany.

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Toxoplasma gondii is an intracellular protozoan parasite that can infect almost all vertebrates, including humans and birds, world-wide. Wild and domestic felids are the definitive host of *T. gondii* shedding the highly infectious, environmentally resistant oocyst stage of the parasite. Felids can acquire *T. gondii* by ingesting already infected prey (such as mice or birds) or by taking up sporulated oocysts. The population structure of *T. gondii* is clonal. Sexual recombination between different *T. gondii* types is rare and can only occur in the intestine of felids. While clonal types I, II and III are predominantly observed in Europe, non-canonical and atypical types dominate in South America, Asia and America. In total, 14 haplogroups are currently recognized world-wide. Genetic diversity seems higher in areas where higher numbers of felids and more feline species exist that can effectively drive recombination events in the wild. Mouse virulence varies considerably between different *T. gondii* types. While infection with only a single parasite of clonal type I is lethal for mice, 10^3 or even more parasites of clonal types II and III are needed to achieve the same effect. *T. gondii* isolated from feline faecal samples and wild animal tissues in Germany were genotyped by PCR-RFLP using nine unlinked independent genetic markers (newSAG2, SAG3, BTUB, GRA6, c22-8, c29-2, PK1, L358 and Apico). The majority of feline faecal, fox and beaver samples isolated from Germany contained *T. gondii* of clonal type II. Additionally, a few specimens with *T. gondii* type III and a small number of mixed *T. gondii* types were observed. Interestingly, a single oocyst sample from a naturally infected cat displayed a combination of type II- and type III-alleles at several different loci. IFN-γ-knockout and BALB/c mice were infected with this oocyst sample. Parasites isolated from infected mice were subsequently cultivated in VERO cells. Tissue culture-derived parasites still showed a combination of type II- and type III-alleles at several loci. Individual, genetically different *T. gondii* clones were obtained by limiting dilution. The majority of these clones were highly virulent for mice, but, interestingly, less mouse-virulent and avirulent clones could also be found. Mouse virulence was also shown to differ between *T. gondii* clones of the same genotype. We present first evidence of sexual recombination and re-assortment of chromosomes of *T. gondii* in a naturally infected cat and show that new *T. gondii* genotypes of high virulence have formed under natural conditions.
This study was funded by the German Federal Ministry of Education and Research (BMBF) grant 01 KI 0765; Toxonet01 and grant 01 KI 1002F; Toxonet02 as part of the Toxonet01 and Toxonet02 Network (National Research Platform for Zoonoses).

OR94 Strategies for assessing genetic diversity in *Eimeria* species parasites of poultry.

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Protozoan parasites of the genus *Eimeria* cause coccidiosis, a severe enteritis that affects many livestock species, most notably poultry. Cost effective recombinant anticoccidial vaccines are becoming a realistic prospect due to the identification of several immunoprotective antigens. However, genetic resistance to anticoccidial drugs can occur rapidly in the field and a similar fate could befall novel anticoccidial vaccines that rely on a small numbers of antigens if these are polymorphic and selectable. To predict the likely efficacy and longevity of such vaccines in the field it is important to know the prevalence of naturally occurring genetic (antigenic) diversity. We aim to determine *Eimeria* species field population structure and investigate the relevance of naturally occurring genetic diversity in two of the world's poorest regions, Africa and India. We have constructed a preliminary molecular phylogeny based on amplification of the ITS-1/-2 region from nuclear ribosomal gDNA to assess genetic diversity in African field samples. In tandem based on an MLST panel of genes of interest, including several immunoprotective antigens, we are developing strain specific genotyping strategies including RFLP and qPCR assays. Our findings will be integrated with the development of novel anticoccidial control strategies. The project forms an integral part of a collaborative project between partners based in the UK and India under the 'Combating Infectious Diseases of Livestock for International Development' initiative.

OR95 Increased *Toxoplasma gondii* positivity relative to age in 125 Scottish sheep flocks; evidence of frequent acquired infection

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Toxoplasma gondii seroprevalence was determined in 3333 sheep sera from 125 distinct sheep flocks in Scotland, which were collected between July 2006 and August 2008. The selected farms are representative for the Scottish sheep flocks. Overall *T.gondii* seroprevalence, at individual sheep level, was determined to be 56.6%; each flock tested, had at least a single positive animal and in four flocks all ewes tested positive. The seroprevalence of sheep increased from 37.7% in one year old stock to 73.8% in ewes that were older than six years, showing that acquired infections during the life of sheep is frequent and that environmental contamination by *T.gondii* oocysts must be significant. The median within-flock seroprevalence varied significantly across Scotland, with the lowest seroprevalence in the South (42.3%) and the highest seroprevalence in the North of Scotland and the Scottish Islands (69.2%). This distribution disequilibrium may reflect the density and survival of oocysts on pasture and lambing areas. A questionnaire accompanying sampling of flocks identified that Toxovax® (commercial vaccine that protects sheep from abortion due to *T.gondii* infection) uptake was low (24.7%) and that it did not significantly affect within flock seroprevalence for *T.gondii*. This study highlights that lamb/mutton maybe a significant source for human *T.gondii* infection.

OR96* Evidence of the three main clonal *Toxoplasma gondii* lineages in British wild carnivores.

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Toxoplasma gondii is a zoonotic pathogen that has the ability to infect all warm blooded mammals including humans. Wildlife can act as reservoirs for *T. gondii* infection. Studying the genotype of *T. gondii* in wildlife provides an indication of strains which can potentially be transmitted to livestock and humans. Three main clonal lineages exist (type I, II and III) within Europe type II is most commonly identified in humans and animals. Currently very little Infomation exists relating to strains in Britain.
DNA was extracted from tissue samples from ferrets, polecats, badgers, foxes, mink and stoats, which had originated from various locations throughout the UK. A PCR, specific for *T. gondii*, was used to detect the presence of the parasite DNA, this was followed by strain genotyping (PCR-RFLP) and sequence analysis.
The prevalence of *T. gondii* within these animals varied from 6% to 44% depending on host species. Type II was the predominant lineage found, however type III and an allele for type I lineages were also identified, though no atypical genotypes were found.
The influence of genotype on human infection is still not fully understood, however certain genetic types may be associated with human clinical toxoplasmosis. This study highlights the presence of alleles for all three lineages with potential for their transmission to humans via infected livestock, or directly by cats.

OR97 Genome sequences of four Eimeria species reveal that most gene sequences are interrupted by homopolymeric amino acid repeats

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Eimeria spp. cause coccidiosis in a range of livestock, causing most serious disease in poultry. We have sequenced the genomes of four *Eimeria* species infecting chickens: *E. tenella*, *E. acervulina*, *E. maxima, E. necatrix*. These genomes contain regions of high repeat content, interspersed with almost no repeats. We found that there is a preference for a particular class of repeat to occur within the coding sequence of genes. These trinucleotide repeats (CAG) are found in 56% of all genes in *E. tenella* and 40% of genes involved in basic cellular processes common to all eukaryotes in up to 88 copies. They are predicted to preferentially code for glutamine, alanine or serine but rarely cysteine or leucine, suggesting selection is acting upon them. These repeats are analogous to those causing human diseases of protein aggregation such as Huntingdon's disease. Homopolymeric amino acid repeats are twice as common in *Eimeria* as in any other genome sequenced to date. We describe the structure of these genomes, aided by optical mapping techniques, and examine the role of repeats in parasite biology.

OR98 Schistosome modulation of inflammatory diseases: the good and bad of a two-sided coin.

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Helminth infection of man evokes profound modulation of the immune system of the host. In experimental animal models, it has also been shown that infection with various helminths induce a modulated state of immunity. Using a range of animals of inflammatory disorders - such as rheumatoid arthritis (CIA), multiple sclerosis (EAE), inflammatory bowel disease (various models) as well as models of allergic information - helminth infections have been shown to influence the course of these diseases. Such studies have prompted the use of live helminths infections as a therapeutic strategy for patients with unrelated inflammatory disorders, in particular inflammatory bowel disease, multiple sclerosis and allergic-like conditions. Schistosome infections are characterized by a propensity to induce potent type 2 allergic-like responses as well as regulatory phenotypes in man and also in mouse models. Schistosome infections of mice can exacerbate and/or reduce inflammation in experiment models of unrelated diseases depending on the model and type of infection used. In this presentation, the potential for Schistosome infections to alter inflammation in the context of a good “protective” or bad “exacerbating” outcome will be examined. Insight gained into novel immune pathways from such studies will be presented.

OR99* Steady state dendritic cells depend on Type I IFN responsiveness for optimal induction of T cell responses against *Schistosoma mansoni*

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Relatively little is known about how dendritic cells (DCs) become activated and function in response to Th2 associated parasitic helminths. Murine bone marrow cultured with Flt3-L differentiates into DC subsets thought to be representative of populations generated *in vivo* in the steady-state. Using FL-DCs we can assess how steady-state DCs respond to soluble egg Ag (SEA, a potent Th2-inducing Ag) from *Schistosoma mansoni*. Although displaying a ‘muted’ phenotype in comparison to bacterial activation, SEA-conditioned FL-DCs capably induced Th2 responses following adoptive transfer into naïve mice. Using FL-DCs we have identified an SEA-specific Type I IFN signature from steady-state DCs. Although Type I IFNs are primarily associated with anti-viral immunity and Th1/17 responses, the role of Type I IFN in Th2 settings is currently unknown. Surprisingly, SEA-pulsed IFNαβR$^{-/-}$ FL-DCs failed to induce Th2 responses, indicating that Type I IFN responsiveness is required for efficient T cell polarisation against helminth Ags by steady-state DCs. Current work is focusing on determining the impact of IFNαβR deficiency on DC phenotypic activation in response to *S. mansoni*, and dissecting the role of Type I IFN during Th2 development against the parasite *in vivo*.

OR100 Immunomodulation during natural and experimental human helminth infection.

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Experimental and field studies have suggested that helminth infection modulates immune responses directed against unrelated antigens. The factors affecting the magnitude and dynamics of the modulation of these immune responses in helminth-exposed people are still poorly understood. We have been conducting studies characterising the relationship between infection with the helminth *Schistosoma haematobium* and immune responses to unrelated antigens, including common allergens (house dust mite), self-antigens (nuclear antigens) and antigen from other pathogens *(Plasmodium falciparum*) as well as investigating the underlying immune responses/mechanisms involved through comparative human studies. In addition to these studies we have also been analysing the immunological outcome of experimental infection of allergic rhinitis suffers with the pig whipworm *Trichuris suis.* Our results highlight the importance of several factors including; current vs. previous parasite infection, infection intensity and antihelminthic treatment in the association between helminth infection and allergic sensitisation as well as autoreactivity.

OR102 Neuropeptidergic signalling in *Caenorhabditis elegans*

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Nematodes include major parasites of livestock, plants and humans in addition to free-living species such as *Caenorhabditis elegans* that can serve as an important model to study neuropeptidergic signalling systems. Bioactive peptides occur in the whole animal kingdom and are involved in most physiological processes. They are derived from large proprotein peptide precursors that require several highly regulated post-translational events to yield the mature peptides. Bioactive peptides are attractive for pharmaceutical use as they can be employed as therapeutics or as indirect targets via their respective receptors or processing enzymes. Understanding the functional role of neuropeptides, however, is unfortunately hindered by the absence of primary sequence information, knowledge of their processing enzymes and identity of their cognate G protein-coupled receptors. Peptidomics aims to identify endogenously present (neuro)peptides by using liquid chromatography and mass spectrometry in a high-throughput way. Using various LC-MS setups, we were able to biochemically characterize neuropeptides from *C. elegans.* Some of the identified peptides display profound sequence similarities with bioactive peptides from other invertebrates, indicating that these peptides have a long evolutionary history and can be considered as attractive targets for drug discovery. We also identified and characterized the main processing enzymes by comparing peptide profiles of deletion mutants deficient in presumed neuropeptide processing enzymes.
Combining peptidomics and reversed pharmacology, where an orphan receptor is used as bait to fish out the activating ligand form a library of synthetic peptides or from HPLC fractions, allowed us to characterise diverse neuropeptidergic signaling systems in *C. elegans*. Here we will focus on the cholecystokinin/gastrin and the pigment-dispersing factor systems that are highly conserved.

OR103 RNA interference as a receptor deorphanisation tool in plant pathogenic nematodes

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Plant pathogenic nematodes (PPNs) impose a significant economic burden on plant cultivation efforts worldwide. Recent estimates predict losses across all sectors of approximately $125 billion annually. Conventionally, an integrated approach to PPN management has relied heavily on various nematicides. As environmental concerns rise over the systemic effects of sustained nematicide use, withdrawal has left a significant shortcoming in our ability to manage this problem and highlights the need for novel and robust control methods. G-protein coupled receptors (GPCRs) are one of the most drugged targets in human medicine, and represent excellent candidates for the development of novel small molecule nematicides. To aid the rational development of next-generation controls, efficient modes of receptor deorphanisation are needed in PPNs. Here we present data which demonstrate the utility of an RNAi-mediated null phenotype matching and co-localisation approach to linking neuropeptide and GPCR function. This study reveals that *Gp-flp-21* is involved in coordinating positive chemotaxis in the potato cyst nematode *Globodera pallida*. Further, using bioinformatic approaches we have identified a candidate *Gp-flp-21* G-protein coupled receptor (GPCR), and show that RNAi reveals matching behaviours and phenotypes following knockdown of either peptide or GPCR, validating receptor control target potential.

OR104 *FMRFamide like peptide*-11 function and localisation in *Globodera pallida*

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Novel control strategies for plant parasitic nematodes, including the potato cyst nematode *Globodera pallida*, are a primary concern due to nematicide withdrawal. The neuropeptide signalling system is an attractive resource in the search for novel chemotherapeutic control targets, as it modulates sensory and motor functions. The FMRFamide-like peptides (FLPs) form one of the largest families of neuropeptides, and are structurally conserved across nematode species, generating potential for a broad-spectrum drug target within the FLPergic system. *flp-11* (RNxLVRFamide) is a commonly expressed FLP, encoding at least one, but up to three distinct peptides, in as many as 12 nematode species across Clades III, IV and V. This study investigates the role of *flp*-11 in *G. pallida* using a range of techniques to show that (i) *Gp-flp-11* encodes a single peptide – AMRNALVRFamide, (ii) *Gp-flp-11* is expressed in the nervous system, (iii) worm movement is increased in *Gp-flp-11* silenced worms, (iv) the ability of *Gp-flp-11* silenced worms to infect potato plants is enhanced, (v) a novel *Gp-flp-11* receptor (*Gp-flp-11 R*) is expressed in *G. pallida*, and (vi) *Gp-flp-11 R* silenced worms also display increased movement. This work indicates that *Gp-flp-11* and *Gp-flp*-11 R interact, and play a neuromodulatory role in *G. pallida.*

OR105* *Macrostomum lignano:* a platform for studying flatworm biology and validating targets for parasite control

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Anthelmintics remain the cornerstone of parasite control. However, due to inadequacies in anthelmintic portfolios and accumulating problems with drug resistance, new chemotherapies are urgently needed. Helminth nerve and muscle systems are attractive sources of targets as they facilitate normal behaviour through the coordination of locomotion, attachment, feeding and reproduction. Further, many leading anthelmintics compromise helminth parasite nerve/muscle function such that these systems appear to be eminently druggable. Unlike the situation in nematodes, flatworms lack a model species that aids studies on putative parasite drug targets. The free living microturbellarian *Macrostomum lignano* is already being used as a model organism for the study of stem cell biology due to its impressive regenerative abilities. It is also an attractive model organism for the study of parasitic helminths due to its basal phylogenetic position within the Platyhelminthes, an entirely sequenced genome, compatibility with a range of molecular tools, ease of laboratory culture and transcriptomic datasets that are enriched for neuronal proteins. Here we report on a programme of research that uses RNA interference in *M. lignano* to validate putative targets from within neuropeptide signalling systems. Specifically, we target selected neuropeptides and peptidylglycine α-amidating lyase (PAL), a key enzyme in the carboxy-terminal α-amidation, and maturation, of neuropeptides.

OR106* Acetylcholinesterase biology of plant parasitic nematodes

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The potato cyst nematode *Globodera pallida* causes an estimated ~£300 million damage to potato crops in Europe. Carbamate and organophosphate-based nematicides have previously been used to control this problem but, due to environmental concerns, their use has been prohibited. We are exploring the potential of RNA interference (RNAi) as a plant parasitic nematode (PPN) control strategy through the validation of putative control targets. We used short-interfering RNAs (siRNAs) to trigger specific transcript degradation of nematode gene transcripts. Acetylcholinesterase (AChE) hydrolyses acetylcholine, the main nematode excitatory neurotransmitter, and has been validated as a control target in PPNs through the enduring success of leading nematicides. The use of gene-specific siRNAs resulted in a 95% reduction of AChE transcripts in infective J2 stage worms with a dominant 'poker straight' phenotype. A sand/glass column assay was used to quantify impact on migratory behaviour and an infection assay was used to assess their ability to invade the host plant. Results revealed that AChE knockdown had a negative impact on J2 migration and that AChE RNAi worms displayed a dramatically reduced ability to infect host plants. The impact of AChE RNAi on those worms which successfully infected host plants was also monitored. The data validate the selection of AChE as a control target and highlight its importance to normal PPN behaviour/movement.

OR107 RNAi approaches to G protein coupled receptor deorphanisation and characterization.

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G protein-coupled receptors (GPCRs) are the largest known superfamily of membrane proteins, and they serve as the target for a remarkably high percentage of pharmaceuticals. There are groups of unique GPCRs in parasites, but they have not yet been exploited as drug targets. We have exploited RNAi-mediated gene suppression to deorphanise and characterize GPCRs in protozoa and helminths. Epinephrine stimulates bacterial engulfment in the model protozoa *T. thermophila*; we found a GPCR (TetEPI-1) that responds to epinephrine. Suppression of TetEPI-1 abrogated epinephrine-induced bacterial engulfment. In flatworms, we used RNAi in conjunction with a biochemical endpoint assay to monitor cAMP modulation in response to the translational suppression of individual receptors. As proof of principle, this approach was used to confirm the neuropeptide GYIRFamide as the cognate ligand for the planarian neuropeptide receptor GtNPR-1. The method was then extended to deorphanise a novel planarian serotonin GPCR, DtSER. These results provide functional data on neurotransmitters central to flatworm biology, while establishing the great potential of an RNAi-based deorphanisation protocol. Future work can help optimize and adapt this protocol to higher-throughput platforms as well as other phyla.

OR108 The microRNAs of *Caenorhabditis elegans* – could they play a role in drug resistance?

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Drug resistance in veterinary nematodes represents a major challenge to the food and livestock industry worldwide. However the molecular mechanisms by which resistance arises in parasite populations are poorly understood. We are investigating the hypothesis that resistance is correlated with alterations in microRNA (miRNA) activity. miRNAs are small non-coding RNAs that bind to target mRNAs and regulate their expression. They are important modulators of drug sensitivity in tumour cells and we propose that they may play similar roles in parasitic nematodes. miRNAs could influence the expression of drug transporters or sub-units of various ion channels and thus affect sensitivity to drug. In a preliminary study, we compared miRNA expression in ivermectin-resistant and wild type *Caenorhabditis elegans.* That study demonstrated that a single miRNA was over-expressed in ivermectin-resistant worms compared to wild-type worms. On-going studies are aimed at defining the role of this microRNA in drug resistance.

OR109 A reverse-genetics approach to control target discovery in liver fluke

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Fasciola spp. liver fluke infection continues to impede agricultural livestock productivity in the UK and worldwide, and is additionally recognised amongst the World Health Organisation's list of Neglected Tropical Diseases of humans in the developing world. Given the current absence of alternative control options, a small cadre of benzimidazole anthelmintics shoulder the burden of *Fasciola* control and underline the resistance threat. Post-genomic reverse genetics technologies promise to accelerate both the discovery of novel control targets and our understanding of fundamental helminth biology, but have not yet been widely applied to *Fasciola* spp. systems. Focusing on some of the most commonly cited vaccine candidate proteins (including cysteine proteases, fatty acid binding proteins, glutathione transferases and leucine aminopeptidases), we have developed RNA interference (RNAi) methodology in *Fasciola hepatica*, permitting the specific 'knockdown' of mRNA and protein targets. While we can demonstrate profound transcript and protein knockdowns, we have seen little indication that doing so results in reduced survival of juvenile *F. hepatica in vitro* during maintenance of up to 28 days. We are currently developing target-specific functional assays, with which to investigate the biological importance, and thus control target potential, of proposed drug and vaccine target candidates. Funded by BBSRC grant BB/H009477/1.

OR110 Identification of essential astacin metalloproteases in parasitic nematodes of veterinary importance

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Parasitic nematodes cause chronic, debilitating infections in both livestock and humans worldwide. *Haemonchus contortus* and *Teladosargia circumcincta* are the major gastrointestinal nematodes infecting sheep, and have developed resistance to the anthelmintics currently available. Hence new targets for anti-nematode drugs need sought urgently. The protective cuticle of these parasites has a key role in nematode development and survival. Cuticle synthesis is a complex multi-step process, involving numerous enzymes, and occurs four times throughout the nematode lifecycle. Nematode AStacin (NAS) metalloproteases are involved in the correct development of the free-living nematode, *Caenorhabditis elegans*, with *nas-34, -35, -36, -37* and *-38* having specific roles in hatching, moulting and cuticle synthesis. Due to the conservation, between *C. elegans* and parasitic nematodes, of the cuticle structure and its development, there will also likely be conservation of these astacin proteases. Genome searches have found homologues of NAS-35, -36 and -38 in the *H. contortus* genome and of NAS-35 and -36 in the *T. circumcincta* genome, showing high homology to the *C. elegans* enzymes. Functional conservation was shown when the *H. contortus dpy-31* orthologue fully rescued a *C. elegans dpy-31* mutant. This interspecies conservation was further demonstrated when the recombinant *H. contortus* DPY-31 enzyme processed the *C. elegans* cuticle collagen SQT-3 at the correct C-terminal procollagen processing site. Thus, nematode astacin metalloproteases may be potential targets for novel anti-nematode drugs.

OR111* Generation of a Recombinant *Teladorsagia circumcincta* Antigen Using *Caenorhabditis elegans*

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Teladorsagia circumcincta is a common sheep parasite of temperate areas, and is the main cause of parasitic gastroenteritis (PGE) in lambs. Strains resistant to common anthelmintics have been described. As repeated exposure to infection can induce immunity, vaccination is a promising alternative to anthelmintics.
Immuno-screening and proteomics studies have identified potential vaccine candidates in *T. circumcincta* Excretory/Secretory (ES) products. One example is Cathepsin F (Tci-CF-1), a cysteine protease. A recombinant version was expressed in the yeast, *Pichia pastoris.* This form lacks enzymatic activity and, compared to native Tci-CF-1, is poorly recognised by host immune response. Possible causes are incorrect folding or altered post-translational modifications (PTMs) of the recombinant yeast version, which may affect its ability to induce protective immune responses.

To express Tci-CF-1 in a form closer to the native protein, we have used the free-living nematode *Caenorhabditis elegans* as a novel expression system. A gene construct coding for Tci-CF-1 was micro-injected into the gonad of *C. elegans* and expressing lines produced. The recombinant protein was purified from the transformed progeny using a combination of Ni^{+2} column followed by immuno-affinity separation. Work is ongoing to assess function and immune recognition of native and recombinant forms of Tci-CF-1. This is the first successful attempt to generate *T. circumcincta* antigens using the *C. elegans* expression system.
OR112 Maria Yazdanbakhsh, Leiden University, Netherlands
Modulation of the immune system by parasitic helminths: data from human studies

OR113 CD11c positive cells are critical for maintenance of Th2 responses and survival during chronic *Schistosoma mansoni* infection

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Dendritic cells (DCs) are key players in induction of immune responses through their ability to activate naive T cells, but are thought to be less necessary for maintenance of T cell responses at effector sites. We have previously demonstrated that CD11c+ cells are necessary for priming of the early Th2 response to the parasitic helminth *Schistosoma mansoni*, using CD11c.DTR mice, which allow depletion of CD11c positive cells including conventional (70-80% depletion) and plasmacytoid (80-90%) DCs. In the current study we have gone on to deplete CD11c+ cells at later stages of *S. mansoni* infection, from a time point where the immune response has been on-going for 3 to 4 weeks, and immunopathology is evident. Surprisingly, depletion of CD11c+ cells at this chronic stage of infection resulted in dramatically impaired Th2 cytokine production, coincident with severe weight loss. Our data point to an unexpectedly important role for CD11c+ mononuclear phagocytes in the maintenance of CD4 T cell responses during chronic helminth infection, and ongoing work is aimed at identifying the mechanism(s) that make CD11c+ cells such a critical component of the Th2 response at this stage of infection.

OR114 Macrophages in helminth infection: Where inflammation is anti-inflammatory

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Macrophages activated by the Th2 cytokines IL-4 and IL-13 are found in high numbers at the site of helminth infection. We have demonstrated that macrophage accumulation in this setting does not require recruitment of blood monocytes but can result from local expansion of the resident F4/80hi population. Proliferative expansion of macrophages occurs in a broad range of tissues in both helminth infection and non-infectious settings such as tissue injury. The canonical Th2 cytokine IL-4, which is responsible for alternative activation and suppression of pro-inflammatory cytokines, also directly drives local macrophage proliferation. Thus, the anti-inflammatory nature of the Th2 response goes beyond the release of downregulatory molecules and is an intrinsic part of the process itself. Although both proliferation and alternative activation are IL-4 induced, they are independently regulated and likely reflect very different functional pathways and evolutionary origins.

OR115* Analysis of phenotype and function of monocyte subsets in human schistosomiasis

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Schistosomiasis is a parasitic disease caused by trematode flatworms of the genus *Schistosoma*. Pathology is a result of cellular infiltration to the site of oviposition leading to inflammation and fibrosis of the liver, bladder or spleen. There have been numerous studies in the murine model regarding the development of the alternatively activated macrophage (AAM) within the Th2 cytokine environment characteristic of parasitic infections. The association of the AAM with wound healing, T cell suppression and controlling the inflammatory response makes them an exciting area of investigation. As a precursor to macrophages, monocytes have been studied in various human infections, and it has become evident that monocytes also play an important role in immune pathology in inflammatory disease. Monocytes can exhibit different effector functions and differentiate into subsets dependant on the cytokine environment, implicating their role in mediating the immune response as well as in disease pathology. Using PBMCs isolated from a cohort of a *Schistosoma haematobium* exposed population in rural Zimbabwe, I have phenotyped monocytes using CD14, CD16, CCR2 and CX3CR1 surface markers. These subsets have been analysed in regard to the expression of functional markers to determine changes with respect to disease status.

OR116 Population genetics of anthelmintic resistance in the small ruminant parasitic nematodes *Haemonchus contortus* and *Teladorsagia circumcincta*

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Anthelmintic resistance in livestock parasites represents a major threat to the sustainability of the industry. The origin and spread of anthelmintic resistance alleles is an important question that is still poorly understood. We have used a number of population genetic approaches to investigate the emergence and spread of benizimidazole resistance alleles for two species of gastro- intestinal nematodes of sheep with contrasting life-histories; *Teladorsagia circumcincta* and *Haemonchus contortus.* Data will be presented that supports a model for both species in which multiple independent benzimidazole resistance alleles recurrently arise and are subsequently spread between locations by animal movement.

OR117 Emodepside is an activator of the calcium-activated potassium channel, SLO-1

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Emodepside is a resistance-breaking anthelmintic. Mode of action studies in *Caenorhabditis elegans* identified a calcium–activated potassium channel SLO-1 as a mediator of emodepside's action and null mutants of *slo-1* are highly resistant to its paralytic effects [1]. *kcnma1*, a close mammalian homologue of *slo-1* can rescue the mutant behavioural phenotypes but the transgenic worms are less sensitive to emodepside that those expressing *slo-1*. This suggests emodepside is 10 to 100 fold more selective for SLO-1 compared to KCNMA1 and provides a molecular basis for its anthelmintic efficacy. Ectopic expression of *slo-1* in the pharyngeal muscle of *C. elegans* confers susceptibility of this tissue to emodepside consistent with the idea that SLO-1 is a direct target of emodepside. To further test this SLO-1 and KCNMA1 were expressed in HEK293 cells. Emodepside modulated SLO-1 and KCNMA1 whole cell currents. This could not be explained by an indirect effect on intracellular calcium levels. Further studies are required to identify the putative emodepside pharmacophore harboured by calcium-activated potassium channels.

OR118* Multidrug resistance protein transcriptional responses to triclabendazole / triclabendazole metabolites in *Fasciola hepatica* newly excysted juveniles

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Due to its efficacy against adult and juvenile liver fluke, triclabendazole (TCBZ) is the drug of choice for the treatment of fasciolosis in both human and veterinary infections. The heavy reliance on TCBZ therapy for liver fluke control coupled with accumulating reports of TCBZ resistance highlight the need to enhance the effective life-span of current drugs, and to identify novel control options. In this study we consider the involvement of multidrug resistance proteins (MDR/MRP) from the ABC transporter family in the susceptibility of *F. hepatica* to TCBZ and its common metabolites. Previous work on *Schistosoma mansoni* implicates the elevated transcription of two MDR proteins (*Sm*MDR2 and *Sm*MRP1) in the reduced susceptibility of schistosomes to praziquantel. Here we identify orthologues of both of these genes in *F. hepatica* newly excysted juveniles (NEJs) and demonstrate a profound, concentration-dependent modulation of *Fh*MRP1 transcript levels following exposure to TCBZ or its therapeutically active sulfoxide metabolite (TCBZ.SO) *in vitro*. We have successfully triggered transcript knockdown of both *Fh*MRP1 and *Fh*MDR2 in NEJs using RNA interference (RNAi) methodology, facilitating examination of any resultant alteration in the phenotypic responses of *Fh*MRP1- and *Fh*MDR2-silenced worms to TCBZ and TCBZ.SO.

OR119 A population genetics approach to ivermectin resistance in *Haemonchus contortus* and *Teladorsagia circumcincta*

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Anthelmintic resistance is a major constraint to the sheep industry worldwide. *Haemonchus contortus*, an intestinal parasitic nematode of small ruminants, is unsurpassed in its ability to develop resistance to the anthelmintics used as the mainstay of its control. The closely related nematode *Teladorsagia circumcincta* is the most common intestinal parasite affecting sheep in the UK. A number of ligand-gated chloride channels and P-glycoproteins have been identified in the partially sequenced genomes of *H. contortus* and *T. circumcincta* and SNPs and/or altered expression levels have been associated with ivermectin resistance in laboratory isolates. However, the importance of these mutations in field isolates remains unclear. We are using a population genetics approach to measure selection at three ligand-gated chloride channel loci (glc-5, gbr-2 and hg1) and three P-glycoprotein loci (pgp-A, pgp-1 and pgp-9) in field populations of *H. contortus* and *T. circumcincta* from farms throughout the UK. Sequencing of PCR amplicons from 25 individual worms from a farm with no reported ivermectin use will provide baseline levels of polymorphism for comparison with worms from farms with high ivermectin use. If the candidate genes are associated with resistance in the field, we expect to see purifying selection at these loci.

OR120* Updated findings from an ongoing cattle parasite survey

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Grazing cattle are infected with a variety of gastrointestinal nematodes and methods of control rely heavily on the use of anthelmintics. There have been a number of reports of anthelmintic resistance in cattle nematodes abroad, but little is known about the prevalence or sensitivity of these parasites to anthelmintics in the UK.
A questionnaire survey examining parasite control practices, in conjunction with faecal egg count reduction tests (FECRTs) to examine nematode sensitivity to ivermectin has been undertaken. Questionnaire results show that UK farmers favour macrocyclic lactones for control, in particular as a pour-on application. To date, ivermectin FECRTs have been performed on first season grazing cattle from 15 farms using an injectable or pour-on formulation, with faecal samples screened for the presence of trichostrongyle nematode eggs and liver fluke eggs. At fourteen days post treatment, mean percentage reductions in trichostrongyle faecal egg counts (FECs) ranged from 61% to 100%. Reduced efficacy (i.e. mean percentage reduction in FECs at day 14 < 95%) was identified on 7 of the farms.
Morphological identification analysis of larvae cultured from pre-treatment samples indicated the presence of a variety of nematode species, such as *Ostertagia, Cooperia, Trichostrongylus*. However, in post-treatment samples, *Cooperia* species predominated larval cultures. This information, together with FECRT data will inform future nematode control strategies on participating farms.

OR121 Wolbachia in Filarial Nematodes: Mechanisms of Symbiosis

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Parasitic filarial nematodes, which belong to the Onchocercidae family, live in mutualism with *Wolbachia* endosymbionts. To better understand the cellular and molecular basis of the symbiosis, we developed whole-mount techniques and new antibodies to follow the segregation patterns of *Wolbachia* through the somatic and germline lineages of several filarial species on one hand, and on the *Wolbachia* contribution to the worm's development on the other hand. *Wolbachia* are transmitted through asymmetric cell division during embryogenesis, from the germline precursors to the hypodermal precursors. In late larval stages, they populate the distal ovaries from the posterior lateral hypodermal chords. We will present these modes of transmission, as well as the use of RNAi to perturb these mechanisms. Wolbachia depletion leads to cumulative apoptosis in the germline and during embryogenesis. We analyzed the fraction of non-apoptotic early embryos, and the germline cells of tetracyclin-treated *Brugia malayi* females. We will present the cellular defects normally repressed by the presence of *Wolbachia*.

OR122 Nematode autophagy regulates *Wolbachia* populations and identifies a novel mode-of-action for anti-filarial treatment

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Filarial nematode parasites are amongst the most important neglected parasitic diseases of humans and animals. Over 150 million individuals are infected with lymphatic filariasis and onchocerciasis and heartworm is an important pathogen of dogs and cats. A new target for anti-filarial treatment is the obligate mutualistic endobacteria *Wolbachia*. Depletion of *Wolbachia* with antibiotics induces defects in nematode development, fertility and viability. In order to identify novel mechanisms to deplete *Wolbachia* as part of the A-WOL drug discovery and development programme, we investigated the role of activating host nematode autophagy to control bacterial populations. Our studies revealed that periods of rapid bacterial population growth and expansion were accompanied by activation of the autophagy pathway and that chemical and genetic manipulation of this pathway could directly regulate bacterial populations at an equivalent level to antibiotic treatment. The activation of the autophagy by using drugs or small-molecules resulted in *Wolbachia* reduction in both *in vitro* and *in vivo* treated *Brugia malayi*. The induction of the host nematode intracellular autophagy defence mechanism can therefore be considered as a novel mode-of-action, which delivers bactericidal activity that can be used to develop improved drugs and regimes for anti-filarial treatment.

OR123 *Schistosoma mansoni* methyl-CpG binding domain protein (SmMBD2/3): a novel component of the schistosome epigenetic machinery

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The fundamental components of the eukaryotic epigenetic machinery are DNA methyltransferases (Dnmts) and methyl-CpG binding domain proteins (MBDs). Dnmts enzymatically convert cytosines into their methylated form, 5-methylcytosine (5mC). MBDs target and bind 5mC and can recruit various co-repressor complexes to these methylated genomic loci, leading to localised chromatin remodeling. Together, these epigenetic components are responsible for gene expression control and heritable alterations in phenotypic diversity. Previous studies in our laboratory have identified transcriptionally co-reguated Dnmt and MBD homologues (SmDnmt2 and SmMBD2/3) in the pathogenic trematode *S.mansoni.* Subsequent functional genomics investigations have shown that SmDNMT2 is indeed a functional cytosine methyltransferase in this organism. Here, using yeast 2-hybrid screens and cell transfections, we present evidence that suggests SmMBD2/3 is also a functional component of the *S.mansoni* epigenetic machinery.

OR124 microRNAs of parasitic nematodes - a role in larval arrest?

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microRNAs (miRNAs) are 22 nucleotide, non-coding RNAs which are important in post-transcriptional gene regulation. These small RNAs bind to complementary sites in the 3'UTR of their target genes resulting in translational repression or destabilisation. miRNAs are essential for correct development in *Caenorhabditis elegans* and other organisms. We are interested in the roles miRNAs may play in regulating development of parasitic nematodes. Using deep-sequencing and bioinformatic homology searching we have identified 104 miRNAs from *Brugia* and 192 miRNAs from *Haemonchus,* in addition to small-interfering (si)RNAs and Piwi-interacting (pi)RNAs. Interestingly, while some of the identified parasite miRNAs are conserved in *C. elegans,* most are novel, which may reflect adaptations to different environments and lifestyles. Microarray analysis and qRT-PCR has confirmed that a number of *Brugia* and *Haemonchus* miRNAs are differentially expressed, with significant differences in level of expression between infective larvae and adults and between male and female worms. We are focussing initially on miRNAs expressed at a high level in infective larvae, to determine any potential role in the arrested larval state.

OR125* Skin- *vs.* Mechanically transformed schistosomula – a transcriptional comparison.

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The process of cercarial invasion and schistosomula migration during the early stages of infection, are relevant in the study of intervention strategies against *Schistosoma* parasites as the schistosomulum stage is considered the most vulnerable of the parasite life cycle. Cercariae transform into schistosomula as they penetrate the skin barrier of the definitive host. This transformation can be mimicked in the laboratory by application of shear pressure to a cercarial sample rendering mechanically transformed schistosomula. However, the transcriptional equivalency between skin-transformed and mechanically transformed schistosomula has never been proven. High throughput gene expression studies (i.e. microarrays) have relied on this equivalency, mainly because obtaining enough material from *in vivo* infections is not always feasible. Therefore, MT schistosomula have been used almost exclusively in high-throughput studies of gene expression, identification of drug targets and identification of effective drugs against schistosomes from a compound library. In our approach to compare these two types of schistosomula preparations, we performed RNA-seq transcriptome profiling of skin-transformed and mechanically transformed schistosomula at 24 hours post transformation. We report genes and pathways that are differentially expressed between these two schistosomula preparations and provide enough data to finally resolve the skin- vs. mechanical long-lasting controversy.

OR126 Modelling the impact of MDA programmes on helminth infections: what do we know about drug effects

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The UK Coalition against Neglected Tropical Diseases (NTDs) has recently subscribed the London Declaration for the control and elimination of ten NTDs by the year 2020. Mathematical models of transmission dynamics and control have a crucial role to play in assessing the feasibility of these goals with current intervention tools. Many large-scale programmes against human helminth infection rely on a handful of drugs whose effects on parasite population biology are poorly understood. Model assumptions are, in part, based on current knowledge about such effects. Since adult worm burden is seldom recorded, cure rates, egg reduction rates, microfilarial repopulation rates, and other measures of drug efficacy depend on our ability to quantify parasite transmission stages with imperfect diagnostics and measurement error. Two examples from onchocerciasis will be discussed. The former illustrates that assumptions regarding whether or not ivermectin reduces irreversibly and cumulatively the production of microfilariae by adult female worms will influence model projections on the feasibility of elimination, yet, these assumptions, based on analyses of limited data, are seldom questioned. The latter discusses how models fitted to clinical trial data on the effect of anti-wolbachial therapy (doxycycline treatment) with frequent follow up and focus on adult worms can help to quantify macrofilaricidal effects, and inform strategies aiming to combine the microfilaricidal effects of ivermectin with the macrofilaricidal effects of doxycycline. Examples of intestinal nematode infections will be discussed in terms of the relative efficacy and expanded delivery of current benzimidazole anthelmintics in integrated control frameworks, highlighting the need for developing a pipeline of replacements as well as tools for the monitoring and evaluation of drug efficacy and resistance

OR127 Long-term impact of large scale community-directed delivery of doxycycline for the treatment of onchocerciasis

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The macrofilaricidal and embryostatic anti-*Wolbachia* treatment with doxycycline has a great potential for therapy and control of onchocerciasis. Nevertheless, the length of the required treatment poses potential logistical and compliance problems to its use in Mass Drug Administration (MDA) strategies. In 2007-2008 a feasibility trial of community-directed treatment with doxycycline was carried out in 17,519 eligible people in two health districts of Cameroon, naive to previous control measures. Therapeutic coverage was 73.8% with 97.5% compliance, encouraging the feasibility of this approach. We evaluated the effectiveness of this community-directed delivery of doxycycline four years after implementation. *Onchocerca volvulus* infection was assessed by skin biopsy and nodule palpation in 375 people who completed the treatment with doxycycline followed by one or two annual rounds of ivermectin MDA and 132 who received one or two annual rounds of ivermectin MDA alone. Significantly lower microfilarial prevalence and load were found in people who received doxycycline plus ivermectin compared to those who received ivermectin alone. This study demonstrates that a multi-week doxycycline treatment delivered with a community-directed strategy is not only feasible but also effective in reducing infection prevalence and burden even when evaluated four years after implementation in an area of ongoing transmission.

OR128 A·WOL drug discovery - screening of focused anti-infective libraries for novel compounds with efficacy against *Wolbachia* endosymbionts of filarial nematodes

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Filarial nematodes are an important group of pathogens infecting 150 million people throughout the tropics with over 1.5 billion at risk. Anti-biotic targeting of filarial *Wolbachia*, an essential bacterial symbiont, provides a novel treatment with macrofilaricidal activity. In order to turn this therapy into a public health tool suitable for filariasis control programmes, the Anti-*Wolbachia* Consortium (A·WOL) utilises *in vitro* cell and nematode assays, followed by secondary *in vivo* assays, to screen both focused and diversity compound libraries against *Wolbachia*. Here we describe the screening of 5399 compounds, from five focused anti-infective chemical libraries, in a *Wolbachia* cell-based assay. We have identified 484 hits, with 117 compounds showing improved efficacy over doxycycline against *Wolbachia*. Hit compounds also show activity against nematode *Wolbachia in vitro* without exhibiting direct anti-nematode activity. Based on our hit criteria, 104 compounds have progressed down the screening funnel and been screened in a *Litomosoides sigmodontis* mouse model, where encouragingly, a number show equivalent or improved efficacy compared with doxycycline against *Wolbachia in vivo*.

OR129 A·WOL drug discovery: Screening diversity libraries to discover novel areas of chemical space with anti-*Wolbachia* properties.

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There is an urgent need to develop new drugs for onchocerciasis and lymphatic filariasis treatment and control. The Anti-*Wolbachia* Consortium (A·WOL) is testing a diverse range of compounds to find new chemical space to meet this demand. 558,000 compounds have been procured from the following libraries: Medicines for Malaria Venture (MMV – 500,000 compounds), BioFocus® (10,000 compounds), and Shanghai Institute of Materia Medica (SIMM - 48,000 compounds). 12,400 of these compounds have already been screened with 130 hits identified as reducing *Wolbachia* levels by >90%. Hits are scrutinised to assess suitability for further assessment of structure-activity relationships and select the best candidates to take forward as part of the drug discovery program. We are exploring a range of cheminformatic approaches to allows us to rapidly identify groups of compounds that show anti-*Wolbachia* activity and reject those where the chemical space is largely redundant.

OR130 Uncertainty surrounding the projections of the long term impact of ivermectin treatment on human onchocerciasis.

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Recent epidemiological and entomological evaluations conducted in Senegal and Mali have indicated that annual ivermectin distribution may be sufficient to locally eliminate human onchocerciasis in certain foci. Mathematical modelling has been used to make projections about the required duration of ivermectin distribution to reach elimination in other African foci. A crucial assumption of these models is that the fecundity of adult worms is reduced irreversibly by 35% with each (annual) ivermectin round. However, other modelling-based analyses have suggested that ivermectin may not have a cumulative impact on fecundity.
We modify a deterministic age- and sex-structured onchocerciasis transmission model, parameterised for savannah areas in Cameroon, to explore the impact of a range of assumptions regarding the effect of ivermectin on fecundity, treatment coverage, compliance and frequency on modelling-based projections of parasitological outcomes due to long-term, mass administration of ivermectin in initially hyperendemic areas.
The projected long-term impact of ivermectin distribution on infection prevalence is strongly dependent on assumptions regarding the drug's effect on worm fecundity and treatment compliance. Results indicate that if ivermectin does not have a cumulative impact on fecundity, elimination of onchocerciasis in areas with a high pre-control endemicity may not be feasible with annual ivermectin distribution.

OR131 Placental hormones alter the outcome of influenza virus infection in female mice

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During seasonal epidemics as well as pandemics (e.g. 1918 H1N1, 1957 H2N2, and 2009 H1N1) of influenza viruses, pregnant women have a 4-18-fold greater risk of being hospitalized or dying from influenza than either age-matched, non-pregnant women or the general population. During the third trimester, inflammatory immune responses are reduced and anti-inflammatory responses are increased to support healthy fetal development. Hormones, including estrogens and progesterone, modulate the immunological shift that occurs during pregnancy. Estradiol is the primary biologically active estrogen found in non-pregnant females of reproductive age. Published data from my laboratory illustrate that treatment of female mice with high doses of estradiol reduces inflammatory immune responses (e.g., TNF-α and CCL2), morbidity, and mortality during IAV infection. Because elevated estradiol protects, rather than harms, females during IAV infection, we hypothesize that other pregnancy-associated hormones might underlie the increased severity of influenza during pregnancy. Estriol (E3) is a placental estrogen that accounts for a majority of circulating estrogens during pregnancy and treatment of female mice with pregnancy-level E3 significantly reduces survival from influenza A virus (IAV) infection and skews Th1/Th2 cytokine responses by suppressing concentrations of TNF-α and IFN-γ and increasing concentrations of IL-4 and IL-6 in the lungs during IAV infection. Progesterone (P4) also is produced in high concentrations by the placenta during pregnancy and treatment of female mice with pregnancy levels of P4 significantly increases survival and production of TGF-β during IAV infection. Manipulation of either E3 or P4 has no effect on IAV replication suggesting that the effects of these placental hormones on the outcome of IAV infection are immune-mediated. We hypothesize that placental hormones affect differentiation and activity of CD4+T cells to alter IAV pathogenesis, which likely contributes to how IAV infection is so severe in pregnant females.

OR132 Identification of new apicoplast proteins, and functional characterization via single step conditional mutants

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Parasite of the phylum Apicomplexa cause diseases that impact global health and economy. This unicellular eukaryotic parasites posses a relic plastid, the apicoplast. This is an essential organelle and a validated source of drug targets. Yet much of its biology remains poorly understood, especially considering its elaborate compartmentalization: four membranes bounding four different compartments.
We hypothesized that enlarging the catalogue of apicoplast proteins will contribute toward identifying new organellar pathways or functions. To this aim we developed a bioinformatic screen based upon post-genomic data sources. We assessed 60 candidate genes by attempting endogenous tagging. This resulted in the identification of 11 novel apicoplast proteins, that are distributed among the different sub-compartments of the organelle. To address their function we developed a robust system allowing rapid generation of mutants via a promoter replacement strategy.
We confirm the feasibility of this system by establishing conditional mutants for five genes. Two are particularly intriguing as they encode hypothetical proteins that is conserved in and unique to apicomplexa and relative-algae. Microscopy and biochemical evidence supports their product localization to the understudied periplastid compartment (PPC). Disruption of both demonstrate that they are essential for parasite survival. Detailed phenotypic analysis raise substantial evidence for their involvement in apicoplast biogenesis and specifically in import of proteins into the organelle, via controlling redox states.
While still running our pipeline of identification-functional characterization, the data generated thus far demonstrate the power of this new strategy to discover novel plastid genes and the efficiency and rapidity by which the single step promoter replacement aids to unravel their function.

OR133* Enterocyte function is compromised by a *Giardia*-secreted mediator.

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Giardia is a major cause of parasitic diarrhoea and inhabits the upper small intestine. It is transmitted by faecal-oral route. Trophozoites attach to the intestinal epithelium, inducing giardiasis. Two genetically distinct lineages (assemblages) cause the human disease. Our work suggests that *Giardia* supernatant influences the short circuit current (Isc) of Colorectal Adenocarcinoma cell line (CaCo-2). The loss of transepithelial flux in response to different inhibitors has raised the possibility of *Giardia*-secreted mediators. By Differential Interference Contrast microscopy (DIC), we observed a concordant modification of the CaCo-2 cells morphology after 24 hours incubation with *Giardia* supernatants at different dilutions. The intestinal cell size significantly decreased when incubated with parasite supernatant, with significant effects being mediated even at high dilution. SDS-PAGE analyses of supernatants identified a number of secreted proteins; showing a majority of such proteins conserved between the two assemblages but also a few assemblage A-specific soluble proteins. Proteomic identification and quantification of these potential mediators of pathology is now underway.
Our results show that *Giardia* secretes soluble mediators which may be able to exert effects even at low concentration and at sites such as the colon which are distal to the infection.

O134 Functionally different subsets of micronemes in *Toxoplasma gondii*

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Apicomplexan parasites differ from other eukaryotic cells by an extra set of specialised secretory organelles (micronemes, rhoptries and dense granules), that are sequentially secreted during invasion of the host cell. Upon host cell contact the apically located micronemes are the first organelle to be released and contain crucial virulence factors that are secreted.
Using *Toxoplasma gondii* we demonstrate that micronemes are organised into functionally independent subsets. We performed an overexpression screen on Toxoplasma Rab-GTPases and show that vesicular transport to these subsets involves distinct trafficking pathways suggesting that they are independent secretory organelles. Furthermore, we show that parasites depleted of specific subsets of micronemes cannot undergo sequential steps of invasion highlighting separable roles for these sub-populations. Our results also indicate that apicomplexan parasites diversified their Rab-GTPase repertoire to allow for this functional separation that is critical for their specialised lifestyle. This work has significant implications for understanding the function and organisation of secretory organelles in the pathogenesis of apicomplexans, such as *Plasmodium falciparum*, the causative agent of severe malaria.

OR135 The ability of progesterone to modulate dendritic cell and macrophage function provides a potential mechanism for the ability of sex and pregnancy to modulate the outcome of *T. gondii* infection.

Fiona M. Menzies, Leigh A. Jones, Shrook Kreem, Fiona L. Henriquez, Shrook Kreem, Muhannad A.M. Shweash, Andrew Paul, James Alexander and Craig W. Roberts
Strathclyde Institute of Pharmacy & Biomedical Sciences, University of Strathclyde, 161 Cathedral Street, Glasgow, G4 0RE, UNITED KINGDOM

The incidence and severity of numerous diseases of infectious and non-infectious etiology not only varies between males and females, but can be affected markedly in females by changes in their hormonal status including pregnancy. Notably, female mice are more susceptible than male mice to *Toxoplasma gondii* infection and are further compromised during pregnancy. Many of these observations can be related to the action of steroid hormones on the immune system. Herein, we demonstrate the ability of progesterone to modulate murine bone marrow-derived dendritic cell (DC) cytokine production (IL-6 and IL-12) and costimulatory molecule expression (CD40, CD80, and CD86) through its ability to sustain IRF3 phosphorylation following TLR-3 but not TLR-4 ligation. In addition progesterone is able to selectively inhibit and augment different aspects of both alternative and classical macrophage function. The results provide a potential basis for the observed ability of sex and pregnancy to modulate the outcome of *T. gondii* infection.

OR136 Geospatial tools in veterinary parasitology: from sampling to modeling

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The application of geospatial tools (e.g. geographical information systems, global positioning system, satellite-based remote sensing and virtual globes) to spatial epidemiology in veterinary parasitology have been nowadays firmly established for geo-positioning, collating, exploring, visualizing and analyzing health data in a spatially explicit manner. These tools have also a great relevance from a practical point of view concerning the sampling procedures to be adopted in cross-sectional surveys of animal parasites. The application of spatial sampling strategies to animal diseases is relatively new and the study of pathogen distribution and abundance at a geospatial scale has focused mainly on vector-borne diseases so far, due to their direct link with the environment. Besides sampling, geospatial tools can be also very useful for spatio-temporal modeling of parasite distribution and abundance at local, regional and area-wide scales. The possibility of using geospatial tools in veterinary parasitology at different levels, from sampling to modelling, represents a very useful way to communicate with field researchers and decision-makers and to address targeting of animal parasite control treatments.

The EU GLOWORM project (FP7-KBBE-2011-5) is kindly acknowledged for supporting researches based on the use of geospatial tools for studying the epidemiology of helminth infections in livestock.

OR137 Ecological Niche Models and the Distribution and Abundance of Hookworms in Bolivia

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The predictive value of Maximum Entropy (MaxEnt) geostatistical models[1] and empirical models based on the growing degree day-water budget (GDD/WB) concept[2.3] were compared as methods of mapping the distribution and abundance of hookworm in Bolivia. Maxent is a general purpose ecological niche modeling software that can be used to predict species geographic distribution when only occurrence data are available for analysis (eg. vector occurrence, case incidence). GDD/WB models are based on known thermal-hydrological preferences and limits of tolerance of a biological system in the environment. A climate grid of Bolivia (18km^2, monthly long term normal temperature, rainfall, evapotranspiration) was used to calculate the annual number of transmission cycles of the free-living stages (egg- L_3) of *Necator americanus* possible in each grid cell using a base temperature of 15° C (below which no development progresses) conditional on a water budget threshold of >0.5 soil moisture and reported mean L_3 longevity. A cumulative value was derived of 260 GDD per transmission cycle (annual GDD if >0.5/260). A risk map based on potential transmission cycles per year revealed an elevation gradient of suitability in Bolivia that ranged from no transmission at high elevation altiplano sites and in arid zones to 13 potential transmission cycles at hot, humid Amazonian sites. Model output was significantly related to 35 municipality level survey prevalence data records (range 0-80%). Maxent geostatistical model analysis yielded a probability surface map that ranged from a 0.0024 to 0.815 probability of occurrence. Maxent threshold analysis was performed by running separate models based on survey points of <2% prevalence and >2% prevalence, revealing a low risk Altiplano zone and a variable predicted probability gradient from the eastern slopes of the Andes to Amazon ecological zones. The potential value and limitations of the two modeling approaches will be discussed.

OR138 Mapping the geographical distribution of schistosomiasis in Nigeria from compiled survey data

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Nigeria is the most populous country in sub-Saharan Africa with over 160 million people. Inadequate basic social amenities, conducive climatic environment, grossly inadequate safe water supplies and weak primary health care system have promoted the transmission of schistosomiasis, a neglected tropical disease, in the country. Presently, there is little or no holistic control programme in place due to lack of funding to generate detailed information on the geographical distribution of schistosomiasis in Nigeria. A systematic review and geo-referencing of records on schistosomiasis in Nigeria was therefore undertaken to create a nationwide geographical information system database suitable for spatial disease risk modelling and control for the country that will aid the national control programme, given the limited resources.
A literature search on schistosomiasis prevalence in Nigeria from peer-reviewed local and international journals was conducted from 2009 until 2010. Additional information obtained from reports of surveys by the Federal Ministry of Health, State ministries of health and Non-Governmental Developmental Organization (NGDOs) were also examined. Relevant schistosomiasis prevalence data were extracted and geo-referenced to create a nationwide geographical information system (GIS) database. These data complied under the open-access Global Neglected Tropical Disease (GNTD) database is online at (http://www.gntd.org). The GNTD dataset supplemented by surveys and reports were analysis to generate point prevalence maps for Nigeria.
Analysis of the compiled records showed that schistosomiasis is endemic in 35 Nigerian states, except for Akwa Ibom State. More specifically, infections were reported at 462 unique locations out of 833 different survey locations. *Schistosoma haematobium* is the most widely distributed *Schistosoma* species in Nigeria endemic in 31 states and present at 368 (79.6%) survey locations. In comparison, *S. mansoni* is endemic in 22 states, 78 (16.7%) survey locations, and *S. intercalatum (guineensis)* only in 2 states and 17 (3.7%) survey locations. Twenty-two states simultaneously observed *S. mansoni* and *S. haematobium* infections, while co-occurrence of the three *Schistosoma* species was solely reported in Rivers State. The averaged prevalence for each species ranged from 0.5% to 100% for *S. haematobium*, 0.2% to 87.1% for *S. mansoni*, and 1.0% to 9.6% for *S. intercalatum* (*guineensis*).
The GIS database on schistosomiasis in Nigeria can be further analysed with disease-related environmental factors to spatially predict prevalence at locations without survey data. This would provide spatially-targeted and evidence-based information for the national control programme for cost-effective operations: planning future survey, targeting and prioritizing control interventions, monitoring and surveillance.

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OR140 Use of personal GPS-dataloggers to infer water contact patterns and social networks that influence transmission of intestinal schistosomiasis among mothers and young children

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Using novel, low-cost GPS data loggers (I-GOTU) coupled with standard parasitological surveillance, water contact exposures and schistosome infections among mothers and their young pre-school aged children were studied at the shoreline village of Bugoigo, Lake Albert. As younger children are not yet formally included within the Ugandan National Control Programme, the levels of daily disease exposure need more formal quantification. At baseline the cohort of 37 mothers, 36 pre-school-aged children had egg-patent infection prevalences of 62% and 67%, respectively, which diminished to 20% and 29%, respectively at 6-month post-treatment follow-up. These subjects wore GPS datalogging devices over a 3-day period shortly after baseline which allowed for an estimation of time spent at the lakeshore as an exposure metric. This metric was later found to be associated with prevalence at follow-up (OR=2.1, P=0.01 95% CI 1.2-3.7 for both mothers and young children and OR=4.4, P=0.01, 95% CI 1.4-14 for young children alone). The spatial patterning of these water contact exposures was also compared against that from a selection of local school-aged children and adult men.

OR141 Adaptation of bacteriophages to natural plant pathogen populations

Brit Koskella
Oxford University, UK

Understanding coevolutionary dynamics, in particular between hosts and parasites, is critical to understanding both biodiversity and ecosystem functioning. Recently, major strides forward have been made due to a burgeoning empirical and theoretical literature that consider how environmental heterogeneity influences the outcome of species interactions. Microbial systems provide an exciting opportunity to examine these complex dynamics with tractable methods both in the field and the laboratory. In addition, microbial communities are of key importance to the health of human, agricultural, and natural populations. A key challenge is to understand how these communities are influenced by interactions with both their eukaryotic hosts and their viral parasites (bacteriophages). In this talk, I first present data on the scale at which bacteriophages adapt to infect their host bacteria within natural populations living in and on their plant host, the horse chestnut tree. I then examine the specificity of these natural phages and the potential consequences of phage-mediated selection on bacterial adaptation to plant hosts, and finally explore the importance of historical contingency in shaping fitness trade-offs. I discuss these findings both in light of phage therapy for regulating bacterial populations and, more generally, to highlight the importance of understanding the spatial scale and biotic complexity of species interactions in successfully predicting the outcome of coevolution.

OR142 Unravelling the within-host dynamics of an acute bacterial infection.
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Despite massive progress in microbiology and immunology, our understanding of the within-host dynamics of bacterial infections remains mostly qualitative. Combining innovative experimental approaches and mathematical modelling, we have started to quantify key aspects of the dynamics of infection by *Salmonella enterica*, the causative agent of typhoid. Our approach consists in applying concepts and modelling tools from ecology to the population dynamics of bacteria. Key to the validation of these models is the use of appropriate statistical techniques in conjunction with experimental data. This requires a constant dialogue between modellers and biologists at all stages of the project. We have used this approach successfully to resolve essential questions about the effect of the host's immune system on the replication, killing and spread of bacteria, across scales from the level of single cells to whole animals. Here I present two specific studies in more detail.

OR143 Manipulating wild, managed population densities to control disease
Ros Porter[1], Lucy Gilbert[2] and Rachel Norman[1]

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Louping ill is a tick borne infection of sheep and grouse. It is a virus which only be transmitted to ticks by a limited number of species (sheep, grouse and hares) but other species are involved in persistence of the disease since they support the tick population (deer are of particular importance here). This disease is of economic importance to sheep farmers and land owners and has a large impact on the rural communities within Scotland.
Since almost all of the species involved in this ecosystem are wild managed populations vaccination is not possible, there have been a number of suggestions for methods of controlling the disease. These include reducing hare populations, adding sheep tick mops and using acaricidal leg bands on the grouse. In this talk we will present a simple mathematical model which describes the interactions of these multiple species and then examines methods of control in order to determine the circumstances under which they would be most effective and how the combinations of species interact to determine disease dynamics.

OR144* Squirrelpox: An Epidemic on Merseyside and its Aftermath

Tim Dale[1], Mike Begon[1], Steve White[2], Colin M[c]Innes[3], Jonathan Read[1] and Julian Chantrey[1]
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The Eurasian red squirrel (*Sciurus vulgaris*) has been in rapid decline in the UK since the introduction of the American grey squirrel (*Sciurus carolinensis*) in the early 1900's. Modeling simulations have shown that the rate of decline cannot be explained by competition alone. Serological studies have indicated a disease caused by squirrelpox virus (SqPV) could be involved in this decline. Disease incidence in red squirrels seems to coincide with grey squirrel incursions. Until now empirical data on the impact of SqPV has been lacking. Here we combine squirrel population data with disease incidence to show that SqPV was responsible for an 80-90% decline in a red squirrel population found in one of the remaining red squirrel strongholds. A post-epidemic serological survey revealed 5/93 red squirrels had ELISA OD values consistent with SqPV exposure. This is the first account of clinically normal wild red squirrels showing they are capable of surviving SqPV exposure in their natural environment. These data show that SqPV is capable of causing dramatic population declines in wild red squirrel populations and is likely to have a strong influence in its decline in the future unless conservation protocols are developed to address the introduction of a novel infectious agent into a naïve population.

OR145 Protozoan infection alters the regulation of host population dynamics – a cockroach-gregarine story

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Parasite infections have been previously shown to have important regulatory effects on avian and mammalian population dynamics. Invertebrate populations are also subject to regulation by parasitic infections, yet a common group of invertebrate parasites, gregarines, have so far been neglected. Gregarine infection (*Gregarina blattarum*) in the German cockroach (*Blattella germanica*) has important life history consequences and here it is shown to also have important consequences for the dynamics of the host population. Infection suppresses host density, which is mediated by both fecundity and survivorship costs. As gregarines are ubiquitous to invertebrates worldwide, future work should consider the impact of such infections in other invertebrate species.

OR146 Evolutionary and ecological dynamics of epidemic rabies

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Rabies virus emergence and spread in Europe (during the 1940s) and the United States (during the 1970s) in terrestrial carnivore hosts has proven a remarkable model system for exploring the joint interaction of ecological and evolutionary forces in shaping patterns of host-pathogen association, spatial and temporal dynamics, and mechanistic strategies for control and abatement. Rabies virus was introduced into naïve populations of raccoons in the central eastern seaboard as a consequence of long distance translocation of virus from an original endemic source in Florida. We have constructed spatially explicit predictive models for epidemic spread of rabies virus in the eastern United States. The epidemic expansion of the rabies virus has left a detectible ecological "signature" within the evolution of viral phylogeny and we are expanding our earlier ecological models of spread to incorporate evolutionary dynamics. These combined eco-evo models can be used strategically to assess the importance of specific ecological processes measurable through their effects on phylogenetic architecture. The specific case of spread with and without Long Distance Translocation (LDT) of pathogen illustrates the general approach.

OR147 Is Co-infection a Key Driver of Inter-Individual Infection Heterogeneity in School Aged Children?

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Co-infection is ubiquitous in people in the developing world but little is known about its relative importance as a driver of infection heterogeneity between individuals. Here we determine the importance of co-infection compared with other potential drivers of infection heterogeneity (*i.e.* host biology and behaviour, socioeconomic status, and environmental conditions). Using generalised linear mixed modelling techniques we simultaneously assessed the proportional effects of these potential drivers of infection heterogeneity on the prevalence of three soil-transmitted helminth species and on self-reported fever. We found that co-infection was a major driver of infection heterogeneity in school-aged children and was associated with between 16 and 67% of explained heterogeneity in individual models. Only one other factor, child's village of residency, had a similarly large influence. Our study demonstrates that co-infection is a key driver of infection heterogeneity and hence, should be a central consideration in future research. Only by taking into account co-infection's strong effects can we fully understand infection heterogeneity and thereby design effective disease control strategies.

OR148 An experimental test for interactions among co-infecting parasites in a wild mammal system

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Co-infection of a single host with multiple parasite species is the norm rather than the exception in nature. Understanding interactions among co-infecting parasites is important, as these will determine how parasite communities respond to disturbance, including drug and vaccine use. Yet the occurrence of interactions within natural host populations, and their consequences for parasite community stability, have rarely been experimentally assessed. Using anthelmintic treatment of wild mice, coupled with longitudinal follow-up of individuals, we tested for parasite interactions and examined parasite community stability in the face of drug-based perturbation. We found clear experimental evidence for strong, but short-lived interactions between nematodes and other gastrointestinal parasites (*Eimeria* spp.). However parasite communities as a whole were surprisingly robust to perturbation. From an applied perspective, this observed stability suggests that similar nematode treatments of humans or domestic animals may have strong, unintended local effects on non-target co-infecting parasites, but few significant effects on the wider within-host parasite community.

OR149 MICRODIVERSITY INSIDE MACROBIODIVERSITY : ZOONOTIC RISK ALONG THE CONGO RIVER

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The year 2010 was celebrated as the Year of Biodiversity and was marked by a series of great expeditions based on the model of the first explorers such as Stanley and Livingstone. The Congo River Expedition, *Boyekoli ebale Congo*, was set up by a consortium of Museums and academic institutions to realize an inventory of the Congo River biodiversity outside protected areas along the Congo River in DR Congo. However the term biodiversity includes not only macroorganisms but also smaller organisms (microbiodiversity) such as helminths, protozoa, bacteria and viruses.
In order to study the microbiodiversity in mammals, animals were trapped in different localities along the Congo River and along three main tributaries (the Lomami, Itimbiri and Aruwimi rivers), and in different habitat types (from domestic to natural rainforest). Bushmeat (either fresh or smoked) was also bought from local markets. Blood, tissues and ecto- and endoparasites were collected from most animals and stored for molecular or serological screening.
We present here the preliminary results on the microbiodiversity in mammals trapped along the Congo river, discussing the anthropozoonotic risk linked with host ecology, local practices and conditions, such as the hunting and consumption of bushmeat, and the lack of efficient diagnostic tools.

OR150 On-going onchocerciasis transmission under long-term ivermectin control

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According to the WHO, human onchocerciasis is potentially eliminable. This requires a thorough understanding of ongoing transmission, population dynamics and control effects. Empirical data on onchocerciasis in areas which have received prolonged vector control and mass annual ivermectin treatments will help parameterize models enabling locality- and vector-specific prediction of *Onchocerca* transmission. Such data will inform control programmes and help quantify for how long treatment must be sustained to achieve elimination. Seven study sites in four regions of Ghana were visited from 2009 to 2011 in both rainy and dry seasons. Host-seeking and host-independent (oviposition traps) blackflies (15,466; 85% *Simulium damnosum* s.l.) were collected, assessed for parity, and stored for molecular and morphological analysis for fly species-, *Onchocerca*- and past bloodmeal-identification. Daily biting rates ranged from 0 to 298 bites/person/day and parity from 18 to 27% (wet season) and 30 to 46% (dry season). The number of L3 larvae per 1000 parous flies was above the WHO threshold for morbidity and transmission control in three of the seven villages (range 1.4 to 115.1 L3/1000 parous flies) despite annual distributions of ivermectin for up to 23 years in one village. Ongoing research will investigate the impact of vector- and bloodhost-density.

OR151 Mechanisms of immune regulation at barrier surfaces

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Employing diverse models of microbial colonization, pathogen infection and chronic inflammation, research in the Artis lab is examining how mammalian host genetics and signals derived from commensal microbial communities influence innate and adaptive immune responses in the skin, lung and intestine. Intestinal epithelial cells (IECs) were recently shown to play a critical role in maintaining the balance of tolerance, immunity and inflammation at barrier surfaces including the gastrointestinal tract. Based on these findings, there are three major research areas in the lab. First, we are employing inducible deletion or overexpression of genes in IECs to interrogate how they regulate the functions of intestinal myeloid and lymphocyte lineages. The long-term goals of these studies are to improve oral vaccination against enteric infections and prevent chronic inflammation associated with diseases including food allergy and inflammatory bowel disease. Second, we are employing gnotobiotic mice to examine the influence of commensal microbial communities on intestinal and peripheral immune cell development and function. Our findings indicate that commensal microbes have a major regulatory influence on CD4+ T cell and granulocyte function associated with susceptibility to multiple inflammatory diseases. To determine if the immune system reciprocally regulates the acquisition and/or composition of commensal microbial communities, we are undertaking high-throughput pyrosequencing analyses of bacterial communities in murine models of health and disease. Third, we are investigating how IECs regulate allergen- or helminth-induced type 2 inflammation at mucosal sites. Secretion of IEC-derived cytokines including IL-25, IL-33 and thymic stromal lymphopoietin (TSLP) appear to be important early events in influencing dendritic cell and CD4+ T cell responses required these responses. Our recent studies suggest that IECs also govern extramedullary hematopoiesis that can influence the development of T_H2 cytokine responses. It is hoped that the results of these studies will advance understanding the pathophysiology of multiple mucosal inflammatory diseases, including asthma, allergy and inflammatory bowel disease and provide a framework to test the therapeutic potential of manipulating IEC responses in these disease states.

OR152* Alternatively activated dendritic cells regulate CD4+ T-cell polarisation *in vitro* and *in vivo.*

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The Th2 cytokine IL-4 has potent effects on multiple cell types of both the innate and adaptive immune system. A wide body of literature details the ability of this cytokine to cause 'alternative' activation of macrophage (MΦ) populations, both *in vitro* and *in vivo*. The importance of IL-4 signaling to MΦ was previously highlighted when it was shown that the presence of IL-4Rα on MΦ populations was required for survival following infection with the parasite *Schistosoma mansoni.* The impact of IL-4 on dendritic cell (DC) populations is, however, less well characterised. This work shows IL-4 dependent up-regulation of alternative activation markers within DC populations *in vitro* and *in vivo*. Furthermore, alternatively activated DCs (AADC) were identified in several tissue sites during *Schistosoma mansoni* infection. We also show a functional role for AADC-derived RELMα in the optimal induction of T cell IL-10 production. This is the first report providing a comprehensive account of alternative activation of DCs by IL-4 both *in vitro* and *in vivo.*

OR153 Metazoan parasites, IgE, immunity and allergy.

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IgE is a major response in just two circumstances, exposure to metazoan parasites, worms and arthropods, and in the allergic diseases that have become epidemic in the developed world. IgE is a late evolutionary adaption in mammals that has been linked with immunity to these parasite infections. In human populations living schistosomiasis endemic areas, the slow development of a partial immunity to re-infection is consistently reported to be associated with the development of IgE against allergen-like worm antigens. Such studies suggest that research into the natural history and host-parasite relationships of worm and arthropod parasites in disease endemic regions can provide unique insights into questions that are central to allergy research, such as, the induction/regulation of IgE and its effector mechanisms and, even: Why do only a small number of protein structural families act as environmental and food allergen targets for IgE-mediated allergic reactions? Multi-disciplinary parasitology studies in disease endemic areas can contribute knowledge that will to help combat disease in both the developing and developed world.

OR154 Phenotypic analysis of colonic macrophages in CX3CR1$^{+/eGFP}$ mice infected with the parasitic nematode *Trichuris muris*.

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Trichuris muris is a nematode parasite of the mouse which dwells in the large intestine. It is a natural mouse model of *Trichuris trichiura*: a prevalent and debilitating parasite of humans worldwide. A Th2 immune response is essential for the expulsion of worms. However, the nature of the ensuing inflammatory response (and its regulation) is not fully understood. Previous studies in this laboratory have shown that macrophages are the predominant type of inflammatory cell in the large intestine post-infection. This study aims to determine the phenotype of these cells. Leukocytes were isolated, by enzymatic digestion, from the large intestine of CX3CR1$^{+/eGFP}$ mice and the macrophages were analysed by multi-colour flow cytometry. In this mouse, cells expressing the chemokine receptor CX3CR1 also express eGFP. Macrophages were defined as F4/80^{+}CD11b^{+}I-A/I-E^{+}Siglec-F^{-}. Two contrasting populations of CX3CR1^{+} macrophages were identified. The first, F4/80^{high}CX3CR1^{high} and predominantly Ly6C^{-} TLR-2^{-}, was relatively abundant in uninfected mice. This phenotype is consistent with resident macrophages. In contrast, the second, F4/80^{low}CX3CR1^{low} and Ly6C^{+}TLR-2^{+} was relatively abundant post-infection. This population is consistent with inflammatory macrophages. Currently, we are further characterising these disparate macrophage populations. Our data describe, for the first time, the changes which occur to resident and inflammatory macrophages following infection with a gut-dwelling helminth.

OR155 Genetic diversity, immunity and resistance to multiple pathogens in a natural rodent population

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Much of what we know about the genetic basis of immunity to infection has come from studies of laboratory animals. However, these animals are kept in conditions very different from those experienced in the natural environment. In order to improve our understanding of the genetic determinants of disease susceptibility, it is therefore important to examine genetic variation and immunity in natural populations. Studies so far have focused almost exclusively on genes of the Major Histocompatibility Complex (MHC). But while the MHC is undoubtedly important in immunity to infection, there are many other genes involved in the immune response that are yet to be investigated. Here we examine genetic variation in cytokines, signalling molecules crucial in the induction and regulation of the different effector arms of the immune response. We use a natural population of field voles, wild rodents related to common laboratory species, and show that variation within cytokine genes is linked to differences between individuals in their immune response and in resistance to multiple pathogens. Some of these genes also show patterns of diversity associated with natural selection. Our results also demonstrate the potential of using wild rodents as a model to discover loci across the genome associated with pathogen resistance.

OR156* Gut microbial diversity in field-caught mosquitoes

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Bacterial symbionts of mosquitoes are thought to play important roles in controlling the rate at which human and animal diseases can be vectored. We investigated the diversity of the bacterial microflora in eight species of mosquitoes collected in Kenya using next generation sequencing. We found that there is surprisingly little diversity in the gut flora of a single mosquito. However, within a species individuals often harbour very different bacteria in their guts, which could potentially result in differences in disease transmission. In contrast, there is little evidence that different species have distinct gut bacteria. Overall, our results highlight the need to understand how variation in mosquito's microbiota affects disease transmission.

OR157 Toll-Like Receptor 2 (*TLR2*) mediates the resistance to *Borrelia afzelii* in a natural reservoir host

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Lyme borreliosis is the most common vector-borne illness in Europe and North America. It is caused by members of the *Borrelia burgdorferi sensu lato* bacteria complex, which are transmitted by ticks to a large number of vertebrate hosts, including rodents, birds and humans. In this study we used a candidate gene approach to investigate genetic mechanisms of resistance to *Borrelia afzelii* in a natural population of one of its main reservoir hosts, the bank vole (*Myodes glareolus*). We show that different genetic variants of Toll-like receptor 2 (*TLR2*), an innate immune receptor that recognises lipoproteins of *Borrelia* and initiates innate immune responses in the host, are associated with high and low levels of *Borrelia* infection. Hosts with one or two copies of the protective *TLR2* variants had a lower *Borrelia* infection intensity, indicating that dominance effects are involved in *Borrelia* clearance. Furthermore, hosts with two copies of the protective *TLR2* variants were less likely to be infected with the most prevalent *Borrelia* strain and they were infected with fewer *Borrelia* strains. These results highlight the important role of *TLR2* in mediating *Borrelia* resistance in a natural reservoir host and indicate that innate immune defence plays an important role in regulating host-*Borrelia* interactions in wild vertebrate populations.

OR158 Molecular epidemiology of ascariasis

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More than 1 billion people are infected with the intestinal roundworm, *Ascaris*. Although the greatest numbers of infected individuals are found in Asia and sub-Saharan Africa, ascariasis shows a cosmopolitan distribution and cases are found in both developing and developed countries. We are using molecular epidemiology techniques to study the population structure of *Ascaris* at a global and local scale. Around 550 ascarid worms were obtained from human and pig hosts in East Africa, Asia and Europe. Genomic DNA was extracted from all worms and a 383 base pair region of the mitochondrial cytochrome c oxidase 1 gene (*cox1*) was sequenced for each worm. Sequences were aligned to identify substitutions, and phylogenetic analysis and assessment of genetic diversity was undertaken. Over 70 different *cox1* barcodes have been identified in *Ascaris* from humans and pigs so far. There is near complete segregation of barcodes between pig and human worms in Africa but in Europe the same barcodes are found in worms from both hosts. Microsatellite analysis of the *Ascaris* DNA using eight loci revealed substantial genetic differentiation between human and pig worms from developing countries but less between human and pig worms from developed countries. These results provide insights into the global transmission dynamics of *Ascaris*.

OR159* Sex and species recognition in *Plasmodium*

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In order to transmit to new hosts, malaria parasites must undergo a round of sexual reproduction in a mosquito vector. However, we still know remarkably little about the ecology and molecular evolution of sex in malaria parasites.

In areas where multiple-species infections are common, there is potential for inter-species interactions at the moment of mating. Consequently, the possibility of hybridization between different *Plasmodium* species should not be discarded.

We developed an experimental approach, using genetically modified parasites in order to test whether hybridization can occur between *P. berghei* and *P. yoelii*. Contrarily to conventional wisdom, our results show that malaria parasites can hybridize. However, this only occurs at significant levels (i.e. no discrimination between con- and heterospecifics) when two important proteins (P230 and P48/45) are absent from the surface of female gametes. Therefore, we suggest that P230 and P48/45 are involved in mate recognition.

In metazoan organisms, mate recognition genes are regularly under positive selection. In order to test for the taxonomic generality of this observation, we have collected an extensive sample of sequence data for P230, P48/45 and P47 (P48/45 paralogue), from all rodent malaria parasite species. Our data reveals the type and strength of selection upon these genes and may be of relevance for the development of transmission-blocking vaccines.

OR160 Monarch butterflies practice herbal medicine: consequences for infectious disease and host-parasite coevolution

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Parasites can dramatically reduce the fitness of their hosts, and natural selection should favour defence mechanisms that can protect hosts against disease. Much work has focused on understanding genetic and physiological immunity against parasites, but hosts can also use behaviours to avoid infection, reduce parasite growth or alleviate disease symptoms. Here, I will describe the phenomenon of trans-generational medication, in which animals actively use medicine to mitigate disease in their offspring. Monarch butterflies are naturally infected with virulent protozoan parasites, and our studies have shown that neither caterpillars nor adult butterflies can cure themselves of disease. Instead, infected adult butterflies preferentially lay their eggs on toxic plants that reduce parasite growth and disease in their offspring caterpillars. These results demonstrate that infected animals may use medicine as a defence against parasites, and that such medication may target an individual's offspring rather than the individual itself. Trans-generational medication directly affects parasite infection and disease, and is likely to act strongly on host-parasite coevolution.

OR161* Weather effects on tick burdens of otters, *Lutra lutra*

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Otters transcend terrestrial, freshwater and marine habitats. Such hosts, with diverse life styles, can be used to investigate the environmental cues important in controlling generalist parasite distributions. We examined the influence of inter-annual variations in large-scale weather and host characteristics on tick prevalence and intensity of Eurasian otters, *Lutra lutra* (N = 575 road kills). Only *Ixodes hexagonus* (prevalence = 24.3%; mean intensity = 7.2; range = 1-122) was recovered from otters in England and Wales. This tick tends to quest within dens, using nocturnal mammals as hosts and is usually associated with hedgehogs. In general, ixodid ticks are most abundant when weather conditions are both warmer and wetter, conditions associated with positive phases of the North Atlantic Oscillation (NAO). For *I. hexagonus*, both prevalence and intensity were positively associated with positive phases of the NAO. Further, tick prevalence had a positive association with higher mean Central England temperatures. Tick prevalence on juvenile otters was higher than sub-adult or adult otters. This association is probably related to the greater length of time spent in the holt (an otter den) by juvenile otters. Otters in poorer condition were found to have higher intensities of ticks indicating that either poorly conditioned hosts are more susceptible to ticks, or tick infestations negatively impact on host condition. Otters are clearly an important and common host for *I. hexagonus*.

OR162 Reshaping the fitness landscape of host species choice in African malaria vectors using interventions: a strategy for evolutionary sustainable control?

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Here we conducted for the first time an experimental investigation of the strength of selection acting on African malaria vectors *An. arabiensis* and *An. gambiae s.s* to preferentially specialize on human instead of animal hosts within natural transmission settings. We used novel semi-field mosquito biosphere within a malaria endemic region of Africa to directly investigate the strength of counterselection placed on vector host species choice by the control measures commonly used in the field: bednets. We experimentally measured the feeding success and subsequent fitness of the two major African vectors (> 16,000 mosquitoes) on humans and other potential host types they co-occur. Experimental data were used to parameterize a stochastic life – history model that combined all measured impacts of host species choice on vector fitness to predict the cumulative effects on the key metric of selection: total lifetime reproductive success. We demonstrated that mosquito fitness depends on host species choice, and the use of bednets substantially reduced the fitness ranking of humans relative to other animal alternatives. For *An. arabiensis*, the vector now dominating in many parts of Africa, the use of bednet widened the fitness differential between humans and cows to an extent where strong selection for zoophily is expected when most people use nets, and cattle are available. This provides proof-of-principle that evolutionary sustainable control approaches incorporating the use of selection for mosquitoes to shift their host choice could enhance the effectiveness of frontline disease control strategies.

OR163 Malaria infection increases bird attractiveness to uninfected mosquitoes

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Among vector-borne diseases, parasite-induced modifications favouring parasite transmission could have a great impact on the epidemiology of the disease. Adaptations aiming at increasing vector-to-host transmission have been well described for *Plasmodium* parasites (e.g. altered vector biting behaviour) but little is still known on the ability of the parasite to alter host-to-vector transmission. Do malaria parasites enhance the attractiveness of their hosts to the vector? To investigate this issue, we assessed how parasite infection modifies the attractiveness of canaries *Serinus canaria* to uninfected *Culex pipiens* mosquitoes, the natural vector of avian malaria parasite, *Plasmodium relictum*.
After an initial assessment to control for inherent differences in attractiveness of pairs of birds, one bird was experimentally inoculated with malaria and vector preference for uninfected vs. *Plasmodium*-infected birds was assessed during both the acute (10 days post-infection) and the chronic (24 dpi) phase of the infection. Mosquito blood meals were analyzed using molecular microsatellite markers to quantify bird attractiveness/vector preference. Our results show that chronically infected birds attract significantly more vectors than uninfected birds, suggesting a manipulation of the host by *Plasmodium* parasites aimed at increasing their transmission.

OR164* The impact of *Toxoplasma gondii* on host behaviour: studies on mechanism of action

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The aim of my PhD is to further elucidate the characteristics of and the mechanisms by which the protozoan parasite *Toxoplasma gondii* may be altering host behaviour.
Using the epidemiologically and clinically applicable rat-*T. gondii* model, and incorporating a battery of both classical and novel non-invasive behavioural and physiological assays, our overall prediction is that the parasite is manipulating its host, at least in part, by elevating dopamine levels.
Results to date indicate that infected females had significantly increased predation risk and altered behavioural profiles relative to their uninfected counterparts. This may be explained by a gender effect, or by the animal's speed of movement. Initial data examining neuromodulator profiles indicated a trend for L-DOPA and serotonin levels to be higher in infected rats, both males and females, particularly in the striatum, to be increased with infection.
These initial results indicate that gender and/ or activity levels interact with infection to increase the host's attraction to definitive host odour. The differences between genders observed support previous human studies which indicate that *T. gondii* may have very differential effects in males and females.
We discuss our results in terms of their theoretical and applied implications.

OR165 Population biology of drug resistance: Comparing viral, bacterial and microparasitic infections

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The emergence of resistant pathogens in response to selection pressure by drugs and their possible disappearance when drug use is discontinued are evolutionary processes common to many pathogens. Despite the existence of generic features that underlie such evolutionary dynamics, different conclusions have been reached about how to best use drugs to minimize the risk of generating high levels of resistance. To which extent these discrepancies in the evolutionary dynamics and treatment recommendations are attributable to specific properties of the pathogen, the host, or the general biological context is currently unclear. Population biological modeling may help in the identification of factors that result in differences in the evolution of resistance between different pathogens. In my talk I will first discuss some of the generic population biological principles underlying resistance evolution and then present some first steps towards a generalized model that allows investigating the benefits and risks of aggressive treatment for different diseases.

OR166 Immune ageing in a wild mammal population

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Immune phenotypes and immune system function change profoundly across the life courses of animals. Age-related variation in immunity could have important consequences for disease resistance, epidemiology and population dynamics of natural populations, although it is rarely studied in such contexts. Our research aims to address this using samples collected from a free-living population of Soay sheep on St Kilda, which has been the subject of a long-term study. We demonstrate cross-sectional variation in a range of immune markers with age in female sheep which are consistent with patterns of immune ageing observed in humans and lab mice. For instance, we provide the first evidence from a wild mammal for decreased proportions of naive T cells and increased acute phase protein levels in older individuals. I will also present ongoing research which aims to understand the links between maternal antibody transfer and growth and survival in newborn animals. Finally, using data on natural antibody levels, I will show that population-level age-related variation in an immune marker is driven by selection rather than an intrinsic change and present new work that delves deeper into the links between survival and antibody levels during adulthood.

OR167 Comparing approaches for inferring the occurrence of interspecific parasite interactions

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There is considerable interest in the occurrence and significance of interspecific parasite interactions. Lab experiments clearly show that co-infecting parasites have great potential to affect each other’s dynamics and abundance. However, the occurrence of such interactions in natural populations remains unclear; some studies suggest that parasite interactions are negligible, while others suggest they are powerful forces underlying parasite dynamics. One potential source of this variability that has yet to be adequately explored is the reliability of the techniques used to detect interactions. Typically studies are observational, often using cross-sectional data, and infer the presence of interactions from patterns of parasite co-occurrence or abundance. However, as is well known in classical community ecology, the best way of detecting interspecific interactions is to experimentally remove one species and follow the response of the other species. We have carried out such an experiment in a naturally-occurring host-parasite system, revealing a strong negative interaction between two co-infecting parasite taxa. Here we test the reliability of various analytical techniques by applying them to the observational data from this experiment, and ask whether they predict the occurrence of the experimentally-revealed interaction.

OR168 Dynamics of reciprocal selective sweeps in an insect-virus system

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Host-parasite coevolution can result in consecutive selective sweeps of host resistance alleles and parasite counter-adaptations. To illustrate the dynamics and outcome of this important but little studied form of coevolution, we have modeled an ongoing arms race between *Drosophila melanogaster* and its vertically transmitted parasite the sigma virus using parameters that we have estimated in the field. We integrate these results with previous work showing that the spread of a resistance allele of the *ref(2)P* gene was followed by the spread of a virus genotype that overcomes this resistance. In line with these observations, our model predicts that there can be rapid selective sweeps in both the host and parasite, and that this can drive large changes in the prevalence of infection. The virus will tend to be ahead in the arms race, due to incomplete dominance slowing down host adaptation and weaker selection for host resistance than for parasites to overcome resistance—the 'life-dinner' principle. This asymmetry in the rate of adaptation results in a partial sweep of the host allele as it loses its advantage part way through the selective sweep. This well understood natural system illustrates how the outcome of host-parasite coevolution is determined by different population genetic parameters in the field.

OR169* Quantifying cross-species transmission from pathogen sequence data: classical swine fever virus in Europe as a case example

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Infectious diseases are often shared by multiple host species but the relative host contributions complicate the transmission quantification. This problem commonly has applied relevance, for example in the case of wildlife species acting as a reservoir for livestock diseases. While relevant information from wildlife are generally limited, pathogen sequence data with saptio-temporal references are commonly collected. Here, we apply phylogenetic tools to such data to gain quantitative insights into the phenomenon of cross-species transmission in a two-host system. We focus on classical swine fever virus (*Pestivirus*), a highly infectious and economically important virus infecting domestic pigs and their wild relatives. We assembled a dataset of 375 sequences of the partial envelope gene *E2,* sampled from domestic pigs (2/3) and wild boars (1/3) across Europe over the past 20 years. An initial cluster analysis identified three spatial groups. Different models of cross-species transmission were then compared, allowing for various amount of transmission heterogeneity within and among groups. The best model supports asymmetrical transmission among species indicates a predominant role of boar to pig transmission. However, accounting for sampling biases by balancing the dataset shows that transmission from the oversampled species is underestimated. This finding is supported by simulation studies and highlights the importance of sampling considerations for estimating cross-species transmission from genetic data.

OR170 Role of *Plasmodium* host cell traversal in the evasion of liver innate immunity

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Plasmodium sporozoites are inoculated in the host skin by a mosquito bite and migrate to the liver, where they invade and develop inside hepatocytes. How sporozoites cross the liver sinusoidal barrier to reach hepatocytes and the *in vivo* contribution of their ability to wound and transmigrate host cells (cell traversal) are long debated and still unresolved questions. Here, we show by intravital imaging in rodents that, in contrast to the established model of sporozoites translocating the liver sinusoidal barrier exclusively through the hepatic macrophage called Kupffer cell (KC), sporozoites use multiple, including KC-independent, crossing paths. Using novel endothelial cell (EC) and KC wounding intravital assays and cell traversal-deficient sporozoites, we show that the sporozoite cell traversal ability is important for crossing the barrier via traversal of EC and/or KC and is crucial for resisting clearance by KC in the sinusoids, ensuring sporozoite survival in the liver.

OR171 Real-time *in vivo* imaging of mice infected with transgenic *Trypanosoma cruzi* expressing 'red-shifted' firefly luciferase

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Various aspects of Chagas disease make small animal models experimentally challenging, including pleiotropism of the causative agent (*Trypanosoma cruzi*), and relatively low and transient parasitaemia, particularly during the chronic stage of disease. Bioluminescence imaging methods therefore represent a potentially valuable tool for the study of disease pathogenesis and for evaluating novel therapeutic compounds. A transgenic *T. cruzi* cell line, constitutively expressing the 'red-shifted' firefly luciferase variant Ppy RE9, was generated by integration of a construct into the ribosomal DNA locus. Luciferase expression levels were similar in epimastigote, trypomastigote and amastigote forms, tightly correlated with parasite number and stable for >3 months continuous culture in the absence of selective drug pressure. A bioluminescent clone was used to image acute infection in an immunocompromised host (SCID mice). The limit of detection was <1000 parasites. As proof of principle for monitoring drug treatment, we showed a significant loss of bioluminescent signal in SCID mice treated with benznidazole compared to untreated controls, with parasite detection sensitivity superior to peripheral blood parasitaemia counts. We were also able to monitor the distribution and intensity of bioluminescence in real-time in a model of chronic disease using immunocompetent BALB/c mice, with specific foci of parasites detectable even several months post-infection.

OR172 Imaging host cell infection by *Leishmania donovani* provides a new view of the early infection process

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L. donovani is the responsible agent of visceral leishmaniasis, one of the major neglected diseases, that is fatal if left untreated. Despite the important impact of this disease in endemic areas, our understanding on how flagellated infectious promastigote parasites enter and colonize vertebrate host cells is limited.
Using high spatio-temporal resolutive microscopy technologies we dissected in real time the initial encounter of promastigotes with phagocytic host cells and provided a complete view of the early infectious process. We revealed that highly polarized and motile promastigote parasites specifically invade host cells through their flagellar tip. Once internalized the parasites re-oriented quickly such that their flagellum faced toward the cell periphery. At this point persistent flagellar activity resulted in a long lasting oscillating movements of the intracellular parasite toward and outward the host cell center. We then demonstrated that *L. donovani* infection was associated with recruitment, docking and exocytosis of lysosomes at the parasite oscillating site. Finally, we correlated focal lysosomal exocytosis at the parasite location with local wounding of the host cell plasma membrane. Our work provides new insights into the mechanism used by *L. donovani* to gain access to the host cell that involve parasite polarity and motility and parasite-mediated host cell injury.

OR173 *In vivo* imaging models of African trypanosomiasis to support drug discovery programs

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Human African Trypanosomiasis (HAT) remains highly prevalent in sub-saharan Africa. Chemotherapy of HAT is associated with toxicity, complex dosing regimens and emerging drug resistance. Current models for assessing drugs against second (CNS) stage disease are cumbersome and time consuming. This project aims to improve drug screening approaches by using fast and robust *in vivo* imaging systems, and enhance our understanding of how and when trypanosomes cross the blood-brain barrier to become established in the brain. We generated strains of *Trypanosoma brucei* expressing bioluminescent proteins for non-invasive *in vivo* imaging of whole mice using a sensitive camera (IVIS). This allows longitudinal monitoring of parasite distribution and burden in infected mice through the full course of infection. Fluorescently-labelled *T. brucei* were imaged through the thinned skull *in vivo* with two-photon microscopy to localise and examine trypanosomes in the brain in high resolution. Using these imaging modalities we monitored disease progression *in vivo* in a second stage GVR35 model. Trypanosomes were detected in various organs including the brain. Two-photon microscopy showed early invasion of the superficial meninges, and that this site was accessible to first stage trypanocidal drugs. We showed effects of known trypanocidal drugs on regional parasitaemia, demonstrating the value of our imaging approach for future drug discovery.

Poster Presentations

P1* Effects of within-host parasite interactions on coinfection treatment outcomes

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Treatments for parasitic infections are often less effective in people infected with multiple parasites. Interspecific interactions between parasites coinfecting the same host may contribute to this increased morbidity. Mathematical models of parasite treatment should include interactions between parasite species within coinfected hosts, and show how interactions within individual hosts scale-up to determine treatment effects at the population level. We used an individual-based model to evaluate treatment outcomes in a coinfected population. The model broadly represents a human community receiving chemotherapy where hosts vary in their susceptibility to two chronic infections. We assessed the effects of treatment under different associations or interspecific interactions between the two parasite species. Results suggest that treatment can have counterintuitive indirect effects on coinfecting parasites depending on the type and direction of the underlying interaction. Modelling the effects of interspecific parasite interactions could therefore have implications for medical programmes for many infections around the world.

P2 Disease dynamics in water vole metapopulations

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Animal populations often exhibit patchy distributions in space as a result of heterogeneous resource distribution or social aggregation. A metapopulation is a set of sub-populations, distributed into discrete patches and linked by dispersal. Metapopulations are distinguished by patch population extinction and re-colonisation events. This patchiness and population turnover can have profound influences on parasite transmission. Host Spatial structure forces more localised transmission, with secondary infections becoming more likely with increasing proximity to an infected host or parasite source. Using a well characterised water vole (*Arvicola amphibius*) metapopulation, this research project explores the extent to which endemic parasite dynamics are influenced by host metapopulation structure. Specifically, for a range of microparasites and macroparasites, I will determine how local population size, population connectivity, and transmission from alternative hosts influence the distribution and prevalence of infected hosts. This study will provide empirical evidence to a field dominated by theoretical modelling, revealing how parasites with different life cycles and transmission modes are influenced by host population spatial structure.

P3 Pharmacologic prevention rather than pharmacologic treatment of chronic toxoplasmosis by a phosphodiesterase-4 (PDE4) inhibitor.

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Toxoplasma gondii is an obligate intracellular parasite responsible for encephalitis in immunocompromised patients. Bradyzoites conversion to multiplication-unchecked tachyzoites initiated and regulated mainly by Th1 cytokines is pathognomonic. Cyclic nucleotides (e.g. cAMP) may be involved in signaling this conversion. As there is no effective treatment of chronic toxoplasmosis, preventing its establishment seems to be a logic approach to avoid the serious consequences of parasite reactivation. Phosphodiesterase inhibitors causing sustained cAMP elevation can inhibit Th1 response. We suggest that rolipram, never used in treatment of parasitic infections, could prevent the establishment of a chronic *Toxoplasma* state. Mice infected with KSU strain of *T. gondii* were treated with rolipram, 10 mg/kg/day, for three weeks, starting day-7 after infection. *Toxoplasma gondii* was never cleared by modulated immune response, nevertheless, rolipram partially prevented the progression to chronic state. The load of *Toxoplasma* brain cysts showed a 74% reduction and the induced steatosis and inflammation were dramatically opposed in liver and brain. Rolipram significantly decreased numbers of *Toxoplasma*-induced inflammatory foci per liver area (57.4%) and of nucleated cells per inflammatory focus (61.3%). Strong inhibition of TNF-α, IFN-γ and IL-12 production (84.6, 76.7 & 71% respectively) was demonstrated. In conclusion, the current study reports, for the first time, a potential application of rolipram as a novel approach of pharmacologic prevention rather than pharmacologic treatment of chronic toxoplasmosis.

P4* Parasites, olfaction and vision in fish behaviour

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Animals use both olfactory and visual information to make decisions. Ethological and physiological evidence suggests that there is cross-modal interaction between these sensory systems in fish. The data presented here are the first to show that ecologically relevant chemical mixtures alter visual behaviour in adult male and female zebrafish, *Danio rerio*, and guppies *Poecilia reticulata*. The effect of *Gyrodactylus turnbulli* parasitism on guppy sensory behaviour was also tested. After being held at a predetermined species-specific irradiance level ('dark'), zebrafish were exposed to food cue or to alarm cue, guppies to food odour. The light intensity was subsequently increased in steps. Videos (IR illuminated) of fish reactions to visual stimuli at different light levels were used to score visual behaviour responses. Adult male and female zebrafish responded to a moving visual stimulus at lower levels of irradiance if they had been first exposed to olfactory stimuli. Guppy sensitivity to both full spectrum 'white' and 'red' light (630nm) was tested in unparasitised and parasitised individuals after exposure to food odour or water, and the observed pattern was similar to that in zebrafish. Further work investigating the effects of parasitism on this sensory interaction in guppy mate choice is described.

P5* Effects of flow dynamics and parasitism on swimming and shoaling behaviour: the guppy *Poecilia reticulata* as a model organism

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Riverine fish commonly experience turbulent and changing flow rates throughout their lifetime, and adapt their swimming behaviour in order to maintain position or to migrate up- and downstream. Flow characteristics in a stream or river are highly variable in space and time, therefore open channel flume experiments provide the opportunity to control flow conditions to identify the important factors influencing fish behaviour. Previous studies have shown that fish can exploit turbulence associated with physical structures in the stream to reduce the locomotory costs of swimming; however infection with parasites may affect the swimming behaviour of the host. For example, heavy rainfall during the wet season in Trinidad causes flushing events which have been shown to disrupt the population ecology of the guppy *Poecilia reticulata*, with males infected with *Gyrodactylus turnbulli* and *G. bullatarudis* being more likely to be swept downstream than uninfected counterparts. Using the guppy as a model host, the current study aims to determine how differing flow rates, placement of obstacles, and the ectoparasite, *Gyrodactylus turnbulli,* affect host swimming and shoaling behaviour using an open channel flume to control flow dynamics.

P6 Mafian schistosomiais is not autochthonous!

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To confirm the local endemicity of *Schistosoma haematobium* on Mafia Island, Tanzania, conjoint parasitological and malacological surveys were undertaken in July 2006 with parasitological investigations supplemented with case-history questionnaires. A total of 238 children (125 girls and 113 boys, mean age of 13.9 years) across 9 primary schools were examined. The prevalence of micro-haematuria and egg-patent infection was 18.1% (CI_{95}=9.6-33.6) and 4.2% (CI_{95}=1.9-7.6), respectively but a strong female bias was observed for micro-haematuria (5.6F:1M) contrasting with a strong male bias for the presence of eggs (1F: 4M). All egg-patent infections were of light-intensity (<10 eggs / 10ml). No clear associations between infection prevalence and local water-contact, by school, were found and all 10 of the egg positive children had a travel history to the nearby mainland or Zanzibar. Inspection of community diagnostic registers at Kilindoni Hospital revealed a low prevalence (< 2%) of egg-patent infection for 20,306 samples tested in the 2000-2005 period. A total of 43 freshwater sites, a third of which were previously sampled in 1999 & 2002, were surveyed and eleven species of freshwater mollusc were found and no collected snail was observed to shed schistosome cercariae. After consideration of molecular DNA typing of parasite material and upon comparison to the African mainland, it seems that Mafian schistosomiasis is an imported infection and not autochthonous.

P7* Mortality among zebu cattle under one year; the role of co-infections

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In natural populations, individuals may be infected with multiple pathogens at a time with varying outcomes on host health, performance and survival. To study effects of pathogens and their interactions on host survival, we followed 548 zebu cattle in their first year of life. Using a combination of clinical signs before death, laboratory, post-mortem, histo-pathology results, and survival analysis statistical techniques, we estimate mortality rates, cause-specific aetiologies, and risk factors associated with calf mortality. In addition, we investigate the role of co-infections in observed mortality patterns. The all-cause mortality rate was 16.1%. Maternal effects were significant predictors of calf survival with bigger, healthier dams associated with lower hazard rates (OR = 0.94, p=0.033). Helminth infections and *Theileria* spps high-intensity infections were associated with higher odds for mortality (OR =1.27, p< 0.001 per 1000 egg per gram increase, and OR = 5.5, p< 0.001, respectively). East Coast Fever (ECF) was the single most important disease associated with calf mortality accounting for 37.1% of all deaths, followed by Haemonchosis accounting for 11.2%. However, the risk of death due to ECF was itself influenced by helminth burden (OR = 1.41, p<0.001). This finding has important implications on disease control strategies suggesting benefits of an integrated approach to worm and ECF disease control.

P8* The effect of nematode infection on reindeer fitness during the Arctic winter.

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There are two main trichostrongyle nematodes that infect Svalbard reindeer: *Ostertagia gruehneri* and *Marshallagia marshalli*. Like most gut nematodes, *O. gruehneri* is transmitted during the summer and has been implicated as a significant factor in regulating population dynamics of Svalbard reindeer. In contrast, we have recently demonstrated that M. *marshalli* is transmitted throughout the Arctic winter, from October to April. During this time Svalbard reindeer are vulnerable due to starvation. Here, we report the results from long-term anti-helmintic capture-recapture experiment where we remove parasite burdens at the start of the winter in order to quantify the impact of *M. marshalli* on Svalbard reindeer fitness.

P9 Genetic Modulation of Trichuris muris infection by loci on Chromosome 4 and 5 in BXD recombinant inbred mice.

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Trichuris muris is a well characterized gastrointestinal nematode of mice. Expulsion of this nematode is facilitated by a polarized Th2 response in infected mice whilst a polarized Th1 response leads to chronic infection. We have utilised a large population of genetically well characterised strains of mice (BXD recombinant inbred strains) to map gene variants that influence worm burden and parasite specific cytokine production. We performed analysis of these phenotypic traits in 20 BXD RI strains and the 2 parent strains (C57BL/6 and DBA/2). All traits were highly variable across the panel and phenotypic values were regressed for each individual against age, parent and batch of eggs used for infection. Analysis was performed separately on male and female mice as gender is a known confounding factor in resistance to *T. muris* infection.
QTL analysis was carried out using WebQTL (http://www.genenetwork.org). Suggestive QTL for the worm burden phenotype in both males and females were identified on chromosome 4. Significant QTL for the interferon-gamma phenotype in male animals was identified on chromosome 5 that also overlapped with the tumour necrosis factor-alpha phenotype in males and the interleukin-6 phenotype in both males and females. Using bioinformatic tools and expression databases available for these strains on genenetwork, we have identified positional candidates in these quantitative trait locus intervals.

P10 Artificial selection on parasitic castration reveals limited evolutionary potential

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The strategy a pathogen implements to exploit host resources in an adequate manner to maximise its own fitness is of particular importance for the evolution of parasite life-history and virulence. Using the crustacean host *Daphnia magna*, we tried to test experimentally for the adaptive significance of castration and gigantism as the main symptoms of infections with the bacterium *Pasteuria ramosa*, using artificial selection. An early study had hinted at the presence of genetic variation underlying these traits, which are involved in coevolutionary processes shaping the expression of virulence. During the course of 5 host generations, parasites where selected for either early or late host castration. According to the 'temporal storage hypothesis', hosts infected by a late castrating strain of *P. ramosa* should display a reduced level of gigantism as correlated response to selection on castration time point and vice versa. However, after five generations of artificial selection, no divergence of treatment groups could be observed. This finding indicates limited possibilities for evolutionary response for this trait. We discuss different possible causes for our finding.

P11 Occurrence of Intestinal parasitic diseases of mice (*Mus musculus*) in Gaza and Rafah, Gaza Strip, Palestine

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Objectives: To study the incidence of intestinal parasites and ectoparasites of mice home in both Gaza and Rafah, the Gaza Strip .
Methods: This study included the digestive tract of 104 mice; the digestive system was removed after dissection from duodenum to the rectum. Stool samples were taken and saved by SAF were intestine was washed by running water into the sieve and the seeing worms were washed with saline solution and then preserved by ethyl alcohol 70%. Stool samples were examined under microscope using saline and iodine using X10 and X40 in the laboratories of the Biology Department in the period November 2010 to April 2011.
Results: It was found that mice were infected with intestinal parasites with prevalence 48.1% and for coccidia 35.6%. It was possible to identify some species of parasites, especially Giardia and some larvae of the worm and Entrobious. The prevalence of parasites for protozoa was 40.4% and for nematode was 16.3%.
It is concluded that the house mouse harboring some types of intestinal parasites and mechanical transmission to human is applicable.
Recommendations: It is recommended to control mice by conventional traps, and by breeding cats.

P12* Using field perturbation experiments to unravel the multi-host dynamics of bacterial parasites in a natural rodent community.

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Many parasites infect multiple host species. This is an important property, relevant to both novel disease emergence and the maintenance of endemic disease in ecological communities. Predicting future disease emergence, and understanding disease maintenance, requires an appreciation of the ecological mechanisms underlying transmission between multiple host species. For example, interactions between hosts and parasites, infection seasonality, and interactions between co-infecting parasites may all be implicated in determining the likelihood, and rate, of cross-species transmission.
Here I present results from a field-scale perturbation experiment in which we aimed to reduce cross-species transmission of flea-borne bacterial blood parasites (*Bartonella* spp.) within a community of bank voles (*Myodes glareolus*) and wood mice (*Apodemus sylvaticus*). At least five species of *Bartonella* naturally circulate within this woodland rodent community, and seasonal prevalence data suggest varying levels of host-specificity and therefore potentially differing levels of cross-species transmission. To test this, we administered an insecticide to all voles caught on experimental grids, to prevent fleabites and therefore vector transmission of *Bartonella* to wood mice. This experimental approach aims to provide novel insights into the way that transmission processes, within and between multiple host populations, interact and underlie emergent epidemiological measures such as prevalence, infection intensity and duration of infection.

P13* Studies on Varroa mites of the honeybee population in Northern Ireland

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Managed honeybees represent the most economically important pollinator species of agricultural plants. Dramatic declines world wide of Western honeybee (*Apis mellifera*) populations are mirrored in Northern Ireland and have largely been attributed to the endoparasitic activities of the *Varroa destructor* mite. Originally, *V. destructor* parasitized the Eastern honeybee (*Apis cerana*) which has evolved to be *Varroa* resistant. *V. destructor* has now shifted to parasitize *A. mellifera* which, due to a much shorter host-parasite association, have not developed *Varroa* resistant behaviours. *Varroa* mites feed on the haemolymph of bee larvae, pupae and adults and act as a mechanical and biological vector for viral pathogens such as deformed wing virus (DWV). Current control methods rely on a restricted number of acaracides, resistance to which has become common and widespread and has recently been documented in Ireland. This threatens to exacerbate the effects of Varrosis and increase the transmission of viral pathogens. This work reports the distribution of acaracide resistant mites across Northern Ireland and also the presence and distribution of viral pathogens among honeybee populations.

P14 Assessment and Management of emerging nematode pests of Northern Ireland grassland and cereals

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Plant parasitic nematode (PPN) damage in pastures and cereals is often subtle and difficult to detect, however when combined with abiotic stresses, crop damage can be significant. There is currently little information on the PPN species present in Northern Ireland pasture and cereal fields, but given the reliance of NI agriculture on these crops and the expected future increases in average soil temperature, the impact of these parasites is of increasing concern. This research project will determine which PPNs are currently causing crop damage and identify those species likely to emerge as more serious pests in the future.
Initial sampling data across a range of soil types has shown root knot nematodes (*Meloidogyne naasi* and *M. minor*) to be present at unexpectedly high levels. The occurrence of these endoparasites appears to be increasing, highlighting them as the most likely threats to future crop production.
Management of PPNs in pasture and cereal crops will be investigated using a range of approaches including plant resistance, biostimulants and fungal endophytes. Initial results on the use of biostimulants in enhancing growth in stressed cereals and grasses will be presented.

P15 Prevalence of coccidiosis in broiler chicken farms in Sirte, Libya

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This study used questionnaires to determine the prevalence of coccidiosis and risk factors associated with coccidiosis by involving 87 broiler farms in the Sirte region of Libya. Secondly, 108 faecal samples were collected from 6 areas of each of the 18 selected broiler farms. The samples were immediately transported to the Sirte University where total faecal DNA was extracted by using the QIAamp DNA Stool Mini Kit protocol.

The initial analysis of the questionnaire referred to the injury of broiler chickens with coccidiosis. The questionnaires were received from 87 broiler-chicken farms during March to April 2010. A total of 87, 540 birds (21%) of the 415, 940 birds were perceived by the farmers to have had coccidiosis at a bird death rate of 6.2%. Overall, the number of infected farms in the Sirte region was 35 (40.2%) of 87 farms. However, the prevalence rate of coccidiosis in birds was 18% (14, 300/79,500) and infection rate of coccidiosis in farms was 61.11% (11/18) during July to August 2010.

Extracted faecal DNA were analysed using a Multiplex PCR with specific primers and sequencing to identify all the *Eimeria* species prevalent among Libyan poultry. The Multiplex PCR detected two species of *Eimeria*; *E. tenella* and *E. acervulina* in the first DNA samples purified from Libyan poultry.

P16 New lineage of *Schistosoma turkestanica* in Eastern Europe: A foreign invader or a reclusive native?

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Schistosoma turkestanica (syn. *Orientobilharzia turkestanicum*) has been reported as a causative agent of human cercarial dermatitis and animal schistosomiasis (previously known as orientobilharziasis) throughout Asia, the Middle East and Turkey. Recently, this parasite has been identified unexpectedly as having natural foci in the Gemenc area of Hungary, utilising red deer as definitive hosts and *Radix auricularia* as an intermediate host. Molecular bar coding methods utilising *cox1* and *ITS* region DNA sequences where used on 10 males harvested from a single deer in order to identify the origin of this parasite assumed to be an exotic invasive species. Several distinct haplotypes were identified in the small sample and phylogenies suggest that the Hungarian isolate is a distinct lineage of *S. turkestanica* when compared to isolates from China and Iran. The unique haplotypes of the Hungarian isolate may be indicative of a longer history of the parasites presence in the Gemenc region and could represent a neglected hotspot of animal schistosomiasis in Europe.

P17* How will climate change alter host-parasite relationships?

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Changes in climate, such as higher temperatures and precipitation levels, are causing increasing ecological disruption. We posit that warmer and wetter conditions associated with climate change may alter the development rate of parasite species with free-living stages, potentially resulting in changes in the parasite community assemblage. To test this hypothesis we used altitude as a natural proxy for climate change and recorded the parasite community of wild rodents at low (warmer and wetter) and high (colder and drier) altitudes. A total of sixty bank voles (*Myodes glareolus*) and yellow-necked mice (*Apodemus flavicollis*) were sampled at high (1400m) and low (500m) altitudes. Upon capture parasites were identified and quantified. Seven species of endoparasites were found at high altitude (prevalence=73.3%, abundance=5.0±1.8), compared with four at low altitude (prevalence=56.6%, abundance=5.4±1.5). Preliminary analyses indicated a negative association between two common parasites (*Heligmosomoides* sp. and *Hymenolepis diminuata*) at high altitude, but this was reversed at low altitudes. One possible explanation could be rapid development of *Heligmosomoides* sp., at low altitudes allowing it to competitively exclude *H. diminuata* whereas at high altitudes, *H. diminuata* may develop at a faster rate and exclude *Heligmosomoides* sp. These preliminary results suggest that climatic variation may play a role in parasite community assemblage.

P18 Phenotypic and genetic associations between immune phenotype, parasite burden and fitness in a wild mammal

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Understanding associations between immunity, parasite infection and host fitness in natural populations is a major challenge for infectious disease ecology. It is often assumed that more vigorous immune responses are associated with lower parasite burdens and higher fitness, despite evidence that this is not always the case. To understand the impact of parasite infection on host fitness, the phenotypic and genetic associations between immune phenotype, parasite burden and host fitness must be established, yet no studies have examined all three associations in a natural vertebrate population. Using a longitudinal data set from a wild population of Soay sheep, we investigated associations between intestinal nematode burden, antibody responses, and body weight. We first examined the phenotypic associations between the traits, before establishing their genetic basis using a quantitative genetic approach. The results suggest that the associations between these traits may not always follow predictions from theories of ecological immunology. The findings also highlight the need for more detailed characterization of the multivariate immune phenotype in natural populations.

P19 Does Host Anaemia promote Malaria Transmission?

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Anaemia is a common health problem affecting women and children in the developing world. Red blood cells are the most important source of protein for mosquitoes, any reduction could impede the ability of vectors to transmit malaria by: influencing mosquito survival, parasite infectiousness and/or the virulence of malaria parasites to mosquito vectors. The aim of this study was to determine how variation in the red cell density of blood characteristic of that associated with anaemia influences the fitness of *Anopheles gambiae* s.s. and its ability to transmit *Plasmodium falciparum*. Human blood containing *P. falciparum* gametocytes of either normal Packed Cell Volume (50%) or that typical of a severely anaemic individual (15%) was fed to *An. gambiae* s.s. females. Mosquitoes feeding on low PCV blood were significantly more likely to become infected than those feeding on blood of normal PCV (X^2_1=9.99,*P*=0.001). However, the oocyst burden and total sporozoite loads in mosquitoes infected from normal or low PCV blood did not vary (X^2_1=1.28,*P*=0.26;X^2_2=0.39,*P*=0.53). Finally, mosquito survival was not significantly reduced after feeding on low relative to normal PCV blood (OR=1.08,95%CI=0.86-1.36). These results demonstrate that human haematological variation of the magnitude likely to arise in malaria endemic settings may have a significant impact of the outcome of vector –parasite interactions, and anaemia which reduces PCV could enhance vectorial capacity by increasing parasite infectivity with no adverse effects on mosquito survival.

P20* Long-term impact of large scale community-directed delivery of doxycycline for the treatment of onchocerciasis

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The macrofilaricidal and embryostatic anti-*Wolbachia* treatment with doxycycline has a great potential for therapy and control of onchocerciasis. Nevertheless, the length of the required treatment poses potential logistical and compliance problems to its use in Mass Drug Administration (MDA) strategies. In 2007-2008 a feasibility trial of community-directed treatment with doxycycline was carried out in 17,519 eligible people in two health districts of Cameroon, naive to previous control measures. Therapeutic coverage was 73.8% with 97.5% compliance, encouraging the feasibility of this approach. We evaluated the effectiveness of this community-directed delivery of doxycycline four years after implementation. *Onchocerca volvulus* infection was assessed by skin biopsy and nodule palpation in 375 people who completed the treatment with doxycycline followed by one or two annual rounds of ivermectin MDA and 132 who received one or two annual rounds of ivermectin MDA alone. Significantly lower microfilarial prevalence and load were found in people who received doxycycline plus ivermectin compared to those who received ivermectin alone. This study demonstrates that a multi-week doxycycline treatment delivered with a community-directed strategy is not only feasible but also effective in reducing infection prevalence and burden even when evaluated four years after implementation in an area of ongoing transmission.

P21 Investigating the symbiotic relationship between *Wolbachia* and *Brugia malayi* during larval development through RNA Seq transcriptome analysis

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The parasitic nematode *Brugia malayi* is a causative agent of lymphatic filariasis, a disfiguring disease affecting over 120 million people worldwide. *B. malayi* exists in a mutualistic symbiotic relationship with the α-proteobacterium *Wolbachia*. Larval development, embryogenesis and adult work survival are the key biological processes, most dependent on this symbiosis. During larval development in the mosquito vector the numbers of bacteria remain static. Once the third-stage larvae infect the mammalian host, a dramatic expansion of the bacterial population takes place, which continues to expand during the first month to reach the highest bacteria/worm ratio seen in any life-cycle stage. Antibiotic targeting of *Wolbachia* arrests the growth and development of L4 larvae, preventing their development to adults. In order to identify factors important for this crucial period of the symbiotic relationship, Illumina RNA Seq was applied to produce a comprehensive transcriptome of four time points spanning the period between early post-infection to mid-L4 development of *B. malayi* in the mammalian host *Meriones unguiculatus*. *Wolbachia/*worm ratios within developing larvae at each time point were also monitored by TaqMan qPCR and fluorescent microscopy. Transcriptomic, together with proteomic and metabolic, data will be integrated in a systems biology approach with the objective of understanding the molecular basis of the *Wolbachia/B. malayi* symbiosis.

P22 An amino acid substitution in *Fasciola hepatica* P-glycoprotein associated with triclabendazole-resistant populations

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Control of infections by the Common Liver Fluke (*Fasciola hepatica*) is threatened by the development of resistance to the anthelmintic of choice, triclabendazole (TCBZ). Enhanced drug efflux by ABC transporters such as P-glycoprotein (P-gp) has been implicated in the development of TCBZ- resistance. A putative full length cDNA coding for a P-gp expressed in adult *Fasciola hepatica* has been constructed and used to design a PCR primer set for amplifying a region encoding part of the second nucleotide binding domain of P-gp. Application of this primer set to genomic DNA derived from liver flukes from TCBZ-resistant and -susceptible field populations shows a significant difference in the alleles present in the two populations, supporting the hypothesis that drug efflux pumps play a role in resistance to TCBZ in the liver fluke. Analysis of an allele present at a three-fold higher frequency in the "resistant" population revealed that it is characterised by a single nucleotide polymorphism (SNP) producing a serine to arginine substitution at residue 1144. Homology modelling studies have been used to locate this site in the P-gp structure and hence assess its potential to modify functional activity. This SNP has the potential to form the basis of a molecular test for TCBZ-resistance based on genomic DNA extracted from fluke eggs.

P23 The Retinoic Acid Receptor agonist Am80 increases mucosal inflammation during an intestinal helminth infection of mice

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Vitamin A metabolites, such as *all-trans*-retinoic acid (RA) act through the nuclear receptor retinoic acid receptor (RAR). RA has previously been shown to have a polarising effect toward the generation of Th2 responses, and to be important for resistance to helminth infections. In order to deduce the role of RA in *T. muris* infection, we treated chronically-infected mice with an RARα/β agonist (Am80). Interestingly, we found that Am80 exacerbated the pathology seen post-infection, demonstrated by an increased colonic crypt length and a significant $CD4^+$ T cell infiltrate. Further, we established that the Am80-driven crypt hyperplasia and $CD4^+$ T cell infiltrate were dependent on IL-6, as both were absent in Am80-treated IL-6 knock-out mice. This study presents novel data showing a role of retinoic acid in *T. muris* infection, and gives further insight into the function of RAR in a biologically-relevant model of gut inflammation.

P24 The relationship between autoimmune reactivity and systemic cytokine levels in an *S.haematobium* endemic population

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Introduction: Helminth infections have been implicated in the protection against autoimmune disease. We have previously shown *S.haematobium* infection in humans is inversely associated with autoimmune reactivity. We further aim to delineate the underlying mechanisms by measuring levels of systemic cytokines within the same population. CD23 was also measured in order to understand the relationship between allergy and helminth immunity.
Methods: 591 Zimbabweans naturally exposed to *S.haematobium* were tested for levels of cytokine IL-10, IL-17, IL-9 and INF-γ using ELISA and correlated with their autoimmune reactivity from the previous study. CD23 was also measured by ELISA and correlated with levels of total IgE and schistosomiasis infection intensity.
Results: Cytokines IL-10 and IL-17 were significantly higher in those who were ANA negative, whilst INF-γ was significantly lower. CD23 was significantly correlated with total IgE in areas of moderate *S.haematobium,* infection, an effect not seen in high infection areas. Conclusion: Autoimmune reactivity is inversely associated with levels IL-10 in a helmith endemic population and hence IL-10 may mediate helminth-induced protection of autoimmune disease. A reduction of CD23 compared to total IgE levels was associated with higher *S.haematobium* infection areas suggesting a role of CD23 in helminth immunity and allergy protection.

P25* Treatment of schistosomiasis in infants and preschool-children with praziquantel: correct dose, side-effects and cure rates

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In large-scale interventions for control of schistosomiasis, use of the World Health Organization dose pole is favoured for mass-drug administration of praziquantel to school-aged children and adults. Application of this simple tool has enabled pragmatic tablet dosing using patient height as a proxy for body weight, allowing control programmes to expand into resource-poor settings. New evidence advocates the immediate inclusion of preschool aged children (≤5 year olds), also at high risk for disease and morbidity, in future control campaigns; therefore, the current WHO pole needs updating. Height and weight data were measured during several epidemiological surveys conducted in Angola (*N*=1067), Mali (*N*=405), Uganda (*N*=3303), Sudan (*N*=137), Zanzibar (*N*=470) and Zimbabwe (*N*=104) to establish and validate an extended PZQ dose pole, which now includes two new height-intervals: 60–84cm for ½ tablet and 84–99cm for ¾ tablet divisions. Anthropometric data from other African countries (Demographic Health Surveys) are now also available and will be analysed in the near future. Treatment has been given to different child cohorts and results show that while treatment cure rates can vary significantly between cohorts (25-100%), side-effects tend to be mild and transient. Theoretical application of the updated dose pole results in >95% of children receiving an acceptable dose (30–60 mg/kg). Using this pole, we suggest that mass-drug administration can be better optimized, streamlining general treatment to reduce drug wastage which could lead to significant programmatic savings and allocation of treatments to younger children with minimal additional cost.

P27* Clocks determine outcome of parasitic worm infection in mice.

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An appropriate immune response is essential for survival in the context of infection. Many chemokines, cytokines and immune cells, fluctuate following a daily rhythm, influencing the ability of the body to mount an immune response to infection. *Trichuris muris* is a nematode parasite dwelling in the large intestine. A Th2-type immune response is required to resolve the infection. A high-dose infection with *Trichuris muris* in C57BL6 mice leads to an acute infection with the expulsion of worms in the majority of mice by day 28. C57BL6 mice were orally infected with a high dose of 200 eggs of *Trichuris muris* at either 7am or 7pm. The progress of infection was monitored in mice on d13, d21, d25 and d28 post infection. All mice exhibited high worm burdens in the first 2 weeks of infection. By d21 mice infected at 7am expelled the worm burden significantly earlier than those infected at 7pm. In line with this, 7am mice exhibited a stronger Th-2 type response in contrast to 7pm infected mice. This experiment shows that the speed of resolution of an acute infection over 3-4 weeks depends on time of day of initial infection, and that this is associated with a Th-2 biased immunological response. Current studies aim to define how long-term programming of immune responses is set by clocks in the first 24-48h of infection.

P28 Intestinal schistosomiasis and contributory risk factors in Nsu, Ehime Mbano L.G.A Imo State, Nigeria.

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Study on the prevalence of intestinal schistosomiasis was conducted in three communities in Nsu, Ehime Mbano L.G.A, Imo State using structured questionnaires for demographic data collection and faecal analysis for detecting the presence of *Schistosoma mansoni* ova. Examination for the presence of *S.mansoni* was done using formol-ether concentration technique and microscopy. Out of the 476 persons examined comprising 268 males and 208 females, 24(5.04%) had their faecal samples positive for *S. mansoni* infection. More males (5.97%) than females (3.85%) were infected. The highest rates of infection occurred in persons aged 15-29years (5.82%) and 0-14 years (5.76%). Students (5.67%) and farmers (5.55%) were mostly affected. There was a significant association between occupation and infection(X^2 51.84, $p>0.05$). Source of water used seemed to play contributory role to infection. Infection occurred most among those that used the stream as their source of water (6.73%). Regarding toilet facilities, those who defaecated in the bush had the highest rate of infection (5.37%). The result of this study has revealed the presence of intestinal schistosomiasis in Nsu. It would be aptly appropriate to include Nsu in the schistosomiasis control programme to prevent further spread of the infection and control possible morbidity and resultant socio-economic effects that may arise there from.

P29 Protection at Mucosal Barriers: The effect of multiple-challenge infections on the susceptibility of Muc5ac mice to Trichuris muris

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We have recently demonstrated that expression of the mucin Muc5ac, normally expressed in non-intestinal mucosa, is highly up-regulated in the intestine following infection with the caecal-dwelling nematode Trichuris muris. Moreover, genetic deletion of Muc5ac leads to complete susceptibility to a high dose infection in an otherwise resistant mouse strain, indicating Muc5ac is a major effector mechanism involved in protection against this parasite. It has also been determined that the expression of Muc5ac is IL-13-dependent since up-regulation of this mucin does not occur in T. muris infected IL-4Rα mice. In nature, multiple-challenge infections represent the typical manner in which host-acquired immunity is generated. Muc5ac null mice were infected repeatedly with small numbers of T. muris eggs to mimic a more natural infection. Accumulative dosing in this manner caused the generation of resistance in Wt mice which correlated with increased expression of IL-13. Work presented here demonstrates how Muc5ac expression levels changed following multiple-challenge infections and utilising Muc5ac null mice, the role of this mucin in this infection scenario was explored. Moreover the immune response that developed in the Muc5ac mice was investigated over the course of infection, with particular focus on the Th2 response and the expression of IL-13.

P30 SCAN: Schistosomiasis Collection at the Natural History Museum

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SCAN, the Schistosomiasis Collection at the Natural History Museum (NHM), is creating a repository of schistosome-related specimens and data to support field studies and future research projects. At present, working in support of the Schistosomiasis Consortium for Operational Research and Evaluation (SCORE), SCAN has accepted around 45000 larval schistosome specimens in the last 12 months from Niger, Zanzibar and Tanzania, and has a growing snail collection. Bringing museum collection management practices into field-sampling regimes is beneficial in considering the handling of samples and data from the outset, and the creation of a well managed collection with accompanying context data will create an accessible resource for longitudinal surveys and comparisons beyond the scope of the original research programme.
NHM has an excellent track record for collections management and their long-term maintenance. With projects such as SCAN and the NHM's new molecular collections facility, the museum is developing this infrastructure to enable collections management support for field-based research projects.
Part-funded by the Wellcome Trust until 2015, SCAN is available to act in support of ongoing and new field schistosomiasis sampling projects additional to the SCORE programme. For further information see http://www.nhm.ac.uk/research-curation/collections/curation-groups/scan/index.html

P31* Investigating dendritic cell subsets in Th2 induction against *Schistosoma mansoni*

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Dendritic cells (DC) are a heterogeneous population of innate antigen presenting cells that are important for the induction and direction of T cell responses, providing an essential link between innate and adaptive immunity. However, the DC subsets involved, and the precise mechanisms they employ, to provoke Th2 responses remains unknown. We have recently shown that Th2 induction during murine *Schistosoma mansoni* infection is dependent on the presence of $CD11c^+$ DCs. To progress our understanding of which $CD11c^+$ DC subsets are important for this process we have used $Batf3^{-/-}$ mice, which are deficient in $CD8\alpha^+$ DCs. $CD8\alpha^+$ DCs are most commonly associated with generation of Th1 immunity and Ag cross-presentation, but their role in helminth infection and Th2 settings is currently poorly understood. Preliminary data using *S.mansoni* egg injection or infection of $Batf3^{-/-}$ mice suggests dysregulated Th2 responses in the absence of $CD8\alpha^+$ DCs and differing roles for this DC subset depending on the tissue site analysed. Ongoing work aims to further understand this important and previously unappreciated role for $CD8\alpha^+$ DCs in orchestration of the immune response against *S. mansoni.*

P32* The role of antibody in resistance to *Trichuris*

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Trichuris muris is a nematode parasite, residing in the ceacum and large intestine. Resistant stains of mouse, including C57BL6, are able to expel their worm burden by producing an appropriate Th2 response. The involvement of antibody in this process is unclear. Activation induced cytidine deaminase (AID) is an RNA-editing deaminase required for somatic hypermutation and class-switch recombination during B cell differentiation. These processes are necessary for antibody class switching in order to exhibit an efficient antibody response. $AID^{-/-}$ mice lack the ability to class switch from IGM to other antibody isotypes. C57BL6 and $AID^{-/-}$ mice were infected with a high dose of 200 *Trichuris muris* eggs and the progress of infection was monitored at day 13, 21 and 35 post infection using serum antibody level analysis, worm burden and mesenteric lymph node cell cytokine responses. Both strains of mouse displayed high worm burdens at day 13. Worm burdens in C57BL6 mice had been completely expelled by day 35. Surprisingly $AID^{-/-}$ mice were unable to expel their worm burden by day 35. The results obtained suggest that antibody may play a role in expulsion of primary *Trichuris muris* infections.

P33* Diversity of the Tegumental Protein Tetraspanin 23 in *Schistosoma mansoni*: potential problems for vaccine design?

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Schistosomiasis is currently second to malaria as one of the world's most virulent and pathogenic parasitic diseases. It is caused by parasitic blood of the genus *Schistosoma* with an estimated 200 million people infected in tropical and subtropical regions of the world. Due to the decreases in the efficacy of praziquantal in a mass drug administration programme the elucidation of a viable vaccine target has been paramount. Tetraspanin 23 (TSP - 23) is considered to be a prime candidate as its expressed on the tegument of the parasite, has an extracellular loop that is directly presented to the host's immune system and the vast majority of antibodies raised by the host are illicit against this protein. However, there appears to be little immune memory to these parasites upon reinfection. DNA sequence analysis of TSP – 23 from several different geographical isolates of *Schistosoma mansoni* show the extracellular loop to be hyper-variable producing allelic isoforms of the protein and causing variation in protein structure and antigenicity. This indicates the establishment of distinct antigenic lineages in *S. mansoni* and could provide problematic for the development of viable anti – schistosomal vaccines.

P34 Investigation of the therapeutic potential of the filarial nematode product ES-62 in a mouse model of chronic asthma

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ES-62, a phosphorylcholine-containing glycoprotein secreted by the rodent filarial nematode *Acanthocheilonema viteae,* is a potent immunomodulator that exhibits therapeutic potential in several mouse models of inflammatory disease including arthritis, acute asthma and systemic lupus erythematosus. We now show that ES-62 is also protective in a mouse model of chronic asthma induced by intraperitoneal injections of 50µg ovalbumin (OVA) in PBS at d0, d3 and d6, followed by weekly intranasal instillations of 20µg OVA from d11 for 9 weeks. Data obtained to date suggest that early (weekly from d11) and mid (weekly from d46) administration of ES-62 (2 µg/dose) prevents development of pathology in the lung in terms of inflammatory cell infiltration and airway remodelling whilst late (d68) administration suppresses the acute OVA-induction of cytokines in the bronchoalveolar lavage fluid. Furthermore, ES-62 exhibits effects on B-cell subsets in the spleen of the animals and the significance of this is being explored. Overall, this study supports the use of ES-62 as the starting point in the development of novel anti-inflammatory drugs for the treatment of asthma.

P35 *Heligmosmoides polygyrus* fourth stages reverse systemic inflammation in animal models of multiple sclerosis

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Primary exposure of mice to the gastrointestinal nematode *H. polygyrus* infection reduces inflammation in an experimental model of multiple sclerosis. In this study, we aimed to evaluate the ability of *H. polygyrus* L4 larvae to reduce the symptoms of ongoing experimental autoimmune encephalomyelitis (EAE) in female C57Bl/6 mice. EAE was induced by myelin oligodendrocyte glycoprotein (p35–55) for 21 days before oral infection with 200 infective larvae (L3) of *H. polygyrus* until the end of the experiment at 21 days post infection. A reduction an EAE symptoms was observed from 3 days post infection and the symptoms were almost completely inhibited at 6 days post infection. This effect was associated with limited leukocyte infiltration into the spinal cord, decreased pro-inflammatory IL-12 concentration and high concentration of regulatory anti-inflammatory cytokines; TGF-b and IL-10 in the spinal cord and serum. The symptoms of EAE was observed again in the enteral phase from 12 days post infection and was associated with leukocyte infiltration into the spinal cord and low level of TGF-b in the presence of high level of IL-12 in the spinal cord and serum. The L4 stage of gastrointestinal nematode can reverse systemic inflammation in animal models of multiple sclerosis by reducing IL-12 and promoting TGF-b and IL-10 production. Work supported by Polish NSC Grant N303 819140.

P36* *In vivo* imaging model of stage II African trypanosomiasis to support drug discovery programmes

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There are 70,000 cases of human African Trypanosomiasis (HAT) annually, with 60 million people at risk. Despite this, HAT is a Neglected Tropical Disease with few significant advances in chemotherapy over the last 50 years. Limited knowledge of the metabolic status and drug sensitivity of trypanosomes in the central nervous system (CNS), and the lack of a useful murine CNS model has limited drug discovery. Here, we report the generation of highly bioluminescent parasites and their use in an *in vivo* imaging model of stage II African trypanosomiasis. Bloodstream forms of the chronic model strain GVR35 were transformed with a construct designed to express "red-shifted" luciferase. The vector was targeted to the rRNA locus, with expression under the control of the endogenous promoter. In microtitre plates, the limit of detection was <1000 parasites using the Caliper IVIS Lumina®. Using the standard 21 day berenil treatment model in CD1 mice, we were able to identify CNS infection. The luciferase signal intensified over the first week of infection and this correlated with peripheral blood parasitaemia. After berenil treatment, there was a rapid clearance of the peripheral signal followed by a gradual appearance of signal in the brain. Trypanosome numbers quantified by qPCR correlated with luciferase intensity.

P37 High-content imaging assays for primary screening of kinetoplastids, the road to increased physiological relevance of screening platforms for intracellular parasites.

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The kinetoplastids *Trypanosoma cruzi* and *Leishmania donovani* pose a major challenge for drug discovery programmes. Their intracellular localisation makes it difficult to set up straightforward high-throughput screening assays. Instead, many primary screening campaigns are conducted using either the free-living insect stage of the parasite or an axenic form of the intracellular stage. The consequence of simplifying the assay in this manner is a significant reduction in physiological relevance of the assay with the potential of high false-positive and false-negative rates compared to the physiologically relevant life-stage. The intracellular life-stages are usually assayed as a follow-up to the primary screen, using a labour-intensive low-throughput microscopy based assay. Here we present the development and use of 384-well imaging-based screening platforms for intracellular *Trypanosoma cruzi* and *Leishmania donovani* which have allowed us to conduct primary small-molecule screens at throughputs of up to 15,000 compounds / week. Parallel screening campaigns using an axenic growth assay were carried out and highlighted the benefits of using the high-content assay as primary platform. The results of a 100,000 compound screening campaign will be presented as well as the lessons learned from our approach.

P38 The development and optimisation of bioluminescent *T.b. brucei* cell lines for *In vivo* imaging of stage II African trypanosomiasis

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Recent advances in bioluminescent reporters and non-invasive imaging have been utilised in a number of *in vivo* models of disease, increasing our understanding of the dissemination of pathogens, drug efficacy and the role of the immune response during infection. However, bioluminescence below 600 nm is decreased by tissue absorbance. Here we report the development of bioluminescent *T.b. brucei* cell lines that constitutively express a novel red shifted *P. pyralis* luciferase variant with a peak emission of 620 nm. Following targeted integration into the ribosomal DNA loci, luciferase expression was stable without drug selective pressure in culture over a period of 3 months. *In vitro* comparisons between cell lines expressing the wild type *Ppy* luciferase, a thermostable red shifted luciferase (Ppy RETS) and a codon optimised red shifted luciferase (Ppy Re9), demonstrated that Ppy Re9 gave a >10 fold increase in bioluminescence over the wild type Ppy and Ppy RETS. Luciferase expression was enhanced further by incorporating an upstream 5' variant surface glycoprotein UTR and a downstream 3' tubulin UTR. It is expected that the use of these cells, in the standard stage II murine model of CNS disease, will accelerate *in vivo* drug screening.

P39 Automated high-content whole organism motility drug screening platform for helminth parasites

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Helminth parasites cause huge disease and economic burdens on humans and livestock worldwide. However few effective drugs are available against these diseases in humans and drug resistance is becoming a major problem in livestock. Whole organism screens are valuable methods for the testing of new compounds against helminths and most involve manual microscopic visualisation of damage to parasites and/or an inhibition of motility. These screening methods are low/medium throughput and only allow screening of small focused compound libraries. Here we report on a high content whole organism-screening (HCS) platform for testing activity of compounds against human and veterinary trematode and nematode parasites. This screen is based on automated image capture and analysis of parasite motility using newly design computer software. Two motility algorithms have been designed to allow for the varied speed, size and motility of the parasites. These algorithms, which measure individual larval motility or total well motility, have successfully been used to screen compounds against a number of helminths including Schistosoma, Onchocerca, Ascaris and Haemonchus. This High Throughput Screening platform makes it feasible for the first time to screen very large compound collections against multiple helminth parasites in parallel.

P40* Glideosome-independent invasion of the host cell by *Toxoplasma gondii*

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Apicomplexan parasites actively invade their host cell. Invasion depends on an arsenal of secreted invasion factors and the ability of the parasite to glide. The Myosin A (MyoA) motor complex, also known as the glideosome, is a multi-subunit complex localised beneath the plasma membrane of the parasite. Conditional knockdown mutants for MyoA demonstrated the essential function of the glideosome for gliding motility in *Toxoplasma gondii* and *Plasmodium*, however, the established gliding mutant did not show the expected complete block in host cell invasion. While background expression of MyoA might be sufficient to drive host cell invasion, it remains possible that the glideosome does not have the widely accepted role in host cell invasion. We generated a novel conditional recombination system based on Cre-recombinase and we employed this system to generate a complete knockout for MyoA in *T.gondii* that, surprisingly, can be maintained *in vitro.* As expected, parasites lacking *myoA* are not able to move by gliding motility and consequently are significantly impaired in host cell egress. However, these parasites are still capable of invading the host, demonstrating that the MyoA-motor complex is dispensable for host cell invasion. Consequently, the link between gliding motility and host cell invasion needs to be re-addressed.

P41* *Leishmania* virulence factors: Inhibitors of serine peptidases

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The *Leishmania* genome has revealed the presence of three ecotin-like peptide inhibitors of trypsin-family serine peptidases (ISPs), enzymes that are absent in *Leishmania*. These ISPs have been proposed to inhibit host serine peptidases, with ISP1 expressed in the vector-borne promastigote stages, and ISP2 also expressed in the infective mammalian amastigote stage. By comparing *L. major* mutants deficient in all three ISP genes (Δ*isp1/2/3*) or ISP2/3 double-null mutants (Δ*isp2/3*) *in vitro*, ISP1 has recently been shown to have a putative function in flagellar homeostasis with regards to differentiation, flagellar pocket flux and interaction with the sandfly host. In addition, ISP2 has been shown to inhibit neutrophil elastase (NE), which mediates a Toll-like receptor 4 (TLR4)-NE pathway during *Leishmania*-macrophage interaction promoting *Leishmania* survival and growth in C57BL/6 murine macrophages due to a downregulation in the production of superoxide. Using fluorescent and bioluminescent *Leishmania* wild-type and ISP2/3 mutants together with flow cytometry, *in vivo* imaging (IVIS) and multi-photon microscopy, we are investigating the role of ISP2 as a virulence factor during *in vivo* infection by examining interactions with innate cells of the early immune response and tracking disease progression.

P42 Modelling the spatial and temporal organisation of transcripts over intergenic regions in *P. falciparum*

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We show that the size of intergenic regions (IGR) in *P. falciparum* (and other apicomplexans with compact genomes) correlates with the types of transcriptional activity that occur over them – two promoters> promoter/terminator> two terminators. Interestingly, there is a spatial feature to this relationship, with the sizes of IGR in subteleomeric regions significantly larger than those in chromosomal internal regions. This suggests that there may be a balancing selection pressure in the subtelomeric region that acts to prevent these regions shortening.

Modelling the apportionment of untranslated regions (UTR) of transcripts would indicate that these are preferentially organised towards the 5' of the open reading frame they encode. The potential for overlapping transcripts, however, is high, even when conservative apportionment models are considered. Whilst our data suggest that the proportion of temporally and spatially cotranscribed transcripts is low, IGR containing two cotranscribed promoters are actually smaller than those of the rest of the genome. These regions (202 in total) represent good candidates for bi-directional promoters during the asexual blood stages of development.

P43 Comparison of different blood collection and DNA extraction methods for malaria diagnosis by PCR in Saudi Arabia.

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Two molecular tests; a *Plasmodium* genus-specific conventional PCR and a species-specific Multiplex-Nested-PCR differentiating between *P. falciparum* and *P. Vivax* were compared for their diagnostic performance using genomic DNA preparations obtained by different methods from 75 confirmed malaria positive blood samples collected in Saudi Arabia endemic areas. DNA preparations were obtained by Qiagen QIAmp® chromatographic mini-columns and Jena-Biosciences® DNA isolation kits from EDTA preserved whole blood samples and by Whatman-FTA® DNA Elution Reagent from dried blood spots onto FTA® cards.
Plasmodium genus-specific PCR showed significantly high sensitivity with DNA templates extracted by QIAmp® and Jena-Bioscience® kits; 97% and 96%, respectively. But, when applied to DNA specimens prepared by Whatman-FTA® reagent from dried blood spots, sensitivity decreased to 55%. *Plasmodium* species-specific Multiplex Nested PCR showed equal sensitivity with QIAmp® extracted DNAs (97%), but only 89% of success was achieved with Jena-Bioscience® isolated DNAs. Curiously, this Nested-diagnostic technique achieved a relative sensitivity of 96% when applied to DNA templates prepared with Whatman-FTA® from dried blood spots. No *P. vivax* cases were detected by the species-specific Multiplex-Nested-PCR. Results indicated that the diagnostic performances of both PCR techniques were highly satisfactory with QIAmp® extracted DNAs. But, if samples are collected onto filter cards as dried spots, the *Plasmodium* species-specific Multiplex Nested PCR would achieve much better results than the conventional direct PCR.

P44* Association of *pfmdr1* and *pfcrt* polymorphisms with slow clearance and recrudescence of *Plasmodium falciparum* after ACT treatment of Kenyan children

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Artemisinin Combination Therapies (ACTs) are now considered the best therapy for the treatment of *Plasmodium falciparum* malaria and have been widely deployed. A decline in efficacy of artemisinin monotherapy in western Cambodia has been reported, characterized by slow parasite clearance. The molecular mechanism of this reduced response to artemisinin is unknown. In this study, *pfcrt* and *pfmdr1,* whose proteins are localized on the membrane of digestive vacuole of malaria parasite, where artesminin is thought to act, have been genotyped for sequence variation in samples collected during a clinical trial of artemether-lumefantrine (AL) and dehydroartemisinin-Piperaquine (DHA-PIP) in Kenya in 2009. The haplotype NFD at positions 86, 184 and 1246 of *pfmdr1* and the wild-type *pfcrt* 76K were associated with slow parasite clearance as measured by qPCR. Both alleles showed evidence of selection on Day-3 and Day-28 after AL and DHA-PIP treatment. Previous published data suggest that the selection of resistant/tolerant parasites which carry the NFD haplotype of *pfmdr1* and *pfcrt* 76K was attributed to the non-artemisinin partner drugs such as lumefantrine. This study provides multiple lines of evidence, for the first time to our knowledge, that NFD haplotype of *pfmdr1* and *pfcrt* 76K are also associated with reduced response to artemisinin *in vivo.*

P45 Natural products; antimalarial propertise of Cryptolepine

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Most of the commonly used antimalarial molecules are based upon plant-derived compounds. *Cryptolepis sanguinolenta* is one of above examples using by traditional healers in the treatment of infectious diseases, amoebiasis, and fevers, including malaria. The major alkaloid of *C. Sanguinolenta* is cryptolepine. Cryptolepine is an inhibitor of the nuclear enzyme topoisomerase II that serves to regulate the topological states of DNA in cells and appears to have apoptotic activity a promising antitumour agent. *In vivo* antimalarial properties of cryptolepine are variable; while a maximum dose of 113mg/kg/day by subcutaneous injection in mice failed to produced a significant reduction of parasitemia, a highest dose 50mg/kg/day given orally to mice infected with *P. berghei berghei* suppressed parasitaemia by 80%. The explanation for the different results obtained may lie in the routes of administration.
In this study using TEM we investigated antiplasmodial activities of cryptolepine *in vitro*. Electron micrographs of *P. falciparum* cultivated in human erythrocytes revealed that cryptolepine easily cross the cell membranes and accumulates selectively in the parasite food vacuol (FV) interveen with parasites feeding mechanism inhibiting the formation of β-haematin. Cryptolepine has dramatic degenerative effects on the entire parasite morphology in general, and on the feeding mechanism in particular. These were shown by parasite cytoplasmic changes including increased vacuolation, FV enlargement, necrosis and disintegration. These facts suggest that the antiplasmodial activity of cryptolepine appears to be due, at least in part, to a chloroquine-like action that does not depend on intercalation into DNA.

P46 Estimating the window of selection for antimalarial drug resistance.

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Drug resistance spreads through a population because parasites can survive direct treatment and/or survive "residual" drug levels i.e. persisting from previous treatment. High levels of drug use and long drug half-lives mean a very high proportion (up to 80%) of the population may have residual drug levels. Most drugs don't kill parasites in the liver stage resulting in a window of selection; a period when drug concentrations allow new infections of resistant parasites to successfully emerge from the liver while infections of sensitive parasites are killed. Field studies typically estimate the window of selection by comparing the earliest day different genotypes become detectable, we are interested in whether this is approximately equal to the first successful emergence of different genotypes from the liver, the latter representing the true window of selection. We use standard pharmacokinetic-pharmacodynamic models to ascertain how accurately the field methods estimate the 'true' window of selection. We found estimates of the window of selection (i.e. observed patency) to be a good match to the 'true' window of selection until parasites are sufficiently resistant to survive very high drug concentrations.

P47 The role of *lipB* in *Plasmodium falciparum* metabolism.

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Temporal expression profiles of genes and proteins during the intra-erythrocytic developmental cycle of *Plasmodium falciparum* form the basis of current metabolic models identifying pathways that may be exploitable for antimalarial drug discovery. However, as many gene products have involvement in cellular pathways, it is important to study their substrates/ reaction products as they govern the physiological state of the parasite cell. Nevertheless, the *P. falciparum* metabolome remains largely unexplored. Recently, targeted metabolomics analysis led to the tentative assignation of some factors involved in carbon metabolism to be of parasite origin. However, information on the overall parasite metabolome is not available.
This study utilises state-of-the-art metabolomics technologies using LC-MS to explore the effect of the deletion of *lipB* on the metabolome of *P. falciparum*-infected erythrocytes using an untargeted approach. LipB (octanoyl-[acyl carrier protein]:protein N-octanoyltransferase) plays a role in lipoic acid biosynthesis. We show that *lipB* knockout leads to specific alterations of the mutant parasite's metabolome. Relative changes in metabolite content aid directed predictions of the involvement of selected gene products and associated metabolites in cellular metabolism, facilitating understanding of the metabolic networks operating in *Plasmodium*.

P48* In silico prediction of antimalarial drug targets

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[2]The University of Melbourne, Australia

The need for new antimalarials is persistent due to the emergence of drug-resistant parasites. Here we aim to identify new drug targets in *P. falciparum* by phylogenomics among *Plasmodium* spp. and comparative genomics to *H. sapiens*. The proposed target discovery pipeline is based on the hypothesis that *P. falciparum* proteins are likely to be essential if there are no similar proteins in the same proteome and while they are conserved across the malaria parasites of mammals. Consecutive filters narrow down the potential target space of *P. falciparum* to proteins that are likely to be essential, matchless in the human proteome, expressed in the blood stages, and druggable. Phylogenomics with fully sequenced Saccharomycetaceae and testing against the *S. cerevisiae* genome-wide deletion dataset supports the working hypothesis on essentiality. Applying stringent filters to the *P. falciparum* proteome, we end up with a list of 40 candidate drug targets comprising proven targets like dihydropteroate synthetase or enzymes of the non-mevalonate pathway, investigational targets such as calcium-dependent protein kinases, and new candidates of potential interest such as phosphomannose isomerase, phosphoenolpyruvate carboxylase, signaling components and transporters. The presented pipeline for essentiality prediction and target identification is largely independent of experimental data and therefore widely applicable. The identified candidates from *P. falciparum* provide insight into biochemical peculiarities and vulnerable points of the malaria parasite.

P49* Application of a bioluminescent assay to determine antimalarial drug activity

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Assays to determine antimalarial drug activity typically rely on a fluorescence based assay of DNA content (eg. Malaria Sybr Green I fluorescence assay, MSF). Whilst cheap and readily scalable for high throughput screening, the MSF assay lacks sensitivity with a low signal/noise ratio and high background signal from DNA in dead and dying parasites. More sensitive assays rely on the use of radioisotopes, although these assays raise issues with safe handling and disposal of reagents.
Here we report the evaluation and application of a bioluminescent assay for measuring drug activity. Here, a stably integrated luciferase reporter cassette in a genetically modified *Plasmodium falciparum* is demonstrated to be temporally expressed during DNA replication. We have evaluated a range of luciferase substrates and lysis conditions and established an assay that is reproducible (high Z' score), with a negligible background signal (dead parasites don't express luciferase) and high signal/noise ratio. We also show a comparison of IC_{50} values for a range of antimalarial drugs using the MSF and bioluminescent assay.

P50* Fluorescence-based analysis of the in-vitro anti-plasmodial activity of methanolic extracts of *Bridelia ferruginaea and Brysocapus coccinus*.

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Multidrug resistant malarial parasites are continuously emerging especially for the most lethal species, *Plasmodium falciparum*. Advances in high-throughput screening technologies and extensive funding have failed to deliver affordable anti-malarial options. The mainstay of drug-based malarial control continues to be largely reliant on the fortuitous discovery of natural compound remedies informed by traditional medical practices. The WHO has recently encouraged African countries to commence the development of Traditional African medicines (TAM) especially in relation to diseases like malaria that are endemic in these regions. One of the main bottlenecks with traditional medicine usage is the lack of standardized dosing, proper extraction and purification methods and hence the need for more research into the scientific validation of these practices.
The present study aims to address this by developing a standardized workflow enabling the screening of some West African extracts for antimalarial activity. Preliminary data from optimised fluorometric *in vitro* assays evaluating the antimalarial activities of two West African herbal extracts *Bridelia ferruginea* and *byrsocapus coccineus* will be presented. The study will also evaluate the efficacy of the extracts as potential candidates for synergy with artemisinin formulations.

P51 *Optimisation of fluorescence-based *in vitro* drug susceptibility assays for malaria

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Out of the plethora of parasitic diseases that afflict mankind, malaria remains the most significant with 200-500 million cases reported annually and 1-3 million fatalities. Treatment and control measures have been hampered by the emergence of drug resistance to most common anti-malarial therapies. Despite significant post-genomic technological advances, drug development has struggled to keep pace with the speed of resistance acquisition. The development of fast, simple, and reliable drug susceptibility assays is an important pre-requisite to enable robust *in vitro* screening. More recently reported fluorescence-based options have afforded significant advantages over traditional hypoxanthine and enzyme-based assays. Data from a range of fluorescence based assays (eg. MTT, flow cytometry and fluorescent microscopy) optimised to report on the in-vitro viability/cytotoxicity of parasite strains exposed to anti-malarial drug combinations will be presented. Optimised methods will be applied to investigate new artemisinin-based synergistic combinations with compounds from the LOPAC library which are reported to show anti-plasmodial activity. Reports of early drug resistance to artemisinins make it imperative that novel candidates for combination therapy are urgently sought in a bid to impede the spread of resistance to this last affordable anti-malarial option.

P52 Functional characterization of *Anopheles stephensi* mosquito target-of-rapamycin and S6 kinase proteins

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The target-of-rapamycin (TOR) pathway is the nutrient-responsive sensor of amino acid in eukaryotes including mosquitoes. Essential amino acids for mosquito ovarian development are obtained from the blood-meal taken from vertebrate hosts to complete development and life cycle. The interference with mosquito feeding and development will implicate malaria parasite development in the mosquito and subsequent transmission. Our objective is the structural-functional analysis of TOR pathway proteins in development of malaria mosquitoes and their role in parasite infectivity to the mosquito. Using BLAST homology we identified two proteins from *Anopheles gambiae*, TOR (AgTOR) and S6 kinase (AgS6K). Genes sequences were used to study their homologues in *An. stephensi* (the malaria vector in Asia), AsTOR & AsS6K. For gene silencing, dsRNAs were designed to the catalytic domains of TOR and S6K. The KD mosquitoes were infected with the rodent malaria *Plasmodium berghei*. The results showed a >30% reduction in parasite oocyst numbers on the mosquito midgut (a key indicator of parasite infectivity to the mosquito) in TOR- and S6K-KD mosquitoes. This might be due to up-regulation of mosquito anti-parasite immune responses or another effect on parasite development. These preliminary results show a possible role of mosquito TOR/S6K proteins in mosquito development and vectorial competence, which require deeper investigations.

P53 Structural-functional analysis of malaria parasite inner-membrane complex (IMC1g) protein

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Malaria is one of the most important human diseases which causes 1-3 million deaths annually. Due to the widespread parasite resistance to antimalarial drugs, there is a continuous to discover new targets and new therapies for prevention and treatment. Malaria parasites possess a family highly-conserved cytoskeletal proteins called the inner membrane complex (IMC1a-h) and related to articulins (alveolins) of eukaryotes. These proteins are essential for cellular mechanical stability and strength. The IMC1 proteins form the membrane skeletons with important roles in parasite motility and invasion of host cells. Here we report on IMC1g of *Plasmodium berghei* that has distinct stage expression in s and localized to parasite ookinete pellicle. To study IMC1g function, we used a transgenic parasite line expressing IMC1g tagged with the Red Fluorescent Protein (RFP). The mCherry coding sequence was amplified from P-DNR-mCherry cassette. A 1.5-kb fragment corresponding to entire imc1g gene plus the 5'UTR was amplified from genomic DNA and inserted in the *SalI*/*Hind*III-digested pDNR-imc1g/mCherry by in-fusion to give the plasmid pDNR-imc1g/mCherry. A 0.7-kb imc1g-3'UTR fragment was amplified and introduced into the *NotI*-digested pLP-TgDHFR2 to give pLP-TgDHFR/imc1g construct. *Cre-lox* recombination was carried out to give the final plasmid pLP-IMC1g/mCherry, which will be introduced into the parasite by double homologous recombination. The phenotype of the transformed parasite will be analyzed regarding mechanical strength, motility and infectivity to both the mosquito and mice.

P54 Functional analysis of a 5'UTR deletion series in *P. falciparum*.

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Despite the impact of microarray and RNAseq data in determining the absolute and temporal profile of *P. falciparum* transcription – the size, apportionment and function of the unusually long untranslated regions (UTR) in this species are still poorly understood. Work in our laboratory suggests that UTRs are predominantly apportioned upstream if the open reading frames (ORF). Further, there appears to be a district spatial enrichment of homopolymeric tracts in sequences that flank ORFs.
To investigate the role of 5'UTR sequences in the control of absolute and temporal transcription, we have created a series of luciferase reporter constructs bearing a nested series of deletions of sequences adjacent to the translational start of PFD0660w. These deletions appear not to affect correct temporal activity of transcription, in that the peak of expression in all constructs occurs, as expected, in trophozoites. Interestingly – whilst we were able to determine an effect on the absolute level of transcription, this was only after deleting some 697bp of 5'UTR.

P55 Haemozoin enhances inflammation-mediated lysozyme release from human monocytes through p38 MAPK- and NF-kappaB-dependent mechanisms

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Malarial pigment (haemozoin, HZ) is a ferroprotoporphyrin IX crystal produced by *Plasmodium* parasites after haemoglobin catabolism. HZ-fed human monocytes are

functionally compromised, releasing increased amounts of pro-inflammatory molecules, including cytokines, chemokines and cytokine-related proteolytic enzyme Matrix Metalloproteinase-9 (MMP-9), whose role in complicated malaria has been recently suggested. The present study investigated HZ effects on release of lysozyme, an enzyme stored in gelatinase granules with MMP-9, focussing on mechanisms underlying its regulation. Results showed that HZ phagocytosis by human adherent monocytes promoted early release of lysozyme, TNFalpha, IL-1beta and MIP-1alpha. HZ-enhanced lysozyme release was abrogated by anti-TNFalpha/IL-1beta/MIP-1alpha blocking antibodies and mimicked by recombinant cytokines. Moreover, HZ precociously activated either p38 MAPK or NF-kappaB pathways by inducing: p38 MAPK phosphorylation; cytosolic I-kappaBalpha phosphorylation and degradation; NF-kappaB nuclear translocation and DNA-binding. Inhibition of both routes through selected molecules (SB203580, quercetin, artemisinin, parthenolide) prevented HZ-dependent lysozyme release. These data suggest that HZ-triggered overproduction of TNFalpha, IL-1beta and MIP-1alpha mediates enhancement of lysozyme release from human monocytes through activation of p38 MAPK and NF-kappaB pathways, providing new evidence on mechanisms underlying HZ-enhanced monocyte degranulation in *falciparum* malaria and suggesting a potential role for lysozyme as a new affordable marker in early diagnosis of complicated malaria.

P56 ITvar9/R29var1 expressing infected erythrocytes demonstrate dual adhesive phenotypes

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Survival of the malarial parasite, *Plasmodium falciparum,* within the human host is facilitated by the phenomenon of sequestration. Infected erythrocytes bind to a variety of receptors such as CD36, ICAM-1 and chondroitin-4-sulphate. The high molecular weight protein, PfEMP-1, which is expressed on the surface on infected erythrocytes, has been shown to mediate this adhesion. Rosetting, the adhesion of infected erythrocytes to two or more non-infected, is another adhesive interaction and strongly correlates with severe disease. Parasite strains that undergo rosetting have been identified as expressing group A var genes and the adhesive interaction is mediated via the NTS-DBL1α_1 domain of the PfEMP-1 molecule. However, as rosettes are absent from the peripheral blood stream, it is suggestive that they also undergo sequestration. Here we demonstrate infected erythrocytes expressing the group A var gene IT/R29var1 are capable of both rosetting via DBL1α_1 and cytoadhesion via the DBL2γ domain to heparan sulphate expressed by the human brain endothelial cell line, HBEC-5i.

P57* Rosetting in a *P. falciparum* field isolate: Identification of PfEMP1 variant associated with rosetting and functional characterization of the antibodies

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Plasmodium falciparum-infected erythrocytes can bind uninfected erythrocytes (rosetting), a phenomenon associated with severe malaria in African children. In many strains, the N-terminal DBL alpha domain of the *Plasmodium falciparum* erythrocyte membrane protein 1 (*Pf*EMP1) molecule has been shown to mediate this adhesion. Previously we examined the effect of antibodies to the extracellular domains of a rosetting variant ITvar9, in R29 parasites. This follow-up study focuses on identification

and detailed analysis of the functional and immunological properties of the rosette-mediating *Pf*EMP1 variant from a Kenyan field isolate. By transcriptional profiling we compared high-level gene expressed between isogenic rosette positive/negative pairs. Using degenerate primers, upstream and downstream of the *Pf*EMP1 regions, we identified the full-length sequence of the *Pf*EMP1 variant. Rabbits were subsequently immunized with the recombinant domains expressed in *E. coli* to produce antibodies. These antibodies recognized the surface of live infected erythrocytes, elicited a dose-dependent inhibition of rosette formation and mediated phagocytosis of homologous parasites. Analysis of the antibodies against a panel of other rosetting lab strains showed minimal cross reactivity with some of the antibodies. Further testing on clinical isolates will assist in the evaluating relevance of rosetting variants in the field and may be helpful in the development of drugs or vaccines to prevent rosetting.

P58* Alterations in the endothelial cells of the blood-brain barrier in cerebral malaria

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Cerebral malaria (CM) is a severe complication caused by *Plasmodium falciparum.* One consistent finding is the alteration of blood-brain barrier (BBB) which is a selective barrier separating the brain parenchyma from blood. The integrity of this barrier is maintained by intercellular junctional proteins such as occludin, claudin-5 and vinculin on its endothelial cells. These proteins are significantly reduced in post mortem brain tissue from CM patients. Human brain endothelial cells (HBEC) were co-cultured for 20 hours with *Plasmodium falciparum* infected red blood cells (Pf-IRBC). Subsequently, analysis of the endothelial cells demonstrated a reduction in the levels of claudin-5, ZO-1 and vinculin in response to Pf-IRBC. The supernatants were analysed and demonstrated the presence of endothelial derived proteases such as metalloproteases and ADAMTS proteases (Patel et al BSP 2011). The supernatant from HBEC/Pf-IRBC co-culture experiment was added to freshly cultured HBEC and incubated for 24 hours. Analysis using cell – based ELISA demonstrated a significant increase in these proteins. This suggested that the loss of HBEC monolayer (BBB) integrity induced by Pf-IRBC/HBEC co-culture supernatant within five hours (Patel et al BSP2011) was not irreversible and eventually recovers.
We suggest that the BBB is disrupted by endothelial-derived mediators during a malaria infection but this recovers, once the infection subsides.

P59* Alterations in the endothelial cells of the blood-brain barrier in cerebral malaria

Mohd Hamzah M Nasir, Bitumani Borah, Adam Sidaway, Monique F Stins* and Srabasti J Chakravorty.
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We suggest that the BBB is disrupted by endothelial-derived mediators during a malaria infection but this recovers, once the infection subsides.

P60* Structure-based mutagenesis to reveal the immunoglobulin M binding site of Duffy-binding-like domains in malarial parasite-encoded proteins.

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Duffy-binding-like (DBL) domains are essential to the function of proteins involved in critical interactions of the malarial parasite with both the mosquito vector and human host. One of these, *Plasmodium falciparum* erythrocyte membrane protein 1 (PfEMP1), found on the surface of infected erythrocytes, plays an important role in generating the antigenic variability that facilitates immune evasion by the parasite. This protein can interact with numerous host ligands leading to diverse disease outcomes. At least fifteen DBL domains are known to bind immunoglobulin M (IgM), allowing the parasite to evade neutralization. We used a structural model to characterise potential binding sites on the DBL domains. We then used site-directed mutagenesis to alter these residues and determined their binding affinities using Surface Plasmon Resonance. By comparing binding affinities of wild-type and mutants we hope to identify the critical residues involved in IgM-binding. To further explore the function of DBL domains we will use these data to develop a bioinformatics tool to predict the structural features that define the propensity of a given DBL domain to engage a particular ligand, which may aid the prediction of system-wide, organism-level outcomes of various mutations.

P61 Integrated control of dengue vector by *Mesocyslops* and *Bacillus thuringiensis* from Lahore, Pakistan

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The present study evaluated the predatory capacity and efficacy of a local strain of copepod *Mesocyclops leuckarti (M. leuckarti)* and a bacterial strain, *Bacillus thuringiensis israelensis (Bti)* for the control of *Aedes aegypti* larvae. The main objective was to develop a cost-effective and environment friendly integrated vector control model in Lahore, Pakistan. *M. leuckarti* was collected from an artificial pond in the Lahore zoo. Single species culture was established in laboratory. *Aedes aegypti* reared in laboratory were used to evaluate the toxic effect of *Bti*. Larval mortality was evaluated singly and both with *Bti* +copepod in the field using 4 litre containers for 10 weeks. *M. leuckarti* and *Bti* showed 100% larval mortality during the first week of field experiments when used singly, which declined to 94 and 64% in the following weeks up to the week 05 respectively. At the end of fifth week *Bti* was not effective to kill larvae and reapplication caused 80-91% mortality by the end of week 10. In an integrated group (*M. leuckarti* + *Bti*), larval mortality was 99.3% by the end of week 5. Reapplication of *Bti* in this group during sixth week caused 100% mortality which

remained 99.6% by the end of week 10. Therefore, an integrated control was found to be an effective strategy for the control of dengue vector in Pakistan.

P62 Fitness cost of disrupting the circadian rhythm of malaria parasites within a resource limited environment.

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Circadian biology assumes that biological rhythms maximise fitness by enabling organisms to co-ordinate with their environment. Our previous work has found that perturbation of *Plasmodium chabaudi* parasite rhythms resulted in a two-fold cost to the production of replicating and transmission stages, revealing a role for circadian rhythms in the evolution of host-parasite interactions. Other models have demonstrated that red blood cells (RBC) may be a limiting resource for parasites. We tested whether the costs experience by parasites of disruption to their rhythms are exacerbated under RBC limitation, generated by anaemia. We investigated the consequences - for parasites - of being temporally mismatched to host circadian rhythms in a resource limited environment using rodent malaria parasite *Plasmodium vinckei* (a synchronous parasite that preferentially invades mature RBC). We demonstrate that limiting the density of preferred RBC significantly reduces the production of replicating stages and temporal mismatch does not have an additional affect. In contrast, the density of transmission stages was more affected by temporal mismatch than resource limitation. We outline a mechanism by which *P. vinckei* may adjust investment in transmission stages to help compensate for the reduction in replication caused by stressful conditions.

P63 Immune and antioxidant defenses in an autogenous *Aedes caspius* mosquito upon infection with *Bacillus thuringiensis kurstaki*

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In this study, the interaction between anti-oxidative response, in terms of glutathione (GSH) titer, and immunity responses, in terms of phenoloxidase (PO) titer, in both larval and adult stages of *Aedes caspius* upon infection with *Bacillus thuringiensis kurstaki* (*Btk*) was investigated. Data from *Btk*-infected larvae showed no or significant lower GSH titer at 12h or 24h post-treatment respectively compared to control larvae. On the other hand, no PO activity was detected at both time points post-*Btk* infection. This may indicate that oxidative stress in larvae was increased while antibacterial response was blocked upon *Btk* infection. Bacterial inoculated adult mosquitoes showed higher GSH activity in both *Btk*- and *E. coli*-inoculated mosquitoes at 12 and 24h post-inoculation compared to control mosquitoes. This activity was more pronounced against inoculated *E. coli*. On the other hand, PO titer showed significant higher PO activity at 12 and 24h post-inoculation with both kinds of bacteria. This response was more pronounced against *E. coli* compared to *Btk.* These results may indicate that antibacterial and antioxidtive responses are more pronounced against *Btk* in adult stage compared to larval stage which may be because of the difference in mode of infection and/or mosquito stage. Moreover, inhibition of both antibacterial and antioxidtive responses upon *Btk* infection in larval stage may explain its high larvicidal activity.

P64* Selection and characterization of a new, non-melanising, line of *Anopheles gambiae* refractory to *Plasmodium falciparum* clone 3D7

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The existing refractory line of *Anopheles gambiae* mosquitoes melanises *Plasmodium falciparum* parasites, a refractory behaviour that is not common under natural transmission. A new, non-melanising, refractory line of *Anopheles gambiae*, denoted GU-REF, was selected for refractoriness to *P. falciparum* clone 3D7 over 9 generations. Gametocyte cultures of 3D7 were used to infect mosquitoes, and eggs were collected from individual mosquitoes two days later, and reared to larvae separately for each female. Mosquitoes were dissected ten days after the infectious blood meal and the number of oocysts on the mosquito midgut counted to establish the level of infection. The larval offspring of the 4 - 10 females with the lowest number or zero oocysts were taken to create the next generation of the selection experiment. This was repeated for nine generations. A control line (GU-CON) was selected at random at the same time as a control for inbreeding effects. The GU-REF line does not appear to exhibit fitness costs or benefits associated with refractoriness, as measured by fecundity, compared to the GU-CON and unselected lines. The GU-REF line is non-melanising, so the refractory mechanism could involve the speed of bloodmeal digestion or non-melanotic immune responses.

P65 Peer education: The effects on knowledge, and preventive practice of pregnancy related malaria in women of reproductive age in Edo-State, Nigeria.

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There is limited uptake of measures to prevent malaria by pregnant women in Nigeria. This is often contributed to limited knowledge of women in child bearing age about the health impact of malaria in pregnancy (MiP) on mother and foetus. A strategy to improve community awareness of MiP is by means of peer-to-peer education. This study explored if peer-to-peer education is an effective tool to raise the level of knowledge amongst women in child bearing age and if increased knowledge translates in improved uptake of preventive measures.
Pre-assessment interviews revealed that knowledge on malaria in general was high in the studied population but knowledge on health risks of MiP and possible preventive measures was limited. The peer education campaign had a significant impact in raising the level of knowledge of women of child bearing age.
Peer education can lead to a significant increase in knowledge on disease transmission and prevention. However, increase in knowledge does not necessarily translates in increased preventive practice. Therefore, health interventions should also focus on addressing other problems influencing preventive practice, such as structural barriers like lack of availability of preventive tools and poor access to health services.

P66 Dispersal ecology of human African trypanosomiasis in a newly affected area of Uganda

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Concerns have been raised by the recent re-emergence of Rhodesian human African trypanosomiasis (HAT) in Uganda and its spread into previously unaffected areas. As is true for many disease vectors and vector-borne diseases, the spatial distribution and dispersal of human African trypanosomiasis can be correlated to environmental factors, including land cover, due to the habitat requirements of the tsetse vector. Despite an understanding of the environmental dependencies of HAT, little research has focused on the environmental factors controlling transmission establishment or the spatially heterogeneous dispersal of disease following a new introduction. Here, an annually stratified case-control study has been used to allow the temporal assessment of correlations between the spatial distribution of HAT and landscape factors. Evidence indicates the establishment of transmission in close proximity to the point of introduction with subsequent spatially heterogeneous dispersal. This dispersal appears to have been guided by environmental cues including elevation and land cover, into areas more suitable for vector survival and parasite transmission. These results provide significant evidence to support targeted control efforts and to prevent further emergence of Rhodesian HAT in currently unaffected areas.

P67* A Pilot Study for Retrospective Screening of Schistosomiasis in Malawi

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Schistosomiasis has been identified as a public health problem in Malawi, with a widespread distribution but heavy infections in specific focal areas. With DFID including Malawi in their ICOSA control programme, mapping and identification of all these areas is needed in a resource effective manner. For this pilot study to try and identify some "hot spot" high infection areas, we proposed to use the residential history of 10 patients that presented at Blantyre's Queen Elizabeth Central Hospital with the chronic manifestations of intestinal schistosomiasis (*S. mansoni*), and trace their history. Two of six identified territorial areas were selected for screening for *S.mansoni*; Blantyre and Jalasi. In total 533 schoolchildren, 251 from Blantyre and 282 from Jalasi, were screened by way of single smear Kato-Katz testing for *S. mansoni* and health questionnaires for *S. haematobium*. Prevalence of *S. mansoni* was found to be as low as 2% (n=13, CI 0.013 – 0.041), with no heavy intensity infection, and *S. haematobium* was 31% (n=166, CI 0.27 – 0.35). Age specific variation was the same for both infections, with the highest frequencies between 10 and 12 year olds. If the low prevalence of *S. mansoni* is a true reflection of the area, then the patients did not get infected there. However it is possible that the transmission pattern has changed due to alterations in the transmission dynamics. Evaluation of sampling methods and effective use of diagnostic tests is needed before recommendations can be made about this novel method of screening.

P68 The relapse period of *Plasmodium ovale curtisi* in travellers differs from that of *P. ovale wallikeri*

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Plasmodium ovale sp. infections are known to relapse, presumably by means of latent hypnozoites in the liver. Travellers with ovale malaria often show delayed presentation months after the last possible date of exposure. We tested the possibility that the two parasite species *P. ovale curtisi* and *P. o. wallikeri*, may differ in their relapse period.

130 blood samples from imported malaria cases (2003-2011) with confirmed *Plasmodium ovale* species were obtained from the HPA Malaria Reference laboratory for analysis. Nested PCR amplification of a repeat region of the gene which encodes for *P. ovale* spp. tryptophan-rich antigen and real-time PCR amplification of reticulocyte-binding protein were used for discrimination between the two species. The latency period for each sample was calculated from data collected from MRL as the number of days between arrival in the UK and presentation with symptomatic microscopy-confirmed ovale malaria. Results were compared with similar figures for *P. vivax* and *P. malariae* infections.
We show that *P. o. curtisi* is much more likely to exhibit long delays in presentation, suggesting a longer liver latency in this species compared to *P. o. wallikeri*. Possible confounders of this association, implications for management of malaria in endemic countries and imported cases will be discussed.

P69* Seroprevalence of Chagas disease in the Gran Chaco: A systematic review of the literature, 20 years post the inception of INCOSUR.

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Background. Bolivia and Argentina have been unable to achieve interruption of vectorial transmission of *Trypanosoma cruzi* despite repeated de-infestations and intergovernmental co-operation. An area traversing the border of these two countries, the Gran Chaco, is believed to have a population with a particularly high proportion of seropositive individuals.
Method. This study systematically reviews the published literature on seroprevalence for Argentina and Bolivia in a 26-year period, and attempts to quantify the true burden of disease for the area through qualitative, quantitative and cartographic analyses.
Results. A pooled estimate of seroprevalence studies from the Gran Chaco since the introduction of intergovernmental co-operation is 37% +/- 6.5 (95% CI) while outside the area it is significantly lower 13% +/- 6.6 (95% CI).
Conclusion. There is compelling evidence to suggest that the Gran Chaco is an area of significant endemicity. Further investigation into the disease prevalence in the region, improved acute surveillance and special provision by INCOSUR should be considered if elimination of Chagas disease is considered a priority.

P70* Towards the development of a rapid diagnostic test (RDT) for detection of anti-schistosome antibodies

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Parasitological methods of diagnosis for schistosomiasis, particularly the Kato-Katz method for *Schistosoma mansoni* and *S. japonicum*, are still widely used in control programs, despite their inherent insensitivity. As many control programs using praziquantel have been and are being initiated, the number of light infections which parasitology often cannot detect is likely to increase. It is therefore envisaged that serological assays involving detection of specific antibodies will become increasingly useful since the sensitivity of these tests is much higher than that of alternative methods, with the proviso that antibody-positivity reflects active infection. Indeed they are already being used in the Chinese national programme for schistosomiasis control.
A rapid diagnostic test (RDT) for detection of anti-schistosome antibodies may therefore be useful and we have begun to develop one that incorporates a novel *S. mansoni* cercarial antigen preparation. Preliminary results with manufactured prototypes of the RDT indicate it could be useful for detection of both anti-*S. mansoni* and anti-*S. haematobium* antibodies. Evaluations in endemic areas are ongoing with the intention that a diagnostic test for schistosomiasis complying with the ASSURED criteria (particularly with regard to **A**ffordability and **U**ser-friendliness) can be brought to market in the near future.

P71 African gyrodactylid monogeneans in phylogenetic and morphological focus

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Representatives of different genera of Gyrodactylidae infect a wide range of hosts from diverse aquatic environments. Recently undertaken parasitological surveys of the monogean fauna of fishes in several countries throughout Africa demonstrate a high morphological diversity and species richness. The aim of this study was to investigate the phylogenetic relationships between gyrodactylid monogeneans of African freshwater fishes and other representatives of Gyrodactylidae, including a wide range of species/genera from both freshwater and marine systems. The phylogenetic analyses, based on SSU and ITS rDNA sequences, point to a polyphyletic origin of African *Gyrodactylus*. Interestingly, African *Gyrodactylus* spp. make up two well-supported clades possibly indicating speciation within host orders: 1) parasites of cichlids (Cichlidae); 2) parasites of catfishes (Siluriformes), consisting of a lineage infecting mochokids and one infecting clariids. *Macrogyrodactylus* spp. firmly cluster into a monophyletic group. The phylogenetic position of *Diplogyrodactylus* and *Afrogyrodactylus* were not well resolved. We found that *Swingleus* and *Fundulotrema* form a clade with *Gyrodactylus* species belonging to the *G. wageneri*-group.
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P72 Tapeworm genomes – the Good, the Bad and the Well-assembled

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The Sanger institute is conducting three tapeworm genome projects; *Echinococcus multilocularis* (the fox tapeworm), *E. granulosus* (the hydatid tapeworm) and *Hymenolepis microstoma* (the rodent tapeworm). Here, I will describe the characteristics and quality of the different genome projects; *E. multilocularis* (114 Mb) is the best assembled, with more than half the genome in chromosome-size scaffolds, *E. granulosus* is similar, and the complete *H. microstoma* (140 Mb) genome is in fewer than 1000 scaffolds. I will highlight our most important findings in these tapeworm genomes, including the finding of large-scale inverted repeats in the genome structure, gene-structures, general gene content, results of comparative transcriptomics across different life-stages, developmental pathways, metabolic pathways, their use of secreted proteins and protease inhibitors for interacting with their hosts, novel domain architectures in the transcription and splicing machinery, and the presence of drug targets in the gene-repertoire. The genomes and transcriptomes of these worms offer many novel insights to the biology of tapeworms, and these genomes have the potential to greatly increase the ease and speed with which new scientific discoveries in tapeworms can be made.

P73* Dynamics of cathepsin B expression in liver flukes

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Liver flukes (*Fasciola hepatica* and *Fasciola gigantica*) cause fasciolosis, a disease responsible for global agricultural losses in excess of US$3.2 billion/year. *Fasciola* vaccine development efforts have focused on virulence target antigens, although such efforts have been hindered by our poor understanding of fluke biology, and the developmental regulation of individual transcripts within multi-gene families. Cathepsin B is one example of such a target: a secreted proteolytic enzyme which appears to be involved primarily in metacercarial excystment and invasion of host tissues by early juvenile flukes. The *Fasciola* cathepsin B superfamily comprises 10 distinct clades, which appear to be differentially expressed during intra-mammalian development. Available data from analyses of the *Fasciola* secretome show defined expression patterns of individual clades in metacercariae, juvenile and adult flukes. Here, we have taken a high-resolution, PCR-based approach to qualifying the expression of all 10 cathepsin B clades across life stages of both *F. hepatica* and *F. gigantica*. Whilst we see largely similar patterns to those previously reported, we have observed the expression of clades B1, B3 and B4 in adult *F. hepatica*. These data suggest a more significant role for cathepsin B in adult flukes than previously recognised.

P74* Reverse genetics characterisation of tegumental proteins in the liver fluke *Fasciola hepatica*

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Fasciolosis, caused by *Fasciola* spp., costs the global agri-food industry an estimated US$3.2 billion per annum and the WHO label it a neglected tropical disease and has estimated that up to 17 million people are infected. With reports of resistance to triclabendazole (the current flukicide of choice), there is a need to validate new control targets for future drugs and vaccines. A recent proteomic study of the adult *F. hepatica* tegument (Wilson *et al*., 2011; *Int. J. Parasitol.* 41, 1347-1359) identified five tegumental proteins of unknown function, displaying limited similarity to proteins from other genera. These proteins represent appealing targets for therapy since they lack any identifiable homology to host proteins, are potentially surface-exposed and function at the host-parasite interface. Here, we aim to evaluate the functional importance of these putative control target proteins using reverse genetics approaches. Analysis of transcript abundance in adult and newly-excysted juvenile (NEJ) *F. hepatica* demonstrates that only three of the genes appear to be expressed in NEJs. RNA interference (RNAi)-based gene silencing experiments have been used to evaluate the impact of gene transcript knockdown on worm phenotype through a series of bioassays aimed at scoring their potential as novel control targets.

P75* *Caenorhabditis elegans* actively resist externally applied cysteine proteinases by blocking their activities with cystatin gene products

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Plant cysteine proteinases (CPs) from papaya are capable of killing parasitic nematode worms *in vitro* and also possess anthelmintic effects *in vivo*. Acute damage reported in gastrointestinal parasites has not been demonstrated in free-living nematodes such as *Caenorhabditis elegans* nor among the free living stages of parasitic nematodes. Dose-response experiments were conducted to assess the effects of papaya latex supernatant (PLS) on wild type (Bristol N2) and cystatin-like null mutant (cysteine proteinase inhibitors 1 and 2[cpi-1 and cpi-2]) *C. elegans*. The effect was dose-, temperature- and time-dependent. Wild type *C. elegans* were not susceptible at room temperature when exposed to doses of CP that are known to destroy parasitic stages of nematodes. However, at low temperatures the wild type was susceptible. Cystatin null mutants were highly susceptible to PLS irrespective of temperature and dose of exposure. We hypothesize that wild type *C. elegans* employ secreted cystatins CPI-1 and CPI-2 in evading CP attack. Coupled with evidence of cuticle damage on both light and scanning electron microscopy, cpi-1 and cpi-2 null mutants (or a double mutant combination of the two) could provide a cheap and effective rapid through-put *C. elegans*-based assay for screening plant CP extracts for anthelmintic activity.

P76 uthenium complexes as possible treatment for Alveolar Echinococcosis

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The current chemotherapy of alveolar echinococcosis (AE) is based on benzimidazoles such as albendazole, and has been shown to be parasitostatic rather than parasiticidal, requiring lifelong duration. Thus, new and more efficient treatment options are urgently needed. By employing a validated assay based on the release of functional phosphoglucose isomerase (PGI) from dying parasites, the activities of 16 organic compounds, some of them rhutenium complexes, were investigated. These complexes have been described as potential anticancer/antimetastatic, and to a lesser extent as antibacterial drugs. Initial screening of compounds was performed at 20 μM, and those exhibiting considerable anti-parasitic activities were tested at lower concentrations. The potential cytotoxicity of the most active compounds was also assessed *in vitro* with two mammalian cell lines, human foreskin fibroblasts and rat hepatoma cells. The complex 10a, a η^6-arene ruthenium (II) phosphate was shown to have high activity even at low concentrations (IC_{50} of 1.37μM) in a dose-response manner. This complex presented high toxicity to rat hepatoma cells, as expected, but not to human foreskin fibroblasts. A pilot *in vivo* experiment was performed with a healthy animal model and showed no adverse effects. In conclusion, complex 10a seems to be a promising potential drug that should be further studied in appropriate *in vivo* models for *E. multilocularis* infection.

P77* Analysis of P-glycoprotein9 Alleles in UK isolates of *Teladorsagia circumcincta*

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The genetic basis of anthelmintic resistance in parasitic nematodes is in many cases still poorly understood.
Recent studies conducted independently at the Hopkirk Research Institute (New Zealand) and the Moredun Research Institute (Scotland) have implicated the involvement of a particular P-glycoprotein (Pgp) gene, *Tci-pgp-9*, in multiple-anthelmintic resistance in *Teladorsagia circumcincta*. In the Hopkirk study, an analysis of allele frequencies across several different candidate genes in “near-isogenic” susceptible and multiple-resistant strains of *T. circumcincta*, revealed a significant shift in the incidence of certain *Pgp-9* alleles in the resistant strain. Furthermore, Real-Time PCR provided strong evidence that resistance was associated with an increase in the copy number of this gene. In the Moredun study, increased expression of the same gene was observed in a multiple-resistant UK field isolate.
The focus of the present study is to further characterise *Tci-pgp-9* and its possible role in ivermectin (and multi-drug) resistance in two UK isolates of *T. circumcincta*: MTci2 which is susceptible to anthelmintics, and MTci5 which is resistant to 3 broad spectrum anthelmintics. High levels of polymorphism in *Tci-pgp-9* have been found in both isolates. Recent work undertaken to assess the relationship between the alleles present and *Pgp-9* gene copy number will be discussed.

P78 Genome sequence and transcriptome analyses of the murine model whipworm *Trichuris muris*

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The whipworm *Trichuris muris* is a gut-dwelling nematode parasite that serves as a laboratory model for investigations of helminth-host interactions and immune responses. We have determined the 84.4 Mb genome of *T. muris* by Illumina high-throughput sequencing. The current genome assembly is in 1,010 scaffolds with an N50 of 1.2Mb. Gene finding and annotation are ongoing and so far comprise approximately 9,400 genes. RNA-seq datasets from L3 larvae, male and female adult worms as well as stichosome vs back-end samples show extensive differential gene expression across life-cycle stages, sex, and worm morphology. We present gene enrichment and comparative analyses, in particular in comparison to other nematode helminths.

P79 A microfluidic tool for electrophysiological analysis of *C. elegans*

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The tractability of *Caenorhabditis elegans* for molecular genetic approaches has made it a highly useful organism for fundamental studies in nematode neurobiology. Combining this with physiological analyses has proven very informative for anthelmintic mode of action studies. Here we describe the development of a new microfluidic based method for making electrophysiological recordings from the pharyngeal circuit which controls feeding behaviour. The activity can be recorded by placing an extracellular recording electrode over the nose of the worm a technique called the electropharyngeogram, or EPG. The EPG provides an experimental platform for defining gene function, the impact of genetic mutations, and the effects of drugs and toxins in a nematode neural network. To date the EPG has been a low-throughput assay as each recording requires a stand-alone experiment in which a single glass microelectrode is used to make the recording. We have designed a microfluidic chip which sequentially traps worms and captures EPG activity combined with the capability to apply drug solutions. We have designed software (AutoEPG) which permits rapid extraction of specific parameters from the EPG trace (see Dillon et al 2009, PLoS One 4:e8482) and thus are progressing towards developing a new tool for consistent, high-quality and high-throughput neurogenetic and neuropharmacological analysis of nematodes.

P80 *Schistosoma mansoni*: implication and validation of kinases as putative drug targets by RNAi

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The reliance on one drug, praziquantel, which is increasingly widely applied, has encouraged efforts to look for new schistosomicides. One approach to this is identification of putative drug targets and to this end we have been applying RNA interference (RNAi) to validate potential targets. Genes for RNAi were selected based on (i) bioinformatic analysis of putative essentiality and (ii) computational prediction of putative targets based on searching the ChEMBL database for compounds found to be active in whole organism screening of focussed compound libraries. As a result, a number of Protein kinases (PKs) were highlighted. PKs are a superfamily of enzymes with key roles in a number of pathways involved in host- parasite relationships and they are considered good potential targets for the treatment of infectious diseases. Here we describe the identification and validation of putative targets using the computational prediction approach. Homologous genes for the kinases identified were mapped in the *S. mansoni* genome and RNAi used to establish the essentiality of 11 such genes. In studies using larval and adult schistosomes consistent gene knockdown has been achieved and significant and distinct phenotypic

P81 Population genetic studies in *Fasciola hepatica:* development of a panel of polymorphic microsatellites

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The liver fluke, *Fasciola hepatica* is an economically important pathogen of sheep and cattle and has recently been described by WHO as a re-emerging zoonosis. Parasite control is heavily reliant on the use of drugs, particularly Triclabendazole (TCBZ) and as a result TCBZ-resistance has now emerged. The population structure of *F. hepatica* is not well known, yet has the potential to impact on parasite control, particularly drugs and vaccines. Microsatellites were identified from 83 Mb of *F. hepatica* genome sequence using msatfinder (www.genomics.ceh.ac.uk/msatfinder) and 2448 potential microsatellites were identified. Twenty new loci were identified and screened and a subset of these markers was developed and optimised for microsatellite PCR, using fluorescent primers. This provided a panel of 11 loci which were found to be polymorphic, with allele numbers ranging from 3-16. Hardy-Weinberg equilibrium and the heterozygote deficiency for each locus were examined and 4 loci (Fh_1, Fh_4, Fh_8 and Fh_11) were found to significantly deviate from Hardy-Weinberg equilibrium, following the Bonferroni's adjustment ($P<0.01$). All 4 of these loci were significant for heterozygote deficiency ($P<0.01$). Several microsatellite pairs showed significant linkage disequilibrium, although this may reflect the small population size studied. This study reports the largest panel of microsatellite markers available to date for population studies of *F. hepatica*.

P82 Molecular Identification of *Austrobilharzia* sp. (Digenea: Schistosomatidae) from Kuwait Bay

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Avian schistosomes belonging to the genus *Austrobilharzia* (Digenea: Schistosomatidae) are among the causative agents of cercarial dermatitis in humans. In this study, ribosomal and mitochondrial DNA sequences were used to study schistosome cercariae from Kuwait Bay that have been identified morphologically as *Austrobilharzia* sp. Sequence comparison of the ribosomal DNA (rDNA) 28S and 18S regions of the collected schistosome cercariae with corresponding sequences of other schistosomes in GenBank revealed high sequence similarity. This confirmed the morphological identification of schistosome cercariae from Kuwait Bay as belonging to the genus *Austrobilharzia*. The finding was further supported by the phylogenetic tree that was constructed based on the combined data set 18S-28S-mtCO1 sequences in which *Austrobilharzia* sp. clustered with *A. terrigalensis* and *A. variglandis*. Sequence comparison of the *Austrobilharzia* sp. from Kuwait Bay with *A. variglandis* and *A. terrigalensis* based on mtCO1 showed a variation of 10% and 11% respectively. Since the sequence variation in the mtCO1 was within the interspecific range among trematodes, it seems that the *Austrobilharzia* species from Kuwait Bay is different from the two species reported in GenBank, *A. terrigalensis* and *A. variglandis*

P83* RNA interference in Adult *Ascaris suum*

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RNA interference (RNAi) is a reverse genetics technique that represents a valuable method of elucidating gene function. In parasitic nematodes, the efficiency of RNAi varies between species and lifestage. Recent successes in invoking RNAi responses in the larval stages of *Ascaris suum* provides an opportunity to validate potential drug target candidates and to further probe the biology of *Ascaris*. This poster reports efforts to establish an RNAi platform in adult *A. suum*. The approach employed includes the examination of four target gene transcripts whose selection was based on: (i) transcript abundance, (ii) expression and localization patterns, and iii) previous success as RNAi targets in animal and human parasitic nematode gene silencing experiments. dsRNAs were injected into the pseudocoelomic cavity of adult *A. suum* and changes in transcript levels post-RNAi were assessed using quantitative real time PCR. Specific, potent and statistically significant transcript knockdown (>70%, n≥4) was induced in two of the four genes targeted; elongation factor (*As-eft-1*) and pyrophosphatase (*As-ppase*). This data demonstrates, for the first time, that adult *A. suum* possesses a functional RNAi pathway and that a simple injection method of dsRNA delivery was sufficient to trigger an RNAi response. These results highlight the potential for the development of a robust platform for the investigation of gene function in *A. suum*.

P84* Attempted characterization by mass spectrometry of a chymotrypsin-like serine protease found in mouse plasma and in extracts of *Schistosoma mansoni* worms grown in mice.

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Extracts of adult *Schistosoma mansoni* worms have been shown to contain a molecule derived from the plasma of their mouse hosts and which has chymotrypsin-like serine protease activity (Darani & Doenhoff, Parasitology, **135**: 467-472, 2008). We have attempted to further characterize this molecule by mass spectrometry after immunoprecipitation with rabbit antisera.
Initial experiments showed that the identities of previously-characterized molecules in *S. mansoni* extracts or from normal mouse serum could be confirmed by mass spectrometric analysis after they had been subjected to immunoelectrophoresis with rabbit antibodies. Following extensive washing to remove non-precipitated material the immunoprecipitin arcs were trypsin-digested and subjected to mass spectrometry. In initial proof-of-principle experiments mouse serum albumin and *S. mansoni* egg antigen k-5 (Schramm et al., Mol Biochem Parasitol, **166**: 4-14, 2009) were two molecules, the previously known identities of which were successfully confirmed by the aforementioned procedures.Preliminary mass spectrometry of trypsin digest-derived peptides from the chymotrypsin-like enzyme in mouse serum indicated they originated from pregnancy zone protein (PZP) and murinoglobulin (MUG). This is paradoxical as PZP and MUG are protease *inhibitors* belonging to the alpha 2-macroglobulin family of molecules. Work is continuing to try and resolve this paradox.

P85* *Schistosoma mansoni* sirtuins as drug targets

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Sirtuins are protein deacetylases that are involved in a wide variety of cellular processes including the regulation of transcription and apoptosis. They are actively investigated as drug targets, particularly in cancer, and in the context of the SEtTReND project financed by the EC, we are investigating the use of sirtuin inhibitors as candidate drugs against schistosomiasis. Five sirtuins, orthologues of mammalian sirtuins (Sirt) 1, 2, 5, 6 and 7, are encoded in the *S. mansoni* genome and we have cloned and characterized their coding sequences. Quantitative RT-PCR shows that all five are expressed at all parasite life-cycle stages tested. Inhibitors of human Sirt1 and 2 induce apoptosis in schistosomula and the separation of adult worm pairs maintained in culture. The production of recombinant *S. mansoni* (Sm)Sirt1, 2 and 6 has been initiated with the aims of obtaining 3D crystal structures and their use in high-throughput inhibitor screening. In order to determine the biological roles of SmSirt1 we are screening for potential protein partners using a yeast two-hybrid library.

P86* Catabolic Enzymes- novel drug targets in *Fasciola hepatica*?

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The common liver fluke, *Fasciola hepatica*, infects both human and animals causing fasciolosis. The World Health Organization has estimated that ~2.4 million people have fasciolosis and another 180 million are at risk of being infected. It is considered to be the most economically damaging trematode disease of livestock, with annual costs estimated at US$2000 million per annum worldwide. An important evolutionary pressure on the parasite is its changing environment throughout its life cycle, requiring its metabolism to move from aerobic to anaerobic as it matures. The fluke's metabolic adaptations include an adaptation of the tricarboxylic acid cycle (TCA), allowing it to maintain energy production in anaerobic conditions.
We are currently working on three enzymes concerned with energy production - triose-phosphate isomerase (TPI), citrate synthase, and galactokinase. TPI from *F. hepatica* has been successfully cloned, sequenced, expressed and purified. The expressed enzyme is active; and its kinetics appears to be complex. Citrate synthase has been cloned and a partial sequence has been obtained; however, expression and purification have proven difficult. Galactokinase has been also successfully cloned, sequenced, expressed and purified; kinetic work is still in the early stages. These current biochemical studies, combined with molecular modelling, will enable us to assess whether catabolic enzymes from *F. hepatica* make plausible targets for the development of novel anthelmintics.

P87* New fluorescence assays for the routine screening of drug susceptibilities of *Trichomonas vaginalis*.

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The sexually-transmitted disease trichomoniasis affects large numbers of people world-wide. It is currently treated with metronidazole, but resistance has developed. Novel anti-trichomonad drug development is therefore urgent and the availability of a reliable *in vitro* drug screening method is essential as *T. vaginalis* is a human-only pathogen and no animal models are available. An attempt to validate a published fluorescence-based assay using resazurin (Alamar blue) dye revealed serious flaws associated with this method. The limitations of the resazurin-based method were attributable to the high reducing potential of the growth media, especially ascorbic acid, which reduces the dye even in the absence of cells. We developed an alternative assay, based on the fluorophore Propidium iodide (PI), which, unlike resazurin, does not need to be metabolically activated by live cells but fluoresces upon binding with nucleic acids. This provides a linear relationship between fluorescent signal and cell numbers when cells are lysed at the end of the incubation with test drugs. An equally good method, not requiring cell lysis, uses the red fluorescent dye resorufin which live *T. vaginalis* trophozoites reduce to non-fluorescent, colourless dihydroresorufin. Both methods allow for easy upscaling and was used to screen a small library of anti-protozoal agents. We recommend this assay for the routine screening of test compounds for anti-trichomonal activity.

P88 *Toxoplasma gondii* clathrin plays a key role in Golgi vesicle formation, but not in receptor-mediated endocytosis

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Apicomplexan parasites are highly polarised eukaryotic cells with a set of unique and specialised organelles, such as micronemes, rhoptries, and dense granules, which are crucial for invasion and modulation of the host cell and linked to the secretory pathway. Trafficking of proteins destined to these secretory organelles is dependent on the dynamin-related protein B, DrpB, with several small Rab GTPases also involved in post-Golgi vesicular sorting. Here, we demonstrate that endogenously tagged clathrin is localised to the trans-Golgi network, indicating a role in vesicle formation at the Golgi. Using a dominant-negative approach and a novel knock down strategy (in which efficient degradation of a gene's mRNA is achieved when a degradation sequence is placed adjacent to the termination codon), we were able to show that ablation of functional clathrin leads to parasites blocked in replication, but not in invasion and egress. Interestingly, these parasites mistarget all secretory proteins and proteins of the inner membrane complex. Based on these findings, we conclude that clathrin is involved in both DrpB dependent and independent vesicle formation at the Golgi, but unlikely in receptor-mediated endocytosis.

P89* Components of the Glideosome are required for intracellular parasite replication

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The glideosome of apicomplexan parasites is a myosin and actin depending machinery anchored between the Inner Membrane Complex (IMC) and the plasma membrane of the parasite. This complex is assembled in a stepwise process. While early components of the glideosome, such as the Gliding Associated Protein 50 (GAP50) are transported to the IMC during its biogenesis, late components (Myosin A, Myosin light chain 1 (MLC1) and GAP45) are assembled during cytokinesis of daughter parasites. It is generally believed that components of the glideosome are not involved in parasite replication, since conditional Knock Downs for MyoA or GAP45 show no effect on intracellular parasite growth. However, we recently established that over-expression of a dominant negative version of MyoA leads to a severe block in intracellular replication in *Toxoplasma gondii*. This block can be attributed to a defect in IMC maturation. We attempted to rescue the phenotype caused by expression of dominant negative MyoA and found that simultaneous over-expression of MLC1 led to a partial complementation of parasite replication. This demonstrates that MLC1 has a dual function in parasite replication and gliding motility and suggests that it is required for at least one additional myosin motor protein in *T. gondii.*

P90 Early and specific diagnosis of toxoplasmosis in sheep in Saudi Arabia

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We have noted in recent years that the mortality rate in births and abortions in sheep and goats have exceeded the 35% spread in sheep disease mystical chronic lead to death of animals within a month or two months. Accordingly, the present is designed to isolate a specific immunodiagnostic antigen from native strain of *T.gondii* tachyzoite to use it in early and accurate diagnosis of toxoplasmosis in sheep in Saudi Arabia.
A total of 360 serum samples were investigated by ELISA for the detection of anti-*Toxoplasma gondii* antibodies. Four tachyzoite antigens were adopted in ELISA. These antigens were RH strain antigen (RHA), local strain antigen (LA) and two fractions isolated from local strain antigen by CNBr-Sepharose 4B affinity column chromatography named; unbound (LAunb) and bound (LAb). LA showed higher diagnostic value (52.8%) than RHA (47.2%). While the highest diagnostic capabilities of toxoplasmosis were associated with LAunb (76.9%), LAb showed the lowest diagnostic potentials (35.6%). The electrophoretic profile of RHA and LA showed comparable separation pattern with molecular weight range of 23-154.5 KDa as revealed by SDS-PAGE. The immunoreactive bands of each of the four antigens with infected sera were identified by immunoblot. In RHA, the immunogenic bands were 154.5, 136, 116, 74, 53, 40 and 31 KDa. While in LA, were 136, 116, 74, 44, 40 and 31 KDa, Four immunogenic bands of 136, 74, 44 and 31 KDa were identified in LAunb and might be responsible for the highest diagnostic capabilities of *Toxoplasma* infection. The current research introduces a partially purified fraction from a local strain tachyzoites antigen, which is utilized for the first time, for the detection of *T gondii* antibodies in Saudi Arabia.

P91 Could our medicines boost pathogens? Increased fitness of antimony-resistant *Leishmania donovani*

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Mathematical models predict that the future of epidemics of drug-resistant pathogens depends greatly on the competitive fitness of drug-resistant versus drug-sensitive strains. Several studies on prokaryotes and eukaryotes have demonstrated that drug-resistance generally confers a reduction in fitness expressed as reduced growth, virulence or transmission. In some cases, compensatory mutations may occur which restore, at least partly, the fitness of drug-resistant mutants to that of wild-type forms. The generally accepted dogma of drug-resistance being associated with a fitness cost is questioned by our recent findings on antimony-resistant (SSG-R) *L. donovani*. A mathematical model showed that the prevalence of SSG-resistance in Bihar could not be explained without assuming a higher fitness of SSG-R parasites. Experimental evidences demonstrated that SSG-R parasites produced more infectious stages, that they better survived in vitro in macrophages and produced higher parasite burdens in vivo. Field studies also revealed a high prevalence of SSG-R parasites in natural populations of India, despite the low SSG pressure nowadays. Mechanisms of SSG-resistance might contribute to explain this higher fitness, but whole genome and metabolome analyses identified other traits not necessarily linked to SSG-resistance but which might affect the parasite's survival skills. We discuss the possible implications of our findings on the control of visceral leishmaniasis.

P92 Efficacy of Miltefosine in the treatment of visceral leishmaniasis in the Indian sub-continent: the current status.

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Miltefosine (MIL) is the only oral drug available for treatment of Visceral Leishmaniasis (VL) which has shown to be safe and effective. Its unrestricted use has raised concern about its continued efficacy. In the context of a large multidisciplinary study, we recruited and followed two cohorts of VL patients in the Indian subcontinent and found a significant decrease in efficacy of the drug in comparison to earlier reported phase IV studies. In India and Nepal, relapse rates were 7.24 % (6-months follow-up) and 21.8 % (12-months follow-up) respectively. DNA fingerprinting of parasites from relapsing patients demonstrated identical patterns as those obtained at the onset of treatment, excluding the occurrence of re-infections. The MIL-susceptibility of clinical isolates was analyzed *in vitro* using promastigote and amastigote assays and did not show so far any clear-cut MIL-resistance in isolates from relapsing patients. However, isolates with a higher tolerance to the drug were observed in some patients. The latter isolates show residual parasites in the spleen upon MIL treatment in infected hamsters, which was not the case with parasites showing low EC50 values. Data on the virulence of the corresponding parasites will also be presented.

P93 *Leishmania* as a paradigm to explore the impact of genome diversity on metabolome variation

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The genome and the metabolome are situated at extreme ends of the modern dogma of molecular biology. The natural diversity of microorganisms provides the context for innovative studies on the functional link between these two '-omes'. We use a Nepalese population of *Leishmania donovani* characterised by a high phenotypic diversity (antimonial drug susceptibility, infectivity…). Full-genome sequencing of 17 *L. donovani* lines revealed a low diversity at the sequence level, but a high diversity at the structural level (aneuploidy, tandemly repeated genes, episomes). We hypothesise that this structural variation drives the evolutionary flexibility and adaptive capacity of *L. donovani*. We are currently investigating how the observed diversity on genome level is manifested on the level of metabolic diversity. A proof-of-principle metabolomics study already demonstrated that 1/3 of all detected metabolites were showing significantly different levels between antimony-resistant and sensitive strains. A similar metabolomic characterisation is currently in progress for all clinical lines which were studied on genome level. The resulting metabolome diversity data will be related to the genomic diversity data for all 17 clinical lines. We anticipate that this integrative approach will give an unprecedented insight into pathogen diversity.

P94* Mitochondrial processing peptidase of *Trypanosoma brucei*

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Trypanosoma brucei has an unusual mitochondrion that undergoes remarkable switches between a fully active and highly repressed form during its life cycle. Very little is known about protein targeting to and maturation in *T. brucei* mitochondria and virtually no data are available comparing these processes between vector (VS) and bloodstream (BS) stages. Herein we investigated mitochondrial processing peptidase (MPP) that plays an essential role in mitochondrial biogenesis by recognizing and cleaving the mitochondrial targeting sequences (MTS) of nuclear-encoded preproteins. Our results showed that MPP is an essential enzyme for viability of *T. brucei*. Recombinant *T. brucei* MPP cleaved targeting sequence of mitochondrial IscU exactly at the cleavage site predicted by PSORT. Immunoblot analysis confirmed presence of α and β subunits of the MPP heterodimer in VS mitochondria. Unexpectedly, dual localization of MPP was observed in BS. In addition to mitochondria, MPP was also detected in BS cytosol. Accordingly, both cytosolic and mitochondrial cellular fractions of BS catalyzed processing of recombinant substrates. Although the role of cytosolic MPP is unclear, it is tempting to speculate that the cytosolic MPP may affects targeting of certain preproteins to BS mitochondria.

P95* Autophagy is Important for Glycosome Turnover during the *Leishmania major* life cycle

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The life cycle of *Leishmania* involves different forms adapted to the distinct environments encountered in the sandfly insect vector and the mammalian host. During differentiation between these forms, the parasites remodel their cellular structure and metabolism. It is thought that glycosomes, which compartmentalise metabolic enzymes, are degraded and new ones synthesised that adapt metabolism to the next life cycle stage. In yeast and mammals, similar organelles, peroxisomes, are degraded by autophagy in response to changing environmental conditions. Autophagy is a catabolic pathway important for cell survival, which involves formation of double-membraned autophagosomes which traffic cargo to the lysosome for degradation. Autophagosome biogenesis requires a set of ATG proteins, including ATG8 which marks autophagosomes from their formation to their breakdown in the lysosome. Throughout the life cycle of *L. major* expressing GFP-ATG8 and RFP fused to a glycosomal targeting sequence, SQL, we observe co-localisation of autophagosomes with glycosomes by fluorescent microscopy. GFP-ATG8 and RFP-SQL can also be seen together in what appear to be lysosomal structures. During differentiation and starvation, in which autophagy increases, more frequent autophagosome-glycosome co-localisation is observed. In addition, autophagy-deficient mutants possess higher numbers of glycosomes when compared to wild-type parasites.

P96* The role of the DNA Repair Enzyme TDP1 in *Trypanosoma brucei*

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The aim of our work is to understand the role of tyrosyl-DNA phosphodiesterase 1 (TDP1) in the bloodstream form of *Trypanosoma brucei brucei*, the parasite that causes African Sleeping Sickness, and to evaluate its suitability as a potential target for anti-parasite therapy. Eukaryotic TDP1 is a DNA repair enzyme that removes covalently trapped topoisomerase from the 3' end of the DNA at DNA strand breaks. Covalent topoisomerase I stalling can be induced by endogenous DNA damage and by anti-cancer drugs such as camptothecin (CPT). A potential approach could be to use TDP1 inhibitors synergistically with CPT in a combined anti-parasite therapy. TDP1 knock out cells are hypersensitive to CPT and accumulate in the G2/M phase of the cell cycle upon treatment with the drug. The CPT hypersensitivity of the TDP1 knock out cells can be fully rescued through ectopic expression of wild type TDP1. The mutant cells appear normal, but exhibit a slight growth delay. TDP1 is the only enzymatic activity present in *T. brucei brucei* blood stream forms that has 3' tyrosyl DNA phosphodiesterase activity. Based on our extensive knowledge of yeast and human Tdp1 we are currently investigating the range of TbTDP1 repair substrates *in vivo* and *in vitro*.

P97 Characterisation of autophagy in *Trypanosoma brucei*

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Autophagy is a widely conserved intracellular mechanism for the degradation of long lived proteins and organelles, and is involved in many fundamental aspects of cellular homeostasis and development. It is a complex multi-protein pathway characterised by the formation of autophagosomes (double membrane bound vesicles) around cargo destined for the lysosome. The ubiquitin-like protein, ATG8, is a key component of the autophagosome membrane and is an established molecular marker for autophagy in many different biological systems.
To investigate autophagy in *T. brucei,* the three candidate ATG8s (ATG8.1, Tb927.7.5900; ATG8.2, Tb927.7.5910 and ATG8.3, Tb927.7.3320) were fused with yellow fluorescent protein and expressed in bloodstream form and procyclic form parasites. Autophagosome-like structures were only detected for ATG8.1 and ATG8.2, suggesting an alternative function for ATG8.3. Subsequent detailed analysis confirmed autophagy occurred in both life cycle stages and was upregulated in response to stress (nutrient starvation in procyclic forms and neuropeptide treatment in bloodstream forms). To validate our approach, we created YFP-ATG8 expressing RNAi-compatible cell lines. Downregulation of specific autophagy pathway genes disrupted the formation of autophagosomes and generated autophagy defective parasites for *in vivo* loss of function analysis.

P98 Characterisation of a putative CDC14 phosphatase in *Trypanosoma brucei*

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CDC14 (Cell Division Cycle 14) phosphatases are highly conserved and present in a wide range of organisms where they fulfil distinct cell cycle regulatory functions. In the yeast *Saccharomyces cerevisiae* for example, the single homologue reverses CDK1 (Cyclin dependent kinase 1) phosphorylation events and thus promotes mitotic exit and cytokinesis. CDC14 functions in other organisms are diverse and include DNA replication, DNA damage repair, nuclear organization, mitotic entry and/or mitotic spindle assembly.
We have depleted a putative *T. brucei* CDC14 phosphatase by RNAi and found it to be essential for proliferation of the bloodstream stage of the parasite. Cells appear to be impaired in cytokinesis as suggested by an accumulation of parasites with either a visible cleavage furrow or unable to undergo abscission. Here we investigate potential interactions of the phosphatase with known cell cycle and cytokinesis regulators and also attempt to identify novel interactions and substrates by tandem affinity purification of CDC14 protein complexes using a CDC14 substrate trapping mutant expressed in the parasite

P99 Kinesins in *Trypanosoma* and *Plasmodium*: essential and novel functions

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In many protozoan parasites, microtubules play essential and peculiar roles, in addition to their role in mitosis. In *Trypanosoma brucei*, the agent of human sleeping sickness, microtubules in the cell corset define cell shape and elongate at the posterior end during the cell cycle. In *Plasmodium*, the agent of malaria, microtubules are required for invasion of host cells. In both cases, microtubules are the basis of the flagellar axoneme, present in all life cycle stages in *T. brucei* and only in the male gametes in *Plasmodium*. Motor proteins, such as kinesins and dyneins, travel along microtubules, transporting various cargoes. Here we present data on the function and localisation of kinesins present either in *Trypanosoma* or in *Plasmodium*.
We show that two proteins belonging to the kinetoplastid specific family 1 (Wickstead et al., 2010) have different localisations and functions. TbKIN1, located at the posterior end of microtubules, is essential for cell survival, whereas TbKIN4, which shows a basal body and flagellar localisation, is not essential for cell survival.
In *Plasmodium*, we focus on KIN8, a member of the kinesin-8 family, a family that is present in many eukaryotes, but intriguingly absent in trypanosomes. We show that KIN8- mutants still assemble flagellar axonemes, however the male gametes cannot exflagellate. We propose that KIN8 could be a motor responsible for exflagellation.

P100 Novel insights in the expression of the Mucin-Associated Surface Proteins (MASP) multigene family of *Trypanosoma cruzi*

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MASPs are the second largest gene family in the *T. cruzi* genome. Due to the big number of genes that is composed, this multigene family must be essential in the complex life cycle of *T. cruzi*. In the present work we found that IgG antibodies specific to the catalytic region of MASP52 protein located at the surface and cytosol significantly reduce the parasite´s capacity to infect the host cells. Using the conserved signal peptide of this family, we found considerable changes in expression during the parasite's intracellular cycle, particularly when *T. cruzi* left the parasitophorous vacuole 24h after infection. Also, we clone 15 transcripts from cDNA of metacyclic trypomastigote forms of three different strains of *T.cruzi.* As a result of a comparative analysis of these sequences, we obtainded 4 groups of paralogy, and three expressed (pseudo)genes as "strain specific". These data support the importance of this family during the invasion of the host cells and the contention that there are strain-specific MASP (pseudo)genes with divergent evolution and other (pseudo)genes groups subjected to a strong positive selection and concerted evolution in their sequences, over the genetic background that represents the MASP family, giving rise to groups of paralogy among them, according to the "birth-and-death" model of evolution.

P101* The flagellum – a novel target for the control of pathogenic protists

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The trypanosome flagellum, with important roles in motility, cell division, cell morphogenesis and host immune evasion could be the target for novel chemotherapeutic applications. The canonical 9+2 axoneme, comprised of an array of conserved and species specific proteins, is the defining structure in most motile flagella of diverse eukaryotic cells and organisms. Analysis of the *T. brucei* flagellar proteome (TbFP) uncovered a 64.8 kDa leucine rich repeat containing protein (TbLRRC48), which is a conserved component of the nexin-dynein regulatory complex (N-DRC); a highly connected regulatory hub essential for flagellar motility. To investigate this important regulatory hub we undertook phenotypic analysis of *T. brucei* RNAi mutants in which TbLRRC48 expression was ablated. These cells displayed profound motility defects. Intriguingly, when expressed in trypanosome cells the human TbLRRC48 orthologue is flagellar targeted, retained within detergent-extract axonemes and at least partially compensates for loss of TbLRRC48 by RNAi. In ongoing work, we are using fluorescently tagged proteins to look for possible dependencies between N-DRC assembly and the assembly/function of other axonemal sub-structures.

P102* Initiation of nuclear DNA replication in *Trypanosoma brucei*: a single factor or a complex?

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Replication of genomic DNA is an indispensable process for life propagation. In *Trypanosoma brucei*, initial studies suggested that initiation of DNA replication may be carried by a single initiator factor, TbORC1/CDC6, and thus may more closely resemble the machinery found in archaea (which posses a single initiator factor, Orc1/Cdc6) than that found in most eukaryotes, where a six-subunit Origin Recognition Complex (ORC) is used. Recently, two studies identified four novel proteins that interact with TbORC1/CDC6. However, these show considerable sequence divergence from other eukaryotes' ORC subunits: while one appears to be a distant ORC4 subunit orthologue, no orthology with any ORC subunits could be found for the others, who may be kinetoplastid-specific. To ask whether these factors function together in a stable complex, or are involved in other functions, we have used immunoprecipitation, immunofluorescence microscopy, subcellular fractionation and RNAi assays to access their function, subcellular localisation and co-localisation. Initial findings suggest that at least some of the factors display dynamic subcellular localisation during the cell cycle, suggesting regulatory functions, whereas others appear exclusively nuclear. We aim to elucidate how DNA replication initiates in *T. brucei* and the topology of interactions amongst these putative ORC factors, thus determining the potentially diverged mechanics of this critical early step in genome propagation.

P103* ***Trypanosoma brucei*** **Polo-like kinase: function and regulation**

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Polo-like kinases play multiple roles across the cell cycle, particularly during mitosis, and are highly conserved among model eukaryotes. This is in contrast to *T. brucei* PLK (TbPLK) where studies characterising its function have found no evidence for a role in mitotic regulation. Instead, TbPLK is essential for golgi biogenesis, basal body duplication and kinetoplast DNA segregation in procyclic form (PCF) parasites and for furrow ingression during cytokinesis in the bloodstream form. Consistent with its lack of mitotic function, TbPLK was not found to localise in the nucleus but at basal bodies, Golgi bilobe and the flagellum attachment zone. Although TbPLK displays low sequence homology to its metazoan/yeast counterparts, it does possess a well conserved N-terminal Ser/Thr kinase domain, complete with a regulatory T-loop, and a C-terminal polo-box domain (PBD) known to mediate PLK activity, localisation and interaction with substrates in other organisms. However, exactly how the activity of TbPLK is regulated is not known. Previously, we have shown that ectopic overexpression of TbPLK results in cell cycle defects, while overexpression of a kinase dead variant does not. Using this overexpression assay, we are testing the activity of various TbPLK mutants to ascertain the importance of T198 within the T-loop and the PBD for TbPLK activity and localisation. Progress with this project will be reported.

P104* Characterisation of ATG24, a protein involved in the controlled degradation of glycosomes in *Trypanosoma brucei*

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Unlike in other organisms, the major part of glycolysis in *Trypanosoma brucei* occurs in peroxisome-like organelles called glycosomes. This compartmentalisation is essential for the bloodstream form of the parasite where 90% of the glycosome content consists of glycolytic enzymes, while in procyclic forms, found in the tsetse fly, this number drops to 40-50%. We postulated that glycosome turnover is inducible and might be important for the parasite's differentiation. Our previous studies have shown increased colocalisation of glycosomal aldolase with the lysosome during differentiation from long-slender to the intermediary bloodstream short-stumpy form, and an even more increased colocalisation during (*in vitro*) differentiation of short-stumpy to procyclic trypanosomes.

Approximately 20 putative homologues of the 40 yeast AuTophaGy-related genes were detected in trypanosomatids. Five of these are, in yeast, pexophagy-specific, including *ATG*24. Typanosome cell lines induced for RNAi to decrease ATG24 levels show that it is not essential for bloodstream nor procyclic forms. Analogous to the situation in yeast, the protein is enriched in endosomal membranes and has no predictable transmembrane region. It associates to PI3P modified membranes via its PX domain as shown by the observation that incubating cells with the PI-kinase inhibitor wortmannin affected the subcellular localisation of ATG24. Interestingly, *in vitro* differentiation of trypanosomes in which ATG24 is knocked down suggests that ATG24 might be a negative controller of autophagy.

P105 Pharmacological validation of the *Leishmania* casein kinase 1 family as therapeutic target and identification of potential inhibitors of CK1a with anti-leishmanial activity.

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Leishmania Casein Kinase 1 (CK1) family members represent promising therapeutic targets. Although *Leishmania* CK1a (LmjF35.1010) has been proposed as a drug target based on studies using promastigotes, chemical validation of this kinase family in amastigotes is lacking. Here we show that the CK1-specific inhibitor D4476 blocks promastigote and axenic amastigote growth in vitro and decreases the number of intracellular amastigotes. In vitro kinase assay using parasite extracts and a canonical CK1 substrate, revealed robust CK1 activity in *L. donovani* promastigotes and axenic amastigotes, which was inhibited by D4476. Utilizing purified recombinant protein, we showed that CK1a is susceptible to D4476 and identified potential inhibitors with IC50 between 0.07 and 9.5 µM after screening of 5,000 compounds. The selected inhibitory scaffold was potent also against extra- and intracellular parasites. Our data reveal important roles of CK1 kinases in intracellular survival and validate CK1a as therapeutic target with good druggability.

P106 Aquaglyceroporin 2 controls susceptibility to melarsoprol and pentamidine in African trypanosomes

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Drug treatment failure is common in Humans African Trypanosomiasis and melarsoprol-resistant trypanosomes often display cross-resistance to pentamidine. Although melarsoprol/pentamidine cross-resistance (MPXR) has been an area of intense interest for several decades, our understanding of the underlying mechanisms remains incomplete. We recently used RNA interference target sequencing (RIT-seq) for genome-scale loss-of-function screening, which specifically linked a locus encoding two closely related aquaglyceroporins, AQP2 and AQP3, to MPXR. RIT-seq profiling also confirmed the role of the *AT1*/P2 transporter in drug uptake and also linked a P-type ATPase (HA1-3) to pentamidine susceptibility. *T. brucei* cells lacking both *AQP* genes were MPXR. Relative to AQP3 and other AQPs, AQP2 has a highly unconventional 'selectivity filter' and we have now found that *AQP2*-specific gene knockout generates MPXR trypanosomes. *AQP2* also reversed MPXR in cells lacking native *AQP2* and *AQP3*. Remarkably, AQP2 was specifically restricted to the flagellar pocket at the bloodstream life-cycle stage, while AQP3 accesses the surface plasma membrane. Thus, AQP2 renders cells sensitive to both melarsoprol and pentamidine and loss of AQP2 function could explain cases of innate and acquired MPXR. A model will be presented to explain how the AQP2, P2 and HA1-3 transporters and the MRPA efflux pump collectively contribute to drug accumulation in *T. brucei*.

P107 Mammalian bloodstream form *Trypanosoma brucei* is totally dependent upon its only choline kinase for survival.

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The major phospholipid species phosphatidylcholine (PC) and sphingomyelin (SM) are essential structural and functional membrane components of the protozoan parasite *Trypanosoma brucei*, the causative agent of human African trypanosomiasis. Unlike man, *T. brucei* lack the ability to synthesise PC via methylation of phosphatidylethanolamine, and thus generate PC exclusively *de novo* via the Kennedy pathway from choline. The limited capability to synthesise PC suggests *T. brucei* may be vulnerable to the inhibition of key enzymes in PC metabolism. We have genetically validated the sole choline kinase as an essential drug target in the bloodstream form, both in culture and in a mouse model. In addition using choline analogues we are able to assess by mass spectrometry, the corresponding metabolic fluxes through the equivalent choline and ethanolamine branches of the Kennedy pathway. Current screening efforts against the recombinant choline kinase are highlighting notable hits, allowing chemical validation and optimisation of potency and selectivity structure-activity relationship studies.

P108* N^5,N^{10}-methylenetetrahydrofolate dehydrogenase/cyclohydrolase and assessment of a potential drug target in Trypanosomatids

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The kinetoplastid protozoan organisms, *Leishmania* and *Trypanosoma sp.* are the causal agents for a range of serious parasitic infections and although there are drugs available for the treatment of these diseases they are toxic, costly and with low efficacy. Increasing levels of drug resistant parasites further complicate this biomedical problem. Folate and derivatives are essential cofactors in the biosynthesis of thymidine, purines, glycine, methionine, initiator fMet-tRNA and also in the metabolism of histidine and serine. Trypanosomatid protozoans are auxotrophic for folates and pterins and are reliant on uptake and salvage mechanisms to maintain the required level of reduced folates and pterins. Three enzyme activities in the protozoan *Leishmania major,* namely N^5,N^{10}-methylenetetrahydrofolate dehydrogenase / N^5,N^{10}-methenyltetrahydrofolate cyclohydrolase (DHCH) and N^{10}-formyltetrahydrofolate ligase (FTL) produce the essential intermediate N^{10}-formyltetrahydrofolate. Although trypanosomatids possess at least one functional DHCH, the same is not true for FTL, which is absent in *Trypanosoma brucei.* Recent work suggests the DHCH activity is essential in *L.major* with knockouts of the disomic copies not possible without the ectopic expression of FTL, thus allowing the alternative route for the synthesis of N^{10}-formyl tetrahydrofolate. We have determined the structures of N^5,N^{10}-methylenetetrahydrofolate dehydrogenase/cyclohydrolase (DHCH) from *L.major* and *T.brucei* to assess and exploit the three-dimensional properties of the active site and to explore the potential for early stage inhibitor development.

P109* Uptake and mechanism of resistance to isometamidium in *Trypanosoma brucei brucei*.

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Resistance to isometamidium (ISM), one of the two main veterinary trypanocides and the only drug currently used for prophylactic protection against the disease in livestock, is a major economic problem in sub-Saharan Africa. We have adapted the standard lab strain *T. brucei* s427 to high levels of isometamidium resistance (ISMRes) by in vitro exposure in order to create an amenable model to study this phenomenon. The resulting strains were slightly cross-resistant to diamidines but not to ethidium. Isometamidium uptake was reduced in resistant strains but not dramatically. In both WT and ISMRes trypanosomes isometamidium was rapidly extruded from cells placed in fresh medium. The LAPT diamidine transporter appears to contribute to isometamidium uptake and showed a reduced V_{max} in the resistant clones. However, currently unidentified plasma membrane transporters contribute to isometamidium uptake. The mitochondrial membrane potential was much reduced in the ISMRes clones compared to the wild type parasites, which were five-fold cross resistance to oligomycin, as well as sensitised to FCCP. The ISMRes cells appear to have lost their kinetoplasts. The findings suggest that the reduced ISM uptake in the resistant clones could have resulted from a reduction in the mitochondrial membrane potential. We are currently investigating the possibility that subunits of the mitochondrial ATPase complex may have been altered in the resistant clones.

P110 Metabolomic and lipidomic profiles of miltefosine-resistant *Leishmania donovani*

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Miltefosine (MIL) is a drug of choice against antimonial-resistant *L. donovani*, thus knowledge on the potential of parasites to develop resistance to MIL and the mechanisms involved is required. This study aims to elucidate both and investigate the impact of inherent differences in antimonial susceptibility to MIL resistance. Three Nepalese and one Indian *L. donovani* isolates with different antimonial susceptibilities were exposed to increasing MIL concentrations in a step-wise manner until all lines grew at similar rates as wild-type parasites in 74 µM MIL. The metabolome and lipidome of clones of each resistant line and its corresponding wild-type were determined by liquid chromatography / mass spectrometry analysis and using mzMatch.R and IDEOM software. Consistent changes correlating with MIL-resistance were identified, although the various lines also displayed specific modifications. Notable changes were discovered amongst lipid compounds, in particular phosphatidylcholines. This study has provided data that will facilitate identification of mechanisms involved in MIL-resistance as well as key biomarkers of resistance with potential field applications.

P111 Ehancing the conformational ensemble of *Trypanosoma brucei* RNA Editing Ligase 1 to improve drug discovery efforts. Jesper Sørensen & Rommie E.

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RNA editing is a unique process, which is essential for the survival of both insect and bloodstream forms of *Trypanosoma brucei*, the causative agent for devastating tropical disease African sleeping sickness. The specific target, RNA editing ligase 1 (TbREL1) is exclusive to trypanosomes, and no close human homolog is known to exist. In addition, the high-resolution crystal structures reveal several unique features of the active site, making this enzyme a promising target for structure-based drug design. In previous work we have found several promising drug-like inhibitors, with micro-molar activity against TbREL1. These compounds were found using a combination molecular docking and classical molecular dynamics (MD) simulations to account for receptor flexibility. Incorporation of conformations from the classical MD simulations alongside the experimental structures helped refine the docked compounds. Notably, a significant structural rearrangement of the enzyme's active site occurs during the simulations without ATP bound, opening an additional cavity adjacent to the ATP binding site that was exploited in the development of the drug-like inhibitors. In this work we have employed a novel method, accelerated MD simulations, to further enhance the conformational ensemble, in an effort to find more promising leads, with the ability to bind to a more diverse ensemble of receptor conformations.

P112* From medieval magic to modern medicine: using natural compounds isolated from frog skin to treat leishmaniasis

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Witch doctors thousands of years ago are known to have used 'eye of newt and essence of frog' in their potions. What is remarkable is that the compounds extracted from the skin secretions of frogs have been proven to be effective treatments for numerous diseases still prevalent today. In particular, some of these compounds have the potential to save millions of people from the neglected tropical disease leishmaniasis. Antimicrobial peptides (AMPs) form part of the immune response in all living systems, and a family of AMPs (the Temporins) extracted from the skin of the European red frog *Rana temporaria* have been found to possess novel antileishmanial activity. The use of peptide-based therapeutic agents is highly advantageous as AMPs are generally non-toxic towards mammalian cells; treatments would be significantly cheaper than the current 'gold standard', Amphotericin B; and they could be administered as a topical application for the cutaneous form of leishmaniasis. AMPs are exciting prospects in this area as they function via a different mode of action to existing drugs. Within the Chemistry Department at Durham University we investigating the potential that these peptides possess, and have carried out the largest study to date into the activity of the Temporin peptides against the amastigote (human) form of *Leishmania mexicana*.

P113* Is arsenic exposure contributing to failure of antimonial treatment of visceral leishmaniasis in India?

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Due to high levels of resistance, use of antimonial drugs for the treatment of visceral leishmaniasis (VL) is no longer recommended in the hyperendemic state of Bihar, India. Since antimony and arsenic are metalloids sharing many chemical properties, we proposed that chronic exposure of the population to arsenic-contaminated well-water contributed to the dramatic decrease in efficacy of antimonials in this region [1]. experiments and field work are being undertaken to test this hypothesis. Mice were exposed to arsenic in drinking-water prior to infection with *Leishmania donovani* amastigotes. Infected mice continued to receive arsenic for 28 days, before amastigotes were isolated and used to infect further groups of arsenic-exposed mice. Sensitivity to Sb^{V} was determined in macrophages in vitro. Following three passages in mice, parasites exposed to the highest concentration of arsenic showed significant resistance to Sb^{V}. These preliminary findings Results from support our hypothesis that cross resistance to Sb^{V} can be generated by exposure to As^{III} in vivo retrospective case control study in the field is underway to compare the level of arsenic exposure in VL patients with their response to treatment with pentavalent antimonials.

*[1] Perry MR, Wyllie S, Prajapati VK, et al. PLoS Negl Trop Dis 2011;5:e1227.

P114 A new ABC transporter in *Leishmania* is involved in sensitivity to antimonials

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ABC (ATP-binding cassette) transporters superfamily in *Leishmania* included 42 genes distributed in 8 subfamilies (A-H), some with relevant biological activities. There is a group of 4 transporters (ABCU, ABCX, ABCY and ABCZ), specific from trypanosomatids with unknown biological function. ABCX is a *half-transporter*, which is expressed in the promastigote and amastigote forms of *Leishmania*. We have determined that overexpression of ABCX transporter in *Leishmania major* promastigote/amastigote forms confers a significant resistance to Pentostam, Sb(III), and to the metal ions As(III) and Cd(II), due to a reduction in their cellular accumulation as a consequence of an increased drug efflux. Also, we have proved that the resistance of parasites to antimony and other metal ions could be partially reversed by the glutathione biosynthesis-specific inhibitor, buthionine sulfoximine. Additionally, we have observed that after treatment with Sb(III), parasites overexpressing ABCX maintain higher values of the mitochondrial electrochemical potential and total ATP levels versus controls. Subcellular localization studies under a confocal microscopy and a surface biotinylation assay using parasites overexpressing ABCX-GFP, suggest that the transporter is localized mainly in the mitochondria and also at the plasma membrane. Altogether, we have shown that this new ABC transporter is involved in drug sensitivity to antimonials and its clinical relevance remain to be determined. *Supported by Ministerio de Ciencia e Innovación (Spain), Proyecto SAF2009-07440 (F.G).*

P115 Monitoring *in vivo* inhibition of *N*-myristoyltransferase by small molecule inhibitors in *Leishmania donovani*

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Myristoyl-CoA: protein *N*-myristoyltransferase (NMT) catalyses the covalent attachment of C14-myristate to the N-terminal glycine residue of a subset of eukaryotic proteins that function in multiple cellular processes. These include vesicular protein trafficking, signal transduction and protein-protein interactions important for transient or permanent localisation of proteins within cells. NMT activity is essential for the viability of all eukaryotic cell types tested to date, including bloodstream stages of *Trypanosoma brucei* and insect stages of *Leishmania* species. A recent study has identified high-affinity NMT-specific inhibitors that inhibit protein *N*-myristoylation in trypanosomes, leading to rapid parasite killing, both *in vitro* and *in vivo,* with the ability to cure acute trypanosomiasis in mice (Frearson *et al*., 2010, Nature 464, 728).
Here, we describe an *in vivo* labelling technique, based on click chemistry, to monitor *N*-myristoylation and the inhibition of NMT activity by small molecule inhibitors, to verify their on-target specificity. Further, this technique allows us to isolate *N*-myristoylated proteins in intracellular amastigotes, which can then be identified by mass spectrometry. This method will generate the most comprehensive analysis of *N*-myristoylated proteins in *Leishmania* species to date, allowing us to better understand and evaluate the downstream effects of small molecule NMT inhibitors on the regulation of signalling processes and vesicular transport in these cells.

P116 RNA editing as a drug target in trypanosomes: development of a high throughput screening assay for RNA editing ligase 1

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RNA editing is essential for mitochondrial gene expression in all trypanosomatids but absent from the host and potentially therefore a powerful drug target. The process is catalyzed by multiprotein complexes, the editosomes, and involves several enzymatic steps. Using a virtual drug screening strategy followed by biochemical validation we had previously identified compounds that inhibit *Tb*REL1 with single-digit micromolar IC_{50} values (Amaro, R., Schnaufer A. et al., 2008). A further eight compounds with low-micromolar IC_{50} values were identified by substructure screening (Durrant, Hall et al. 2010, PLoS, Neglected Tropical Diseases 4(8): e803).
Our current efforts are focused on expediting compound library screens by developing a high throughput fluorescence-based (FRET) REL1 assay. We have systematically optimized the assay with respect to reaction chemistry; recombinant enzyme production; fluorophores and enzyme kinetics. This has significantly improved reaction rate, sensitivity and dynamic range. We will implement large scale compound library screens utilizing this improved FRET assay in association with the Drug Discovery Unity at the University of Dundee.

P117 Effects of artemisinin on *Leishmania major,* cellular apoptosis and INF-γ and IL-4 production

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Leishmaniasis is one of the significant causes of morbidity and mortality in several countries worldwide and is among important problems in endemic areas such as Iran. Due to problems made by the therapeutic already used, the aims of the present study were to evaluate the effect of Artemisinin in various doses to treat the disease The drug was evaluated in vitro firstly; followed by the effective IC50 dose on the standard suspension promastigotes produced the *leishmania major* (MRHO/IR/75/ER) equal to 25 mg/ml. Then the BALB/c race mice were infected by (0.1 ml/ with 2×10^6) lives promastigote *L. major* as subcutaneous injection in tail base. When the wound appeared, the mice were divided in five group, including two under-treatment groups were received ointment and intraperitoneal injection and three control groups (healthy, infected without treatment and Vaseline control groups). Their treatment began with specific dose (25mg for each gram weight) for 6 weeks locally and twice a day (each 12 hours) and in injection form each day. The wounds' diameter and the mice's weight, the mice death rate and the Cytokines' amount were measured. The results proved that ointment compounds healed the wounds more effectively and this group was considerably different from the control groups; regarding the death rate. The findings of the Donken research shows that IFN-γ in ointment treated group are higher than those of other groups, the IL-4 is lower than other groups($p<0.05$). Artemisinin is an appropriate and effective drug, Thus we propose that it can be applied to treatment of cutaneous leishmaniasis.

P118 ANTILEISHMANIAL ACTIVITY OF SUB-2, A SEMI-SYNTHETIC MOLECULE

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Leishmaniasis, zoonotic protozoan diseases caused by Leishmania spp., are still considered a major health problem. The number of effective drugs available against the disease is extremely limited. Bioassay-guided approach has been used in our laboratory in the search for bioactive agents of plant origin. We have already tested 346 compounds obtained from different plant families against *L. amazonensis* promastigotes. Here we report the evaluation *sub-2* citotoxicity against macrophages J774 culture and its leishmanicidal activity. To investigate the *sub-2* effects on *L. amazonensis* promastigotes and amastigote forms, the MTT colorimetric assay was realized. Amastigotes were obtained from adherent macrophages infected with metacyclic promastigotes forms at a macrophage to parasite ratio of 1:10. The infected cultures were incubated for 24hs in RPMI medium. *Sub-2* was added at different concentrations (3.0 to 0.19μg/mL) after 24hs of infection and each concentration was screened in triplicate. The coverslips were Giemsa stained and microscopically analyzed. *Sub-2* showed potent activity against promastigote forms (IC_{90}=1.8μg/mL) when compared to pentamidine (IC_{90}=6.5μg/mL). *Sub-2* does not show to be cytotoxic to J774 macrophages, presenting selectivity index of 80. S*ub-2* activity against amastigotes showed to be specific against parasites (Phagocyte index=21.47%). Sub-2 might be interesting as lead in the search for new antileishmanial drugs.

P119 *Leishmania donovani* is able to induce resistance to drug combinations

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Chemotherapy is the only current treatment option for leishmaniasis. Recently, the WHO has recommended combination therapy as a strategy to be implemented in clinical trials. However, for the future long life of this therapeutic strategy it is important to determine how easy it is to induce resistance experimentally to drug combinations. Thus, we have generated *Leishmania donovani* lines resistant to drug combinations through a step-wise adaptation process to increasing drug concentration. The drug combinations generated were: amphotericin B (AmB) + miltefosine (MLF), AmB + paromomycin (PMM), AmB + antimony (Sb), MLF + PMM, and Sb + PMM. Our results show that the response of the parasite is variable depending of the drug combination used. *L. donovani* induces an easier resistance to MLF + PMM than AmB + PMM combination, being the latter a combination that could be recommended for future use in clinical trials; these results were validated in intracellular amastigotes. Similarly, *L. donovani* lines resistant to a specific drug combination have significant cross-resistance profile to other unrelated drugs, but the resistance was unstable after six months without drug pressure. Additionally, the resistant lines showed significant differences versus wild-type lines in drug uptake, plasma membrane composition, levels of thiols, ATP, and mitochondrial membrane potential, among other drug resistance markers. *Supported by Plan Andaluz de Investigación, Proyecto de Excelencia (CTS-7282), Junta de Andalucia (Spain).*

P120* The impact of induced paromomycin resistance on the fitness and metabolomic profile of *Leishmania donovani*

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The increase in incidence of antimonial resistance in *Leishmania donovani* is shifting the focus for treatment of visceral leishmaniasis towards other anti-leishmanial compounds. Paromomycin (PMM) has recently been approved for the treatment of visceral leishmaniasis in the Indian sub-continent. We investigated the fitness and metabolic profile of experimentally generated paromomycin resistant (PMM-R) parasites. Resistance to PMM was induced in *L. donovani* promastigotes by step-wise exposure to drug pressure over a number of weeks. Paromomycin resistant clones were selected and the ability of promastigotes and intracellular amastigotes to resist macrophage killing and oxidative stress was assessed *in vitro* and compared to wild-type (WT) parasites. In addition, the metabolomic profile of both wild type and PMM-R clones was determined. Resistance to PMM appears stable, even in the absence of drug pressure, in cultured promastigotes. Promastigotes of PMM-R clones were more resistant to the effects of nitric oxide and superoxides whereas intracellular amastigotes were less resistant to nitric oxide killing. Metabolomic profiling revealed that a number of metabolites were either up or down-regulated in PM-R parasites compared to WT controls.

P121* Assessment of NMT as a Drug Target in *Trypanosoma cruzi*

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Trypanosoma cruzi the causative agent of Chagas disease continues to be a major health problem in Latin America, with an estimated 10 million infected individuals. The current drugs (Benznidazole and Nifurtimox) are often associated with adverse side effects and poor efficacy against the chronic stage of the disease. With only a single treatment currently in clinical trials, there is a need to validate new drug targets and to develop new treatments to target the disease. One prospective target is the enzyme *N*-myristoyltransferase (NMT) responsible for catalysing the addition of myristic acid onto the *N*-terminal glycine of specific proteins, a ubiquitous process in eukaryotes. Using the NMT inhibitor DDD85646 we present evidence that NMT is essential for growth and that it can be specifically inhibited within the parasite.

P122* Comparative genomics of drug resistance in *Trypanosoma brucei rhodesiense*

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We are investigating the molecular mechanisms of drug resistance in African trypanosomes, the causative agents of sleeping sickness. Two bloodstream-form *T. b. rhodesiense* lines have been selected *in vitro* for resistance against the clinical drugs melarsoprol and pentamidine, respectively. Both lines exhibit cross-resistance to either drug as well as to other diamidines and adenosine analogues. We applied next generation sequencing to find the mutations causing drug resistance. Comparative transcriptomics and genomics revealed the complete absence of the gene *TbAT1* in the melarsoprol-selected line and a non-synonymous point mutation in *TbAT1* in the pentamidine-selected line. *TbAT1* encodes the aminopurine transporter P2 which has long been known to be involved in drug uptake. However, the selected lines exhibit higher resistance factors than explainable by loss of *TbAT1* alone, so additional mechanisms must contribute the multidrug-resistance phenotype. Both resistant lines have a deletion at the locus of the aquaglyceroporin gene *TbAQP*2, which is orthologous to a known resistance gene from *Leishmania*. Thus *TbAQP2* is likely to play a major role in melarsoprol/pentamidine cross-resistance also in trypanosomes.

P123* Magnesium can support the *in vivo* activity of phosphoglycerate mutase from trypanosomatid parasites, but at a low level

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The cofactor-independent phosphoglycerate mutase (iPGAM) from kinetoplastid protozoa is an attractive drug target because it shares no common properties with the corresponding enzyme in humans. Purified iPGAMs from *Trypanosoma brucei* and *Leishmania mexicana* display maximum activity in the presence of cobalt, but recent measurements of metal concentrations in *T. brucei* cytosolic fractions by ICP-OES showed that the concentration of Co^{2+} is too low to support the activity of iPGAM. These measurements instead indicated that potentially only Mg^{2+} and Zn^{2+} were present in sufficient concentrations. We have recently developed a multimode plate reader discontinuous assay whereby it is possible to measure the activity of iPGAM in the presence of divalent metal ions without interference by the metals with the activities of the three coupling enzymes enolase, pyruvate kinase and lactate dehydrogenase. Our results confirm that Co^{2+} does indeed support a high level of iPGAM activity. Of the biologically relevant metals, only Mg^{2+}, can support iPGAM activity, but at about 10% of the level of Co^{2+}. By contrast Zn^{2+} was strongly inhibitory. The mechanism and behaviour of iPGAM in its *in vivo* surroundings is yet to be explored in detail, but it now seems possible that it has one of the lower *in vivo* specific activities of all glycolytic enzymes.

P124* Potently trypanocidal curcumin analogues bearing a mono-enone linker motif act by depleting *T. b. brucei* of trypanothione

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Some curcumin analogues have been identified as having potent antiparasitic activity and low toxicity. In particular analogues with a C_7 linker bearing a C_4-C_5 olefinic linker with a single keto group at C3 (enone linker) displayed mid-nanomolar activity against the bloodstream form of *Trypanosoma brucei*. The effect of curcumin and the enone lead compound, ASHK014, on cellular parameters were investigated to elucidate the mechanism of action. We found no effects on signal transduction (intracellular calcium, cAMP, membrane potential), mitochondrial membrane potential, cell cycle progression or DNA integrity - arguing against apoptosis. An AS-HK014-resitnat line was developed by in vitro exposure to the drug. A metabolomic analysis was performed on WT and resistance line after exposure to AS-HK014, which revealed that rapid depletion of glutathione and trypanothione in the WT but not in the resistant line. Indeed, trypanothione was barely detectable in the drug-exposed WT cells, though almost all other metabolites were unchanged relative to control. In resistant cells drug exposure instead increased glutathione levels. An adduct of AS-HK014 and trypanothione was identified. Protein levels of glutathione synthetase and γ-glutamylcysteine synthetase showed no change in resistant trypanosomes and no mutations were found in the GS and γGCS open reading frames.

P125* Evaluation of pyrimidine biosynthesis by African trypanosomes as a drug target

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The quest for drug discovery in trypanosomiasis involves targeting biosynthetic pathways essential to the parasite. The essentiality of the pyrimidine biosynthesis pathway was studied by knocking out orotidylate decarboxylase (ODC) and characterising the resulting phenotype. The resulting *ODC*$^{-/-}$ strain was unable to grow in pyrimidine-free media and in pyrimidine-free media supplemented only with thymidine but normal growth in uracil-supplemented media. *ODC*$^{-/-}$ showed very slow initial growth in uridine-supplemented media, but increased growth rate after several days, suggesting an upregulation of either uridine transport or uridine phosphorylase under pyrimidine-limiting conditions. These observations were consistent with our findings that uracil was efficiently salvaged by bloodstream trypanosomes, whereas uridine uptake was barely detectable in wild-type trypanosomes and low affinity. However, uridine can be used as a pyrimidine source, by intracellular conversion to uracil, which is phosphoribosylated to UMP. Uracil uptake appeared somewhat increased in ODC$^{-/-}$ in a uracil time course assay but no evidence was found for the expression of a different uracil transport entity. Indeed, the uracil K_m and V_{max} values were not significantly changed in the pyrimidine auxotrophic line. Interestingly, *ODC*$^{-/-}$ became significantly sensitised to 5-Fluorouracil, 5-Fluoro-2'deoxyUridine and 5-Fluoro-2'deoxyCytidine, as well as fully resistant to 5-Fluoroorotic acid. We are currently investigating the infectivity of *ODC*$^{-/-}$ trypanosomes in rodents to determine whether pyrimidine biosynthesis might be a viable drug target.

P126* Episomal plasmid stability in *Leishmania chagasi*

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Objective: Evaluate the fluorescence stability provided by the pX63-Gfp plasmid in the absence or presence of drug pressure.
Methods: *Leishmania chagasi* were transfected with pX63-Gfp plasmid using geneticin for drug selection. We selected clones with highest and lowest Fluorescence Intensity (FI). Fourteen successive passages were made of each clone with the different conditions of drug pressure.
The clone with highest FI was inoculated in Hamster. After 54 days the animal was euthanized and parasites recovered from liver had their FI analysed by FACS.
Results: After 14 passages in the absence of drug the Leishmania had only 3% of its initial FI. After 14 passages with 30ug/ml of G418 the FI was equal to 46% of its original. With a gradual increase of drug pressure, we observed an increase in the FI, obtaining the maximum concentration value of 500µg/ml of G418, which corresponded to 138% of initial FI. In the case of the passage thought hamster, the leishmania had only 5% of its initial FI.
The compared clones with lower and higher initial FI showed maintenance of the fluorescence in a similar pattern. These results suggests that the plasmid pX63-Gfp can be routinely used to express fluorescent proteins in *L. chagasi,* since the acquisition time without drug pressure doesn't exceed a few months.

P127* Inhibitors of kinetoplastid sphingolipid synthases

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The protozoan kinetoplastid parasites *Leishmania* spp, *Trypanosoma brucei* and *Trypanosoma cruzi* are responsible for potentially fatal diseases that affect over 22 million people worldwide, with an estimated 450 million at risk. Current therapies are expensive and not widely accessible. In addition, drug toxicity and emerging resistance are major concerns. Previous work has identified the essential kinetoplastid sphingolipid synthase (SLS) as an attractive pharmaceutical target due to the divergence of function compared with the mammalian orthologue. A high-throughput compatible screening assay was developed to test a library of potential inhibitors against the *Leishmania major* enzyme. Current work is ongoing towards the synthesis of analogues of identified hits. This will allow the elucidation of structure-activity relationships and facilitate future synthesis directed towards highly active, target-specific compounds. In parallel, this work is being extended to include the *Trypanosoma brucei* and *Trypanosoma cruzi* orthologues. Of particular interest from the preliminary results is the observation that different kinetoplastid SLSs show varying sensitivity to a known fungal SLS inhibitor. This multidisciplinary approach, targeting a conserved enzyme of the kinetoplastid pathogens, will allow significant advancement towards a solution for an increasing global threat.

P128 CNS Invasion by African trypanosomes

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Trypanosoma brucei are kinetoplastid parasites responsible for human African trypanosomiasis (HAT). Parasites invade the CNS leading to coma and death if untreated. An estimated 60 million people are at risk of African trypanosome infection across sub-Saharan Africa. There is an urgent need to identify late stage HAT diagnostic markers and new drug targets that could lead to less toxic late stage treatments. Understanding how trypanosomes and drugs cross the blood-brain barrier (BBB) is crucial for therapeutic development.

Advances in molecular and imaging technology now allow us to address key questions such as the route of *T. brucei* CNS invasion and the sequential movement of parasites within the brain as disease progresses. In this study I demonstrate a sensitive method for detection of parasites in the brain using transgenic fluorescent organisms. I am also using a human brain microvascular endothelial cell (HBMEC) based BBB model to study the mechanism of *T. brucei* CNS invasion and testing its capacity for rapid drug screening. This work addresses the main obstacle to generating effective late stage treatments for clinical trial.

P129 Disctinct gene expression profile by live *Leishmania amazonensis* amastigotes-hosting C57BL/6 and DBA/2 dendritic leucocytes.

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The inoculation of a 10^4 *Leishmania/L. amazonensis* metacyclic promastigotes into the dermis of ear pinna of C57BL/6 and DBA/2 mice results in distinct outcome as assessed by i) parasite load values and ii) ear pinna macroscopic features monitored from days 4 to 22-phase 1- and from days 22 to 80/100-phase 2-. While in C57Bl/6 mice, the onset of the amastigote population size increase is slow and progressive, in DBA/2 mice, the onset of the amastigote population size increase is rapid, as is its sustained control. The aim of this study was to provide insights about immune processes which could account for the distinct outcome during the phase 1, namely, *when* phagocytic dendritic leucocytes/DLs have been subverted as live amastigotes-hosting cells. With this objective in mind, bone marrow-derived C57BL/6 and DBA/2 DLs were generated and exposed or not to live *Ds*Red2 expressing transgenic *L. amazonensis* amastigotes. The four DL populations were compared by flow cytometry and Affymetrix-based transcriptomic analysis. In contrast to live amastigotes-hosting C57BL/6 DLs, the DBA/2 ones display transcriptional signatures and markers that are consistent with immune regulatory functions and rapid amastigote establishment in both the ear pinna and ear- draining lymph node.

P130 TbScd6 is a translational repressor involved in regulating EP procyclin

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Members of the Scd6 protein family have been identified in many organisms as conserved components of translation repression complexes in mRNA-containing granules such as processing bodies (PB) and maternal mRNA storage granules. All family members contain an N-terminal Lsm domain, an FDF motif of unknown function and clusters of RGG motifs, but it is not known which domains are required for translational repression.

We have studied the function of *T. brucei* Scd6 and performed a detailed analysis of its domains. As in other organisms, Scd6 is a general repressor of translation initiation. While steady state levels of most proteins are unchanged, depletion of Scd6 by RNAi causes an increase in surface-expressed EP procyclin, suggesting that Scd6 specifically affects proteins targeted to the plasma membrane. This effect could be seen when another parasite surface protein, *T. congolense* GARP, was used as a reporter, but not for cytosolic GFP, and was independent of the EP 3' UTR. Tethering of Scd6 directly to mRNA results in reduced levels of translation without affecting transcript levels. We show that the Lsm domain is essential for translation repression, and the Lsm plus an asparagine-rich domain (Lsm-N) are sufficient to perform this function. Studies using Scd6 mutants fused to GFP showed that the Lsm-N domains are essential and sufficient for localization of Scd6 to PB.

P131* The zinc finger protein ZC3H11 stabilizes heat shock protein transcripts

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In Kinetoplastids, virtually all control of protein-coding gene expression is post-transcriptional, with mRNA degradation playing a central role. Unlike other eukaryotes, trypanosomes do not transcriptionally induce the expression of heat shock proteins upon elevated temperatures. It has previously been shown that heat shock transcripts are selectively stabilized under stress conditions, mediated by sequences in their 3'UTR, but the molecular mechanism had not been resolved.
We show here that the *Trypanosoma brucei* CCCH zinc finger protein ZC3H11 controls the levels of various stress-related mRNAs. ZC3H11 binds to mRNAs that encode homologues of the major heat shock protein families. These transcripts share a consensus recognition motif of AUU repeats in the 3'UTR. Several of these targets, including the major cytoplasmic *HSP70* mRNA, are also decreased upon ZC3H11 depletion. ZC3H11 regulation of, and binding to, *HSP70* mRNA depends on its AUU repeat region. Furthermore, tethering of ZC3H11 to a reporter mRNA increases reporter expression. ZC3H11 interacts with a trypanosome homologue of MKT1, which in turn interacts with a trypanosome PBP1. In yeast, Mkt1 also interacts with Pbp1, which in turn binds to the poly(A) binding protein Pab1. We propose that ZC3H11 stabilizes *HSP70* and other stress-related mRNAs by recruiting MKT1 and PBP1 and thereby promoting retention of poly(A) binding protein on the mRNA.

P132 Comparative proteomic and phosphoproteomics analysis of bloodstream and procyclic form *Trypanosoma brucei.*

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The protozoan parasite *Trypanosoma brucei* has a complex digenetic lifecycle between a mammalian host and an insect vector, and adaption of its proteome between lifecycle stages is essential to its survival and virulence. We have optimized a procedure for growing *Trypanosoma brucei* procyclic form cells in conditions suitable for stable isotope labeling by amino acids in culture (SILAC) and describe a comparative proteomic and phosphoproteomic analysis of cultured procyclic form and bloodstream form *T. brucei* cells. In total we were able to identify 3959 proteins and quantify SILAC ratios for 3553 proteins with a false discovery rate of 0.01. A large number of proteins (10.6 %) are differentially regulated by more the 5-fold between lifecycle stages, including those involved in the parasite surface coat, and in mitochondrial and glycosomal energy metabolism. Our proteomic data is broadly in agreement with transcriptomic studies, but with significantly larger fold changes observed at the protein level than at the mRNA level. The comparative phosphoproteomic analysis used strong cation exchange chromatography and TiO2 to enrich for phosphopeptides prior to analysis by mass spectrometry. Phosphosite data were normalised for changes in protein abundance, and showed widespread variation between lifecycle stages. Data analysis and validation are ongoing.

P133* RNA Editing Ligases in Trypanosomes: Buy Two, Get One Free?

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Uridylyl insertion/deletion mRNA editing is essential for mitochondrial gene expression in *Trypanosoma brucei* and governed by multiprotein complexes called editosomes. The final step in this post-transcriptional process is that of religating the edited mRNA fragments. The ~20S RNA editing core complex contains two RNA editing ligases. While REL1 is clearly essential for RNA editing, REL2 knockdown by RNAi has not resulted in a detectable phenotype. To explain these findings, alternative scenarios have been suggested: (a) REL2 is not functional *in vivo*; (b) REL1 can function in both insertion and deletion editing, whereas REL2 can only function in insertion editing; (c) REL1 has an additional role in repairing erroneously cleaved mRNAs.
To further investigate respective functions of the two RELs we used genetic complementation with chimeric ligase enzymes, and limited sequencing of the editing intermediates that accumulate *in vivo* after REL1 knockdown. Expression of a chimeric ligase providing a REL2 catalytic domain at REL1's position in the deletion subcomplex did not rescue the growth defect caused by REL1 ablation. This indicates that specific catalytic properties of REL1 rather than its position within the deletion subcomplex make it essential. Trapping of unligated editing intermediates by 5' linker ligation, in conjunction with limited sequencing, suggests that REL1 functions in both insertion and deletion editing as well as in the repair of mis-cleaved mRNAs.

P134* Density-dependent developmental gene regulation in transmissible stumpy forms of *Trypanosoma brucei*

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The transmission of *Trypanosoma brucei* depends upon the development in the bloodstream of transmissible "stumpy forms" from proliferative "slender forms". Although slender and stumpy forms share the same environment of the mammalian bloodstream, they show differential gene expression. Many mRNAs are downregulated in stumpy forms and translation is generally repressed. However, a small subset escape this repression and are upregulated, presumably as an adaptation for transmission. To understand the basis of this, regulatory sequences within the 3'UTR of a major stumpy-enriched transcript (an ESAG9 gene) have been characterised. This has identified a 33-nucleotide sequence that contributes strongly to gene silencing in slender forms. Analysis of gene expression when monomorphic slender cells develop to stumpy-like forms when exposed to 8-pCPT-2'-O-Me-cAMP has determined that this short sequence also mediates responsiveness to this signal. This, therefore, represents the first identification of an mRNA sequence responsive to a chemical signal believed to intersect with the stumpy-induction signalling pathway. The importance of this regulatory sequence in density-dependent developmental gene regulation in the mammalian host is currently being investigated in pleomorphic slender and stumpy forms and this data will be presented.

P135 TbFACT plays a major role in chromatin remodeling affecting histone dynamics in *Trypanosoma brucei*

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Chromatin structure plays an important role in transcriptional regulation of VSG expression sites (ESs) in *Trypanosoma brucei*. RNA Polymerase I (Pol I) transcribes one of ~15 VSG ESs in a monoallelic fashion. Depletion of the TbFACT (FAcilitates Chromatin Transcription) subunit Spt16 results in a cell cycle arrest during G2/M, which is accompanied by derepression of silent VSG ES promoters. We find that ES derepression in G2/M phase does not appear to be a simple consequence of the G2/M cell cycle arrest itself. In contrast, depletion of Cyclin6 results in a strikingly similar G2/M cell cycle arrest; however, silencing of VSG ESs is not affected. Comparison of overall histone levels also reveals a significant decrease in histone H3 and histone H2A after knockdown of the FACT subunit Spt16, but not after knockdown of Cyclin6. This reduction in general histone abundance is consistent with depletion of nucleosomes at silent VSG ES promoters and Pol II transcription units. An interesting exception to this is the active VSG ES where levels of histone H3 increase after Spt16 knockdown, which is correlated with ES transcription shutting down. These data indicate that TbFACT plays a major role in chromatin remodeling affecting histone dynamics in *T. brucei*, which in turn influences chromatin structure, transcription and the onset of mitosis.

P140 TDP1 is an architectural chromatin protein important for transcription control in *Trypanosoma brucei*

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The high mobility group B (HMGB) protein family constitutes a large and abundant class of non-histone chromatin associated DNA-binding proteins which play a role in chromatin architecture in a wide range of eukaryotes. In *T. brucei*, the HMGB protein TDP1, which contains two HMG boxes and one DEK C terminal DNA-binding domain, was first identified as binding to *VSG* expression site (ES) promoter sequences. We report that TDP1 is an essential nuclear protein enriched in the nucleolus and VSG expression site body, and facilitates transcription. Blocking TDP1 synthesis using RNAi results in approximately 40-90% reduction in transcription of RNA polymerase I (Pol I) transcribed genes. Using chromatin immunoprecipitation (ChIP), we find that TDP1 is enriched in the rDNA and on the active *VSG* ES in bloodstream form *T. brucei*. Additionally, ChIP of the core histones after RNAi mediated knock-down of TDP1 in bloodstream form *T. brucei* revealed a statistically significant increase in histone occupancy at transcriptionally active Pol I transcribed regions but no significant change in relatively silent Pol I regions or in regions transcribed by Pol II or Pol III. Lastly, we performed TAP experiments with TDP1 and found that TDP1 interacts with the core histones. These results indicate that TDP1 is an architectural chromatin protein involved in control of Pol I transcription in *T. brucei*.

P141 RNAseq applied to *Leishmania donovani* reveals key molecules potentially involved in parasite virulence

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Leishmania donovani is the infectious agent of visceral leishmaniasis (VL), causing fatal hepato-splenomegaly if left untreated. The severity of this disease is driven by virulence factors enabling the pathogen to resist to and subvert host immune functions. We previously identified important proteomics differences between *in vitro* generated avirulent axenic, and hamster-derived virulent splenic *L. donovani* amastigotes. With the aim to initiate a systems-wide analysis, we used high-throughput quantitative RNAseq technology in order to gain novel insight into the nature, the expression, and the regulation of virulence factors by investigating the expression profiles of these *L. donovani* virulent and avirulent samples. Four libraries of 50bp-long reads were generated using directional sequencing, including two biological replicates. The 30 million reads generated per library covered about 8000 genes per sample. Our analysis revealed 2345 differentially expressed genes in virulent *vs* avirulent parasites, with more than 200 transcripts showing a two-fold or higher difference in abundance. We identified deep expression differences in several transcripts, including folate/biopterin transporters, which are playing crucial roles in *Leishmania* intracellular growth. Ongoing genomic analyses investigate if these differences are due to gene deletions or copy number variations.

P142* Comparative Transcriptomics and Proteomics of *Trypanosoma brucei* and Investigation of its mRNA Methyl Cap

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Trypanosoma brucei (T. brucei), a protozoan parasite transmitted by tsetse flies, causes sleeping sickness in humans and nagana in cattle. Contrasting physiological requirements for parasite survival between procyclic (vector) and bloodstream (mammal) forms necessitate different molecular processes and therefore changes in protein expression. Transcriptional regulation is particularly interesting in *T. brucei* because arrangement of genes is polycistronic, however genes which are transcribed together are cleaved into separate mRNAs by trans-splicing and are individually regulated. While multiple stage-specific transcripts were identified, studies using RNA-seq and microarrays found marginal changes in overall transcript levels suggesting that *T. brucei* predominantly regulate gene expression by post-transcriptional mechanisms. Our working hypothesis is that RNA recruitment to ribosomes plays a critical role in gene expression regulation. Intercalating proteomic data (SILAC) with the transcriptomic data will allow us to approach this question in an integrated manner and maximize the data produced.
Trypanosomal gene regulation will be examined in the context of 7-methylguanosine cap formation. In addition to the established capping enzymes, trypanosomes have a unique bifunctional transferase enzyme. We propose to study the structure and essentiality of this enzyme in bloodstream form *T. brucei* and investigate its therapeutic potential.

P143* The effect of buthionine sulphoximine on the survival of different *Leishmania* species.

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Glutathione (GSH) is widely distributed in animal cells, and is the major intracellular thiol antioxidant in cells. Buthionine sulphoximine (BSO) is irreversible inhibitor of gamma glutamyl cysteine synthetase (g-GCS), an enzyme which catalyses the rate-limiting step in GSH synthesis. Previous studies have shown that BSO can inhibit the survival of *Leishmania donovani* both *in vitro and in vivo* (Carter *et al.*, 2003). In this study the effect of BSO treatment on the survival of promastigotes and intracellular amastigotes of *Leishmania donovani*, *L. mexicana* and *L. major* was determined using luciferase expressing parasites. In addition the activity of g-GCS in all three parasites was determined using a spectrophotometric assay. Results showed significant differences in the survival of all three parasites with *L. donovani* having the highest susceptibility to BSO at both the promastigote and intracellular amastigote stage. For example, the IC_{50} for the promastigote stage was 1.4mM for *L. donovani* 1.8mM for *L. major* and 4.5 mM for *L. mexicana* respectively.

P144* Purification of RNA Binding Proteins in *Trypanosoma brucei*

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Trypanosomes transcribe the majority of their genes as polycistronic units, regulating gene expression mainly at the post transcriptional level. A specific mRNA can be stored, degraded, or translated due to the presence of *cis*-regulatory motifs on its untranslated regions and the association of RNA binding proteins. In order to find these regulatory elements two different approaches for the isolation and further characterization of messenger ribonucleoproteins were designed. Both methods rely on the purification of specific polyribosomes via the nascent peptide. The first aims at the isolation of the Variant Surface Glycoprotein using polyclonal antibodies. The second involves the purification via the synthesis of a *Streptavidin*-tag at the N-terminus of a specific protein. While these methods are being optimized, we have analyzed the proteomes of free and membrane-bound polyribosomes, revealing a number of associated RNA binding proteins. Interesting results from the mass spectrometry will be presented.

P145* NUP-2, an intermediate filament-like protein in *Trypanosoma brucei* is a second component of the trypanosome nucleoskeletal lamina

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Lamins are required for a diverse array of cellular functions, from chromatin organisation and transcriptional regulation to nuclear structure and physical support for the cytoskeleton. Yet orthologs with high sequence similarity have been found in few non-metazoan lineages. Previous work has demonstrated that NUP-1 is a protein at the nuclear periphery in African trypanosomes with functionally lamin-like roles, encompassing nuclear structure, chromatin organisation and transcriptional regulation. Here we describe NUP-2, which is a predicted coiled-coil protein, and is restricted to the Kinetoplastida. NUP-2 interacts with NUP-1 and the nuclear pore complex, either directly or indirectly, as demonstrated by co-immunoprecipitation and MALDI-MS. NUP-2 is located in a punctuate distribution at the nuclear periphery throughout the cell cycle. Puncta of NUP-2 and NUP-1 are in close proximity but do not co-localise. Similarly, NUP-2 is also in close proximity to the telomeric ends of the chromosomes. Knockdown of NUP-2 leads to rapid and severe defects in proliferation, nuclear structure and whole cell morphology. We suggest that NUP-2 is a second component of the highly unusual nuclear lamina possessed by trypanosomes.

P146* Characterization of PUF2 RNA binding protein in *Trypanosoma brucei*

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PUF proteins, a conserved family of 3'UTR regulatory RNA-binding factors, are important regulators of mRNA translation and stability across the eukaryotic kingdom. The aim of this project is to functionally characterize *Tb*PUF2 (*Tb*927.10.12660), focusing on the elucidation of its mRNA targets.
Endogenous *Tb*PUF2 protein is found in discrete foci in the cytoplasm of bloodstream form cells of *Trypanosoma brucei.* *Tb*PUF2 knockdown leads to a growth defect in bloodstream cells, showing its importance for the normal growth. A minor fraction of V5-tagged *Tb*PUF2 protein is associated with polysomes, which suggests a role of *Tb*PUF2 in translational regulation. RNAseq analysis of the *Tb*PUF2 RNAi bloodstream cells showed decreases in the expression of many cytoskeletal mRNAs, which has been confirmed by northern blotting. Moreover, CAT (Chloramphenicol acetyl transferase) reporters carrying the PFR1 and PFR2 (paraflagellar rod proteins) 3'UTRs showed a decrease in the CAT protein and mRNA levels upon *Tb*PUF2 RNAi, suggesting a role of *Tb*PUF2 in the stability of these mRNAs. However, *Tb*PUF2 tethering to a CAT reporter mRNA caused a two-fold decrease in the CAT protein and mRNA levels. The results so far suggest that *Tb*PUF2 might be either stabilizing its cytoskeletal mRNA targets or destabilizing other targets, which in turn, stabilize these cytoskeletal mRNAs. Currently, I am verifying some of the putative mRNA targets, obtained by *Tb*PUF2 CLIP (Cross linking and immunoprecipitation).

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In trypanosomatids, gene expression is primarily regulated at the post-transcriptional level rather than at initiation, allowing up-regulation of expression by copy-number amplification. This leads to genome plasticity, a mechanism used by several pathogens to adapt to changing environments. Next-generation sequencing methods now allow high-throughput studies of this phenomenon in natural populations. We recently sequenced over 100 clinical lines of *Leishmania donovani* from the Indian subcontinent and developed new methods for calling structural diversity. Little sequence variation was observed in contrast with a high degree of genome structural variation. Changes in gene dosage were achieved by four mechanisms: aneuploidy, amplifications of large chromosomal stretches, tandem array expansions and extra-chromosomal episomes. The extent and ubiquity of aneuploidy are particularly striking in *L. donovani*: about 95% of the lines showed an altered and unique karyotype and up to 27/36 chromosomes were tri- or tetrasomic. We propose a general model of gene dosage and discuss its potential biological impact as well as practical consequences for further research.

c regions of linear chromosomes are subject to elevated mutation rates compared with chromosome cores, and hyperevolution of subtelomeric genes is considered to be key in generating diversity in organisms. The kinetoplastid parasite *Trypanosoma brucei* depends for its antigenic variation on a subtelomeric archive of genes encoding the variant surface glycoproteins (VSG) that form its cell-surface coat. The archive's subtelomeric location exposes it to a hypermutational environment that could contribute greatly to diversification. We are using the trypanosome *VSG* archive as a model to study subtelomere evolution.

We are examining the archives of two isolates of the same trypanosome strain sampled from the field 17 years apart. The two genomes have been deep-sequenced and assembled, with good coverage of subtelomeres. Comparison of these genomes indicates that the mutation rate is three- to four-fold higher in subtelomeres than chromosome cores. We are annotating conserved and novel *VSG* genes and pseudogenes, and are cataloguing differences between isolates. Comparisons so far have revealed insertions, deletions, base substitutions, and putative segmental conversions. This detailed quantification of changes provides raw data which will be used in modelling the contribution of various mutation processes and selection pressures in *VSG* archive evolution.

P148* Structural variation and genome diversity in natural populations of *Leishmania donovani*.

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[1] Institute of Tropical Medicine, Antwerp, Belgium; [2] Wellcome Trust Sanger Institute, Hinxton, United Kingdom, [3]Banaras Hindu University, Varanasi, India, [4]BP Koirala Institute of Health Sciences, Dharan, Nepal

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P149 Evidence for the existence of a new group of *Leishmania* parasites, the *Leishmania* enriettii complex

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Over 30 species of *Leishmania* have been described to date, including several that are significant diseases of humans and the subject of much research effort. However, to fully understand this group of parasites those that primarily infect non-human vertebrate hosts also require investigation, including their classification and phylogenetic relationships with the parasites of medical importance. Such studies help us to understand those features of the human parasites that are relevant to their pathogenicity, and are particularly important in a genus such as *Leishmania* where many of the human-infective parasites are zoonotic. Two well established sub-groups of *Leishmania* have been described previously and gained acceptance as subgenera: the *Leishmania* (*Leishmania*) and *Leishmania* (*Viannia*). There has been debate about the position and validity of a third grouping, the *Sauroleishmania* or lizard *Leishmania*. To this we now add a new group: the *Leishmania enriettii* complex. Sequence data and phylogenetic analysis places four species in this group, *L. enriettii* and three as yet unnamed species from Australia, Martinique and Namibia. These findings have implications for our wider understanding of vector-parasite relationships, transmission mechanisms, pathogenicity and classification of the genus *Leishmania*.

P150* Following evolution and diversification of antigens encoded in *Trypanosoma brucei* subtelomeres

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[1]Wellcome Trust Centre for Molecular Parasitology, University of Glasgow; [2]Wellcome Trust Sanger Institute, Wellcome Trust Genome Campus, Cambridge; [3]Institute of Genetics, Queen's Medical Centre, University of Nottingham

The subtelomeric regions of linear chromosomes are subject to elevated mutation rates compared with chromosome cores, and hyperevolution of subtelomeric genes is considered to be key in generating diversity in organisms. The kinetoplastid parasite *Trypanosoma brucei* depends for its antigenic variation on a subtelomeric archive of genes encoding the variant surface glycoproteins (VSG) that form its cell-surface coat. The archive's subtelomeric location exposes it to a hypermutational environment that could contribute greatly to diversification. We are using the trypanosome *VSG* archive as a model to study subtelomere evolution.
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P151* Recent loss of polynucleotide kinase phosphatase in *Leishmania*

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The inherent instability of the primary structure of DNA entails that detection and repair of DNA damage is an essential process. Normally, the greatest burden of DNA damage occurs from endogenous sources such as reactive oxygen species. Polynucleotide kinase phosphatase (PNKP) possesses both 5'-kinase and 3'-phosphatase activities and functions in the repair of discontinuities in the phosphodiester backbone arising through oxidation. We cloned and characterised the PNKP homolog from *Trypanosoma brucei* and determined that it is a bifunctional DNA repair enzyme. However, we find that in *Leishmania* parasites PNKP is now merely a pseudogene. Interestingly, sequence analysis reveals that the kinase domain in the artefactual gene has undergone substantially greater degeneration than the phosphatase domain, suggesting the ancestor of *Leishmania* possessed polynucleotide phosphatase. Synthesis of an artificial open reading frame with one of the stop codons from the *L. major* pseudogene removed and two highly conserved sites reconstituted resulted in active phosphatase following *in vitro* folding of recombinant protein. The reason(s) why PNKP is retained in *Trypanosoma* species but not *Leishmania* is not yet clear but procyclic *T. brucei* mutants that are null for PNKP are viable. Given the phenotypes associated with PNKP loss in some other eukaryotes, we will discuss the unexpected evolutionary degeneration of PNKP in *Leishmania* in the context of functional redundancy within DNA repair pathways.

P152 Making sense of suggested mutases in trypanosomatid parasites

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Due to their biochemical tractability our understanding of trypanosomatid metabolism was good prior to the availability of nuclear genome sequences for trypanosome and *Leishmania* species. With annotated genome sequences at hand, clearer pictures of central intermediary metabolism in trypanosomatids emerge: species- and stage-specific differences between these metabolic maps reflect differences in niche adaptation. Homology-led classification of novel genes, however, readily leads to mis-annotation. Thus, we were intrigued by predictions for several isoforms of the glycolytic enzyme phosphoglycerate mutase (PGAM) in *Trypanosoma brucei*, despite published evidence that a single experimentally characterised PGAM isoform maintains glycolytic flux. Here, we show predicted PGAMs include a novel alkaline phosphatase or belong to a diverse cohort of histidine phosphatases. They are conserved across the Trypanosomatidae, but are only sparsely distributed across eukaryotic and prokaryotic kingdoms. In addition to a *bona fide* PGAM and the gluconeogenic enzyme fructose-1,6,-bisphosphatase, the trypanosomatid histidine phosphatases include homologues of the broad specificity phosphatase PhoE, the recently discovered animal Ser/Thr phosphatase PGAM5, and two novel paralogous mitochondrial proteins that arose from gene duplication prior to trypanosomatid radiation. Aside from the experimentally characterised PGAM and fructose-1,6-bisphosphatase, the metabolic or signalling advantages resulting in retention of an unexpectedly diverse repertoire of histidine phosphatases and a novel alkaline phosphatase in trypanosomatid parasites are unclear.

P153 Comparative genomics of ion transporters in unicellular eukaryotes

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Transporters are fundamental to parasitic metabolism since all parasites import nutrients from their hosts. Parasite nutrient transporters are of pharmacological interest as potential drug targets or for the specific delivery of toxic compounds to the parasite. The availability of complete genome sequences from various parasites allows systemic surveys for transporters resulting in 'transportomes'. We have constructed hidden HMM-based profiles for all known families of channels, porters, and pumps. This was achieved by a semi-automatic pipeline, starting from the experimentally validated transporters in UniProt and their Transporter Classification Database (TCDB) accessions. The set of transporters obtained for each TCDB entry was redundancy reduced, subjected to multiple alignment, and a HMM profile was constructed. The resulting HMM library was particularly useful for the identification and comparative genomics of ABC transporters and ion transporters. Constructed HMM profiles were run over the predicted proteomes of parasites and free-living eukaryotes, and for each species a vector of 73 components was built, i.e. the scores of the best hit against each transporter profile. When these vectors were hierarchically clustered, unrelated parasites co-assembled. These data allow to identify transporter families based on their phylogenetic information content such as the ion channels that are present in trypanosomatids but absent in other parasites.

P154 Episomal gene amplification and copy number variation in natural populations of *Leishmania donovani*

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Structural variation has gained attention as contributor to genomic diversity in addition to sequence variation. This is particularly prevalent in *Leishmania donovani* from the Indian subcontinent, where the large amount of copy number variation (CNV) contrasts with the high level of sequence conservation. One mechanism responsible for CNV is the formation of extra-chromosomal circular episomes. Such episomes were previously evidenced in experimental conditions, e.g. drug resistance induction, and found to be unstable in the absence of drug pressure. We present a bioinformatics method to predict the occurrence of episomes from whole-genome sequencing data. We applied our method on a population of 17 drug resistant and sensitive *L. donovani* clinical lines, detected two potential episomes and experimentally verified our approach. The first episome was already described (H-locus containing the ABC-thiol MRPA gene), the second one harbored 4 genes, among which a MAPK homologue. We expanded the analysis of these loci in >100 clinical lines and found that (i) both episomes were present in most lines and (ii) the copy number of the MAPK-locus was linked with treatment failure of visceral leishmaniasis in the corresponding patients. We also tested in vitro and in vivo episome stability.

P155 GeneDB: an annotation database for pathogens.

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J. Myler[4], David Roos[5], Christiane Hertz-Fowler[3], Mark Carrington[6], Deborah F. Smith[2], Matthew Berriman[1].
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GeneDB (http://www.genedb.org) is a database of annotated prokaryotic and eukaryotic pathogen genomes. We focus on the rapid release and simple, rapid access, to reference genomes, with a depth of annotation and curation that is not found on other databases. Sequence updates are made available to the user community on a regular basis, with new bioinformatic tools being developed in-house to enable rapid changes to be made to sequences. The development of the database in recent years has focused on providing database-driven annotation tools and pipelines, as well as catering for increasingly frequent assembly updates. The website has been significantly redesigned to take advantage of current web technologies, and improve usability. The current release stores 41 datasets, of which 17 are manually curated and maintained by biologists, who review and incorporate data from the scientific literature, as well as other sources. GeneDB is primarily a production and annotation database for the genomes of predominantly pathogenic organisms.

P156* Investigation of hemidesmosomes and their roles in the attachment and transmission of *Leishmania*

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During the *Leishmania* life cycle the organism differentiates into distinct forms within its hosts. The parasites use their flagella to attach and anchor themselves to the surface of the gut wall in their sand fly vectors. On cuticular surfaces the flagellar tip forms hemidesmosome-like structures, which are proposed to be an important element of the transmission mechanism. The identity of *Leishmania* hemidesmosomal protein molecules and their function in the attachment mechanism is not known. In order to investigate this, *Leishmania* promastigotes were cultured in the presence of various materials in attempts to replicate this attachment phenomenon in vitro. The aim was to find a substance that could provide a good quantity and high quality of attached parasites to facilitate biochemical and molecular characterization, and identify those proteins and their functions in formation and regulation of the hemidesmosome structure.

P157* Ergosterol – the Achilles' heel of Trypanosomes: Comparative Genomics of Sterol Biosynthesis

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[1] Swiss Tropical and Public Health Institute, Basel, Switzerland[2] University of Basel, Basel, Switzerland

There is an urgent need for new drugs against trypanosomatids. Sterol biosynthesis is one distinct pathway that harbours potential drug targets. Cholesterol, the most important sterol in vertebrates, is an essential constituent of cellular membranes and a precursor to fat-soluble vitamins and steroid hormones. Pathogenic fungi and certain protozoa require the presence of different endogenous sterols, typically ergosterol and other 24-alkylated sterols, which cannot be replaced by the vertebrate or plant host's sterols. Ergosterol synthetic enzymes lacking orthologues in vertebrates are potential drug targets. We aim to find the most promising of these targets by comparative genomics, evaluating sterol metabolism in different species. A specific profile is generated for each enzyme in the metabolic pathway and run against proteomes of parasites and reference organisms. For each species, the scores of the best hit against each profile are clustered hierarchically. Organisms with a similar set of enzymes co-assemble in the resulting heat map, revealing convergent evolution in unrelated parasites such as Giardia and Apicomplexa, which have lost sterol metabolic enzymes. Based on these data, known selective sterol biosynthesis inhibitors are tested in vitro against several parasites. Good correlation between in silico and in vitro data indicates that the examined compound acts on the target under investigation.

P158* Trypanosome Cathepsin-L Increases Calcium Waves in Cardiomyocytes

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Clinical signs in trypanosomiasis classically include neurological symptoms, but cardiac alterations are increasingly recognised (70% sleeping sickness patients). Calcium fluxes in blood brain barrier models have been shown to be induced by *Trypanosoma brucei* Cathepsin-L (TbCatL). Given the importance of intracellular Ca^{2+} dynamics in normal cardiac function this study aimed to examine whether TbCatL could alter cardiac function via a similar mechanism to the brain.
We assessed adult rat ventricular cardiomyocytes for induction of spontaneous contractions resulting from spontaneous store-mediated intracellular Ca^{2+} release when incubated with *T.brucei* culture supernatant. The percentage of cells exhibiting spontaneous contractions was significantly increased in supernatant versus control. In *ex vivo* perfused whole rat hearts an increase in arrhythmic events which are linked to spontaneous release of intracellular Ca^{2+} was observed in hearts perfused with supernatant versus media.
In single cardiomyocyte studies, addition of CA074, a Cathepsin-B inhibitor, showed no effect on percentage of cells waving. However, waving was reduced significantly with K11777, a TbCatL inhibitor. Cardiomyocytes incubated with recombinant TbCatL also showed a significant increase in waving. RNA interference of TbCatL reduced waving to levels comparable with controls. These data suggest TbCatL is responsible for these contractile events in cardiomyocytes, which may contribute to the arrhythmias observed in the whole heart.

P159 Disctinct gene expression profile by live *Leishmania amazonensis* amastigotes-hosting C57BL/6 and DBA/2 dendritic leucocytes.

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The inoculation of a 10^4 *Leishmania/L. amazonensis* metacyclic promastigotes into the dermis of ear pinna of C57BL/6 and DBA/2 mice results in distinct outcome as assessed by i) parasite load values and ii) ear pinna macroscopic features monitored from days 4 to 22-phase 1- and from days 22 to 80/100-phase 2-. While in C57Bl/6 mice, the onset of the amastigote population size increase is slow and progressive, in DBA/2 mice, the onset of the amastigote population size increase is rapid, as is its sustained control. The aim of this study was to provide insights about immune processes which could account for the distinct outcome during the phase 1, namely, *when* phagocytic dendritic leucocytes/DLs have been subverted as live amastigotes-hosting cells. With this objective in mind, bone marrow-derived C57BL/6 and DBA/2 DLs were generated and exposed or not to live *Ds*Red2 expressing transgenic *L. amazonensis* amastigotes. The four DL populations were compared by flow cytometry and Affymetrix-based transcriptomic analysis. In contrast to live amastigotes-hosting C57BL/6 DLs, the DBA/2 ones display transcriptional signatures and markers that are consistent with immune regulatory functions and rapid amastigote establishment in both the ear pinna and ear- draining lymph node.

P160* ***Leishmania mexicana* influences the migration of immune cells towards draining lymphatic vessels**

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The intracellular protozoan *Leishmania mexicana* has the capacity to modulate the expression of various chemokines and their receptors during infection. This manipulation of the host immune response exerts a profound effect on immune cell functionality and migration. It is essential to determine the interaction between dendritic cells, macrophages and the draining lymph node upon infection with *L. mexicana*, to better understand the immune response elicited during infection with the parasite. We have therefore used a combination of cutting-edge microscopy techniques to visualise cell interaction and migration following infection with *L. mexicana*. Here, we demonstrate that immune cell migration is significantly altered by *L. mexicana*, with a failure of dendritic cells to co-localise with lymphatic vessels. These data suggest that *L. mexicana* is capable of reducing the migratory capacity of immune cells, specifically antigen presenting dendritic cells, into the draining lymph node. The observed failure of dendritic cells to enter the lymphatic vessels suggests a consequential reduction in T cell-dendritic cell interactions, affecting the generation of T cell mediated immunity.

P161* Human African trypanosomiasis: a review of non-endemic cases in the past 20 years

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Human African trypanosomiasis (HAT) is caused by sub-species of the parasitic protozoan Trypanosoma brucei and is transmitted by tsetse flies, both of which are endemic only to sub-Saharan Africa. Several cases have been reported in non-endemic areas, such as North America and Europe, due to travelers, expatriots or military personnel returning from abroad or due to immigrants from endemic areas. In this paper, non-endemic cases reported over the past 20 years are reviewed; a total of 68 cases are reported, 19 cases of Trypanosoma brucei gambiense HAT and 49 cases of Trypanosoma brucei rhodesiense HAT. Patients ranged in age from 19 months to 72 years and all but two patients survived. Physicians in nonendemic areas should be aware of the signs and symptoms of this disease, as well as methods of diagnosis and treatment, especially as travel to HAT endemic areas increases. We recommend extension of the current surveillance systems such as TropNetEurop and maintaining and promotion of existing reference centers of diagnostics and expertise.

P162* *myo*-Inositol Uptake is Essential for Bulk Inositol Phospholipid Synthesis in *Trypanosoma brucei*

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myo-Inositol is an important osmolyte and an essential precursor for the production of inositol phosphates and inositol phospholipids in all eukaryotes. Intracellular *myo*-inositol is generated by *de novo* synthesis from glucose-6-phosphate or provided from the environment via *myo*-inositol symporters. It has previously been reported that in *Trypanosoma brucei de novo* synthesis of *myo*-inositol is necessary for normal growth of parasites in culture. We now show that *myo*-inositol in *T. brucei* is also taken up via a specific proton-coupled electrogenic symporter (TbHMIT) and that this transport is essential for parasite survival in culture. In a phylogenetic analysis, TbHMIT and its homologues in other trypanosomatids group as a separate clade clearly distinct from their nearest neighbors, the HMITs from plants and mammals. Down-regulation of TbHMIT in procyclic forms using RNA interference inhibited uptake of *myo*-inositol and blocked the synthesis of the *myo*-inositol-containing phospholipids, phosphatidylinositol and inositolphosphoryl ceramide; in contrast, it had no effect on glycosylphosphatidylinositol production. This together with the unexpected localization of the *myo*-inositol transporter in both the plasma membrane and the Golgi demonstrates that metabolism of endogenous and exogenous *myo*-inositol in *T. brucei* is strictly segregated.

P163* How does iron get into the cytoplasm of African trypanosomes?

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In infected mammals, African trypanosomes obtain iron from host transferrin, which is endocytosed and trafficked to the lysosome. At the acidic pH of the lysosome, Fe^{3+} is released. To access the cytosol it must then be reduced to Fe^{2+} by a ferric reductase and transported through a divalent cation channel. We report the characterisation of *Trypanosoma brucei* genes which may fulfil these requirements. Mucolipin 1 (MCOLN1) is an endolysosomal cation channel in humans which is permeable to Ca^{2+}, Fe^{2+} and Zn^{2+}. *T. brucei* has an orthologue (*TbMLP*) with a conserved pore domain. *TbMLP* mRNA is expressed in bloodstream and procyclic forms and the protein localises to the lysosome. TbMLP expression is essential as the corresponding gene can only be deleted in the presence of an ectopic copy. RNAi-mediated knockdown results in a growth defect and enhances susceptibility to the iron chelators deferoxamine and SHAM. This phenotype is recapitulated in conditional null mutants, with an even greater susceptibility to deferoxamine. *T. brucei* also has an orthologue of the ferric reductase cytochrome b_{561} (TbCytb_{561}). TbCytb_{561} is localised to the endomembrane sytem and RNAi knockdown has a pronounced effect on susceptibility to deferoxamine. Thus the properties of TbCytb_{561} conform to those predicted of the ferric reductase required for iron uptake from transferrin.

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P165 Biosynthesis of unsaturated fatty acids is an essential process in both procyclic and bloodstream form *Trypanosoma brucei*

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Both procyclic and bloodstream form *Trypanosoma brucei* are capable of *de novo* synthesis of fatty acids and the process is essential for parasite survival. Polyunsaturated fatty acids (PUFAs) are synthesized by enzymes known as desaturases. Two desaturase enzymes were identified in *T. brucei*: △9 desaturase that synthesizes oleate from stearate and △12 desaturase that converts oleate into linoleate. Knocking down these desaturase enzymes, in both procyclic and bloodstream form *T. brucei*, caused a growth phenotype and also exerted a significant effect on the total fatty-acid composition of the parasite. Isoxyl and 9-thiostearate, known Δ9 desaturase inhibitor, showed an inhibitory effect on the growth of bloodstream form trypanosomes with EC_{50} of 0.1 μM and 1 μM, respectively. Two Δ12 desaturase inhibitors, 12- and 13-thiostearate, totally inhibited parasite growth with EC_{50} of 2μM and 7μM, respectively. The results suggest that Δ9 and Δ12 desaturase are essential for both procyclic and bloodstream form *T. brucei*. The complete absence of Δ12 enzyme activity in mammalian cells and the significant structural differences between trypanosome and mammalian Δ9 desaturases, highlight these enzymes as promising targets for selective chemotherapeutic intervention against the parasitic disease.

P166 *myo*-Inositol Uptake is Essential for Bulk Inositol Phospholipid Synthesis in *Trypanosoma brucei*

Amaia Gonzalez-Salgado[1], Michael Steinmann[1], Eva Greganova[2], Pascal Mäser[2], Erwin Sigel[1] and Peter Bütikofer[1]
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myo-Inositol is an important osmolyte and an essential precursor for the production of inositol phosphates and inositol phospholipids in all eukaryotes. Intracellular *myo*-inositol is generated by *de novo* synthesis from glucose-6-phosphate or provided from the environment via *myo*-inositol symporters. It has previously been reported that in *Trypanosoma brucei de novo* synthesis of *myo*-inositol is necessary for normal growth of parasites in culture. We now show that *myo*-inositol in *T. brucei* is also taken up via a specific proton-coupled electrogenic symporter (TbHMIT) and that this transport is essential for parasite survival in culture. In a phylogenetic analysis, TbHMIT and its homologues in other trypanosomatids group as a separate clade clearly distinct from their nearest neighbors, the HMITs from plants and mammals. Down-regulation of TbHMIT in procyclic forms using RNA interference inhibited uptake of *myo*-inositol and blocked the synthesis of the *myo*-inositol-containing phospholipids, phosphatidylinositol and inositolphosphoryl ceramide; in contrast, it had no effect on glycosylphosphatidylinositol production. This together with the unexpected localization of the *myo*-inositol transporter in both the plasma membrane and the Golgi demonstrates that metabolism of endogenous and exogenous *myo*-inositol in *T. brucei* is strictly segregated.

P167 Mass spectrometry and phosphoproteomics for database improvements – a *Leishmania mexicana* case study

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Knowledge about protein function, sequence and post-translational modifications is required to advance the understanding of *Leishmania* biology. We constructed a pipeline for large-scale proteome and phosphoproteome analyses of *Leishmania mexicana*, employing a 6-frame DNA-based translation library as well as a predicted protein list for database searches. This led to the identification of 1,945 proteins, 1,029 of them phosphorylated on a total of 3,079 unique residues. 561 of the phosphorylation sites were found in protein kinases (87 proteins, 270 unique sites), phosphatases (22 proteins, 84 unique sites) and membrane protein transporters (58 proteins, 207 unique sites). Within these groups of proteins, we validated 9 phosphorylation sites that were located outside the predicted protein sequences. Screening our entire dataset, we confidently identified a total of 154 peptides with 77 phosphorylation sites located outside the predicted protein sequences of 85 proteins in the current TriTrypDB *Leishmania mexicana* database. Furthermore, we have 41 validated phosphorylation sites in 23 translated sequences showing no homology to any predicted *Leishmania* protein sequences. Our findings have implications for the annotation of the *Leishmania* databases in general, and highlight the value of mass spectrometry as a valuable tool to improve the quality of genome databases.

P168 Metabolomic analysis of *Trichomonas vaginalis* identifies novel thioethers as antioxidants.

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Trichomonas vaginalis, the causative agent of the sexually transmitted disease trichomoniasis which has been identified as a co-factor in the transmission of HIV/AIDS, is a microaerophile with a fermentative energy metabolism that nevertheless metabolises oxygen. The parasite has antioxidant defence mechanisms to protect against oxidative stress and these include a peroxiredoxin, that forms a redox couple with thioredoxin and thioredoxin reductase to detoxify peroxides, and cysteine, which is produced from phosphoserine and H_2S using cysteine synthase and is the major intracellular thiol.
We have now carried out a metabolomic analysis of *T. vaginalis* to provide a baseline metabolite profile and to investigate further sulphur amino acid metabolism and antioxidant defence. We used growth conditions shown previously to affect cysteine synthase enzymic activity and intracellular thiol levels. LC-MS analysis identified S-methyl-cysteine (SMC) as a novel metabolite and its structure was confirmed by fragmentation analysis. SMC is a thioether analogue of methionine, known in several leguminous plants and the protozoon *Entamoeba histolytica*, that can function as an antioxidant in scavenging hydroxyl radicals. Several other thioethers were also identified putatively in *T. vaginalis* and their different functional groups suggest that they have different antioxidant activities. The data from this analysis suggest that *T.* vaginalis has an unusual array of antioxidants.

P169* The hydrophobic region of the *Leishmania* peroxin 14 is important for the formation of the transient pore that mediates protein import into the glycosomal matrix

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In *Leishmania*, glycolysis and a number of other vital metabolic pathways are segregated in the glycosome, a specialized organelle that is related to the peroxisomes of other eukaryotes. Proteins targeted to the glycosome typically contain either a PTS1 or PTS2 signal sequence that is recognized and tightly bound by the receptor proteins peroxin 5 (LdPEX5) and peroxin 7 (LPEX7), respectively. These cargo-receptor complexes are escorted to the glycosome surface where they bind to peroxin 14 (LdPEX14) a constituent of the docking/translocation machinery. Genetic studies in the related parasite have demonstrated that PEX5, PEX7, PEX14 are all essential components for glycosome biogenesis and parasite viability. However, little is known about the protein-lipid bilayer interactions involved in the formation of a pore structure that mediates the translocation of PTS1 and PTS2 cargo proteins across the glycosomal membrane. Here we show that the *Leishmania* LdPEX14 forms macromolecular structure on the glycosome surface resembling a rosette or ring-like structure with a diameter of ~30-40 nm. Studies using large unilamellar liposomes established that an amphipathic region spanning residues 149-179 is critical for membrane binding. Moreover, dye release assay demonstrated that a ldpex14 fragment encompassing this amphipathic structure is sufficient to pores formation in liposomes. These results suggest a model in which the binding of cargo loaded LdPEX5 and LdPEX7 to LdPEX14 triggers structural changes that promote insertion of the LdPEX14 amphipathic helix into the glycosomal membrane and formation of a transmembrane pore through which glycosomal matrix proteins are imported.

P170 *myo*-Inositol Uptake is Essential for Bulk Inositol Phospholipid Synthesis in *Trypanosoma brucei*

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myo-Inositol is an important osmolyte and an essential precursor for the production of inositol phosphates and inositol phospholipids in all eukaryotes. Intracellular *myo*-inositol is generated by *de novo* synthesis from glucose-6-phosphate or provided from the environment via *myo*-inositol symporters. It has previously been reported that in *Trypanosoma brucei de novo* synthesis of *myo*-inositol is necessary for normal growth of parasites in culture. We now show that *myo*-inositol in *T. brucei* is also taken up via a specific proton-coupled electrogenic symporter (TbHMIT) and that this transport is essential for parasite survival in culture. In a phylogenetic analysis, TbHMIT and its homologues in other trypanosomatids group as a separate clade clearly distinct from their nearest neighbors, the HMITs from plants and mammals. Down-regulation of TbHMIT in procyclic forms using RNA interference inhibited uptake of *myo*-inositol and blocked the synthesis of the *myo*-inositol-containing phospholipids, phosphatidylinositol and inositolphosphoryl ceramide; in contrast, it had no effect on glycosylphosphatidylinositol production. This together with the unexpected localization of the *myo*-inositol transporter in both the plasma membrane and the Golgi demonstrates that metabolism of endogenous and exogenous *myo*-inositol in *T. brucei* is strictly segregated.

P171 Disparate phenotypic effects from the knockdown of various *Trypanosoma brucei* cytochrome c oxidase subunits

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The *Trypanosoma brucei* cytochrome *c* oxidase (cIV) is a very divergent complex containing a high number of hypothetical trypanosomatid-specific subunits. To gain insight into the functional organization of this large respiratory complex, the expression of three novel subunits were down-regulated by RNAi. We demonstrate that all three subunits are important for the proper function of cIV and the growth of procyclic *T. brucei* cells. These phenotypes were manifested by the structural instability of the complex when these indispensible subunits were repressed. Overall, the absence of cIV resulted in other severe mitochondrial phenotypes, such as a decreased mitochondrial membrane potential, reduced ATP production via oxidative phoshorylation and redirection of oxygen consumption to the trypanosome-specific alternative oxidase. Interestingly, a dissimilar effect of the studied subunits was observed regarding the activity of cytochrome bc1 (cIII). While the activity of cIII was down-regulated for two of the cIV knockdowns, RNAi of the third subunit actually exhibited higher levels of cIII activity and elevated levels of ROS formation. This suggests that the studied subunits may have different functional roles within *T. brucei* cIV, perhaps involving the ability to communicate between sequential enzymes in the respiratory chain.

P172 Binding properties of the *Trypanosoma brucei* F_oF_1ATPase inhibitory peptide *in vivo* and *in vitro*

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Trypanosoma brucei F_oF_1-ATPsynthase works in reverse in the bloodstream stage, hydrolyzing ATP to maintain the essential mitochondrial (mt) membrane potential in the absence of a cytochrome-mediated respiratory chain. This hydrolytic activity of the ATPsynthase in higher eukaryotes has only been demonstrated in rare cases during hypoxia when the complex switches to its hydrolytic function to temporarily sustain the membrane potential, but in doing so it depletes the tissue of ATP and leads to cell death. This aberrant activity is inhibited by the small mt protein, IF1. Importantly, a homolog of this protein was identified in the *T. brucei* genome and its expression was detected only in the insect stage of the parasite. Predictably, the over-expression of TbIF1 in the bloodstream and dyskinetoplastid cells significantly inhibited the hydrolysis activity of the ATPsynthase, leading to the death of the parasite. Furthermore, it appears that the recombinant TbIF1 has a slightly acidic pH optimum for binding to the purified Tb-F_1-ATPase. Although the sequence of the TbIF1 is related to that of bovine IF1, the bovine peptide does not inhibit the Tb-F1-ATPase, presumably reflecting changes in the sequence and the regions with which it interacts in F1-ATPase. Therefore, a series of truncated and mutated TbIF1s were prepared to map its interaction interface with Tb-F1-ATPase.

P173 Iron uptake in procyclic *Trypanosoma brucei*

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Iron is an essential nutrient for *T. brucei*, and both bloodstream and insect stages must have developed ways to acquire it from their respective hosts, mammals and tsetse flies. A receptor for host transferrin (ESAG-6, ESAG-7) is expressed in the bloodstream stage, allowing an effective uptake of protein-bound iron. While procyclic insect stage of *T. brucei* possess a fully active mitochondrion with iron-sulfur cluster proteins, ESAG genes are not expressed at this stage and nothing is known about the mechanism of iron uptake.
Our goal was to determine whether procyclic forms of *T. brucei* employ a reductive mechanism to take up iron from ferric complexes. We incubated procyclic *T. brucei* with ^{55}Fe(III)-citrate in the presence or absence of Fe(II) trapping agent, bathophenantroline, or ascorbate as a reducing agent. Levels of cell-associated radioactivity were determined by scintillation counting. Ferric reductase activity was measured using a bathophenantroline-based colorimetric assay, and blue native polyacrylamide gel electrophoresis was performed with mitochondrial proteins isolated from procyclic *T. brucei* incubated with ^{59}Fe(III)-citrate.
We showed that procyclic *T. brucei* efficiently takes up iron from ferric complexes. Ferric iron is reduced prior to uptake, and is subsequently transported to and used in the mitochondrion. Further, we would like to identify a putative ferric reductase or other components of the reductive mechanism, and Fe(II) transporters on the cell membrane.

P174 Cysteine biosynthesis in *Leishmania*

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Cysteine is a key building block of trypanothione, an antioxidant unique to trypanosomatids that plays a pivotal role for the survival of the parasites. *Leishmania* can obtain cysteine in two ways, using the sulphydrylation and trans-sulphuration pathways. Humans lack an equivalent sulphydrylation pathway, thus this, and especially cysteine synthase (CS), of *Leishmania* could provide a good drug target. This study aims to determine the relative importance of these pathways and CS for supplying cysteine in *Leishmania* during its life cycle. The levels of a range of thiols at different stages of promastigote growth of wild-type and mutants lacking CS (?*cs*) were determined. Further, the sensitivity wild-type and ?*cs* mutants to metal, peroxidation, and progargylglycine (PAG; an inhibitor of pyridoxal 5'-phosphate- dependant enzymes) was analysed. CS forms a complex with the second enzyme of the sulphydrylation pathway serine acetyltransferase (SAT), inactivating CS and activating SAT. Peptides correponding to the SAT C-terminus from different organisms were tested for inhibition of *Leishmania* CS, as an approach to inhibitor and drug discovery.

P175 Laboratory mouse dendritic leucocytes harboring live *Leishmania* amastigotes display unique remodeling of their lipid metabolism

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The aim of this study was to investigate the potential modification of lipid metabolism of phagocytic dendritic leukocytes /DLs, once they have been subverted as live *Leishmania/L. amazonensis* amastigotes-hosting cells. Bone marrow-derived C57BL/6 and DBA/2 DLs were generated and exposed or not to live *Ds*Red2 expressing transgenic *L. amazonensis* amastigotes. The four DL populations were compared by cytometry and Affymetrix-based transcriptomic analysis. Our results indicated that live amasigotes-hosting DLs displayed unique modulations of genes involved in the metabolism of neutral lipids, *i.e.* the coordinated increase of: i) triacyl-*sn*-glycerol synthesis and storage, ii) long chain fatty acid uptake and transport, and iii) cholesterol uptake and esterification to cholesteryl esters. All together the unique transcriptional signatures led us to further explore the presence of cellular storage organelles known as lipid bodies/LD in the amastigotes-hosting DLs. Numerous LBs were displaying more or less intimate contact with amastigote(s)-loaded parasitophorous vacuole (PV) membrane, the latter ranging from PV membrane curvature changes to PV membrane rupture. Whether accumulation of the glycerolipids could be favoured by processes that maintain Phosphatidyl Choline /PC homeostasis at the expanding LD monolayer will be also discussed.

P176* Extending a dynamic model of trypanosome metabolism: challenges for iterative systems biology

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Systems biology is widely described as an iterative process, where models are used to form hypotheses predicting biological behaviour, which can be tested by wet lab experiments, and the obtained data is subsequently used to improve the initial model leading to new hypotheses and the next round of experimentation.
Here we demonstrate the surprising challenges faced in the modular extension of a successful and well-curated model, the Bakker model of energy metabolism in the tropical parasite *Trypanosoma brucei*, by a single additional pathway.
Incorporating the pentose phosphate pathway, partly localized in a unique organelle called the glycosome, into a model of *T. brucei* glycolysis, caused a lethal imbalance in bound phosphates in the glycosome. Two mechanisms to relieve this problem are explored: (i) the presence of a glycosomal ATP:ADP antiporter, and (ii) the presence of a ribokinase enzyme working in the direction of ribose production. The ribokinase hypothesis was tested experimentally, and catalytic activity and reverse genetics support a previously unexpected essential role of ribokinase in *T. brucei*.
Additionally, this method of modular extension is used to extend this model further by incorporating the trypanothione pathway, essential in oxidative stress protection.

P177* Functional and structural interactome of the major ADP/ATP carrier in procyclic *Trypanosoma brucei* mitochondria

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The highly conserved ADP/ATP carriers (AAC) are key energetic links between the mitochondrial (mt) and cytosolic compartments of all aerobic eukaryotic cells. By exchanging cytosolic ADP with ATP generated inside the mitochondria by oxidative phosphorylation, these essential mt transmembrane transporters supply energy to consuming biosynthetic pathways throughout the cell. The *T. brucei* genome encodes two AAC proteins that are related to the human and yeast ADP/ATP carriers. Importantly, TbAAC1, the more conserved of the two homologues, partially complements the function of yeast AAC2, the only essential carrier. Furthermore, RNAi silencing of TbAAC1 in the procyclic form results in a severe growth defect that coincides with a significant reduction of mitochondrial ATP synthesis by both substrate and oxidative phosphorylation. This data suggests that TbAAC1 is the major ATP/ADP carrier and its function can‘t be substituted by TbAAC2. Further experiments demonstrate that the elimination of TbAAC1 has no impact on the function and structure of respiratory complexes III, IV and ATP synthase. However, glycerol gradient sedimentation experiments performed under different detergent conditions and co-immunoprecipitaion studies suggest that in contrast to yeast, but similar to mammals, TbAAC1 seems to be associated with ATP synthase. Further validation of the presence and composition of the *T. brucei* ATP synthasome is under investigation.

P178* A simple colorimetric assay for high-throughput screening of drugs against *Leishmania* intracellular amastigotes.

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Development of safe and efficacious therapies able to counteract the significant problems posed by current anti-leishmanial drugs has been strongly advocated. Crucial to the search for new anti-leishmanial drugs is the availability of high-throughput methods to screen chemical compounds against the relevant stage for disease pathogenesis, the intracellular amastigotes. Recent progress in automated microscopy and genetic recombination have produced powerful tools for drug discovery. However, a simple and efficient test for measuring cytotoxicity against *Leishmania* clinical isolates is still lacking.
Here, we describe a quantitative colorimetric assay, whereby the activity of a *Leishmania* native enzyme is used to assess its viability. Enzymatic reduction of disulphide-trypanothione, monitored by a microtiter plate reader, was used to quantify the growth of *Leishmania donovani* intracellular amastigotes. An excellent correlation was found between the optical density, as measured at 412nm, and the number of parasites inoculated. Validation of this assay was performed with a selected drug-panel against standard microscopy. The activity of several anti-leishmanial reference drugs, as measured by this assay, corroborated with microscopy results and previously published data. This simple and relatively inexpensive assay provides a reliable, accurate method for screening anti-leishmanial agents, at high-throughput. The basic equipment and manipulation required to perform the assay makes it easy to implement, simplifying the methodology for scoring inhibitor assays

P179* PEX4 and its role in glycosomal matrix protein import of *Trypanosoma brucei*. Melisa Gualdrón-López, Nathalie Chevalier & Paul Michels. “de Duve Institute”, Université catholique de Louvain, Brussels, Belgium Glycolysis in trypanosomatids is compartmentalized in peroxisome-like organelles called

glycosomes. The import of proteins into the glycosomal matrix involves the cytosolic receptor PEX5, which recognizes the C-terminal peroxisomal-targeting signal PTS1 of these proteins. In yeast and mammalian cells the cargo-loaded PEX5 associates with the peroxisomal membrane, delivers its cargo and is then ubiquitinated, a modification that serves as signal for retrieval of PEX5 that may subsequently be used for further cycles of import (monoubiquitination) or degraded by proteasomes (polyubiquitination). We have found stable monoubiquitinated PEX5 in cytosolic fractions of wild-type bloodstream and procyclic *Trypanosoma brucei*. We have identified the *T. brucei* homologue of PEX4, the ubiquitin-conjugating (UBC) enzyme responsible for PEX5 ubiquitination in yeast. It is expressed in bloodstream and procyclic forms and associated with the cytosolic face of the glycosomal membrane. TbPEX4 knockout procyclic cells show that this peroxin is involved in TbPEX5 monoubiquitination. Surprisingly, live cell imaging of this mutant expressing GFP-PTS1 shows no defect in glycosomal matrix protein import contrary to RNAi cell lines for TbPEX12 and TbPEX6, peroxins also involved in PEX5 cycling. qPCR analysis of the KOPEX4 mutant shows that other enzymes of the putative UBC repertoire are upregulated which could cause aberrant ubiquitination in other cellular processes and explain the observed growth and morphological defects, unrelated to glycosome biogenesis.

P180* Pyrimidine salvage in *Trypanosoma brucei* bloodstream forms

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Pyrimidine uptake has previously been investigated in *Trypanosoma brucei* procyclics and here we report a study of pyrimidine salvage and metabolism in bloodstream forms. Uptake of ^{3}H-uracil and ^{3}H-tymidine were mediated by separate transporters, designated TbU3 and TbT1, respectively. TbU1 is a procyclic high affinity uracil transporter, with a K_m value of 1.5±0.3 μM, similar to the value for U3 (1.1±0.3 μM) but, unlike TbU1, TbU3 is not competitively inhibited by uridine. Thymidine uptake is slow in BSF but detectable at 10 μM; it was not inhibited by uracil which indicates that this is mediated by a separate thymidine transporter (T1). The trypanocidal activity of pyrimidine analogues was tested; uridine analogues showed no effect against BSF up to 1 mM, whereas pyrimidine nucleobase analogues and 2'-deoxynucleosides display micromolar activity. We have induced resistance to 5-fluorouracil (137-fold), 5-fluoroorotic acid (125-fold) and 5-F2'deoxyuridine (830-fold) by *in vitro* exposure of BSF to stepwise increased concentrations, although pyrimidine transport was essentially unchanged in the resistant clones. However, metabolomic analysis of fluorinated pyrimidines in resistance cell lines showed a significant reduction in the level of 5-F-UDP-glucose. In addition, 5-fluoroorotic acid resistant cells show a significant reduction in 5-fluorouridine nucleotides. Cells treated with 5-F2'deoxyuridine show an increase in dUMP, which suggest a block in thymidylate synthase or possibly thymidylate kinase. Extracellular thymidine protected trypanosomes against 5F-2'deoxyuridine but not 5-fluorouracil.

P181 The Kinetoplast: Adapting to Loss and Other Tales of Resistance

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In trypanosomes, mitochondrial DNA takes the form of the kinetoplast; replication and expression of which is normally essential in procyclic and bloodstream form *Trypanosoma brucei*. A number of current and lead chemotherapies are known to accumulate in the parasite mitochondrion where they bind to kinetoplast DNA (kDNA); a long-standing question in the field is whether this property is related to their mode of action. Using polymorphisms in ATP synthase gamma previously identified (1, 2), we have generated transgenic *T. brucei* bloodstream forms capable of surviving kDNA loss. The otherwise isogenic character of our kinetoplast-dependent and -independent strains makes them ideal tools to resolve questions of mitochondrial DNA targeting by current drugs as well as new lead compounds. We show that a single point mutation in ATP synthase gamma gives rise to significant, sometimes dramatic cross-resistance to key compound classes, including the diamidines (pentamidine, diminazene and DB compounds) and phenanthridines (isometamidium, homidium). Preliminary data on the extent to which this is due to changes in drug transport will be presented. Measuring differential sensitivities to specific inhibitors of mitochondrial activities also allowed us to shed light on the molecular mechanism allowing cell survival in the absence of kDNA.

(1) Schnaufer, A et al (2005) *EMBO J* **24**:4029-4040
(2) Lai, D-H et al (2008) *PNAS* **105**:1999-2004

P182* Inhibitors of *T. b.* brucei cAMP phosphodiesterases: promising therapeutic lead and valuable experimental tool.

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The role of cAMP in the regulation of cellular activities in kinetoplastid parasites is still not well understood. Numerous adenylyl cyclases have been identified and the phosphodiesterases PDEB1 and PDEB2 have been shown to be essential but further progress has been hampered by a lack of experimental tools to manipulate cellular cAMP levels in trypanosomes. Inhibitors of the TbPDEBs were identified through a high-throughput screen. A selection of the most potent PDEB inhibitors displayed similarly potent antitrypanosomal activity and increased cellular cAMP content and the lead compound, CpdA, was characterised further. The effects on cAMP were dose dependent and cAMP levels increased rapidly over extended periods, leading to growth arrest and eventual cell death. Cell cycle analysis and DNA content flow cytometry showed that DNA synthesis and division/separation of kinetoplasts and nuclei progressed as normal, but that cytokinesis was not completed before the start of another round of DNA synthesis and cell division. The high cAMP level appeared to specifically disrupt the final cytokinesis stage of abscission. CpdA-resistant cells were generated, but did not display altered cAMP response to CpdA. Instead, the cells had become impervious to elevated cAMP levels.

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P184 Novel insight into molecular mechanisms relevant for *Leishmania donovani* intracellular survival by comparative proteomics

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Leishmania parasites are important human pathogens that differentiate inside host cells into an amastigote life cycle stage responsible for the pathogenesis of *leishmaniasis*. We previously compared morphology, infectivity and protein expression of *Leishmania donovani* either grown in culture (axenic), or exclusively propagated in hamsters, with the aim to reveal parasite traits absent in axenic amastigotes but selected for in hamster-derived parasites through leishmanicidal host activities. Axenic and hamster-derived amastigotes showed striking differences in virulence and the ability to cause experimental hepato-splenomegaly in infected hamsters. Quantitative 2D-DIGE analysis revealed statistically significant differences in abundance for 7% of the detected proteomes. Various enzymes either implicated in protein and amino-acid metabolism or linked to intracellular parasite survival showed increased abundance in hamster-derived amastigotes. We previously showed that in absence of transcriptional control, post-translational regulation through differential protein phosphorylation may play an important role in *Leishmania*. We currently perform quantitative analysis of axenic and hamster-derived amastigote phosphoproteomes using LC-MS/MS in combination with biosynthetic and chemical labeling procedures with the aim to identify signaling events specifically selected for parasite intracellular survival and proliferation. The application of these technologies and the biological significance of obtained results for *Leishmania* virulence and pathogenicity will be discussed.

P185* The essential *Leishmania major* MAP kinase LmaMPK4 regulates differentiation of virulent metacyclic promastigotes

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We studied the role of the *L. major* MAP kinase LmaMPK4 using a novel knock out system based on the episome pXNG that renders transgenic parasites sensitive to the drug ganciclovir (GCV). LmaMPK4 null mutants established in pXNG-MPK4 transgenic parasites retained pXNG-MPK4 during negative GCV selection despite the toxic effect of the drug, showing that LmaMPK4 expression is essential for L. major promastigote viability in culture. Structure/function analysis of MPK4 mutants using a plasmid shuffle approach allowed us to establish viable mpk4-/- parasites expressing kinase dead MPK4_K59R. These parasites were normal in promastigote growth and morphology, but showed strong virulence attenuation in macrophage infection assays as a result of reduced metacyclic differentiation during stationary culture. The genetic approaches presented here allow new insight into the function of an essential *Leishmania* protein kinase, which escapes classical knock out analyses due to the lethal null mutant phenotype. Our data dissociate kinase-dependent and -independent MPK4 functions and demonstrate an essential role for MPK4 expression in parasite viability and MPK4 phospho-transferase activity in metacyclic differentiation and virulence.

P186* Analysis of non activation lip phosphorylation sites in LmxMPK1

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LmxMPK1 is a MAP kinase homologue from *Leishmania mexicana* that is essential for the mammalian stage of the life cycle. This class of signalling molecules are present in almost every eukaryote and are canonically regulated by phosphorylation (to activate) and dephosphorylation (to inactivate) on both the threonine and tyrosine residues in the TXY motif of the activation lip. Recombinant LmxMPK1 was phosphorylated on the tyrosine of the activation lip but not the threonine, however a threonine residue 48 amino acids downstream (threonine 224) was found to be phosphorylated (the same residues were phosphorylated in the amastigote stage of the parasite). Co-expression of LmxMPK1 with one of three phosphatases (the dual-specificity Lambda-phosphatase, human Protein-Tyrosine Phosphatase-1β and a novel *Leishmania* protein tyrosine phosphatase homologue, LmxPTP) resulted in differential dephosphorylation of LmxMPK1, affecting but not ablating the ability of the kinase to autophosphorylate and phosphorylate myelin basic protein (MBP). Hence, phosphorylation of further residues outside the phosphorylation lip like threonine 224 has a modulating effect on kinase activity.

P187 Chemical inducers of stumpy formation in *Trypanosoma brucei.*

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During the bloodstream stage of the *Trypanosoma brucei* lifecycle, the parasite exists as the proliferative slender-form or the non-proliferative, transmissible, stumpy-form. The transition from the slender to stumpy-form is stimulated by a density-dependent mechanism and is important in infection dynamics, ordered antigenic variation and disease transmissibility.
Here, we use a monomorphic reporter cell line in a whole-cell fluorescence-based assay to screen over 6000 kinase inhibitors for their ability to induce stumpy-like formation in a high-throughput screening programme. The primary screen generated over 190 hits, most of which were validated in titration assays. After removing a number of auto-fluorescent compounds, the chemical structures of the remaining hits were compared in order to reveal common chemical series, which are now undergoing further analysis.
The identification of chemical inducers of stumpy formation offer two opportunities: Firstly, compounds able to induce stumpy formation may have anti-virulence potential and, secondly, identification of the targets of stumpy-inducing compounds provides tools to aid in the dissection of the stumpy induction pathway.

P188* Functional genetics characterization of the *Leishmania* MAP kinase MPK10

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We previously showed amastigote-specific activity and phosphorylation of the *Leishmania major* MAP kinase MPK10. Here we used genetics approaches in combination with mutagenesis analysis to gain insight into regulation and function of this protein kinase in environmentally-induced parasite differentiation. We first expressed various GFP-tagged MPK10 mutants in transgenic *L. donovani* and tested activity of these derivatives by in vitro kinase assay using affinity purified proteins obtained from promastigote and axenic amastigote extracts. This transgenic strategy allowed us to confirm the essential role of the conserved THY motif in kinase activation, and revealed an unexpected auto-inhibitory role of the parasite-specific C-terminal domain. By utilizing a bottom up proteomics approach we identified a unique phospho-serine residue in this domain and demonstrated by mutagenesis analysis its requirement for auto-inhibition. We next investigated this protein kinase by loss-of-function, deleting the endogenous *L. major* and *L. donovani* MPK10 alleles. The resulting *L. donovani* mutants showed normal growth and morphology at the promastigote stage but failed to convert into axenic amastigotes and underwent massive cell death in response to acidic pH and elevated temperature that trigger amastigote differentiation. Thus *Leishmania* MPK10 is regulated by auto-inhibitory intra-molecular interactions, and is essential for the development of the pathogenic amastigote stage.

P190* Trafficking and functional characterization of a novel family of type I trans-membrane proteins in *Trypanosoma brucei*

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Extensive studies on the major surface type I trans-membrane proteins, invariant surface glycoproteins 65 and 75 (ISG65/75), revealed highly conserved lysine residues in the cytoplasmic region, demonstrated to be involved in ubiquitin-mediated trafficking and controlling copy number. Here, we have analysed trafficking constraints and signals required for targeting of a novel family of type I trans-membrane domain proteins distinct from ISG65/75. These trypanosome-specific proteins are highly N-glycosylated with short, lysine-rich cytoplasmic domains and can be grouped into three distinct subfamilies, designated as IGP48, IGP40 and IGP34. Members of each sub-family are expressed at the mRNA level in both major life-cycle stages, with up-regulation seen in the short stumpy stage shown by quantitative real-time PCR (qRT-PCR). Trafficking was examined by generating chimeric proteins containing the C-terminal portion of representative proteins from the two larger subfamilies IGP48 and IGP40 (encompassing 23 C-terminal residues of the extracellular domain plus the trans-membrane and cytoplasmic domains) fused to the N-terminal domain of BiP (BiPN). Immunofluorescence analysis shows epitope-tagged versions of the full-length protein are associated with the endoplasmic reticulum. However, BiPN versions co-localise with endosomes and biotinylation assays show this construct accesses the cell surface. RNA interference (RNAi) indicates the family is required for robust growth and normal cellular proliferation.

P191* *Trypanosoma brucei* BRCA2: BRC-mediated RAD51 interaction and genome stability

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BRCA2 is a key factor in homologous recombination, interacting with Rad51 via conserved BRC repeats that mediate Rad51-directed homologous strand exchange. *T. brucei* BRCA2 displays a striking expansion in BRC repeat number, which appears not to be needed for the activation of intact *VSG* genes during antigenic variation. Bloodstream stage *T. brucei brca2-/-* mutants of strain Lister 427, examined after prolonged growth, display chromosomal rearrangements resulting from the loss of genetic material from the megabase chromosomes, including copies of at least one *VSG*. To ask if BRCA2 functions to maintain the *VSG* subtelomeric archive, and to investigate the basis of chromosomal rearrangements, *brca2*-/- mutants were made in procyclic form *T. brucei* of strains TREU927 (where the *VSG* archive has been positionally annotated) and Lister 427. Surprisingly, the *brca2*-/- mutants are deficient in DNA damage repair, but do not display detectable chromosomal rearrangements. It is therefore possible that *T. brucei* BRCA2 acts in a bloodstream stage-specific process that is suppressed in procyclic form cells. In addition, we have made BRCA2 variants, in both life cycle stages, possessing BRC repeat numbers varying from one to twelve, and will discuss how these function in DNA repair and in RAD51 subcellular dynamics. Finally, we will discuss the surprising complexity of BRCA2-RAD51 *in vivo* interactions in *T. brucei*.
propose that IGP48 represents an essential ER-localized protein family, likely with specialized roles associated with differentiation.

P192 Sand fly fauna (Diptera: Psychodidae) of Tajikistan, Central Asia, an endemic country of leishmaniasis

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Introduction: Tajikistan, an ex-soviet Republic, suffered a civil war after becoming independent in 1991. Due to political and economic instability vector control programmes were interrupted. There is scarce data about the actual situation of leishmaniasis and sand fly vectors in the area. **Methods:** In August 2010, six districts of Tajikistan were sampled, using CDC mini-light traps, sticky traps and mouth aspirators. Specimens were morphologically identified (Artemiev,1978) and a fragment of *Cytochrome b* (mitDNA) (Parvizi, 2006) was amplified and sequenced for species confirmation and population analysis.
Trypanosomatidae infection was studied by RT-PCR targeting *ssrRNA* gene (Prina, 2007). Further characterisation was performed using primers for *kDNA minicircle1*, *L.amazonensis kDNA 1*, *Leptomonas GAPDH2* and *MAG-1* (Weirather, 2011).
Results: 395 specimens were caught, 241 males and 154 females. *Phlebotomus sergenti* was the most prevalent species (49.3%) followed by *P.keshishiani* (17.4%), *P.angustus* (13.92%), *P.papatasi* (11.39%), *Sergentomyia* spp(6.3%), *P.caucasicus* (1.01%) and *P.alexandri* (0.5%). Phylogenetic analysis showed two different lineages of *P.angustus*, three of *P.keshishiani* and a homogeneous population among *P.sergenti* and *P.papatasi* specimens. 10% of the specimens analysed were positive for Trypanosomatidae and 5% for *Leishmania* spp.

P193* Functional genetics characterization of the *Leishmania* MAP kinase MPK10

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We previously showed amastigote-specific activity and phosphorylation of the *Leishmania major* MAP kinase MPK10. Here we used genetics approaches in combination with mutagenesis analysis to gain insight into regulation and function of this protein kinase in environmentally-induced parasite differentiation. We first expressed various GFP-tagged MPK10 mutants in transgenic *L. donovani* and tested activity of these derivatives by in vitro kinase assay using affinity purified proteins obtained from promastigote and axenic amastigote extracts. This transgenic strategy allowed us to confirm the essential role of the conserved THY motif in kinase activation, and revealed an unexpected auto-inhibitory role of the parasite-specific C-terminal domain. By utilizing a bottom up proteomics approach we identified a unique phospho-serine residue in this domain and demonstrated by mutagenesis analysis its requirement for auto-inhibition. We next investigated this protein kinase by loss-of-function, deleting the endogenous *L. major* and *L. donovani* MPK10 alleles. The resulting *L. donovani* mutants showed normal growth and morphology at the promastigote stage but failed to convert into axenic amastigotes and underwent massive cell death in response to acidic pH and elevated temperature that trigger amastigote differentiation. Thus *Leishmania* MPK10 is regulated by auto-inhibitory intra-molecular interactions, and is essential for the development of the pathogenic amastigote stage.

P194 *Trypanosoma cruzi* trans-sialidase activity stimulates G-protein regulated uptake of microparticles.

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Imunologia e Parasitologia, Universidade Federal de Santa Catarina, Florianópolis, Santa Catarina, Brazil.

Trans-sialidase activity catalyses the transfer of alpha-(2-->3)-sialic acids from host cell glycoconjugates to the cell surface of *Trypanosoma cruzi*, a process previously associated with invasion of the parasite. Here we have coated active (TcTS) and inactive (TcTS2V0) recombinant trans-sialidase onto latex beads and followed their uptake by MDCK II cells. Both proteins induce cholesterol-dependent, actin-mediated entry of beads. Laurdan microscopy showed increased liquid order at the bead-cell interface. Fluorescence imaging showed accumulation of caveolin-1 in the region of the bead but siRNA of cav1 does not functionally affect uptake. TcTS coated beads showed higher levels of attachment and entry than the TcTS2V0 coated beads. This increased entry was ablated by pertussis toxin, identifying parasite trans-sialidase activity as a modulator of the host cellular response via G protein signalling. Our results suggest that active and inactive trans-sialidase share a common method for attachment requiring raft formation and that active trans-sialidases trigger a supplemental G-protein dependent internalisation. This evidence clarifies the role of trans-sialidases in *T. cruzi* invasion and reinforces their importance as a therapeutic target for the future.

P195 Use of pyrosequencing to identify SNPs in the ß-tubulin gene associated with benzimidazole resistance in *Teladorsagia circumcincta* and *Haemonchus contortus* isolated from UK field samples

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A survey of ovine nematodes present on UK farms was carried out from 2008-2010. This project involved gathering questionnaire data detailing farming practices and the collection of faecal samples from ewes and lambs from farms across the UK. From these faecal samples, first stage larvae (L1) were cultured and stored in ethanol to form a biobank. Farms with high numbers of *Teladorsagia circumcincta* and *Haemonchus contortus* were identified for each study year. Pyrosequencing analysis was used for SNP detection to identify whether individual worms carried the polymorphism at codon 167, 198 or 200 on the ß-tubulin gene, which is the major genetic determinant for resistance to benzimidazoles (BZ). Initial results showed that BZ resistance-associated polymorphisms were found in *T. circumcincta,* primarily at codon 200 and in *H. contortus,* primarily at codon 167, on the selected study farms. Both heterozygous and homozygous genotypes were observed. By combining these results with information on farming practices (including anthelmintic treatment history) from the questionnaire data, we can begin to build a picture of the patterns of BZ resistance in the field in the UK.

P196 Cryptosporidiosis in Scottish beef suckler herds

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There have been reports of increased cryptosporidiosis in calves on Scottish beef suckler farms. Information about *Cryptosporidium* species infecting cattle in Scotland and the effectiveness of control measures is scarce. A study was conducted to indicate the extent of the problem, identify *Cryptosporidium* species present and to describe farmer practices used to manage neonatal diarrhoea.
A questionnaire was administered to 41 farms in Aberdeenshire and Caithness in spring 2011 regarding husbandry practices, preventive & therapeutic disease control strategies and calf mortality and morbidity rates. Faecal samples from calves, 7-14 days old; cows and heifers prepartum and at weaning and diarrheic calves were tested for *Cryptosporidium* species and genotypes by PCR amplification and sequencing. A follow-up questionnaire was conducted at the end of calving.
Questionnaires were completed by 39/41 farmers and 28 farms submitted 191 faecal calf samples. There were no significant differences in management practices so data were pooled. The median incidence of diarrhoea in calves was 6% (0 to 80%). Median mortality was 0.7% (0 to 10.5%). No farm characteristics or management strategies were significantly associated with the diarrhoea-associated morbidity or mortality. *Cryptosporidium parvum* was confirmed by 18S rRNA sequencing on 19/23 farms. The dominant GP60 genotype was IIaA15G2R1, which was found in samples from all 17 farms on which GP60 was sequenced.

P197 Stage-specific suppressive activity of *Teladorsagia circumcincta* ES components on ovine peripheral blood lymphocyte proliferation

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Teladorsagia circumcincta is an important pathogenic nematode of sheep in the UK. We have recently demonstrated that excretory-secretory (ES) products derived from fourth stage larvae of *T. circumcincta* (*Tci*-L4-ES) are capable of suppressing both antigen-specific and mitogen-induced proliferation of ovine lymphocytes. The identity of the immunosuppressive component(s) within *Tci*-L4-ES is unknown. In this study, the effect of stage-specific *T. circumcincta* ES on ovine peripheral blood lymphocytes was investigated.
ES products were generated from third stage larvae (*Tci*-L3-ES), fourth stage larvae (*Tci*-L4-ES) and adult *T. circumcincta* (*Tci*-adult-ES) and their effect on mitogen-induced proliferation of ovine lymphocytes assessed *in vitro*. Suppression of proliferation was observed with each stage-specific ES, with *Tci*-L3 ES being most suppressive and *Tci*-L4 ES least suppressive. Suppression by all stage-specific ES was abolished upon heat-inactivation, suggesting the effects were protein-mediated. Identification of the immunosuppressive components of *Tci*-ES is being investigated using size fractionation of whole *Tci*-L4-ES by gel filtration chromatography. One fraction was observed to retain the majority of the suppressive effect on ovine lymphocytes. Analysis by SDS-PAGE and silver stain of this fraction demonstrated the presence of four unique proteins ranging in size from 6-15 kDa. These are currently being analyzed by mass-spectrometry and may represent crucial factors by which *T. circumcincta* modulates the host adaptive immune response.

P198 The Genomics and Phenomics of the Fatty Acid Binding Protein Superfamily of *Fasciola hepatica* and *Fasciola gigantica*

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The Fatty Acid Binding Protein (FABP) superfamily from the temperate and tropical liver flukes, *Fasciola hepatica* and *F. gigiantica*, have been the subject of many recent scientific studies as potential immune-diagnostic/therapeutic candidates. Despite initial promise, development of a Fascioliasis vaccine based upon FABP has stalled. An incomplete understanding of the FABP superfamily in *Fasciola* sp. may be responsible for the lack of success. Therefore, with the aid of modern molecular biology we have investigated the FABP superfamily from both *F. hepatica* and *F. gigantica* combining high resolution 2DE proteomics and next generation sequencing projects to aim to gain a complete picture of the genomics and phenomics of this vaccine candidate superfamily. To this end, two new FABP isoforms, present in both species, have been identified; deemed FABP IV and FABP V. The antigenic profile of the complete FABP superfamily has also been investigated. Recombinant forms of both FABP IV and V have been produced to allow a biochemical analysis of each.

P199* Phylogenetic Analysis of Lymnaeid Snails from Northern Ireland and Southern India Based on 18s rDNA and Mitochondrial Sequences

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Snails were collected from different farms of Northern Ireland and Southern India where outbreaks of Fasciolosis had been reported. As the traditional use of morphological features to identify snails requires expertise and is sometimes error-prone we have used the 18s rDNA and mitochondrial COI sequences of these snails. These sequences were selected since they are useful in elucidating phylogenetic relationships and subgroup distinctiveness due to their high degree of variation and high copy number. Sequence analyses revealed the presence of variable species of snails in the different farms and also within the same farm. Three genuses (*Galba, Stagnicola* and *Radix*) were observed in Ireland with *Galba* being the most prevalent. Climatic status of the region and the intensity of the trematode infection in the farm were important factors. The results from Ireland where *F. hepatica* is present have been compared to those from two different climatic regions in India where the infecting trematode is *F. gigantica*. Such phylogenetic tracking is essential to our understanding of the genetic compatibility in such host parasite relationships.

P200* Transcriptomic analysis of *Ascaris suum* larvae during their hepatopulmonary migration.

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Common roundworms are important intestinal nematodes of man (*Ascaris lumbricoides*) and pig (*Ascaris suum*). During the first stages of the infection, the larvae of these parasites undergo a hepatopulmonary migration. This migration is likely to require tightly regulated transcriptional changes in the parasite. We explored this aspect in *Ascaris suum* by characterizing the transcription profiles of infective L3s from eggs, liver- and lung-L3s and intestinal L4s by next generation sequencing approach. When the most abundant transcripts per life stage were investigated, results showed that in the egg-L3s, transcripts associated with the regulation of translation and transcription, mainly ribosomal proteins, were most abundant. From the liver-L3s onwards, high transcription levels were seen for cuticle collagens, indicating the growth of the larvae during their migration. Interestingly, the type of highly expressed cuticle collagens in the intestinal L4s differed with those present in the liver- and lung-L3s. Apart from collagens, potentially important molecules for host-parasite interaction like C-type lectin-4 and Mucin-5 were in the top 5 of most abundant transcripts in the lung-L3. Unfortunately, a great number of transcripts that are specific for certain larval stages did not show any homology to other proteins within the NCBI database, suggesting that many biologically interesting molecules from this parasite are still to be investigated.

P201* Prevalence of Trypanosomiasis within small ruminant species in the Kachia Grazing Reserve, Central Nigeria.

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Small ruminants are known reservoirs of African animal trypanosomiasis. The effect of this reservoir was studied within the Kachia Grazing Reserve, Kaduna State, Central Nigeria. This region has been set aside by the Nigerian government for Fulani settlement. The predominant livestock species are White Fulani cattle which are still regularly taken on migration for grazing outside the reserve. It is hypothesised that although tsetse levels are low within the reserve the animals may contract trypanosomiasis when on migration. Sheep and goats are seen as having secondary importance in relation to cattle and are unlikely to receive treatment when showing signs of trypanosomiasis as a result of their perceived lower importance.
This study will assess how differential husbandry factors between these two species, and when compared to cattle, may affect the epidemiology of the disease. For example sheep are often involved in migratory movements whereas the goats are kept in the vicinity of the household.
The study was conducted at the end of the rainy season, October 2011. A total of 717 Yankasa sheep and 752 Sokoto Red goats were randomly selected for blood sampling. Blood samples were stored on FTA cards and transported to the University of Edinburgh, for analysis. Trypanosomes were identified using an internal transcribed spacer (ITS) PCR. This technique produces amplicons of differing lengths depending on trypanosome species present.
The prevalence data from this study will be presented. The outcomes will be discussed in terms of planning future treatment interventions.

P202 Study of rLc36 gene as a new antigen for canine visceral leishmaniasis serodiagnosis

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Visceral leishmaniasis is the most severe clinical form and can be fatal if untreated. A specific and accurate diagnosis is required for identification of infected dogs, which are important *Leishmania sp* reservoirs, to provide better epidemiological control in order to interrupt the disease transmission to humans. Parasite demonstration and serological tests present limitations due to low parasitemia and false positive (use of entire parasite as antigen causing cross reactions), respectively. So that, there is urgent need of development of trustworthy diagnosis tests. This work reports isolation and characterization of one specific *Leishmania chagasi* gene, named rLc36, and its product as a hypothetical antigen for a new diagnosis method. On-line tools were used to obtain *L. chagasi* sequences (http://www.genedb.org). Partial rLc36 gene was amplified with specific primers, cloned in pET28a, and expressed in *E. coli* BL21 DE3 strain. The rLc36 recombinant protein was purified by nickel column. Different concentrations of the protein were tested by *ELISA* using serum from infected dogs and from health dogs. The recombinant protein concentrations of 7μg/mL and 8μg/mL were detected in the *ELISA* tests, and it strongly reacted with infected dogs´ serum in preliminary results, so it has shown potential to be used in diagnosis tests.

P203* Detection of *Rickettsia africae* in *Amblyomma variegatum* (Fabricius, 1794) ticks from Nigeria

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Rickettsia africae is the causative agent of African tick-bite fever (ATBF), an emerging infectious disease endemic in rural sub-Saharan Africa (SSA), the most important rickettsioses in travel medicine. The disease consists of a febrile illness, with influenza-like symptoms including headache, prominent neck myalgia, inoculation eschars, regional lymphadenitis, and apthous stomatitis. *Rickettsia africae* is transmitted by *Amblyomma* ticks (i.e. *Amblyomma variegatum* and *Amblyomma hebraeum*), which serve as both vector and reservoir of the pathogen. The present study aims to detect the presence of *R. africae* DNA in *A. variegatum*, the most widespread *Amblyomma* species in SSA. In June and October 2010, adult and immature *Amblyomma variegatum* ticks were collected from indigenous Zebu (i.e. *Bos indicus*) cattle of various sex and age in Plateau State, Nigeria. All ticks were morphologically identified using taxonomical keys and preserved in 70% ethanol from the time of collection until the molecular processing. DNA of washed ticks was extracted using DNeasy blood & tissue kit (Qiagen®), and polymerase chain reactions (PCRs) for rickettsial citrate synthase (gltA), 16s rRNA, and outer membrane protein (ompA) genes were carried out. DNA from positive samples was purified using the QIAquick PCR purification kit (Qiagen®), and later on sequenced by the use of an automated sequencer. All obtained sequences were compared to those available in GenBank. Preliminary results from the molecular screening and sequence analysis will be conveyed.

4. **P204* The relationship of large intestinal mast cell numbers to cyathostomin burden in horses**
5. Ruth Clements[1], Tom McNeilly[1], Kirstie Pickles[2], John Keen[2] and Jacqui Matthews[1]
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7. Cyathostomins are potentially life-threatening parasitic nematodes of horses and are highly prevalent worldwide. Infected animals may be asymptomatic or show clinical signs of weight loss, diarrhoea and colic. Third and fourth stage larvae spend a large proportion of their lifecycle encysted in the large intestinal wall where they cannot be detected.
8. Mast cells and their associated proteinases have been implicated in the protective host immune response against nematode infections. Mast cell infiltration in the large intestine of horses with cyathostomin infection has been demonstrated previously. Here, to test association of these cells with levels of infection, equine tissue samples from the caecum, right ventral colon and rectum were collected from an equine abattoir (n=16) and the R(D)SVS (n=6). Faecal egg counts, luminal counts, trans-mural illumination and pepsin digestion were performed to enumerate cyathostomin burden. Tissue samples were collected in Carnoys fixative and mast cells were enumerated following rehydration and overnight staining with 0.5% Toludine Blue in 0.5M HCL, pH 0.5 and counterstaining with 1% eosin in 70% ethanol. A variation in cyathostomin burden was observed in the samples collected. Correlations between cyathostomin burdens and mast cell numbers will be presented. This study will further define the role of mast cells in cyathostomin infection and will investigate their utility as diagnostic markers of infection.
9.
10. **P205* Genetic variability in beta-tubulin genes in benzimidazole-resistant and susceptible strains in *Haemonchus contortus***
11. Shamaila Irum, Michael Stear, Mazhar Qayyum
12. University of Glasgow
13. Infections caused by gastrointestinal nematodes are a major threat to livestock industry all over the world. They cause production losses each year and result in mortality in extreme conditions. There has always been search for methods to control parasitism especially helminthiosis. Use of synthetic drugs is being practiced for a long time and various groups of anthelmintic drugs are available in market. However, there is widespread emergence of anthelmintic resistance to almost all groups of anthelmintics presently available. There have been reports from various parts of world about resistant strains emerging especially in Haemonchus contortus, a highly pathogenic nematode. Benzimidazole is among the prominent anthelmintic groups against which resistance is emerging very fast. The mechanism of benzimidazole resistance appears to be most common in many species ranging from fungi to nematodes and involves alteration in gene encoding β tubulin. Present study was carried out to find the variation existing in β tubulin isotype-1 gene. Adult nematode Haemonchus contortus were subjected to DNA isolation according to manufacturer's instruction using DNA isolation kit. Amplification reaction was performed according to parameters mentioned in the literature using specific primers for β tubulin isotype-1. Sequencing was carried out using the bio-sequencer and the analysis was done using Chromas for chromatogram analysis and CLC genomics was used for BLAST search. Out of 50 individuals analyzed 37 showed benzimidazole susceptible gene while 13 were resistant indicating single nucleotide mutation at amino acid 200 TTC/TAC. In 12 organisms several regions of consistent difference were recognized indicating single nucleotide polymorphism at various positions in coding region. The sequences for β tubulin for Haemonchus contortus also showed varying degree of similarity with different organisms in BLAST search. This was the first study carried out for β tubulin isotype-1 with samples from Rawalpindi, Pakistan and the experiment was conducted at Veterinary School, University of Glasgow

P206 PROTOZOAN PARASITES OF GAZELLES AND ORYX IN SAUDI ARABIA

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Protozoan parasites in Arabian gazelles and oryx are mainly gut dwelling and cyst forming coccidia. Four species and subspecies of gazelles, *Gazella gazella* ssp (idmi),

Gazella gazella erlangeri (erlangeri), *Gazella subgutturosa marica* (reem) and *gazella dorcas* together with the Arabian oryx (*Oryx leucoryx*) are raised at King Khalid Wildlife Research Centre (KKWRC) for breeding and research purposes. Coccidian parasites of the genus *Eimeria* has been reported from gazelles, *Eimeria rheemi* and *Eimeria idmii* and *Eimeria dorcadis*. The only eimerian parasites reported from the oryx, *Eimeria saudiensis*, appeared to be non pathogenic. *Cryptosporidium parvum* has been reported only from the Arabian oryx and it was associated with mortalities in calves. Cyst forming coccidia infecting gazelles included *Sarcocystis* spp, *Toxoplasma gondii* and *Hammondia triffittae*. *Sarcocystis* parasites have been detected in 54.9% of idmi, 74.4% of reem, 35.7% of erlangeri and 83.9% of dorcas gazelles. *Toxoplasma gondii* was reported from 5.9% of idmi, 4% of reem, 4.3% of dorcas and 3.3% of erlangeri gazelles. *Hammondia triffittae* has been detected indirectly from an idmi gazelle as a result of feeding foxes meat from gazelles infected with *Sarcocystis* parasites when investigating the life cycle of *Sarcocystis* parasites in gazelles. Of the cysts forming coccidian parasites only *Sarcocystis* parasites have been detected microscopically from the Arabian oryx.

P207* Antinematode effects of Cyclosporin A in *Caenorhabditis elegans*

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Cyclosporin A (CsA) is an immunosuppressant that produces antinematode effects as demonstrated in *C. elegans*, disrupting the formation of the collagen-rich nematode cuticle and preventing its correct moulting between life cycle stages. This has been related to the binding of CsA to cyclophilins, inhibiting their peptidyl-prolyl isomerase (PPIase) activity, the rate-limiting step of collagen formation. As a vital structure to the body of the nematode, disruption to the cuticle causes severe morphological defects, as demonstrated by mutations in related genes and produced by CsA, such as short, fat “dumpy” worms, unshed cuticles and malformed guts. Novel, synthetic cyclophilin-inhibitors have previously been developed to mimic the inhibitory activity of CsA, and these have been shown to produce similar effects in *C. elegans* and *Haemonchus contortus, in vitro.* Microarray and proteomics approaches were utilised to study the effects of these compounds on gene and protein expression in *C. elegans*. Chaperones, nuclear hormone receptors and xenobiotic metabolising enzymes, amongst others, were identified to be differentially regulated, and may therefore be involved with the action of the compounds or their metabolism and clearance. Common *C. elegans* methods, such as mutagenesis, antibody staining, GFP reporters and RNAi are being utilised towards identifying and characterising the genes and proteins involved with the uptake, action, metabolism or clearance of the compounds.

P208* Regulation of parasitic nematode development by microRNAs

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The gastrointestinal nematode *Haemonchus contortus* causes major economic and welfare problems in small ruminants worldwide. As with other parasitic nematodes of sheep and cattle, resistance of *H. contortus* to the major anthelmintics has increased significantly, thus identification of novel control targets is urgently needed.
The *H. contortus* genome sequencing project is nearing completion at the Sanger Institute. Using the genome data combined with small RNA sequencing and homology-based discovery, we have identified 192 microRNAs from *H. contortus*. microRNAs are 22 nucleotide non-coding RNAs that regulate post-transcriptional gene expression and are essential for correct programmed development in the free-living nematode *Caenorhabdits elegans* and in higher organisms. Importantly, a number of *H. contortus* miRNAs show differential expression in infective L3 larvae and adult worms and between male and female parasites, suggesting important roles in development and reproduction. The expression patterns of selected miRNAs of interest have been confirmed by quantitative RT-PCR and pull-down assays are being developed to identify miRNA-target gene interactions. Several developmentally regulated miRNAs of *H. contortus* are conserved in *C. elegans* and bioinformatic prediction programmes combined with mutant analysis are being used to characterise their potential roles.
Our goal is to identify miRNAs regulating key developmental pathways and to determine whether interfering with essential miRNA function offers a novel approach to parasitic nematode control.

P209* Genetic mapping and transient transgenesis - tools supporting identification of anti-apicomplexan vaccine candidates in *Eimeria* genomes

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Coccidiosis is a disease caused by the apicomplexan *Eimeria* species. Control of these parasites has largely been based upon chemotherapy or live vaccination. Genes underlying susceptibility to immune or chemical killing have obvious relevance to the development of novel anticoccidial control strategies, but their identification is demanding. Sustainable alternatives are being sought but, in common with other apicomplexan parasites, differentiating immunoprotective from immunogenic antigens has proven difficult.
Studies with *Eimeria maxima*, the most immunogenic of the *Eimeria* species that infect the chicken, have previously identified a selectable strain-specific ability to induce sterile immune protection against secondary challenge that characterises some inbred chicken lines. Building on these studies a mapping panel created by crossing antigenically-distinct *E. maxima* strains has been analysed using a population-based genetic mapping strategy. Application of our strategy has revealed that the strain-specific immune response targets just six discrete regions of the *E. maxima* genome absolutely. In the absence of an annotated *E. maxima* genome sequence locus screening based upon genome fragmentation and transient parasite-transfection has identified apical membrane antigen-1 (AMA-1) and immune mapped protein-1 (IMP-1) as genuine anticoccidial vaccine candidates. The conserved vaccinal potential of AMA-1 described for many apicomplexan genera, and the identification of putative IMP-1 homologues in other coccidial genomes, promotes the use of our strategy to identify novel anti-apicomplexan vaccine candidates.

P210* N-linked glycosylation and oligosaccharyltransferase essentialities in *Trypanosoma brucei*

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N-linked glycosylation is a ubiquitous post translational protein modification. *Trypanosoma brucei*, the causative agent of African sleeping sickness, has many essential glycoproteins and a dense glycocojugate coat. The mammalian oligosaccharyltransferase has 8 subunits whereas the trypanosome has only the catalytic subunit STT3, suggesting a possible therapeutic target. A further difference between host and parasite is that mammals have only one N-glycan precursor whereas *T. brucei* has two, $Man_5GlcNAc_2$ and $Man_9GlcNAc_2$, which are co- and post-translationally transferred, respectively. Trypanosomes have three very similar STT3 genes - *Tb*STT3A, *Tb*STT3B and *Tb*STT3C - in tandem array. *Tb*STT3A and *Tb*STT3B are expressed in bloodstream form (in human or cattle hosts) and procyclic form (in the tse-tse fly) but *Tb*STT3C is not expressed in either life cycle stage. Knockdown experiments showed *Tb*STT3A and *Tb*STT3B are essential *in vivo* but previous knockout experiments were unsuccessful.
To better understand the function of *Tb*STT3A and *Tb*STT3B we are using gene replacement strategy. To overcome the knockout difficulties with similar genes and their repetitive flanking regions, our new strategy is to insert an ectopic *Tb*STT3A copy into bloodstream form using pLEW100 then *in-situ* tag the endogenous *Tb*STT3A gene with YFP-3xHA under HYG selection. This unique *in-situ* tag should allow knockout of *Tb*STT3A and subsequently *Tb*STT3B in addition to localization studies and further investigation of this important enzyme.

P211 Histological Changes in Organs of Experimental Rats Infected with *Trypanosoma congolense*

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Different experimental trypanosomiasis research have been carried out successfully and widely established in areas such as pathophysiological changes, chemotherapeutic investigations and other biological functions. Experimental trypanosomiasis in animals has made immeasurable contributions to the knowledge of the disease in literature. This paper aims to identify the effects of experimental *Trypanosoma congolense* infection in some organs of albino rats. It demonstrates features observed in this study and those earlier studied. The studied organs of infected rats showed marked histological changes that contribute to chronic debilitation when compared with uninfected rats that revealed normal morphology.

P212 Application of the Happy Factor™ Targeted Selective Treatment approach on a commercial sheep farm.

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Targeted Selective Treatment (TST) using the Happy Factor™ decision support system has been shown to be an effective means of maintaining productivity of sheep under challenge from gastrointestinal nematodes under experimental conditions, but has not yet been tested in a commercial environment.
Two hundred lambs were divided into three groups balanced for weight and sex and co-grazed for a 10 week period. The Routine Treatment (RT) group (n=60) were treated with Zolvix at manufacturers recommended dose rate under the advice of the farm veterinarian using faecal egg count to monitor infection. TST animals were treated individually with Zolvix™ (monepantel, n=60) or Oramec™ (ivermectin, n=80) at manufacturers recommended dose rate with treatment decisions being dependent upon each lamb achieving the weight target. Targets were calculated using the Happy Factor™ (Greer *et al.* 2009) programme which uses previous weight, temperature and available nutrition to calculate treatment threshold weight targets.
RT animals were given one treatment of Zolvix during the study period, while the TST animals received only 0.68 treatments per lamb. No difference in weight gain was observed between groups over the study period. Forty percent of the TST lambs required no treatment during the study. This study demonstrates the potential for TST strategies in a commercial environment as TST animals were equally productive with reduced anthelmintic input.

14. P213 Association between rodent control and flock-level prevalence of *Cryptosporidium* spp. shedding in dairy cattle farms in Al-Dhulail district, Jordan

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Cryptosporidiosis is an important disease frequently associated with enteric disease in calf, lamb, goat, Man and other species. The prevalence of *Cryptosporidium* spp. infection in cattle was studied in Al-Dhulail district, Jordan. A total of 910 fecal samples were obtained from 91 farms (455 from calves, 455 from cows). Fecal samples were tested using flotation technique (Sheather's solution) and stained by acid-fast stain. Univariable and multivariable analyses were used to test for associations between six management practices and herd-level *Cryptosporidium spp.* prevalence in calves, cows and cattle farms. The herd-level prevalences of *Cryptosporidium spp.* infection were 81% (95% CI: 72, 88), 85% (95% CI: 76, 91), and 90% (95% CI: 82, 95), in calves, cows and farms respectively. While individual-level prevalences were 36% (95% CI: 32, 41), 40% (95% CI: 36, 45) and 39% (95% CI: 35, 42) in calves, cows and farms respectively. Of the six variables tested, none were associated with herd-level *Cryptosporidium spp.* infection in calves, while controlling rodents in the farm associated with low odds ratio (0.2) of herd-level *Cryptosporidium spp.* Infection in cows.

P214 You'll do it my way: reprogramming of host cell gene expression by *Theileria annulata*

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Infection of bovine leukocytes by *Theileria annulata* results in establishment of transformed, infected cells. Infection of the host cell is known to promote constitutive activation of pro-inflammatory transcription factors that have the potential to be beneficial or detrimental. In this study we have generated gene expression profiles representing bovine leukocytes (BL20 cells) and their *Theileria*-infected counterpart (TBL20) following activation with lipopolysaccharide (LPS). While stimulation with LPS induced cell death and activation of NF-κB in BL20 cells, the viability of *Theileria*-infected TBL20 cells was unaffected. Analysis of expression networks showed that the parasite establishes tight control over pathways associated with cellular activation. This includes modulated expression of receptors that perceive inflammatory stimuli and alteration of the expression outcome of target genes of infection-activated transcription factors, including NF-κB. Killing the parasite with drug was unable to fully reverse the infection-associated changes to host cell gene expression, and resulted in cell death. Our results provide evidence that *T. annulata* irreversibly reconfigures host cell gene expression networks associated with inflammatory disease and cancer to generate an outcome that promotes survival and propagation of the infected leukocyte.

P215 Identification of immunodiagnostic markers for cyathostomin infection in horses

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The Cyathostominae are an important cause of disease in horses. This group consists of many species, all of which have similar life cycles that involve encystment of larvae in the large intestine. Encysted larvae (EL) can persist for months and large numbers accumulate and emerge to cause diarrhoea, weight loss and colic. This can be fatal in up to 50% cases. There is no diagnostic method that enables detection of EL burden. Previously, we described two native antigen complexes that showed utility as markers for EL burden. We identified a protein component of one of the complexes. The protein, cyathostomin gut-associated larval antigen (Cy-GALA), was isolated by immunoscreening a mixed-species, EL cDNA library using sera from experimentally-infected horses. The resultant recombinant, rCy-GALA-1, was shown to be a target of serum IgG(T) in infected horses. Transcription of *Cy-gala-1* was restricted to EL and the native protein was limited to EL. The recombinant exhibited no reactivity to serum from horses infected with other helminth species. Sequence analysis of PCR products derived from single worms indicated that Cy-GALA-1 was derived from *Cyathostomum pateratum*. To address species coverage, GALA was cloned and expressed from five additional species. These were combined to produce a cocktail and subjected to ELISA using serum from horses with known EL burdens. ROC curves derived from the data, using varying burdens as cut-offs, indicated that these proteins can discriminate well between levels of EL infection.

P216* **An investigation of differences in outcome following *Theileria parva* infection of cattle**

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East Coast Fever (the disease caused by *Theileria parva*) is a major restriction on cattle production in East Africa. It is widely observed that indigenous cattle breeds ,such as the short horn zebu, have a tolerance to the disease, but they do suffer significant calf mortality and morbidity. This work investigates *T.parva* in a cohort of 548 short horn zebu calves that were recruited at birth and examined and sampled frequently through their first year of life. 72% of the calves were exposed to *T.parva* within that year, but only 35 died from the infection. This work explores the distribution of *T.parva* through that cohort, investigates the differences in clinical outcomes between individuals and identifies exposures associated with these differences, with particular interest in concomitant infections and previous exposures. *T.mutans* (a usually non-pathogenic *Theileria* species) was found to be significantly associated with survival when preceding infection with T.parva. This work is an interesting example of the interaction of pathogens and how this interaction can effect clinical expression.

P217* **Evaluation of a composite sampling strategy to determine flukicide treatment outcome in the field, using coproantigen ELISA and faecal egg counts**

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Fasciolosis in sheep is a cause of significant economic loss to UK farmers. Convenient, standardised tests are needed to help diagnose fluke infections in the field and to monitor the efficacy of flukicide treatment. Farmers across Great Britain were recruited to collect faecal samples according to a set protocol from two groups of sheep pre- and post-treatment. A composite was formed from each group, and both individual and composite samples tested by FEC and coproantigen ELISA. Of the 36 farmers sent sampling kits, 18 returned complete pre- and post-treatment samples. A variety of flukicides was used, both triclabendazole- and closantel-based, and a variety of methods employed to determine dosage. A wide range of values was observed for pre-treatment FEC (range 0 to 140epg, average 12epg), with differences in distribution seen both within and between groups. It would appear that average FEC is more sensitive for the detection of infection pre-treatment than an average ELISA, composite FEC or a composite ELISA. Discrepancies were seen when comparing treatment outcome by average FEC and average ELISA, composite FEC and composite ELISA, average FEC and composite FEC and by average ELISA and composite ELISA. Treatment failure was seen in nine groups based on average FECRT, and paramphistome eggs were detected on a number of farms.

P218* The distribution of strongyle eggs in horse faeces

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Faecal egg counts (FEC) are routinely used to determine treatment requirements or for assessing anthelmintic efficacy. There are several underlying factors leading to variation in FEC , which present difficulties in interpretation, potentially leading to the misclassification of resistance and to under-/over-estimating the requirement for treatment. The spatial distribution of eggs in faeces was investigated. A single, entire motion was collected from three horses once a day for three consecutive days. Each motion was divided into individual boli and two, 1g samples were taken from each and analysed using a FEC method (sensitivity 1 epg). The distribution of eggs within each motion, between boli and between samples was tested for overdispersion by multiplying the variance to mean ratio by the degrees of freedom, and comparing the result with the chi-square distribution. Where overdispersion was confirmed ($p = < 0.05$), the negative binomial distribution was fitted to the data using maximum likelihood estimation. Bonferroni correction was used to determine the chi-square critical value to test for overdispersion between samples within each bolus and at cuvette level.
Results demonstrate that eggs were overdispersed ($p<0.01$) within motion. There was no overdispersion between boli, between samples or between transects within the cuvette. Thus, homogenisation prior to sub-sampling and the size of subsample should be considered to minimise variation in FEC and account for overdispersion.

P219 Assessment of Molecular Bovine Trypanosomiasis in the Kachia Grazing Reserve, Central Nigeria.

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African animal trypanosomiasis is a parasitic disease caused mostly by *T. vivax*, *T. congolense* and to some extent by *T. brucei,* affects a wide range of domestic livestock and results in 3 million cattle deaths annually in Africa and millions of dollars lost in terms of low productivity.
This study analyses the prevalence of trypanosome infections in White Fulani cattle belonging to the Fulani settlement of the Kachia Grazing Reserve in Kaduna State, Central Nigeria. It is been suggested it could produce 8 million dollars annually in terms of milk and meat production with an adequate animal health programme.
A total of 3703 randomly selected blood samples of White Fulani cattle were collected during the dry and wet season of 2011. Samples were obtained by puncture in the jugular vein and blood was spotted onto FTA cards which were transferred for downstream analysis to the University of Edinburgh. The identification of the trypanosome species was done by means of PCR using primers targeting the ITS region. Posterior species specific PCR was done to confirm the initial results.
The overall trypanosomiasis infection rate was 11.04%. The most prevalent trypanosome species was *T. vivax* (87.04%) which is in agreement with previous reports done in the area however, an increase in the presence of other trypanosome species was recorded. Further work will investigate the importance of migratory movement in relation to these observations.

P220* **Immune phenotyping of placentas following experimental inoculation with *Neospora caninum* in cows at mid gestation**

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Despite *Neospora caninum* (NC) being a major cause of bovine abortion worldwide, its pathogenesis is not completely understood. NC infection stimulates host cell-mediated immune responses, which may be responsible for the placental damage leading to abortion. The aim of our study was to characterise placental immune response following an experimental infection at day 140 of gestation. Cows were culled at 14, 28 and 42 days post inoculation (dpi). Placentomes were examined by immunohistochemistry using antibodies against macrophages (CD68), T-cells (CD3, CD4, CD8, γδTCR), natural killer (NK) cells (CD335) and B cells (CD79). Inflammation was generally moderate and mainly characterised by presence of $CD3^+$ and $CD4^+$ cells; whereas $CD8^+$, γδTCR and NK-cells were less numerous. Macrophages were labeled in increasing numbers through the subsequent time-points after infection. The immune system cell subset distribution observed in this study was similar to those seen at different stage of gestation. However, cellular infiltrates were less severe than those seen during the first trimester NC infections and more severe when compared to third trimester infections. This may explain the milder clinical outcome observed when animals are infected at mid or late gestation.

P221* ***Caenorhabditis elegans*** **as a Model for the Assessment of Nanomaterial Toxicity**

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Nanomaterials, having at least one dimension less than 100nm, have unique physico-chemical properties due to their small size, large surface area and surface reactivity. Manufactured nanomaterials have enormous potential in industrial, pharmaceutical and biomedical applications but there is still concern among the public regarding their safety. Our research indicates that the soil nematode, *Caenorhabditis elegans* can be used as a model to determine the toxicity of nanomaterials. *C.elegans* is widely used in the laboratory due to its simple structure (translucent), fast reproduction (3 days), ease of culture and low cost maintenance. This free living nematode has the capability of providing an excellent medium – to high- throughput model and has been used to test the toxicity of polystyrene beads, silver, zinc oxide and poly-lactic-*co*-glycolic acid (PLGA) bulk/nano materials; focusing on the effects that these particles have on life-span, viability, reproduction and regulation of genes related to stress and cell death. The *C.elegans* genome was sequenced in 1998 and so allows for the investigation of various genes using methods such as RNA interference (RNAi) and real-time PCR. A promising development from this research could be the use of nanomaterials in drug targeting for parasitic nematodes. A growing incidence of anthelminthic resistance shows that novel treatments are desperately needed and preliminary research using *C.elegans* and nanomaterials could be a step forward.

P222* Purification of larval antigens from *Teladorsagia circumcincta* L3 by IgA-immunoaffinity chromatography

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Teladorsagia circumcincta, an abomasal nematode of small ruminants, is widespread and economically important in temperate regions. Infection is primarily controlled with anthelmintics; however, resistance is widespread in *T. circumcincta* populations. As immunity can be acquired through prolonged trickle-infection, vaccination is a possible alternative control method. Parasite-specific, mucosal IgA responses have been implicated in inhibition of larval development. Here, we purified IgA from abomasal mucus derived from animals that had been subjected to repeated trickle administration with infective larvae (iL3). IgA was purified by gel filtration following removal of IgG by affinity chromatography. A custom antibody-affinity column was then created using the purified IgA. This column was used to purify antigens from a *T. circumcincta* iL3 somatic extract, with elution of the IgA-bound fraction using a decrease in eluant pH. Eluted antigens were identified using liquid chromatography-electrospray ionization-tandem mass spectrometry (LC-ESI-MS/MS). Analysis generated peptide sequence data enabling identification of IgA-reactive antigens; including galectin-1, thrombospondin, paramyosin and protein disulphide isomerase. Due to their reactivity to local IgA these antigens are valid candidates for a sub-unit vaccine for control of teladorsagiosis. This is the first report of this approach for identifying *T. circumcincta* antigens. It has allowed identification of antigens which are immunoreactive in their native state, which is preferable to techniques involving denaturation as it more accurately reflects natural interactions of antigen and host immune responses.

P223* Identification of immunodiagnostic markers for cyathostomin infection in horses

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The Cyathostominae are an important cause of disease in horses. This group consists of many species, all of which have similar life cycles that involve encystment of larvae in the large intestine. Encysted larvae (EL) can persist for months and large numbers accumulate and emerge to cause diarrhoea, weight loss and colic. This can be fatal in up to 50% cases. There is no diagnostic method that enables detection of EL burden. Previously, we described two native antigen complexes that showed utility as markers for EL burden. We identified a protein component of one of the complexes. The protein, cyathostomin gut-associated larval antigen (Cy-GALA), was isolated by immunoscreening a mixed-species, EL cDNA library using sera from experimentally-infected horses. The resultant recombinant, rCy-GALA-1, was shown to be a target of serum IgG(T) in infected horses. Transcription of *Cy-gala-1* was restricted to EL and the native protein was limited to EL. The recombinant exhibited no reactivity to serum from horses infected with other helminth species. Sequence analysis of PCR products derived from single worms indicated that Cy-GALA-1 was derived from *Cyathostomum pateratum*. To address species coverage, GALA was cloned and expressed from five additional species. These were combined to produce a cocktail and subjected to ELISA using serum from horses with known EL burdens. ROC curves derived from the data, using varying burdens as cut-offs, indicated that these proteins can discriminate well between levels of EL infection.

P224* Spotlight on Babesiosis in Belgium

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Infection of humans with species of *Babesia* can have serious implications for human health and blood transfusion. However the risk of human infection in many European countries is not known with accuracy. This study aimed to assess the general prevalence of *Babesia* parasites in Belgium, with particular emphasis on species with zoonotic potential. Investigation for evidence of *Babesia* spp. in ticks identified species previously unreported in Belgium, *Babesia* sp. EU1 and *Babesia capreoli*, and confirmed the presence of potential zoonotic species. Evaluation of infection rate in ticks collected from respective vertebrate hosts and the environment was found to be between 1.3 and 14.6%. A seroprevalence of 14.3% for *Babesia* spp. was estimated in bovines from Southern Belgium and a prevalence of between 9 and 40%, against three known zoonotic *Babesia* spp., was obtained using samples representing a human "at risk" population. Co-infections with *Babesia* and *Borrelia* spp. or *Anaplasma phagocytophilum* were identified.
We conclude that babesiosis should be considered as a threat for susceptible livestock and humans, especially the elderly and immunocompromised. Preventive action can be implemented to minimize the risk of acquiring tick-borne disease, but the most useful strategy remains dissemination of relevant risk information to the medical community and the general public.

P225 The chemokine CXCL12 is essential for the clearance of the filaria *Litomosoides sigmodontis* in resistant mice.

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Litomosoides sigmodontis is a cause of filarial infection in rodents. The outcome of infection is dependent on the parasite's modulatory ability and on the host genetic background. The goal of this study was to determine whether the chemokine axis CXCL12/CXCR4, which notably participates in the control of immune surveillance, can influence the outcome of the infection. We compared *L. sigmodontis* infection of C57BL/6 resistant strain and BALB/c susceptible strain of mice. We showed that rapid parasite clearance was associated with a *L. sigmodontis*-specific CXCL12-dependent cell response in C57BL/6 mice and that CXCL12 was produced mainly by pleural mesothelial cells. Futhermore, interfering with the CXCL12/CXCR4 axis in both strains of mice delayed filarial development, as evidenced by the postponement of the fourth molting process. Moreover, the *in vitro* growth of stage 4 filariae was favored by the addition of low amounts of CXCL12. The CXCL12/CXCR4 axis thus appears to have a dual effect on the *L. sigmodontis* life cycle: by acting as a host-cell restriction factor for infection, and as a growth factor for worms.

P226 Antigen persistence of Rapid Diagnostic Tests (RTDs) and its implications in the diagnosis of malaria in pregnancy

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Diagnosis of malaria during pregnancy is often complicated by absence of parasites in peripheral blood, due to sequestration in the placenta. There is a need for different manners of diagnosis, and RDTs may offer such an alternative. The aim of this study was to evaluate RDTs for antigen persistence in pregnant women after malaria treatment.
In total, 35 pregnant women with malaria were followed 28 days after treatment. At days 0,1,2,3,7,14 and 28, a blood sample was collected. Two RDTs were used: SD Bioline Malaria Antigen P.f (HRPII) and (ADVANTAGE MAL CARD, Pf and PAN (pLDH). Thick and thin smears were prepared and blood is spotted on filter paper for RT-PCR. Preliminary results show that on average, microscopy was negative in 1.2 days, pLDH RDT in 0.9 days, and HRPII test in 7.5 days. The average time to become negative for PCR is 2 days.
HRPII RDTs stay positive much longer after treatment than other tests. When using this test in diagnosing malaria in pregnant women, this issue should be considered, especially if the patient has recently received IPTp. There is a need for RDTs with a better detection threshold than pLDH RDTs, but fewer problems with antigen persistence.

P227 Development and evaluation of simplified molecular diagnostic devices for malaria

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Microscopy is the gold standard for malaria diagnosis, but at low parasitaemia it becomes less sensitive. Molecular tools allow for specific/sensitive diagnosis, but current formats, such as PCR with gel-electrophoresis, are difficult to implement in resource poor setting. Therefore, a simple, fast, sensitive and specific detection system, nucleic acid lateral flow immunoassay (NALFIA) to detect amplified PCR products of Pan-*Plasmodium* and human GAPDH (internal control) was developed and evaluated under laboratory conditions and in a multi country ring trial.
Analytical sensitivity/specificity of PCR-NALFIA in a single laboratory evaluation was >95% and able to detect 1 parasite/μl blood. All laboratories in the ring trial reported ease of use of the system and could successfully perform the protocol. Overall laboratory inter variability was low and the agreement of reported results was high. Overall k value was 0.89 (95% CI: 0.83 – 0.94; p<0.001). The overall test sensitivity and specificity in the different laboratories was >95% with very small confidence intervals.
PCR-NALFIA for malaria diagnosis conducted well in all laboratories and further phase II and III evaluations in disease endemic countries is justified.
Funding EU FP7 grant 201889 Multi drug resistance in malaria under combination therapy: assessment of specific markers and development of innovative rapid and simple diagnostics (MALACTRES).

P228 Evaluation of novel antigen detecting tests, RTD, microscopy and PCR for the diagnosis of malaria in Pregnancy in Burkina Faso

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During pregnancy, infection of the placenta with *Plasmodium falciparum* is related to poor birth outcome and it adversely affects maternal health. Diagnosis is often complicated by the absence of parasites in peripheral blood, due to sequestration in the placenta. Rapid diagnostic tests (RTDs) are considered an alternative but have not been systematically studied for their ability to detect placental malaria. Therefore, different RTDs and newly developed prototype antigen detection tests were tested for their diagnostic accuracy under field conditions.
Two commercially available RTDs (based on HRP2 or pLDH detection) were compared to ELISA format tests based on monoclonal antibodies against new target antigens (HDP and DHFR) at the district hospital in Nanoro, Burkina Faso. PCR analysis and microscopy was performed as “gold standard” tests. In total 418 pregnant women were screened and tested.
For the diagnosis of malaria in pregnant women, SD-Bioline, an HRP2-based RDT, had the best sensitivity compared to microscopy. Results obtained with PCR, ELISA will be presented.

P229 Peer education: The effects on knowledge, and preventive practice of pregnancy related malaria in women of reproductive age in Edo-State, Nigeria.

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There is limited uptake of measures to prevent malaria by pregnant women in Nigeria. This is often contributed to limited knowledge of women in child bearing age about the health impact of malaria in pregnancy (MiP) on mother and foetus. A strategy to improve community awareness of MiP is by means of peer-to-peer education. This study explored if peer-to-peer education is an effective tool to raise the level of knowledge amongst women in child bearing age and if increased knowledge translates in improved uptake of preventive measures.
Pre-assessment interviews revealed that knowledge on malaria in general was high in the studied population but knowledge on health risks of MiP and possible preventive measures was limited. The peer education campaign had a significant impact in raising the level of knowledge of women of child bearing age.
Peer education can lead to a significant increase in knowledge on disease transmission and prevention. However, increase in knowledge does not necessarily translates in increased preventive practice. Therefore, health interventions should also focus on addressing other problems influencing preventive practice, such as structural barriers like lack of availability of preventive tools and poor access to health services.

P330 Phosphodiesterase inhibitors for the treatment of leishmaniasis and/or trypanosomiasis

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Neglected diseases, have a severe impact on human health and represent a major economic burden on developing countries. An estimated 70.000 new cases of human African trypanosomiasis occur each year, resulting in the death of approximately 40.000 people. Leishmaniasis has a much higher incidence with approximately 2 million new cases per year. The clinical presentation of leishmaniasis can vary from self-healing lesions, affliction of mucosal membranes and infection of the internal organs. The latter manifestation, visceral leishmaniasis, is fatal with 100.000 victims each year. Treatment is with toxic drugs (having serious side effects) and often failing due to emerging drug resistance and new drugs are not in the pipeline. A consortium comprising academia, industrial partners and the Drugs for Neglected Diseases initiative are searching for new compounds to fight these diseases. The project aims to develop solutions to these diseases by targeting parasite specific forms of the enzyme phoshodiesterase (PDE) throught the development of specific phosphodiesterases inhibitors that block one or more of the five enzyme subtypes, therefore preventing the inactivation of the intracellular second messengers cyclic adenosine monophosphate (cAMP) and cyclic guanosine monophosphate (cGMP) by the respective PDE subtype(s). At present >120 compounds have been screened in parasite specific assays with varying rates of inhibitory activity.

P231 Immunophilin-Protein Interactions in *Plasmodium falciparum*

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Probing for protein-protein interactions in *Plasmodium falciparum* is predicted to lead to the identification of novel antimalarial targets. To this end we are investigating protein–protein interactions of three *P. falciparum* immunophilins: two cyclophilins PfCYP19A and PfCYP19B and an FK506 binding protein (FKBP) PfFKBP35. Immunophilins are an important class of proteins that are involved in a rate-limiting step of protein folding, peptidyl-prolyl *cis-trans* isomerisation, and also act in some cases as molecular chaperones. Immunophilins have been shown to play roles in virulence in several protozoa, and host immunophilins are crucial in hepatitis C and HIV infection as well as being the receptors for major immunosuppressive drugs such as cyclosporin A and FK506. To date one whole-cell method, co-immunoprecipitation (co-IP), and one whole-genome method, yeast-2-hybrid screening (Y2H), were used to find possible interactions. Co-IP was performed against PfCYP19B while Y2H was used to find interactors for PfFKBP35. Subsequent mass-spectrometric analysis of the co-IP proteins has identified seven novel, unconfirmed protein–protein interactions for PfCYP19B, while Y2H returned eleven putative interactions for PfFKBP35. While some of these interactions were predicted, based on data from other organisms, others were completely novel and may provide starting points for new investigations into functional roles of immunophilins and potential protein–protein interaction modulating drugs.

P232 Azithromycin displays a dual mode of action

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Azithromycin (AZ) is a well characterized antibacterial macrolide with an orally effective route of administration, a long elimination half-life and a safety profile that enable its use in pregnant women and young children. AZ exerts its antimicrobial mode of action by interacting with ribosome and inhibiting protein synthesis.
Azithromycin manifests reasonable efficacy as antimalarial and appears promising for being used in combinations directed against drug-resistant parasites. Azithromycin activity against *P. falciparum in vitro* is moderate but potency increases considerably after a second generation of parasite intraerythrocytic growth (96 hours assay), phenomenon known as "time delayed phenotype". Azithromycin antimalarial mode of action is related to the inhibition of protein synthesis in the apicoplast, as has been demonstrated with well characterized resistant parasites with mutations in ribomal proteins of this organelle (bacterial-like protein synthesis).
AZ shows the delayed death phenotype characteristic of organelle protein synthesis inhibitors.
Using a well characterized plasmodium resistant strain, harbouring mutations in the apicoplast ribosomal proteins, we have demonstrated that the moderated activity of azithromycin against *P. falciparum* in the standard 48 hours assay is not related to the inhibition of apicoplast protein synthesis.

P233 Identification of a new Flagellar Pocket Collar protein in *T. brucei*

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The *T. brucei* flagellum exits the cytoplasm via the Flagellar Pocket (FP). The FP is an essential organelle and is the unique site for endo- exocytosis. The Flagellar Pocket Collar (FPC) component of the FP maintains a ring/horseshoe structure at the flagellum exit site. The FPC contains uncharacterised proteins, but also the first identified FPC component - BILBO1. BILBO1 is essential. (PLoS Biol. 2008 May 6;6(5):e105).
Two-hybrid *T. brucei* genomic screens using BILBO1 as bait has revealed a number of BILBO1 partners that localize to the FPC. Here we present data on FPC4, a hitherto uncharacterised FPC protein, which is insoluble, cytoskeletal, FPC located, and syntenic in kinetoplastids. FPC4 forms a hook-like structure in the FPC of procyclic cells when observed by GFP-tagging and indirect Immunofluorescence microscopy, and is thus a new marker for FPC formation and biogenesis.
Functional analysis of FPC4 in *T. brucei,* using GFP fusion protein over-expressed in procyclics, produces long insoluble polymers. The formation of the polymers is ultimately toxic. Toxicity may also be linked to a dominant negative effect. Surprisingly RNAi knockdown in procyclic forms does not influence cell growth. We are currently investigating the role of FPC4 in bloodstream forms and expressing it in mammalian cells to assess its role in these forms, and also its polymer forming properties.

P234 Genome evolution and the origins of parasitism in Kinetoplastids

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Bodo saltans (Eubodonidae: Kinetoplastida) is a freshwater bacteriovore, and the closest known free-living relative of the trypanosomatid parasites (*Trypanosoma, Leishmania, Leptomonas, Crithidia*). Trypanosomatids are adapted for parasitism through developmental pathways linked to complex life cycles, specialized metabolism, and cell surfaces that facilitate cell invasion and intracellular survival and antigenic variation. *B. saltans* offers our best model of the ancestral Trypanosomatid that made the transition from a free-living life strategy to parasitism. Exploring this ancestral model will enable us to identify the features of trypanosomatid genomes that are uniquely associated with parasitism. Here we compare the draft *B. saltans* and trypanosomatid genomes to identify their principal differences. Our analysis of gene ontology terms shows that genes present in *B. saltans* but missing in trypanosomatids are significantly enriched for processes associated with cellular membranes and macromolecular metabolism. These genes are conserved in another bodonid (*Trypanoplasma borreli*), indicating that these represent the major gene losses experienced by the ancestral trypanosomatid. Changes on the cell surface also accounted for some evolutionary gains in the parasites. Phylogenetic analysis of gene families such as amino acid transporters and nucleotide transporters show that these have diversified substantially, providing examples of the innovations that have adapted the Trypanosomatid genomes to a parasitic life strategy.

P235 MSP3.3C-specific antibodies block the intraerythrocytic development of *Plasmodium falciparum* and induce apoptotic features within the parasite

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Merozoite surface antigen-specific immunoglobulin Gs (IgGs) inhibit the growth and development of the parasite *P. falciparum* in vitro, although the functional mechanisms of this inhibition are not fully understood. In this study, antibodies raised by immunization with a recombinant antigen derived from the C-terminal region of merozoite surface protein 3.3 (MSP3.3C) showed novel properties in *P. falciparum* inhibition assays.

P. falciparum blood-stage parasites were cultured in the presence of anti-AMA-1 specific, anti-MSP3.3C specific or naïve rabbit IgG. Parasite DNA content and morphology, antibody localisation and the presence of apoptotic markers were monitored over the parasite life cycle by microscopy, flow cytometry and indirect immunofluorescence antibody tests.

MSP3.3C-specific IgG was found to be highly inhibitory in growth inhibition assays. This activity appears to be caused by inhibition of the intraerythrocytic development of the parasite and not by inhibition of merozoite invasion. Notably, we have shown that antibodies to MSP3.3C can access the intraerythrocytic parasite post merozoite invasion and effectively block further development of the parasite within the host erythrocyte. Our data indicates that specific IgG to MSP3.3C can prevent the export of MSP3.3 through the parasitophorous vacuole membrane into the erythrocyte cytoplasm. In addition, anti-MSP3.3C antibodies induce several characteristic features of programmed cell death within the parasite.

P236 Vitamin B_5 biosynthesis in *Toxoplasma gondii* - genome analysis to drug target

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Toxoplasma gondii is an ubiquitous obligate intracellular protozoan parasite that causes lifelong infections in 10-30% of humans worldwide. It is an opportunistic parasite in AIDS patients and can cause eye disease and potential neurological effects, and has a major veterinary importance. The metabolic enzyme pantothenate synthetase has been studied that acting as a key enzyme in pantothenate biosynthesis producing the precursor to Coenzyme A, which is required by 4% of enzymes in eukaryotic cells. A system for testing pantothenate synthetase inhibitors in *T. gondii* has been developed using a fluorescent parasite strain. The EC50 of each inhibitor was calculated based on fluorescence measurements analysed using Prism software. Known Toxoplasma drug (Pyrimethamine) was used as a control with EC50 similar to published data. Of the fourteen pantothante synthetase inhibitors screened with different growth-inhibiting kinetics for their effects on *T. gondii* YFP strain, two of them namely SW404 and SW413 were found to show the higher inhibition rate according to their EC50s. Confirmation that pantothenate synthesis is being targeted specifically has been proved by testing inhibitors in the presence of exogenous pantothenate. This will increase our knowledge of growth and metabolism in this important parasite and highlight a new drug target.

P237 *Trypanosoma vivax:* a new model to study *Nagana*, the animal sleeping sickness

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Trypanosoma vivax is one of the most important causes of Animal African Trypanosomosis (AAT) in West Africa and Latin America. However, there have been little reports about the parasite biology and the immunopathogenesis it induces in mammalian host and few laboratories have attempted to develop a reference model for *Trypanosoma vivax*.

We developed such a model of infection in mice and used it to study the immunobiology of the infectious process and characterize some of the key players in the immunopathology of *Nagana*. Robust and reproducible infections were obtained with Outbred mice. Hallmarks of livestock trypanosomosis were also observed, namely severe acute anemia, thrombocytopenia and a reduced number of B lymphocytes.

In addition, we established several tools to better approach the impact of gene function *in vivo*. We developed epimastigote axenic culture and determined transfection conditions allowing selection and maintenance of genetically modified parasites *in vitro*, and their return into immunocompetent mice. Using specific integrative vectors, we generated *T. vivax* strains stably expressing either GFP or luciferase reporter genes. These strains represent powerful and promising tools to characterize *in vivo* the *T. vivax* infectious process.

P238 Large scale immunological characterisation of *Schistosoma mansoni* Tropomyosins

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Several invertebrate tropomyosins have been identified as clinically relevant allergens – most notably as the major food allergens in some crustaceans. The hygiene hypothesis suggests that parasitic infections can modulate host immunity and affect atopy, and plausible correlations between helminth infections and inflammatory diseases have been established. Given the high prevalence of schistosomiasis – a parasitic infection that causes chronic morbidity in an estimated 200-300 million people worlwide – we believe that a thorough characterisation of *Schistosoma mansoni* (*Sm*) tropomyosins may contribute to our understanding of the evolutionary relationship between allergy, autoimmunity and helminth infections, and may also enrich the current view of host-pathogen interactions during the course of the infection. Therefore, we have identified the life cycle expression patterns of the predicted alternatively spliced isoforms of *Sm* tropomyosin by PCR, and expressed & purified four phenetically distinct splice variants in *E. coli.* We performed circular dichroism spectroscopy on the purified recombinant proteins to confirm that they were correctly folded; this analysis also revealed that *Sm* tropomyosins can refold *in vitro* even after being heated to 90°C. Finally we have measured the levels of anti-tropomyosin IgE, IgG4 and IgG1 using high throughput ELISA in >200 individuals from Uganda chronically infected with schistosomiasis, and are currently investigating the relationships between these responses and epidemiological determinants, as well as previously characterised responses to several other *Sm* antigens, and whole-worm extracts in the same population.

P239 Assessing Population Variation of Vaccine Candidates in *Fasciola gigantica*

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Vaccines for neglected parasitic diseases are of paramount importance. An understanding of the basic biology underpinning vaccine target expression within parasite populations is one of the pre-requisites for vaccine discovery and development. *Fasciola gigantica* is one of the most important helminth infections of ruminants in Asia and Africa and is most prominent in poorer regions impacting on individual and small farming communities; inflicting significant losses in cattle, buffalo, goats and sheep. Fasciolosis induces major economic losses in terms of milk yield, with average yields falling ~30 % with *Fasciola* infection, thus warranting research into sustainable vaccines. To this end we are looking into the plasticity of the sub-proteome of current vaccine targets, including thioredoxin glutathione reductase and leucine amino peptidase, in *F. gigantica* populations.

P240 *In vitro* screening of bisnaphthalimidopropyl derivatives on *Trypanosoma brucei*

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Trypanosoma brucei found in sub-Saharan Africa is a protozoan parasite, transmitted by the bite of tsetse flies. The subspecies *Trypanosoma brucei rhodesiense* and *Trypanosoma brucei gambiense* cause the debilitating disease human African trypanosomiasis (HAT). The urgent need to develop new drugs is supported by inadequate standard therapies.
The anti-leishmanial properties of bisnaphthalimidopropyl (BNIP) derivates have been described and are composed by two naphthalimidopropyl groups linked a carbonated chain, varying in size and presence of amines. To further evaluate their application in the treatment of infections caused by trypanosomatids as *Trypanosoma brucei (T. brucei*), this study describes a cell-based drug screening towards HAT disease.
The bloodstream form of *T.brucei was* used to optimize an *in vitro* 96-well resazurin-based assay for drug screening. Mouse fibroblast L929 cells were used as a counter-screen for non-selective inhibitors determined by tetrazole 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay.
As accordingly with the literature pentamidine and suramin exhibited IC_{50} (half maximal inhibitory concentration) values against *T.brucei* ~4 nM and 70 nM, respectively. From the small BNIP library, of 15 compounds, 5 exhibited IC_{50} value below 100 nM. It was clear that BNIP compounds with more than 8 carbons in the linker chain loss their anti-*T.brucei* activity. The presence of two cyclohexane or benzene rings in the linker chain will increase rigidity in the molecules, which also hampers its anti-*T.brucei* activity.
BNIPDabut was the most potent compound with ~3 nM activity, closed to the IC_{50} of pentamidine and with a selectivity index for *T.brucei* over fibroblasts about 8000-fold higher.

P241 New Insights into the Glutathione Transferase Protein Superfamily of *Fasciola gigantica*

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The glutathione transferase (GST) protein superfamily of *Fasciola* species have long been the subject of scientific studies for their potential as immuno- and chemotherapeutic targets. To date, 3 GST classes (Mu, Sigma and Omega) are known in *Fasciola hepatica*, the temperate liver fluke. Currently however, there is incomplete understanding of the GST family in *Fasciola gigantica*, the tropical liver fluke, potentially hindering vaccine formulation progress. Therefore, utilising modern molecular and biochemical methods we have investigated the GST family from *F. gigantica* combining high-resolution 2DE proteomics with next-generation sequencing data to gain greater insight to this vaccine candidate family. In doing so, both Omega and Sigma class GSTs are now confirmed in *F. gigantica* and a new Zeta class GST identified *in silico*.

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This year, BioMed Central are generously offering three prizes for the best talks and poster on behalf of ***'Parasites and Vectors'*** and '***Malaria Journal***'. Please complete the following tear-out form and return to the voting box at the Registration desk to cast your vote.

Best student talks

1st choice: Abstract

No……………………………………………….

Name…………………………………………..…..

2nd choice: Abstract

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Best poster

Abstract

No.………………………………………………

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