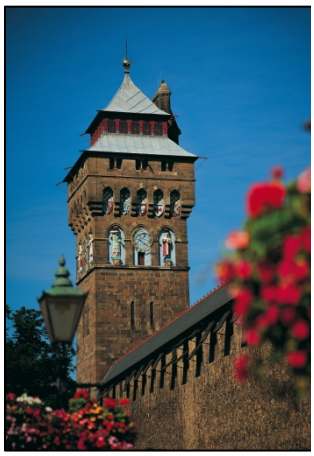
British Society for Parasitology
Spring Meeting and
Trypanosomiasis & Leishmaniasis Seminar






Cardiff University, Wales, UK March 29th – April 1st

BSP 2010

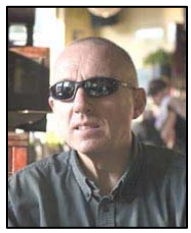
Welcome from Cardiff University Parasitologists
Croeso oddi wrth Parasitolegwyr Prifysgol Caerdydd!

Dr. Jo Cable - Parasite ecology CableJ@cardiff.ac.uk

Dr. Jo Lello - Co-infection dynamics and host-pathogen evolution LelloJ@cardiff.ac.uk

Dr. Sarah Perkins - Networks, helminth vectors & immunogenetics of invasions Perkinss@cardiff.ac.uk

Prof. David Lloyd - Biochemist and microbiologist



Dr. Mark Jervis - Life history and evolution of insect parasitoids

Dr. Rhys Jones - Reptile parasites, public understanding of science

Dr. Corallie Millet - Metabolism and chemotherapy of *Spironucleus vortens*

Dr. Mireille Johnson - Coevolution of Host-Parasite interactions

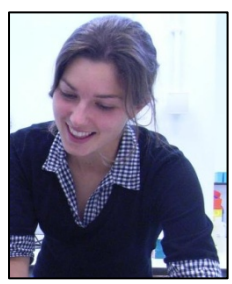
Linda Erlandsson - Fish parasitology

Loys Richards - Impact of parasites on fish foraging behaviour and personality

Joanna Rumsey - The effects of co-infection on host population dynamics

Rui Sa - Transmission of parasites from humans to chimpanzees

Bettina Schelkle - Gyrodactylid biology and control



Ellie Sherrard-Smith - Epidemiology of parasites in the Eurasian otter

Catrin Williams - Antimicrobial effects of garlic against fish parasites

We will be on hand to assist you during the conference

CONTENTS

Welcome to Cardiff for BSP 2010

Dear Delegates,

We take great pleasure in welcoming you to the city of Cardiff and especially to Cardiff University for the Spring Meeting of the British Society for Parasitology, and the Trypanosomiasis and Leishmaniasis Seminar, 2010. This is the first time in ca. 40 years that the meeting has been held in Cardiff and we hope that you are going to enjoy this exciting event. Whether your interests lie with our special focus sessions on ecology or with other areas of our programme, we are confident that there will be plenty to stimulate you.

Cardiff is a beautiful and compact city which is very easy to get around but with plenty to do. The University is just minutes away from the museum, castle, theatres, cafes and restaurants of the city centre. Slightly further a field (extra 2 min) you can find the Millennium Stadium, and a short bus ride will take you to Cardiff Bay; host to the National Assembly for Wales and the iconic Welsh Opera House. The city is also renowned for its beautiful parklands and is within easy reach of some of the most stunning areas of the Welsh countryside including the Brecon Beacons and the Pembrokeshire coastline.

We are extremely privileged this year, to have Prof. Sir Roy Anderson as our plenary speaker on Wednesday afternoon and our programme will kick off on Monday evening with a public understanding of science event with the home-grown talent Dr Rhys Jones, followed by Dr. McCrumble interviewed by Dr. Mark Booth. Within the remainder of this booklet we hope to answer any of the questions you may have about the conference, but should you require further information please do not hesitate to contact any of the Cardiff University organising committee and helpers.

Best wishes,

Jo Cable	(Cardiff University)
Jo Lello	(Cardiff University)
Sarah Perkins	(Cardiff University)
Paul Horrocks	(Keele University) Malaria
Karen Grant	(Lancaster University) Trypanosomiasis & Leishmaniasis
Graham Coombes	(Glasgow University) Trypanosomiasis & Leishmaniasis
Julian Fuller	(BSP secretariat)
Cathy Fuller	(BSP secretariat)

Venue

The conference oral presentations will be held in the School of Biosciences, Main Building, Museum Avenue (please see maps in delegate bags and maps on pages 8 & 9) while all the breaks and the majority of the social functions will be held in the Students' Union area (see maps on pages 8 & 9). The Students' Union area will host the trade stands, tea/coffee, lunches, poster and social events. The trade stands have been carefully selected to be of interest to the BSP delegates – please be sure to visit them all.

Safety - Fire Evacuation Procedures

Please take note of the location of fire exits, break-glass call points and instruction notices in your surrounding area.

- The Fire Alarm for the Main Building is a continuous sound.
- If the alarm sounds for longer than 10 seconds you must evacuate using the nearest available exit.
- Fire exits are indicated by the green "running man" signs.
- You should follow any instructions for evacuation from Fire Wardens/ or Security staff.
- Please make your way to the assembly point on the lawn in front of the building where you will be informed when it is safe to re-enter the building.

If you have impaired mobility, please inform the event organisers who will provide additional guidance to help you.

Identification

We would like to remind delegates that it is important to wear name badges at all times in order to identify themselves to Cardiff University staff. The conference committee and helpers at Cardiff University can be identified by name badges which display the Cardiff University logo. Please also see our photos on the inside cover of this booklet.

Reception and Registration

The conference reception desk will be located in the VJ Gallery in the Main Building from 2:00 pm- 8:00 pm (see maps on pages 8 & 9) on Monday, March 29th. Thereafter the reception desk will be located in the Students' Union (see maps on pages 8 & 9).
Facilities provided by the reception desk include:

- Conference registration
- Urgent message pickup
- Conference Organising Committee contacts
- Internet access codes

Information for Speakers

Loading of your presentations will take place in the lecture theatre appropriate to your 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. Each room will be manned by an AV assistant in case of any problems with equipment. We have a very busy programme and we politely request that speakers ensure that they 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 8:30 am on Tuesday 30th and must be removed by 1 pm Thurs 1st 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 Students' Union. Please note the use of blue tack and drawing pins is strictly prohibited and only Velcro may be used on the boards, which is available at the conference reception desk in the Students' Union. 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 prize competitions are marked with an asterisk (*) throughout the programme and abstracts.

Food, Refreshments and Evening Bar
With the exception of the Welcome Reception and the Conference Dinner, all coffee breaks and all meals will be provided in the Students' Union across the road from the Main University Building. Delegates are reminded to bring their meal tickets to lunches and to the dinner on Tuesday evening. In addition to the refreshments and meals provided by the conference, the Kitchen Bar in the Students' Union area will be open throughout the conference period for purchasing of refreshments and light snacks.

Social Programme
Mon 29th March: The social programme starts with a Welcome Reception from 6:30 pm to 8 pm on Monday the 29th of March, in the VJ Gallery of the Main University Building. Viriamu Jones was the first principal of the University and the gallery provides the perfect venue to welcome you all. Immediately following the Welcome Reception there will be a public understanding of science event in the Large Shandon Lecture Theatre (see maps on pages 8 & 9). This event will start with Dr Rhys Jones talking about 'Polyticks and the Media' followed by Dr Mark Booth presenting 'Dr Joseph McCrumble's Guide to Engaging with Parasites'. After this event there will be a further opportunity to meet and greet your fellow delegates in the Taf Bar across the road in the Students' Union from nine until late.

Tuesday 30th March: Poster Event with a Wine Reception, starting at 5:30 pm in the Students' Union. This event will be followed by the 'acappella group' the 'Spectrum Singers' at 7.30 pm. A hot buffet dinner will be available in the same venue from 8.00 pm. The Students' Union bar will be open until 2:00 am to give you plenty of time to catch up with old friends, make new contacts and enjoy our Welsh hospitality.

Wednesday 31st March: Conference dinner at the Hilton Hotel in the centre of Cardiff, which is five minutes walk from the University. The evening commences with a welcome drinks reception (6:30-7:30 pm) accompanied by the internationally renowned Welsh harpist Eleri Darkins. Dinner will begin at 7:30 pm and at 9:00 pm there will be a short interval (coffee in the Atrium) in which we will clear the ballroom to make way for our evening's main entertainment - the Gypsy Kings tribute band "Andalus". To encourage delegates onto the dance floor Cardiff University's Dancesport Society will show us how it's done with their display of classics such as the Passo Doble, Cha-Cha-Cha and Rumba.

For anyone not involved in the conference dinner event, your conference delegate packs include a City Guide which will help you find the many excellent pubs, bars and restaurants to keep you entertained for the evening.

Internet facilities
Delegates can access the internet via several different wireless networks. Delegates who have registered with 'eduroam' at their own institution can access this network with their own password. Alternatively Cardiff University has guest Wi-Fi passes valid for the duration for the conference. Desktop computers with internet access will also be available in the Library in Main Building (see maps on pages 8 & 9). Access codes to sign onto desktops and 'CU-Guest-WiFi' can be picked up from the reception desk in the VJ gallery (on Monday only) and the Students' Union at all other times.

Left Baggage Facilities
On Thursday, delegates wishing to do so may leave their luggage in 'left luggage' located in the Main Building up until 2:00 pm. Please see maps on page 8 & 9 for location.

Details of the Plenary and Wright Medal Talks
We are privileged to present Professor Sir Roy M. Anderson FRS, FmedSci, Chair in Infectious Disease Epidemiology at Imperial College, London, as our plenary speaker. During his illustrious career Prof. Anderson has made key contributions to global infectious disease control through his seminal research and via educational/advisory roles. His role have been as diverse as University lecturer and former Chief Advisor to the Ministry of Defence, and Prof. Anderson continues to provide advice to national and international governments, and to public and private sector bodies. Furthermore, he has been integral in setting up a range of new activities including the Institute for Global Health. Prof. Sir Roy Anderson will speak on Wednesday March 31st at 2.15 pm in the Great Hall, Students' Union Building. His talk title is 'Our changing world and the emergence of novel infectious agents'.

This year's Wright Medalist is Professor Mark C. Field, Professor of Cell Biology and Parasitology at the Department of Pathology, University of Cambridge and Fellow and Director of Studies for Natural Sciences (Biological) at St Edmund's College. Prof Field focuses on genomic research on the parasitic protozoan *Trypanosoma brucei* with particular emphasis on cell trafficking. Prof. Mark Field will speak on Wednesday March 31st at 3.30 pm in the Great Hall, Students' Union Building. His talk title is 'Membrane trafficking systems; trypanosomes, surfaces and genomes'.

PROGRAM OVERVIEW

Monday March 29th	
14:.00-20.00	**REGISTRATION - VJ Gallery, Main Building** **BSP Council meeting (Room 0.51, Vice Chancellor's Board Room, Main Building, 15:00 onwards)**
18.30-20:00	**WELCOME RECEPTION - Drinks and finger buffet** **In the VJ Gallery, Main Building**
20:00-21:00	**Public Understanding of Science Lecture, Large Shandon Theatre - Room 1.64, Main Building** **Dr Rhys Jones (BBC Wales Presenter: www.bbc.co.uk/wales/mid/sites/royal_welsh/pages/rhysjones.shtml) – 'Polyticks and the Media'** **Dr. Mark Booth, Durham University – 'Dr Joseph McCrumble's Guide to Engaging with Parasites'**
21:00 -late!	**Bar Open in Students' Union - The Taf**

Tuesday March 30th					
	Session A **MALARIA and GENERAL PARASITOLOGY** **Large Shandon Theatre**	**Session B** **ECOLOGY** **Large Chemistry Theatre**	**Session C** **GENERAL PARASITOLOGY** **Small Chemistry Theatre**	**Session D** **TRYP/LEISH SEMINAR** **Wallace Theatre**	**Session E** **TRYP/LEISH SEMINAR** **Beverton Theatre**
	1A. Molecular Biology and Genomics	1B. Parasite and Host Life History Traits	1C. Helminth Immunology	1D. Trypanosomatid Systems Biology	
10:30-11:00	**Coffee break in the Students' Union** **(Posters on display in Students' Union throughout the meeting)**				
11:00-12:30	2A. Malaria: New Blood	2B. Host Parasite Co-evolution	2C. Disease Control	2D. Cell Biology I	2E. Comparative Genomics
12:30-14:00	**Lunch in the Students' Union**				
14:00-15:30	3A. Malaria: Immunology and Pathogenesis	3B. Parasite Transmission	3C. RNA Interference in Parasitic Nematodes	3D. Cell Biology II	
15:30-16:00	**Coffee in the Students' Union**				
16:00-17:30	4A. Malaria: A Historical Perspective	4B. Parasite Community Ecology	4C. Helminth Neurobiology / RNAi	4D. Nuclear and Chromatin Structure	
17:30-19:30	**Poster session in the Students' Union with drinks**				
19:30-late	**Buffet Dinner, Acappella Singers and Bars open until late in Students' Union**				

PROGRAM OVERVIEW

Wednesday March 31st					
09:00-10:30	5A. Protozoa: Molecular Pathogenesis	5B. Wildlife Disease and Invasion	5C. Schistosomes I	5D. Chemotherapy	5E. Immunology
10:30-11:00	**Coffee break in the Students' Union**				
11:00-12:30	6A. Host and Helminth Genetics	6B. Population Dynamics and Theoretical Ecology	6C. Schistosomes II	6D. Cell Death and Autophagy	6E. Mixed Session I
12.30-14.00	**Lunch in the Students' Union**				
14:00-14:15	**Welcome address** **Professor Ken Woodhouse, Pro Vice-Chancellor for Engagement, Cardiff University** **In the Great Hall, Students' Union Building**				
14.15-15:15	**BSP PLENARY LECTURE** **Our changing world and the emergence of novel infectious agents** **Prof. Sir Roy Anderson** **In the Great Hall, Students' Union Building**				
15:15-15:30	**Short Coffee break in the Great Hall, Students' Union Building**				
15:30-16:30	**CA WRIGHT MEDAL LECTURE by Prof. Mark C. Field** **Membrane trafficking systems; trypanosomes, surfaces and genomes** **In the Great Hall, Students' Union Building**				
16:30-	**BSP Annual General Meeting, Council Chambers, Main Building**				
18.30 -02:00	**CONFERENCE DINNER AND PARTY** **Hilton Hotel - Welcome Drinks & Welsh Harpist, Dinner followed by Latin Music And Dance**				

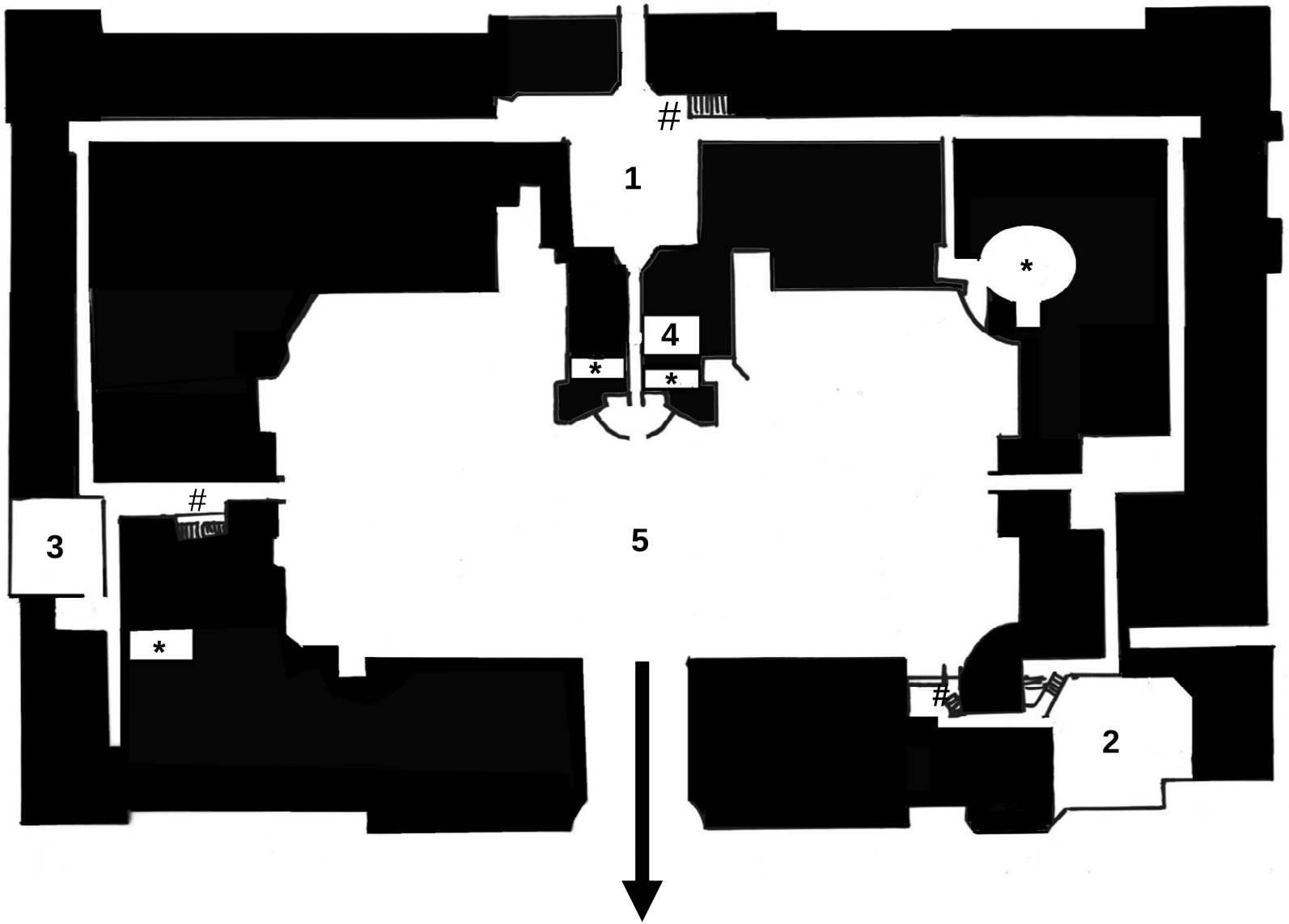
Thursday 1st April					
09:00-10:30	7A. Malaria: Mosquito's Innate Defence Against Malaria	7B. Environmental and Social Influences on Parasite Epidemiology	7C. Veterinary Parasitology	7D. Biochemistry	7E. Molecular Epidemiology and Population Genetics
10:30-11:00	**Coffee in the Students' Union**				
11:00-12:30	8A. Malaria: Control and Drug Discovery	8B. Ecology Meets Immunology	8C. Aquatic Parasitology	8D. Mixed Session II	8E. Sex in the Vector and in the Field
12:30-	**Lunch in the Students' Union & Conference Close**				

Map 1: Ground Floor Main Building

1. VJ Gallery
2. Shandon Lecture Theatre
3. Wallace Lecture Theatre
4. VC boardroom & left luggage
5. Car Park

* Toilets

\# Stairs to 1st floor



Map 2: First Floor Main Building

6. Council Chambers

7. Computer Suite

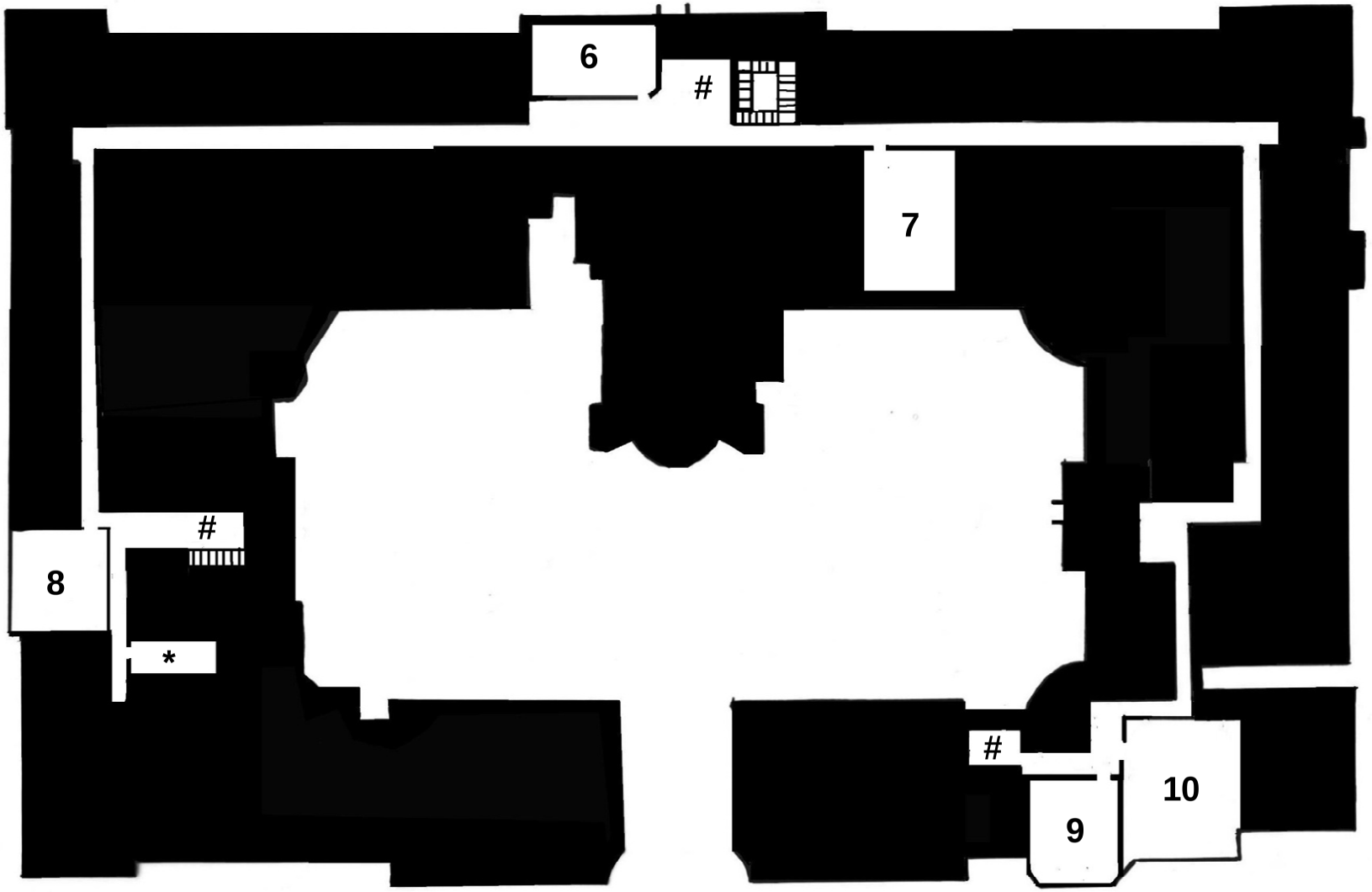
8. Beverton Lecture Theatre

9. Small Chemy Lecture Theatre

10. Large Chemy Lecture Theatre

* Toilets

\# Stairs to ground floor



Tuesday March 30th					
	Session A **MALARIA** **Large Shandon Theatre**		**Session B** **ECOLOGY** **Large Chemistry Theatre**		**Session C** **GENERAL PARASITOLOGY** **Small Chemistry Theatre**
09.00-10.30	**1A. Molecular Biology and Genomics** (Chair Paul Horrocks, Keele)	**09.00-10.30**	**1B. Parasite and Host Life History Traits** (Chair Mike Begon, Liverpool)	**09.00-10.30**	**1C. Helminth Immunology** (Chair Joseph Jackson, Liverpool)
09:00-09:30	M1 Invited Speaker No place to hide? Gene discovery in malaria parasites in the age of genomics. Sandra Cheesman	09:00-09:30	S34 Invited Speaker Parasite life history traits and environmental interactions Mark Viney	09:00-09:15	S74 *Fasciola hepatica*: investigation of triclabendazole (TCBZ) resistance in field cases of fasciolosis using histopathological and immunocytochemical methods to identify apoptosis in the reproductive structures of TCBZ-sensitive flukes. R. E. B.Hanna
				09:15-09:30	S75* Proteomic and immunological comparative studies of different trematode species Melissa Higón
09:30-09:50	M2 Unravelling the unique CSA binding mechanism of *Plasmodium falciparum* causing placental malaria Madeleine Dahlbäck	09:30-09:45	S35 Sex ratio and morphological polymorphism in an isolated, endemic *Teladorsagia circumcincta* population Barbara H. Craig	09:30-09:45	S76* Investigation into the mechanisms underlying the slow development of human anti-schistosome immunity Kate M. Mitchell
09:50-10:10	M3* It is all in your head: a model for cerebral malaria Antoine Claessens	09:45-10:00	S36* Consequences of parasitism for survivorship and fecundity in the cockroach host Joanna Rumsey	09:45-10:00	S77 Imbalance of regulatory and activated T cells in human *Schistosoma haematobium* infections Norman Nausch

10:10-10:30	M4 Organelle-specific localisation of the Fe-S cluster assembly protein NifE in *Plasmodium falciparum* Ingrid B. Müller	10:00-10:15	S37 Modes of egg production and larval development as 'organisers' of life-history in parasitoid wasps and plant-parasitic Lepidoptera Mark Jervis	10:00-10:15	S78 Targeting of host lipoproteins by the parasitic worm *Schistosoma mansoni* Saskia deWalick
		10:15-10:30	S38 Stress, drugs and the evolution of reproductive restraint in malaria parasites Sarah E. Reece	10:15-10:30	S79 Eosinophil degranulation against adult *Onchocerca ochengi* during macrofilaricidal chemotherapy is dependent on depletion of *Wolbachia* Benjamin L. Makepeace
10:30-11:00	**Coffee break in the Students' Union** **(Posters on display in Students' Union throughout the meeting)**				
11:00-12:30	**2A. Malaria: New Blood** (Chair Mike Blackman, NIMR)	**11:00-12:30**	**2B. Host Parasite Co-evolution** (Chair Phil Harris, Oslo)	**11:00-12:30**	**2C. Disease Control** (Chair Rupert Quinnell, Leeds)
11:00-11:30	M5 Invited Speaker Drowsy chaperones for a lively parasite Alexander G. Maier	11:00-11:30	S39 Invited Speaker Evolutionary arms races between Drosophila and its pathogens Francis Jiggins	11:00-11:30	S80 Invited Speaker Paradigm lost: How parasite control may alter pattern and process in human helminthiases MarÍa-Gloria Basáñez
11:30-12:00	M6* Invited speaker Stage estimation and lineage commitment in *Plasmodium falciparum* Jacob E. Lemieux	11:30-11:45	S40 * Geographic mosaic of the Red Queen: more sex in coevolutionary hotspots Kayla C. King	11:30-11:45	S81 Porcine parasites in Northern Ireland: incidence, distribution and correlation with management and control strategies J. Black
		11:45-12:00	S41 Decoupled coevolutionary cycles under two-step infection genetics Andy Fenton	11:45-12:00	S82* Detection of *Echinococcus granulosus* in farm dogs in South Powys, Wales using coproELISA Wai-San Li

12:00-12:30	M7 Invited speaker Systematic analysis of vesicular traffic in apicomplexan parasites Markus Meissner	12:00-12:15	S42 Antagonistic coevolution drives extremely rapid and divergent molecular evolution Michael Brockhurst	12:00-12:15	S83* A novel nicotinic acetylcholine receptor subunit of parasitic nematodes Hayley Bennett
		12:15-12:30	S43 Virulence evolution and competition Rob Knell	12:15-12:30	S84 Vaccination of rats against fasciolosis by a multivalent vaccine of stage-specific cathepsin proteases induces significant protection Terry W. Spithill
12:30-14:00	**Lunch in the Students' Union**				
14:00-15:30	**3A. Malaria: Immunology and Pathogenesis** (Chair Chris Newbold, Oxford)	**14:00-15:30**	**3B. Parasite Transmission** (Chair Jo Cable, Cardiff)	**14:00-15:30**	**3C. RNA Interference in Parasitic Nematodes** (Chair Angela Mousley, Belfast)
14:00-14:30	M8 Invited Speaker *Plasmodium falciparum* AMA1: Allelic diversity in asymptomatic, mild and severe malaria and allele-specific immunity Faith Osier	14:00-14:30	S44 Invited Speaker Transmission characteristics of *Protopolystoma xenopodis* (Monogenea) in a population of African *Xenopus laevis* isolated in Wales for over 40 years Richard C. Tinsley	14:00-14:30	S85 Invited Speaker Developing an alternative RNA interference protocol to study druggable targets in parasitic nematodes Michael J. Kimber
		14:30-14:45	S45* Getting out of the host: Transmission strategies in protozoan parasites Laura C. Pollitt	14:30-14:45	S86 An eye on roundworm RNAi Aaron G. Maule
14:30-14:50	M9 Fine specificity of vaccine induced *Plasmodium falciparum* CSA-adhesion blocking antibodies Sisse B. Ditlev	14:45-15:00	S46***** To boldly go where no fish has gone before: impact of host personality on parasite transmission Loys Richards	14:45-15:00	S87 Investigating the efficiency of RNA interference in the clade III nematode parasite *Ascaris suum* N. Warnock
14:50-15:10	M10* The effect of adjuvants in the immune phenotype induced by a *Plasmodium falciparum* DBL4-VAR2CSA based vaccine Vera Valadão Pinto	15:00-15:15	S47 Do parasites fit into Lindeman's classic biomass pyramid pattern of food webs? Alexander D. Hernandez	15:00-15:15	S88 In vitro and in vivo RNAi of *Haemonchus contortus* H11 Dave Knox

15:10-15:30	M11 Maternally acquired immunity and protection from malaria infection Vincent Staszewski	15:15-15:30	S48 *Heligmosomoides polygyrus* and *H. bakeri*: host shifts, host dispersal and co-evolution Phil D. Harris	15:15-15:30	S89* RNAi reveals a role for Mi-che-3, Mi-osm-3 and Mi-flp-21 in the plant host finding response of the root knot nematode *Meloidogyne incognita* Johnathan J. Dalzell
15:30-16:00	**Coffee in the Student's Union**				
16:00-17:30	**4A. Malaria: A Historical Perspective** (Chair: Carol Reeves, UCL)	**16:00-17:30**	**4B. Parasite Community Ecology** (Chair Andy Fenton, Liverpool)	**16:00-17:30**	**4C. Helminth Neurobiology / RNAi** (Chair Aaron Maule, Belfast)
16:00-16:30	M12 Invited Speaker 'All patients were clinically cured': The discovery and development of artemisinin as an effective anti-malarial drug Tilli Tansey	16:00-16:30	S49 Invited Speaker Patterns and processes of microparasite coinfection in a wildlife host Mike Begon	16:00-16:15	S90 The use of siRNAs to probe cathepsin L function in *Fasciola hepatica* E. Cameron
				16:15-16:30	S91* Tissue-specific sensitivity to RNA interference in the plant parasitic nematode, *Globodera pallida* M. Stevenson
16:30-17:00	M13 Invited Speaker Exploring old ground: Greece and the malaria pandemic of 1905 Katerina Gardikas	16:30-16:45	S50 Co-infection causes variation in transmission Sandra Lass	16:30-16:45	S92 Using *Caenorhabditis elegans* to study drug targets from parasitic nematodes Susan Glendinning
		16:45-17:00	S51 Resolving the *Wolbachia* paradox Greg Hurst	16:45-17:00	S93 A PAL for *Schistosoma mansoni* PHM Louise E. Atkinson
17:00-17:15	M14* Pills, parasites and profits Mark Honigsbaum	17:00-17:15	S52 Do worms promote or prevent malaria? Using meta-analysis to assess the evidence in mice and men Sarah C. L. Knowles	17:00-17:15	S94 Neuronal RNAi in *Schistosoma mansoni* Paul McVeigh
17:15-17:30	M15* Burdwan fever and the making of a malarial locality, 1863-1875 Rohan Deb Roy	17:15-17:30	S53 Individual based measure of interactive/isolationist degree of infracommunities: towards an assessment of the role of intrinsic and extrinsic factors Nicola Ferrari	17:15-17:30	S95 Voltage-operated calcium currents in *Schistosoma mansoni* are enhanced by FMRFamide-like peptides (FLPs) Tim A. Day
17:30-late	**See Programme Overview**				

Wednesday March 31st					
	Session A **GENERAL PARASITOLOGY** **Large Shandon Theatre**		**Session B** **ECOLOGY** **Large Chemistry Theatre**		**Session C** **GENERAL PARASITOLOGY** **Small Chemistry Theatre**
09.00-10.30	**5A. Protozoa: Molecular pathogenesis** (Chair Markus Meissner, Glasgow)	**09.00-10.30**	**5B. Wildlife Disease and Invasion** (Chair Sarah Perkins, Cardiff)		**5C. Schistosomes I** (Chair Joanne Webster, Imperial)
09:00-09:30	M16 Invited Speaker From transcriptomes to systems: do we need a reality check? Jonathan Wastling	09:00-09:30	S54 Invited Speaker Managing a most unusual parasite: Tasmanian devil facial tumour Hamish McCallum	09:00-09:30	S96 Invited Speaker Contrasting reservoirs or transmission patterns for *Schistosoma japonicum* between marshland and hilly regions of China Da-Bing Lu
09:30-10:00	M17 Invited Speaker The molecular evolutionary immunoecology of *Toxoplasma gondii* Jonathan Howard	09:30-09:45	S55* Patterns of stable isotope signatures and malaria infections in *Ficedula* flycatchers Katarzyna Kulma	09:30-09:45	S97 New insights into the epidemiology and interactions of *Schistosoma haematobium* group species in Senegal Bonnie Webster
		09:45-10:00	S56* Molecular phylogenetics of *Thelohania muelleri* like parasites infecting gammarid amphipods Toby J Wilkinson	09:45-10:00	S98* IgE and IgG4 responses in human *Schistosoma haematobium* and atopy Nadine Rujeni
10:00-10:15	M18 A soluble mediator from *Giardia Lamblia* disrupts human gastrointestinal epithelial cell function Suha Al-Naimi	10:00-10:15	S57* Spatial distribution of *Pseudamphistomum truncatum* and *Metorchis albidus* in England and Wales using ArcMap GIS Ellie Sherrard-Smith	10:00-10:15	S99* The *Schistosoma mansoni* cercarial elastase: investigating its potential as a vaccine for schistosomiasis Marwa El Faham
10:15-10:30	M19* Investigation of putative species specific markers of *C. hominis* and *C. parvum* identified by comparative genomics tools Maha Bouzida	10:15-10:30	S58 Parasites alter the predatory interactions between native and invading species. Alison M. Dunn	10:15-10:30	S100 Development and validation of a quantitative, high-throughput, fluorescent-based bioassay to detect *Schistosoma* viability E. Peak
10:30-11:00	**Coffee in the Students' Union**				
11:00-12:30	**6A. Host and Helminth Genetics** (Chair Steve Paterson, Liverpool)	**11:00-12:30**	**6B. Population Dynamics and Theoretical Ecology** (Chair Jo Lello, Cardiff)	**11:00-12:30**	**6C. Schistosomes II** (Chair Mike Doenhoff, Nottingham)

11:00-11:15	S20 An examination of VAL diversity throughout the Platyhelminthes Iain W. Chalmers	11:00-11:30	S59 Invited Speaker Modelling Dengue: Epidemic Patterns and Persistence through Vector Transmission Mike Boots	11:00-11:15	S101 *Schistosoma mansoni* Annexin B2: characterization, immunolocalization and function studies Cibele A. Tararam
11:15-11:30	S21 Expression profiling and immunological characterization of the *Schistosoma mansoni* VAL protein family Samirah Perally			11:15-11:30	S102 Investigation of Venom Allergen Like Proteins (VALs) from *Schistosoma mansoni* as vaccine candidates Luciana C. C. Leite
11:30-11:45	S22* DNA plasmids encoding modified parasite proteins overcome parasite-driven suppression of host's immune responses HongLin Luo	11:30-11:45	S60 Geographical variations in elimination criteria for global helminth control programs Manoj Gambhir	11:30-11:45	S103* Whole Blood Cytokine Responses in Urinary Schistosomiasis: from pathway immunology to human disease Claire Bourke
11:45-12:00	S23 GTP-Cyclohydrolase in parasitic nematode development Rachael Smith	11:45-12:00	S61 Epidemic malaria and warmer temperatures in recent decades in an East African highland David Alonso	11:45-12:00	S104* The Effects of *Schistosoma mansoni* Haemozoin on Macrophage Activation in a Changing Cytokine Milieu Martha Truscott
12:00-12:15	S24 Genetic changes associated with the selection of an ivermectin-resistant isolate of *Haemonchus contortus.* Adrian Wolstenholme	12:00-12:15	S62 Integrating theory and experiment to understand variation in malaria parasites Nicole Mideo	12:00-12:15	S105 Observed Reductions in *Schistosoma mansoni* Transmission from Large-scale Administration of Praziquantel in Uganda: a Mathematical Modelling Study M. D. French
12:15-12:30	S25 The role of host genetics in predisposition to human hookworm and *Ascaris lumbricoides* infection Rupert Quinnell	12:15-12:30	S63* Density-dependent mortality of the human host in onchocerciasis: relationships between microfilarial load and excess mortality Martin Walker	12:15-12:30	S106 Systemic antibody and cytokine levels differ with infection status and age in people exposed to *S. haematobium* Francisca Mutapi
12.30-14.00	**Lunch in the Students' Union**				
14:00-onwards	**See Programme Overview**				

Thursday 1st April					
	Session A **MALARIA** **Large Shandon Theatre**		**Session B** **ECOLOGY** **Large Chemistry Theatre**		**Session C** **GENERAL PARASITOLOGY** **Small Chemistry Theatre**
9.00-10.30	**7A. Malaria: Mosquito's Innate Defence Against Malaria** (Chair Janet Hemmingway, LSTH)	**9.00-10.30**	**7B. Environmental and Social Influences on Parasite Epidemiology** (Chair Mark Booth, Durham)	**9.00-10.30**	**7C. Veterinary Parasitology** (Chair Oliver Sparagano, Newcastle)
09:00-09:30	M26 Invited Speaker: Mosquito's innate defence against malaria George K. Christophides	09:00-09:30	S64 Invited Speaker Epidemiological and evolutionary effects of intensive farming Arne Skorping	09:00-09:30	S107 Invited Speaker An evolutionary perspective on gastrointestinal nematodes of sheep Michael Stear
09:30-09:50	M27 Genetic control of mosquito populations to combat malaria: modelling approach A. Deredec	09:30-09:45	S65* Estimating the Global Burden of Disease of the Neglected Tropical Disease Onchocerciasis Simon O'Hanlon	09:30-09:45	S108 Reduced efficacy of ivermectin-pour-on on English cattle farms K. A. Stafford
09:50-10:10	M28 Modelling the impacts of climate change on malaria transmission Paul E. Parham	09:45-10:00	S66* Lymphatic Filariasis mapping using Bayesian spatial techniques Hannah Slater	09:45-10:00	S109* Effects of mixed grazing on nutrition and growth of grazing goats infected by gastrointestinal nematodes S. D'Alexis
10:10-10:30	M29 * Impact of anaemia on the feeding and fitness of the malaria vector *An. gambiae s.s.* S. N. Emami	10:00-10:15	67* Environmental conditions and senescence in parasite resistance of a wild mammal Adam D. Hayward	10:00-10:15	S110 Using lectins to identify "hidden antigens" in the liver fluke, *Fasciola hepatica* Heather McAllister
		10:15-10:30	S68 Effects of temporal environmental variation on host-parasite dynamics in the *Paramecium caudatum - Holospora undulata* system Alison B. Duncan	10:15-10:30	S111 Molecular detection of haemopathogen infections of dogs, cats and horses using the reverse line blot hybridization assay Oliver Sparagano
10:30-11:00	**Coffee in the Student's Union**				
11:00-12:30	**8A. Malaria: Control and Drug Discovery** (Chair Colin Sutherland, LSHTM)	**11:00-12:30**	**8B. Ecology Meets Immunology** (Chair Mark Viney, Bristol)	**11:00-12:30**	**8C. Aquatic Parasitology -** Sponsored by Environment Agency (Chair Iain Barber, Leicester)

11:00-11:30	M30 Invited Speaker The Malaria Parasite Mitochondrion: Bioenergetics and Drug Development Giancarlo Biagini	11:00-11:30	S67 Invited Speaker The use of predator-prey-like models to assess the action of the immune system on parasite infections Minus van Baalen	11:00-11:30	S112 Invited Speaker Host-parasite interactions in variable environments Otto Seppälä
11:30-11:50	M31 New alleles of pfmdr1 and other markers of *P. falciparum* resistance in the post-chloroquine era in Africa Rachel Hallett	11:30-11:45	S70 Acellular pertussis vaccination facilitates *Bordetella parapertussis* infection in a rodent model of Bordetella Infection Gráinne H. Long	11:30-11:45	S113 Parasites as agents of selection in perch (*Perca fluviatilits*)? MHC variability and parasite host dynamics Jasminca Behrmann-Godel
11:50-12:10	M32 Development and Evaluation of a Novel Magneto-Optical Test (MOT) for the Diagnosis of Malaria Henk Schallig	11:45-12:00	S71* Why do antibodies induced by malaria and helminths cross-react? Karen J. Fairlie-Clarke	11:45-12:00	S114* Growth, metabolism and ultrastructure of *Spironucleus vortens* Coralie Millet
12:10-12:30	M33 Who transmits malaria? Petra Schneider	12:00-12:15	S72 Post-genomic strategies allow the analysis of immunological signalling networks in wild animals: a case study on immunodynamics in the field vole, *Microtus agrestis* Joseph Jackson	12:00-12:15	S115* Sex and hybridization in gyrodactylid monogeneans Bettina Schelkle
		12:15-12:30	S73* Immunogenetics, selection and parasite resistance in a natural population of field voles (*Microtus agrestis*) Andrew Turner	12:15-12:30	S116 *Anisakis simplex* associated with 'red vent syndrome' in wild adult Atlantic salmon *Salmo salar* in Great Britain Melinda Beck
12:30-	**Lunch in the Student's Union & Conference Close**				

Tuesday March 30th			
	Session D **TRYP/LEISH SEMINAR** **Wallace Lecture Theatre**		**Session E** **TRYP/LEISH SEMINAR** **Beverton Lecture Theatre**
09:00-10:30	**1D. Trypanosomatid Systems Biology** (Chair Mike Barratt, Glasgow)	**09:00-10:30**	
09:00-09:30	T/L117 Invited Speaker Trypanosome Systems Biology Rainer Breitling		
09:30-09:50	T/L118 Integrated spectroscopic time-trajectory assessment of *Trypanosoma brucei brucei* in the mouse Jasmina Saric		
09:50-10:10	T/L119 Relevance of 2,4-dienoyl-coa reductase of Leishmania for virulence Daniel Paape		
10:10-10:30	T/L120 * The *Trypanosoma brucei* RNA-binding protein RBP10 is required for maintenance of glycolytic energy metabolism in the bloodstream form M. Wurst		
10:30-11:00	**Coffee break in the Students' Union** **(Posters on display in Students' Union throughout the meeting)**		
11:00-12:30	**2D. Cell Biology I** (Chair Tansy Hammarton, Glasgow)	**11:00-12:30**	**2E. Comparative Genomics** (Chair Christiane Hertz-Fowler, Liverpool)
11:00-11:30	T/L121 Invited Speaker A novel phosphatase cascade regulates differentiation in trypanosomes via a glycosomal signaling pathway Keith R. Matthews	11:00-11:30	T/L158 Invited Speaker i-seq profiling in trypanosomes: next-generation sequencing for genome-scale RNA interference studies David Horn

11:30-11:45	T/L122 The RNA helicase DHH1 is central to the correct expression of many developmentally regulated mRNAs in trypanosomes Susanne Kramer	11:30-11:45	T/L159 Genome sequencing project has begun on two plant trypanosomatids ("Phytomonas") Michel Dollet
11:45-12:00	T/L123 Transcripts and Cofactors of a Post-Transcriptional Regulator Complex Pegine B. Walrad	11:45-12:00	T/L160 Aneuploidy and mosaicism in Leishmania strains as adaptative means to changing host environments Patrick Bastien
12:00-12:15	T/L124 TAO is imported into mitochondria via unique pathway in the bloodstream form of *Trypanosoma brucei* Minu Chaudhuri	12:00-12:15	T/L161 Whole comparative genome sequencing reveals several levels of genomic diversity among *Leishmania donovani* strains in Indian subcontinent. Hideo Imamura
12:15-12:30	T/L125 The assembly of iron-sulfur clusters in *Trypanosoma brucei* Julius Lukeš	12:15-12:30	T/L162 Functional characterization of cyclopropane fatty acid synthetase in Leishmania Samuel O. Oyola
12:30-14:00	**Lunch in the Students' Union**		
14:00-15:30	**3D. Cell Biology II** (Chair Keith Matthews, Edinburgh)	**14:00-15:30**	
14:00-14:15	T T/L126 * The regulation of the sub-cellular localisation of GPI-PLC Jack Sunter		
14:15-14:30	T/L127* The N-terminus of phosphodiesterase TbrPDEB1 of T. brucei contains the flagellar targeting information Edith Luginbuehl		
14:30-14:45	T/L128* AIR9 is a cytoskeleton-associated protein required for organelle positioning and cytokinesis in *Trypanosoma brucei* Sophie May		
14:45-15:00	T/L129 *Trypanosoma brucei* Rab28 mediates late endocytic processes including sensitivity to trypanosome lytic factor Jennifer Lumb		
15:00-15:15	T/L130 Functional characterisation and drug target validation of a mitotic Kinesin-13 in *Trypanosoma brucei* Klaus Ersfeld		
15:15-15:30	T/L131 Regulation of NDR kinase activity in *Trypanosoma brucei* Corinna Benz		
15:30-16:00	**Coffee in the Students' Union**		

16:00-17:30	**4D. Nuclear and Chromatin Structure** (Chair Mark Field, Cambridge)	**16:00-17:30**	
16:00-16:30	T/L132 Invited Speaker: Nuclear architecture and chromatin interactions underlying VSG epigenetic regulation in *Trypanosoma brucei* Miguel Navarro		
16:30-16:45	T/L133* Genome-wide mapping of Orc1/Cdc6 DNA binding sites and replication origins in Trypanosoma brucei Calvin Tiengwe		
16:45-17:00	T/L134 NUP-1, a large coiled-coil protein in *Trypanosoma brucei,* performs analogous functions to lamins of Metazoa Kelly N. DuBois		
17:00-17:15	T/L135 Elongator protein 3b regulates ribosomal RNA transcription in trypanosomes Sam Alsford		
17:15-17:30	T/L136 Centromere-associated topoisomerase activity in bloodstream form *Trypanosoma brucei* John M. Kelly		
17:30-late	**See Program Overview**		

Wednesday March 31st			
09:00-10:30	**5D. Chemotherapy** (Chair Simon Croft, LSHTM, London)		**5E. Immunology** (Chair Jim Alexander, Strathclyde)
09:00-09:30	T/L137 Invited Speaker Targeting the Leishmania kinome for the development of novel anti-parasitic strategies Gerald Späth	09:00-09:23	T/L163 Invited Speaker Neutrophil-derived CCL3 is essential for the rapid recruitment of dendritic cells to the site of Leishmania inoculation in resistant mice Fabienne Tacchini-Cottier
09:30-09:45	T/L138 Anti-leishmanial activity of betulinic acid derivatives in *Leishmania* (L.) *braziliensis*. Comparison of statistical methods to evaluate the IC50 of best candidates Alicia Ponte-Sucre	09:23-09:46	T/L164 Invited Speaker B cell dysfunction in African trypanosomiasis Stefan Magez1,
09:45-10:00	T/L139 Dissecting the essentiality of the bifunctional trypanothione synthetase-amidase in *Trypanosoma brucei* using chemical and genetic methods Susan Wyllie	09:46-10:01	T/L165 Modulation of dendritic cell antigen presentation by *Leishmania mexicana* Owain R. Millington
10:00-10:15	T/L140 Unraveling the trypanocidal mechanism of nifurtimox Belinda Hall	10:01-10:16	T/L166 Complement 3 deficiency influence lesion progression and immune response during *Leishmania major* infection in C57BL/6 mice Rami M Mukbel
10:15-10:30	T/L141 Cyclic nucleotide phosphodiesterases of kinetoplastids: new targets for drug development? Thomas Seebeck	10:16-10:30	T/L167 Interleukin-13 reduces hyperalgesia and the level of Interleukin-1β in BALB/c mice infected with *Leishmania major* and upregulates the level of Interleukin-6 Marc C. Karam
10:30-11:00	**Coffee break in the Students' Union**		
11:00-12:30	**6D. Cell Death and Autophagy** (Chair Nicolas Fasel, Lausanne)	**11:00-12:30**	**6E. Mixed Session I** (Chair Karen Grant, Lancaster)
11:00-11:30	T/L142 Invited Speaker Cell death mechanisms in protozoan parasites Michael Duszenko	11:00-11:15	T/L168 Dyskinetoplastic trypanosomes: how to live without mitochondrial DNA Achim Schnaufer
		11:15-11:30	T/L169 Characterization of the Trypanosome Orthologue of DIP13, a Protein Implicated in Sjögren's Syndrome Helen P Price

11:30-11:45	T/L143 Autophagy in Leishmania Roderick A. Williams	11:30-11:45	T/L170 Assessing stumpy formation in *Trypanosoma brucei* Paula MacGregor
11:45-12:00	T/L144 Neuropeptides kill trypanosomatids by targeting intracellular compartments and inducing autophagic-like cell death Jenny Campos-Salinas	11:45-12:00	T/L171 Blocking synthesis of the FACT subunit Spt16 causes cell cycle specific derepression of the ES-promotor in Trypanosoma brucei Viola Denninger
12:00-12:15	T/L145* Processing of metacaspase into a cytoplasmic catalytic domain mediating cell death in *Leishmania major* Habib Zalila	12:00-12:15	T/L172 Evolutionary reconstruction of the retromer complex and its function in *Trypanosoma brucei* V. Lila Koumandou
12:15-12:30	T/L146 Contribution of cathepsin B in the cell death pathway of Leishmania parasites Nicolas Fasel	12:15-12:30	T/L173 Ubiquitin-mediated mechanisms for turnover of invariant surface glycoproteins in *Trypanosoma brucei* Ka Fai Leung
12.30-14.00	**Lunch in the Students' Union**		
14:00-onwards	**See Program Overview**		

Thursday 1st April			
09:00-10:30	**7D. Biochemistry** (Chair Michael Ginger, Lancaster)	**09:00-10:30**	**7E. Molecular Epidemiology and Population Genetics** (Chair J-C Dujardin, Antwerp)
09:00-09:30	T/L147 Invited Speaker Sugar-kinases and anomerization of sugar-phosphates in trypanosomes Paul Michels	09:00-09:30	T/L174 Invited Speaker Epidemiological and population genetic studies in *the L. donovani* complex Gabriele Schönian
09:30-09:45	T/L148 Distinctive biochemistry in the mitochondrial intermembrane space of trypanosomatids James W. A. Allen	09:30-09:45	T/L175 Diversity within *Trypanosoma (Duttonella) vivax* revealed by Fluorescent Fragment Length Barcoding, FFLB Emily Adams
09:45-10:00	T/L149* On the role of glutaredoxins in *Trypanosoma brucei*, an organism that lacks the classical glutathione/glutathione reductase system Sevgi Ceylan	09:45-10:00	T/L176 Re-assessment of role of domestic animals in the epidemiology of kala-azar in Nepal Jean-Claude Dujardin
10:00-10:15	T/L150* Functional genomics of amino acid permeases from *Leishmania donovani*: The story of proline transport Ehud Inbar	10:00-10:15	T/L177* Is vector-parasite interaction a determining factor for the population structure of *L. donovani* in East Africa? Tesfaye Gelanew

10:00-10:30	T/L151* *Trypanosoma brucei* metacaspases – surprises from the fourth family member Will Proto	10:00-10:30	T/L178* The effectiveness of a targeted re-treatment intervention in reducing the prevalence of trypanosomiasis in cattle in Uganda. Louise Hamill
10:30-11:00	**Coffee in the Students' Union**		
11:00-12:30	**8D. Mixed Session II** (Chair Graham Coomb, Strathclyde)	**11:00-12:30**	**8E. Sex in the Vector and in the Field** (Chair Wendy Gibson, Bristol)
11:00-11:15	T/L152* Leishmania infantum IZT3 is an inducible zinc transporter Sandra Carvalho	11:00-11:30	T/L179 Invited Speaker Demonstration of a Leishmania Sexual Cycle in the Sand Fly Vector David Sacks
11:15-11:30	T/L153 TbMLP1: A putative iron transporter in *Trypanosoma brucei* Martin C. Taylor		
11:30-11:45	T/L154* Biophysical characterization of the *Leishmania donovani* peroxin 14 and its role in glycosomal protein import Normand Cyr	11:30-11:50	T/L180 Visualizing meiosis in trypanosomes Lori Peacock
11:45-12:00	T/L155* Cyclosporin A treatment of *Leishmania donovani* reveals stage-specific functions of cyclophilins in parasite differentiation, proliferation, and viability Wai-Lok Yau	11:50-12:10	T/L181 Origins of natural *Trypanosoma cruzi* hybrid lineages Michael Lewis
12:00-12:15	T/L156 The trypanosomatid sphingolipid synthases: common enzymes with divergent functions Paul W. Denny	12:10-12:30	T/L182 *Trypanosoma cruzi* population genetics: progress and new perspectives from multilocus microsatellite typing (MLMT) Martin S Llewellyn
12:15-12:30	T/L157 In vitro activity and host cell dependence of anti-leishmanial drugs Karin Seifert		
12:30-	**Lunch in the Students' Union & Conference Close**		

ORAL PRESENTATION PLANNER					
Tuesday March 30th					
	Session A **MALARIA and GENERAL PARASITOLOGY** **Large Shandon Theatre**	**Session B** **ECOLOGY** **Large Chemistry Theatre**	**Session C** **GENERAL PARASITOLOGY** **Small Chemistry Theatre**	**Session D** **TRYP/LEISH SEMINAR** **Wallace Theatre**	**Session E** **TRYP/LEISH SEMINAR** **Beverton Theatre**
09.00-10.30	1A. Molecular Biology and Genomics	1B. Parasite and Host Life History Traits	1C. Helminth Immunology	1D. Trypanosomatid Systems Biology	
10:30-11:00	**Coffee break in the Students' Union** **(Posters on display in Students' Union throughout the meeting)**				
11:00-12:30	2A. Malaria: New Blood	2B. Host Parasite Co-evolution	2C. Disease Control	2D. Cell Biology I	2E. Comparative Genomics
12:30-14:00	**Lunch in the Students' Union**				

ORAL PRESENTATION PLANNER					
Tuesday March 30th					
	Session A **MALARIA and GENERAL PARASITOLOGY** **Large Shandon Theatre**	**Session B** **ECOLOGY** **Large Chemistry Theatre**	**Session C** **GENERAL PARASITOLOGY** **Small Chemistry Theatre**	**Session D** **TRYP/LEISH SEMINAR** **Wallace Theatre**	**Session E** **TRYP/LEISH SEMINAR** **Beverton Theatre**
14:00-15:30	3A. Malaria: Immunology and Pathogenesis	3B. Parasite Transmission	3C. RNA Interference in Parasitic Nematodes	3D. Cell Biology II	
15:30-16:00	**Coffee in the Students' Union**				
16:00-17:30	4A. Malaria: A Historical Perspective	4B. Parasite Community Ecology	4C. Helminth Neurobiology / RNAi	4D. Nuclear and Chromatin Structure	

ORAL PRESENTATION PLANNER					
Wednesday March 31st					
09:00-10:30	5A. Protozoa: Molecular Pathogenesis	5B. Wildlife Disease and Invasion	5C. Schistosomes I	5D. Chemotherapy	5E. Immunology
10:30-11:00	**Coffee break in the Students' Union**				
11:00-12:30	6A. Host and Helminth Genetics	6B. Population Dynamics and Theoretical Ecology	6C. Schistosomes II	6D. Cell Death and Autophagy	6E. Mixed Session I
12.30-14.00	**Lunch in the Students' Union**				

ORAL PRESENTATION PLANNER					
Thursday 1st April					
09:00-10:30	7A. Malaria: Mosquito's Innate Defence Against Malaria	7B. Environmental and Social Influences on Parasite Epidemiology	7C. Veterinary Parasitology	7D. Biochemistry	7E. Molecular Epidemiology and Population Genetics
10:30-11:00	**Coffee in the Students' Union**				
11:00-12:30	8A. Malaria: Control and Drug Discovery	8B. Ecology Meets Immunology	8C. Aquatic Parasitology	8D. Mixed Session II	8E. Sex in the Vector and in the Field
12:30-	**Lunch in the Students' Union & Conference Close**				

SPRING MEETING ORAL ABSTRACTS

M = Malaria presentations
S = Spring Meeting presentations
T/L = Trypanosomiasis/Leishmaniasis Seminar presentations
Student presentations are marked with an asterisk (*)

Column A – Malaria

TUESDAY 30th MARCH

Session 1A - Malaria: Molecular biology and genomics (Chair: Paul Horrocks)

M1 Invited Speaker
No place to hide? Gene discovery in malaria parasites in the age of genomics
Sandra Cheesman
School of Biological Sciences, Institute of Immunology and Infection Research, The University of Edinburgh, EH9 3JR.

The huge recent technological advances in genomics are having a big impact on malaria research. One area that has benefited from this is the molecular genetics methodology, Linkage Group Selection (LGS), which was designed to identify parasite genes controlling biologically or medically important selectable phenotypes of the parasites. LGS works by applying a selection pressure that represents the property of interest, for example, fast or slow multiplication in the red blood cells of the host, to the progeny of a genetic cross between parasites that differ in that property. By screening the selected cross progeny with quantitative genome-wide markers representing the parents of the cross, locations within the genome can be identified where the markers have come under the effects of selection, leading towards the identification of specific genes that determine the difference in blood-stage multiplication rate between the two parasite lines. In this presentation I will describe how the application of LGS to identify genes controlling specific biological phenotypes of malaria parasites is providing new insight into their biology.

M2 Unraveling the unique CSA binding mechanism of *Plasmodium falciparum* causing placental malaria
Madeleine Dahlbäck[1], Pongsak Khunrae[2], Morten A. Nielsen[1], Gorm Andersen[1], Sisse B. Ditlev[1], Mafalda Resende[1], Vera V. Pinto[1], Thor G. Theander[1], Matthew K. Higgins[2], Ali Salanti[1].
[1]Centre for Medical Parasitology, University of Copenhagen and Copenhagen University Hospital, Denmark. [2]Department of Biochemistry, University of Cambridge, Cambridge, UK.

In pregnant women *P. falciparum*-infected erythrocytes (IEs) express a unique member of the PfEMP1 family named VAR2CSA, which is associated with the ability of the IEs to adhere to chondroitin sulphate A (CSA) in the placenta. Understanding the mechanism behind this specific CSA interaction is important for the optimal design of a vaccine against placental malaria. CSA-binding of single domains of VAR2CSA has appeared to not reflect the specificity of the native IE binding. Therefore, to get a clearer picture of the role of VAR2CSA in this interaction we have for the first time expressed the complete extracellular region of VAR2CSA (~325 kDa) and analyzed the function of this protein. The 'full-length' VAR2CSA, as well as truncated variants, were expressed in Baculovirus-infected insect cells. Our results demonstrate that VAR2CSA alone can bind with nano-molar affinity to placental CSA and this binding shows a similar specificity to that observed for IEs. At least four domains of VAR2CSA seem to be required to maintain the specific binding site. Antibodies raised against 'full-length' VAR2CSA completely inhibit parasite binding to CSA. These results suggest that native VAR2CSA binds directly to CSA in the placenta and that the binding site is comprised of parts from several domains, which will have implications for vaccine development.

M3* It is all in your head: a model for cerebral malaria

Antoine Claessens, Zbynek Bozdech & Alex Rowe.
IIIR, University of Edinburgh, Ashworth Laboratories, Edinburgh, UK.

Cerebral malaria is characterised by a blockade of brain microvessels due to an accumulation of infected erythrocytes. *P. falciparum* infected erythrocytes have been shown to bind to a Human Brain Endothelial Cell line (HBEC-5i), however, the parasite adhesion ligands necessary to anchor the infected erythrocytes onto HBEC-5i have not been identified. We aimed to identify parasite variant surface antigens (VSA) that are differentially transcribed after selection for cytoadherence to HBEC-5i.
The *P. falciparum* laboratory strains 3D7, HB3 and IT/FCR3 were selected using a panning assay for binding to HBEC-5i. In order to analyse their transcriptome using a microarray chip based on the 3D7 genome, VSA sequences (var, rif, stevor) were extracted from the sequenced HB3 and IT genomes and added to the 3D7-based microarray chip.
Microarray data indicate significant changes in var gene transcription (but not other VSA) between unselected and HBEC-selected parasites. The unselected parasite populations express almost uniquely centromeric var genes (group B and C), while HBEC-selected express mostly a single Group A var gene. Reverse transcriptase-PCR confirm these finding, suggesting that group A var genes are the main parasite ligand for binding to HBEC-5i. Interestingly, our findings are consistent with previous work showing an association between Group A var genes and cerebral malaria. The HBEC-5i receptors mediating this interaction with infected erythrocytes are currently under investigation.

M4 Organelle-specific localisation of the Fe-S cluster assembly protein NifE in *Plasmodium falciparum*

Ingrid B. Müller, Sabine Butzloff, Sandra Massmann, Rolf D. Walter and Carsten Wrenger
Biochemistry, Bernhard Nocht Institute for Tropical Medicine, Hamburg, Germany

Iron-sulfur (Fe-S) clusters act as enzymatic cofactors in various metabolic reactions and are essential for electron transport in mitochondrion and chloroplast. The biogenesis of Fe-S clusters is highly regulated in pro- and eukaryotes. Four different assembly systems have been described so far. The mitochondrial Nif- (nitrogen fixation) and Isc- (iron-sulfur-cluster) assemblies, the chloroplast-localised Suf- (sulfur mobilisation) machinery and the cytosolic iron-sulfur protein assembly (Cia). In all syntheses molecular sulfur is taken from cysteine by a specific cysteine desulfurase, e.g. NifS or SufS, and is subsequently transferred by NifU or SufE on molecular iron, held by scaffold proteins, which then transport the cluster to the target apoprotein. Each organelle has its own cysteine desulfurase. However, in plants the assembly protein SufE is present in both the mitochondrion as well as the chloroplast. The human malaria parasite *Plasmodium falciparum* holds two Fe-S cluster syntheses; the Nif- and the Suf- system, but only one SufE-like sequence is found in the genome. We show that the plasmodial NifE is functional, that it localises in both organelles and that this localisation is regulated in a posttranslational manner.

Session 2A - Malaria: New blood (Chair: Mike Blackman)

M5 Invited Speaker
Drowsy chaperones for a lively parasite

Nick Proellocks[1], Andy Mikkonen[1], Matt McKenzie[1], Ping Cannon[2], Melanie Rug[2] & Alexander G. Maier[1]
[1]Department of Biochemistry, La Trobe Institute for Molecular Science, La Trobe University, Melbourne Victoria 3086 Australia. [2]The Walter and Eliza Hall Institute, 1G Royal Parade, Melbourne Victoria 3050 Australia

The malaria parasite *Plasmodium falciparum* exports molecules across its own confines into the cytoplasm of its host cell. Some of these proteins are virulence factors displayed on the surface of infected red blood cells, which are crucial for the survival of the parasite.

During the export process the parasite molecules have to be translocated across several membranes and assembled into higher order functional complexes. Chaperones are assisting in this process and DNAJ molecules together with heat shock protein (HSP) 70 represent one chaperone system of particular interest. Most eukaryotic cells have 1-5 genes encoding DNAJ molecules, whereas *P. falciparum* contains at least 43 members of the DNAJ family. Some of these proteins are exported. The absence of exported falciparum HSP70 suggests that these molecules use host HSP70 as a functional partner. In our work we investigate the role of exported DNAJ proteins and their interaction with host molecules.

M6* Invited speaker
Stage estimation and lineage commitment in *Plasmodium falciparum*
Jacob E. Lemieux [a,1,] Natalia Gomez-Escobarb,[1], Avi Fellerc,[1], Celine Carretd,[2], Alfred Amambua-Ngwa[b], Robert Pinches[a], Felix Daya,[3], Sue A. Kyes[a], David J. Conway[b,1], Chris C. Holmes[c,e,1] & Chris I. Newbolda,[1,4]
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The cell-cycle dependent transcriptional pattern of the majority of genes in *Plasmodium falciparum* presents analytical difficulties for studying gene expression. Asynchrony between samples can reduce the power of statistical comparisons, and subtle differences in temporal development can violate the independence assumption of many tests. Identifying differences between expression profiles also requires some care due to their periodic nature. We present statistical and morphological approaches to estimate the temporal development, synchronization and lineage commitment of *P. falciparum*. In studies from our own lab as well as publicly available datasets, we use these models to study parasite populations from patients with falciparum malaria. At the level of gene expression, we find a shift to gametocyte-like expression profiles in a fraction of the parasite population which varies continuously among patients and appears to be attenuated in *ex vivo* culture. We demonstrate that this diversity is non-temporal in nature and is consistent with a model in which each patient is considered as a mixture of asexual and sexual stages.

M7 Invited speaker
Systematic analysis of vesicular traffic in apicomplexan parasites
Markus Meissner
Division of Infection & Immunity and Wellcome Centre for Parasitology, Faculty of Biomedical and Life Sciences, University of Glasgow, Glasgow, UK.

In order to invade a host cell apicomplexan parasites evolved a whole set of unique organelles, such as the secretory organelles (micronemes, rhoptries and dense granules) or the Inner Membrane Complex (IMC). While the contents of these organelles have been shown to be essential for invasion of the host cell, our knowledge about their evolutionary origin and the mechanisms involved in their biogenesis is still embryonic. Apicomplexa belong to the recently recognised group of protests referred to as Alveolata. Despite their large morphological differences these protists share an endomembrane system underneath the plasma membrane which comprises of membranous sacks named alveoli (or IMC in case of apicomplexan parasites). In addition most alveolata contain specialised secretory organelles. Therefore we speculated that alveolates share common, unique trafficking factors that are required for the specific vesicular traffic to the alveoli and the secretory organelles. Indeed we identified the small GTPase Rab11B and the mechanoenzyme Dynamin related protein B (DrpB) as highly specific alveolate proteins in a phylogenetic analysis. Using *Toxoplasma gondii* as a model system, we show that Rab11B is required for the biogenesis of the IMC and DrpB for the biogenesis of the specialised secretory organelles. In addition we characterised the role of other Rab-GTPases and trafficking factors and will present a model considering the organisation of the secretory traffic in *T.gondii.*

Session 3A - Malaria: Immunology and pathogenesis (Chair: Chris Newbold)

M8 Invited Speaker

***Plasmodium falciparum* AMA1: Allelic diversity in asymptomatic, mild and severe malaria and allele-specific immunity**

Faith Osier[1], Gareth Weedall[2], Federica Verra[3], Linda Murungi[1], Kevin Tetteh[3], Pete Bull[1], Ed Remarque[4], Alan Thomas[4], Kevin Marsh[1] & David Conway[3,5].

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Although *Plasmodium falciparum* apical membrane antigen 1(AMA1) is a leading malaria vaccine candidate, extensive allelic diversity may compromise its vaccine potential. To assess the impact of allelic diversity on naturally-acquired immunity, we: a) sequenced the ectodomain of *P. falciparum* AMA1 from patients with asymptomatic, mild and severe malaria and, b) measured antibodies to three allelic AMA1 proteins, using competition ELISA to analyze allele-specific antibodies. Seventy-eight unique haplotypes were identified from 129 alleles. No clustering of allelic types with disease severity was observed, but allele frequency distributions were indicative of balancing selection. Antibodies to three allelic AMA1 proteins were highly correlated, and associated with protection from clinical malaria. Over 50% of selected children with anti-AMA1 IgG (n = 106) did not have detectable reactivity to allele-specific epitopes, and the prevalence of allele-specific antibodies did not differ between susceptible and protected children. Despite the extensive antigenic diversity, antibodies to conserved epitopes in AMA1 may be sufficient to contribute to clinical protection.

M9 Fine specificity of vaccine induced *Plasmodium falciparum* CSA-adhesion blocking antibodies

Sisse B. Ditlev, Mafalda Resende, Madeleine Dahlbäck, Thor G. Theander, Morten A. Nielsen & Ali Salanti. Centre for Medical Parasitology at Department of International Health, University of Copenhagen, Copenhagen University Hospital (Rigshospitalet)

Pregnancy-associated malaria (PAM) is a major cause of maternal anemia, stillbirth and delivery of low-birth-weight children in malaria endemic areas. The disease is caused by accumulation of *P. falciparum*-infected erythrocytes (IE) in the placenta, mediated through VAR2CSA. We have previously shown that antibodies raised against the DBL4ε domain of VAR2CSA FCR3 effectively inhibit IE binding to CSA, which has made the DBL4ε construct a promising PAM vaccine candidate. To define the targets of these inhibitory antibodies and understand how these interfere with IE adhesion to CSA we analyze the antibody response induced by DBL4ε using a peptide array covering the entire domain. The peptide reactivity of DBL4ε induced inhibitory and non-inhibitory IgG were analysed and we identified a number of peptides reacting with the inhibitory antibodies and to a much lesser extend with the non-inhibitory IgG. This region was mapped onto a structural model of DBL4ε and showed to locate to a flexible loop region. The corresponding peptides were subsequently used for immunization of rats and we found that the peptides induced IgG reacting with the native VAR2CSA expressed on IE and IgG is now evaluated for the ability to inhibit the CSA-binding of the IE.

M10* The effect of adjuvants in the immune phenotype induced by a *Plasmodium falciparum* DBL4-VAR2CSA based vaccine

Vera Valadão Pinto[1], Ali Salanti, Lars M. Joergensen[1], Else Marie Agger[2], David E Arnot, Thor G. Theander[1] & Morten A. Nielsen[1]
[1]Centre for Medical Parasitology at Department of International Health, University of Copenhagen, Copenhagen University Hospital (Rigshospitalet)
[2]Department of infectious Disease Immunology Statens Serum Institut

Pregnancy Associated Malaria (PAM) is a major public health problem for both mother and child in the Sub-Saharan Africa. A future vaccine against PAM may be based on the VAR2CSA antigen or fragments hereof. The DBL4 domain of VAR2CSA induced antibodies, which inhibited the parasite binding to chondroitin sulfate A (CSA) present on the placental surface. To enhance the immune reactivity of this vaccine candidate we formulated the antigen with different adjuvants currently used in malaria related clinical trials or research: Freunds complete and incomplete adjuvant, Montanide® ISA 720, Alhydrogel® and CAF01. Groups of rats immunized with each of the adjuvant and DBL4-VAR2CSA combination all induced antibodies that inhibited parasite binding to CSA from 82-99% with CAF01 being the most potent of the tested adjuvants. Although the direct correlation to in vivo protection is unclear the inhibition rate suggests that the DBL4-VAR2CSA antigen could be used in a vaccine formulation with any of the tested adjuvants. To further understand what a tailored protective immune response against PAM should aim for, the resulting antibodies from these immunizations were analysed regarding titre, specificity, epitopes bias and affinity.

M11 Maternally acquired immunity and protection from malaria infection

Vincent Staszewski, Emma J.A. Cunningham & Sarah E. Reece
Centre for Immunity, Infection and Evolution, School of Biological Sciences, University of Edinburgh, Edinburgh EH9 3JT, Scotland, UK

Despite the partial protection against malaria observed for young infants, the evolutionary and ecological consequences of maternally transferred antibodies remains poorly understood. Vaccine development has revealed the importance of antigenic variation and genetic diversity for immune protection but the impact of this diversity for maternally transferred protection is yet to be tested.
We used the rodent malaria model *Plasmodium chabaudi,* to test whether maternally transferred immunity is: (a) strain specific; (b) influenced by drug treatment of mothers; and (c) differs according to genetics of hosts and parasites. To do so**,** we compared infection dynamics and virulence experienced by pups of three different mouse strains during homologous (challenged with the same strain as their mothers) or heterologous infections. We observed a higher mortality for pups from non infected mothers or mother infected and drug treated (i.e. not exposed to the full repertoire of malaria antigens) compared to pups from infected mothers. We also observed strain specificity of maternally transferred protection as the protective effect of maternal antibodies was stronger during homologous infections. Our results open perspectives for a better understanding of the evolution and ecology of maternally transferred immunity but also to determine how maternal drug treatment might affect the protection conferred to young children.

Session 4A - Malaria: A historical perspective (Chair: Carol Reeves)

M12 Invited Speaker
'All patients were clinically cured': The discovery and development of artemisinin as an effective anti-malarial drug

Tilli Tansey
The Wellcome Trust Centre for the History of Medicine at UCL, 183 Euston Rd, London NW1 2BE

In 1979 a remarkable paper appeared in the Chinese Medical Journal, by the 'Qinghaosu Anti-malaria Coordinating Research Group', describing anti-malaria trials *in vitro* in rodents and humans of a traditional herbal remedy, qinghao. It detailed early successes and promise of an

extract, qinghaosu. All 2099 malarious patients treated with qinghaosu (later named artemisinin) were pronounced 'clinically cured', and good results were also noted in patients with cerebral malaria and chloroquine resistant falciparum malaria. This talk will discuss the history and impact of this paper and the development of artemisinin and artemisinin-based combination therapy (ACT).

M13 Invited Speaker
Exploring old ground: Greece and the malaria pandemic of 1905
Katerina Gardikas
Department of History, School of Philosophy, University of Athens, Ilissia Campus, 15784 Athens, Greece

The paper will examine the malaria pandemic of 1905 in Greece, the most malarious country in Europe. The pandemic, the first since the discovery of the transmission mechanism of malaria, found the country's physicians already mobilised against the disease. Nonetheless it is estimated to have affected one third of the population. A stream of publications and nation-wide surveys that range between 1901 and 1907 and the descriptive data they provide set the pandemic outbreak in the context of the country's fragmented geography and its history of endemic malaria.

M14* Pills, parasites and profits
Mark Honigsbaum
The Wellcome Trust Centre for the History of Medicine at UCL, 183 Euston Rd, London NW1 2BE

A course of Coartem, the Artemisinin-derived Combination Therapy (ACT) manufactured by Novartis, clears the malaria parasite from the bloodstream of infected patients within 48 hours. But in Uganda – as in other parts of Africa – ACTs are not getting to hospitals and clinics in time. Doctors are having to resort to Orodar (sulfadoxine-pyrimethamine), an old-line malaria drug that is useless against many strains of the parasite. Kodjo Edoh, the head of the Medicins Sans Frontieres mission in Madi Opei, a former rebel stronghold, investigated the shortages of ACTs in public hospitals and found that officials were stealing the medications and selling them on the black market. With the help of Interpol, the Ugandan government is now cracking down on thefts and prosecuting corrupt officials. But with parasite resistance to artemisinin - the key component of ACTs - now being reported in Cambodia, and a growing worldwide trade in counterfeit malaria medications, time is running out. Why it is that drugs that could save lives and for which, to date, the Global Fund for Aids, TB and Malaria has provided $250 million in donor funding, are still failing to reach people in need? Is this a new problem or history repeating itself?
This presentation is based on the research for a new film 'The Killing Season', produced following my book, *The Fever Trail: The Hunt for the Cure for Malaria* (Macmillan, 2001).

M15* Burdwan fever and the making of a malarial locality, 1863-1875
Rohan Deb Roy
The Wellcome Trust Centre for the History of Medicine at UCL, 183 Euston Rd, London NW1 2BE

This presentation will not probe into the causes or effects of the Burdwan fever, an epidemic attributed to malaria in British Bengal (in British India) in the 1870s. Instead it will explore how a series of dispersed and dissimilar debilities came to be represented as a single, continuous epidemic of malaria in Bengal and beyond. This paper will argue that the making of the Burdwan fever epidemic cannot be ascribed to conveniently locatable intentions or a straightforward series of causes, but to a 'game of relationships': between diagnostic protocols and pharmaceutical interests; codes of bureaucratic reporting and information gathering; medical relief, land control and commercial priorities; indenture labour market and medical geography; between the colonial government and different layers of landed proprietors.

Session 5A - Protozoa: Molecular pathogenesis (Chair: Marcus Meissner)

M16 Invited Speaker
From transcriptomes to systems: do we need a reality check?
Jonathan Wastling
Department of Comparative Molecular Medicine, School of Veterinary Science, University of Liverpool, Liverpool L69 7ZJ, UK.

Recent advances in transcriptomics, proteomics and metabolomics have raised expectations that we might soon be able to provide a total "systems-view" of host-parasite relationships. Such a detailed understanding of how parasites and hosts interact at the molecular (and population) levels would clearly be of enormous benefit, not just in advancing basic biological knowledge, but also in the practical development of more effective vaccines and therapies. But how realistic are these expectations? And is there a danger that we seriously underestimate the complexity, diversity and unpredictability of the systems we are studying? This paper examines how one of the key components of the "molecular system", protein expression, can now be analysed in considerable depth and breadth in parasites and their hosts, using advanced protein separation techniques and mass spectrometry. We have already shown how proteomics can be used alongside other functional genomic tools to help understand fundamental mechanisms of host-cell invasion and the modification of host-cell function to sustain successful parasitism. However, despite the huge progress that has been made toward describing the proteomes and sub-proteomes of some parasites, extending these studies beyond the merely descriptive raises considerable technological difficulties. A major challenge is that of exploiting the next generation of quantitative proteomic technologies to help model the key relationship between mRNA transcription and protein expression in parasites. Only when this has been achieved will we feel more confident in interpreting both transcriptional and proteomic data in the context of a "systems biology" understanding of parasitism.

M17 Invited Speaker
The molecular evolutionary immunoecology of *Toxoplasma gondii*
Jonathan Howard
Institute for Genetics, University of Cologne, Germany

Unlike the genus *Plasmodium*, *T. gondii* is a single species world-wide. All cats are "mosquitoes" for *T. gondii* and all warm-blooded animals are the intermediate hosts. I will discuss how the recent history of mankind has altered the evolutionary trajectory of *T. gondii*, and how the consequences of this transformation have played out on the immune system of the mouse. The mouse possesses a powerful cell-autonomous resistance system against *T. gondii* based on a dedicated family of GTPases, the IRG proteins, that are essential for the control of parasite virulence.

M18 A soluble mediator from Giardia Lamblia disrupts human gastrointestinal epithelial cell function
Suha Al-Naimi, John P. Winpenny, Paul R. Hunter & Kevin Tyler
Biomedical Research Centre, School of Medicine, Health Policy and Practice, University of East Anglia, Norwich NR4 7TJ, England, UK.

Giardia lamblia, an important cause of diarrheal disease, resides in the small intestinal lumen in close apposition to epithelial cells. Since the disease mechanisms underlying giardiasis are poorly understood, elucidating the specific interactions of the parasite with the host epithelium is likely to provide clues to understanding the pathogenesis. We have used the Ussing chamber system to investigate the effect of both giardia co-cultures and giardia supernatants on ion transport in monolayers of the human colonic epithelial cell line, CaCo-2, grown on permeable supports. There

was a reduction in both the forskolin-stimulated, GlyH101 inhibitable short circuit current (Isc) and the uridine 5'-triphosphate (UTP)-stimulated Isc from CaCo-2 monolayers co-cultured with giardia. No difference was observed between different giardia isolates. Importantly however, in all cases transepithelial electrical resistance (TEER) of the CaCo-2 monolayers decreased after 24h of co-culture with the parasite suggestive of a disruption to the epithelial monolayer.
Addition of giardia culture supernatants alone to the apical side of the CaCo-2 monolayers resulted in a reduction in the forskolin-stimulated, GlyH101 inhibitable Isc. This study raises the possibility of the presence of a soluble mediator whose activity abrogates CaCo-2 epithelial cell function. This disruption might contribute to gastrointestinal symptoms in infections involving *Giardia* strains which do not express well-established enterotoxins.

M19* Investigation of putative species specific markers of *C. hominis* and *C. parvum* identified by comparative genomics tools

Maha Bouzid[a], Rachel Chalmers[b], Paul R. Hunter[a], Kevin Tyler[a]
[a] Biomedical research centre, School of Medicine, Health Policy and Practice, University of East Anglia, Norwich NR4 7TJ, England, UK. [b] UK Cryptosporidium Reference Unit, NPHS Microbiology Swansea, Singleton Hospital, Swansea SA2 8QA, UK.

Two species of Cryptosporidium, *C. parvum* and *C. hominis*, are of public health importance. Genome representatives of *C. parvum* and *C. hominis* showed only 3-5% sequence divergence. Using comparative genomic tools, we identified over 250 putatively specific genes, the majority corresponding to hypothetical proteins. In order to investigate this apparent specificity, we used PCR to amplify twelve of these genes in a panel of UK clinical isolates, previously genotyped at the Cryptosporidium Reference Unit. In addition, 3 reference strains IOWA, Moredun and TU502 were also tested. As the amount of DNA available for testing was limited, we used and validated whole genome amplification (WGA) for archiving of these isolates. While the majority of the tested genes were present in both species, sequence analysis provided a uniquely unbiased selection of coding sequences for multilocus analysis and allowed discovery of a wide variety of novel SNPs, thus enabling more robust genotypic analysis. Interestingly, our secondary screen eliminated all but 2 of the putative species specific genes. The biological function of these genes (Cop-1 and Chos-1) is currently under investigation, in addition to their potential as species determinant, epidemiologic and diagnostic marker.

Session 6A - Host and helminth genetics (Chair: Steve Paterson)

S20 An examination of VAL diversity throughout the Platyhelminthes

I. W. Chalmers & K. F. Hoffmann
IBERS, Aberystwyth University, Aberystwyth, SY23 3DA, UK.

Members of the Sperm-coating protein/Tpx-1/Ag5/PR-1/Sc7 (SCP/TAPS) superfamily have been identified in several important parasitic nematode species such as *Necator americanus* (*Na*-ASP-2) and *Onchocerca volvulus* (*Ov*-ASP-1). Importantly, characterization has shown several of these SCP/TAPS superfamily members to have roles in the establishment and maintenance of infection (*Na*-ASP-2, *Ov*-ASP-1 & *Ac*-NIF).
In the trematode parasite *Schistosoma mansoni,* previous work in our group led to the identification of 28 SCP/TAPS family members named *Schistosoma mansoni* venom allergen-like (SmVAL1-28). A range of analyses provided strong evidence for the division of the SmVAL family into two distinct subfamilies; the group 1 proteins which are likely to be secreted/excreted from the parasite and the group 2 proteins which are likely to function intracellularly. Here, we present data on the identification, protein features and evolutionary relationships of identified VAL homologs within the phylum Platyhelminthes.
Using new 454 sequencing data combined with existing genomic and transcriptomic data, novel VAL homologs were discovered in over 20 platyhelminth species. Phylogenetic and protein feature analyses highlight the existence of both group 1 and group 2 members in all four major platyhelminth classes (Trematoda, Monogenea, Cestoda and Turbellaria). Within the

schistosomes, the identification of highly conserved SmVAL orthologs in both *S. japonicum* and *S. haematobium*, suggests a common function. However, our analysis also suggests that species-specific homologs exist within the genera, signifying that some VALs may have evolved to perform additional roles in host/parasite interactions or developmental biology, particular to the specialized lifestyle exhibited by each species.

S21 Expression profiling and immunological characterization of the *Schistosoma mansoni* VAL protein family

Leonardo P. Farias[1], Iain W. Chalmers[2], Samirah Perally[2], Paul Hensbergen[3], Ron Hokke[3], Luciana C.C. Leite[1] & Karl F. Hoffmann[2]
[1]. Centro de Biotecnologia, Instituto Butantan, 05503-900 São Paulo, SP, Brazil. [2]. Institute of Biological, Environmental and Rural Sciences, Aberystwyth University, SY23 3DA, UK. [3]. Centre of Infectious Diseases, Leiden University Medical Centre, Leiden, The Netherlands.

The *Schistosoma mansoni* venom-allergen-like proteins (SmVALs) are members of a diverse protein superfamily containing a highly conserved SCP/TAPS (Sperm-coating protein/ Tpx-1/Ag5/PR-1/Sc7) domain. SCP/TAPS proteins may be important in key biological processes including host-pathogen interactions and defense mechanisms, however their specific role in trematodes has not yet been fully elucidated.
Real-time RT-PCR analysis of the entire *S. mansoni* VAL-complement (SmVAL1-29) was performed to gain a better understanding of these molecules during schistosomiasis. Here, distinct expression patterns were observed, with many of the SmVALs being stage-specific and some displaying constitutive patterns of expression. Mapping the transcript profiles to SmVAL phylogeny provided an insight into their evolution and their putative function within the parasite.
Proteomics and nano LC-MS/MS were next exploited to examine the immunological properties of the SmVAL family in *S. mansoni* and other *Schistosoma* spp. Using antibodies raised against recombinant SmVAL4, -5 and -26, we show antibody cross-reactivity within phylogenetically-related SmVALs, for example anti-SmVAL5 recognition of SmVALs -26/28, -27, and -9/29. Together, our results are discussed in light of the anticipated need for alternative strategies to combat schistosomiasis.

S22* DNA plasmids encoding modified parasite proteins overcome parasite-driven suppression of host's immune responses

HongLin Luo, David W. Taylor, Neill Storrar, Judith E. Allen & Simon A. Babayan
Institute of Immunology & Infection Research, University of Edinburgh, Edinburgh, UK

Filarial nematodes are tissue-dwelling parasites that are able to withstand immune attack and reproduce for many years in their host by modulating its immune response with a variety of excreted/secreted products. Consequently, the efficiency of a vaccine against filariasis will depend on how well it circumvents filaria-driven immunosuppression. We developed a vaccine strategy that specifically targets filarial excretory products involved in immunosuppression, while enhancing antigen processing and type-2 adaptive immune responses. The *L .sigmodontis* Abundant Larval Transcript-1 (Ls-ALT) and Cysteine Protease Inhibitor (Ls-CPI) gene was cloned and genetically engineered to ablate their immunomodulatory properties. In order to enhance antigen processing of the target protein by the host, we fused the target with an anti-DEC205 antibody fragment. Plasmids encoding the Th2 cytokine IL-4, and APC-activating MIP-1α and Flt3L were co-administered. Mice immunised with mutated forms of parasite proteins produced more specific antibody post-challenge and showed strongly increased lymphocyte stimulation above controls. The immune response was further enhanced when plasmids encoding FLt3L, MIP-1a or anti-DEC-205 were co-administered, with IL-4 skewing the response towards a Th2/IgG1 phenotype. In conclusion, we have designed vaccines that circumvent parasite-induced immunosuppression, enhance antigen-processing and skew the immune response towards a protective phenotype. Additional benefits of this strategy are that the effects of these vaccines are long-lived, and cost-effective.

S23 GTP-Cyclohydrolase in parasitic nematode development

Rachael Smith[1,2], Alasdair Nisbet[1] & Jacqui Matthews[1,2]
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Parasitic nematodes require the expression of different sets of genes at different stages of their lifecycles. Previous work has shown that the gene encoding the enzyme GTP-Cyclohydrolase (GTP-CH) is much more highly expressed in the 3rd (free-living, L3) stage of the nematode *Teladorsagia circumcincta* compared to the 4th (parasitic, L4) stage. This observation has also been made in several other Clade V nematode species. As the rate limiting enzyme in the production of tetrahydrobiopterin, there are a number of different pathways which could require such high levels of GTP-CH. A number of different products are generated by these pathways, including dopamine, serotonin and melanin. To examine which products and pathways are critical for L3 stage worms, investigations have been undertaken in *T. circumcincta* and, to examine the potential influence on hypobiosis, *Dictyocaulus viviparus*, using both real-time quantitative PCR and *in vitro* bioassays employing GTP-CH inhibitors. Analysis of gene expression in *D. viviparus* showed that GTP-CH gene expression was more than 40 times higher in the 1st and 3rd larval stages compared to eggs and each of the parasitic stages of the lifecycle. Analysis using hypobiotic and non-hypobiotic larvae showed that GTP-CH gene expression was similar in both, implying that the enzyme is not directly involved in the larval arrest of *D. viviparus*.

S24 Genetic changes associated with the selection of an ivermectin-resistant isolate of *Haemonchus contortus.*

Sally Williamson[1,2], Gerald Coles[3], Samantha McCavera[2] & Adrian Wolstenholme[1,2]
[1]University of Georgia, USA, [2]University of Bath, UK, [3]University of Bristol, UK.

Ivermectin resistance has become a serious concern in veterinary parasitology, yet the genetic basis of this resistance has yet to be determined, though changes in both the P-glycoproteins and ligand-gated chloride channels have been reported. Part of the reason for this is the high level of genetic diversity seen between parasite isolates. In an attempt to overcome this, we selected a new ivermectin-resistant isolate of *H. contortus* from the inbred ISE isolate. Worms fully resistant to the normal therapeutic dose of ivermectin (0.2 mg/kg) were selected in only 4 generations, providing a population predicted to genetically similar in all other respects to ISE. The ivermectin-resistant population was also resistant to thiabendazole in the egg-hatch test and possessed an increased level of benzimidazole resistance-associated alleles (17.7% vs 0.3 % in the parent population) at position 200 of the β-tubulin gene; no benzimidazoles were used during the selection process. We are examining the sequences of mRNAs encoding ligand-gated chloride channel subunits sensitive to ivermectin. We have identified several genes homologous to P-glycoprotein in the *H. contortus* genome sequence and are measuring the expression of mRNAs derived from those genes in the resistant and parent nematodes. These data will identify candidate ivermectin resistance-associated genes in this parasite.

S25 The role of host genetics in predisposition to human hookworm and *Ascaris lumbricoides* infection

Rupert Quinnell, Rachel Pullan, Lutz Breitling, Stefan Geiger, Bonnie Cundill, Rodrigo Correa-Oliveira, Simon Brooker & Jeffrey Bethony
Institute of Integrative and Comparative Biology, University of Leeds, Leeds, UK; London School of Hygiene and Tropical Medicine, London, UK; René Rachou Research Centre FIOCRUZ, Belo Horizonte, Brazil; The George Washington University, Washington DC, USA; Malaria Public Health and Epidemiology Group, KEMRI-Wellcome Trust Research Programme, Nairobi, Kenya

Predisposition to heavy or light human helminth infection is consistently reported in treatment-reinfection studies, but the factors responsible for generating predisposition are not well understood. Here we use bivariate variance components analysis to investigate the relative roles of host genetics, shared household environment and known risk factors in determining predisposition to hookworm and *Ascaris* infection. Infection intensity (faecal egg counts), pedigree information and socio-economic and remote-sensed environmental risk factors were measured in

a treatment-reinfection study of 1300 people in Brazil. Results showed significant predisposition to hookworm and *Ascaris* infection. The heritabilities of hookworm and *Ascaris* infection intensity were 0.17-0.22 and 0.22-0.29 respectively, controlling for household and other risk factors. There was a high positive genetic correlation between pretreatment and reinfection *Ascaris* intensity, suggesting that host genetics accounted for the majority of the observed predisposition to *Ascaris*. However, the genetic correlation for hookworm infection was lower, with household and environmental factors playing a larger role in predisposition to hookworm infection.

BSP Plenary Lecture and CA Wright Medal Lecture

Plenary Lecture: Our changing world and the emergence of novel infectious agents

Prof. Sir Roy Anderson
Department of Infectious Disease Epidemiology, Imperial College London, Old Medical School, St. Mary's Hospital, Norfolk Place, London, W2 1PG, UK.

The lecture will discuss how changes in the world we live in over the past few decades have influenced the pattern of the emergence and spread of infectious agents.

Increased mobility, changing demography and increased urbanisation - and also scientific advances that enable new pathogens to be indentified and tracked - will be examined. Growth in human population size and the increase in megacities with over 10 million inhabitants, promotes both the transmission and evolution of infectious agents. How these spread around the world is very much influenced by air traffic flow between the world's major cities. Novel ways of examining the distribution of human population density and interactions within and between centres of high and low population density will be discussed. These include satellite sensing, simulation methods, digital communication and pathogen genome sequencing.

The talk will use examples of recent epidemics including H1N1, SARS and HIV-1 to illustrate how our changing patterns of habitation, travel and interaction have influenced pathogen evolution and spread.

CA Wright Medalist: Membrane trafficking systems; trypanosomes, surfaces and genomes

Prof. Mark C. Field
Department of Pathology, University of Cambridge, Tennis Court Road, Cambridge, CB2 1QT

Intracellular transport is the process by which macromolecules are delivered to the organelles constituting the endomembrane system and also provide mechanisms for secretion to the environment, for uptake of nutrients and control of signaling pathways. In protozoan parasites this process is vital as the cell surface represents the host-parasite interface with the host immune system. In African trypanosomes immunoglobulins and other immune effectors recognizing or bind ing the parasite surface are removed by efficient endocytosis, proteolysis and recycling, maintaining the variant surface glycoprotein density at the plasma membrane. Ensuring a constant surface protein composition is also vital to additional cellular functions. Our contributions towards understanding mechanisms of selective surface protein turnover will be discussed. More recently, parallels between membrane transport and distinct systems involved in membrane deformation have emerged. Specifically, strong similarities in the structures and architectures of vesicular transport factors and the nuclear pore complex have emerged. These findings have allowed the development of a holistic paradigm for the evolution of membrane transport, and an emerging model for the origins of these systems back to the last eukaryotic common ancestor and beyond. Specific examples exploiting broad genome sequence information, proteomics and functional studies focused on African trypanosomes will be presented, in the context of reconstructing the order in which the organelles of the endomembrane system arose and furthering understanding of trypanosome cell biology.

Session 7A - Malaria: Vector biology (Chair: Janet Hemingway)

M26 Invited Speaker: Mosquito's innate defence against malaria

George K. Christophides
Division of Cell and Molecular Biology, Imperial College London, South Kensington Campus, Exhibition Road, SW7 2AZ, London, UK

Malaria parasites must accomplish a complex developmental lifecycle in the mosquito in order for them to be transmitted to a new human host. Throughout this process, especially during ookinete traversal of the mosquito midgut epithelium, the mosquito immune system kills the vast majority of parasites. Intestinal epithelial immunity forms the first line of defence against *Plasmodium*, followed by a second line of humoral defence in the mosquito hemolymph. The first line is controlled by an NF-[kappa]B signalling pathway mostly triggered by symbiotic bacteria that reside in the mosquito gut and drastically proliferate soon after a female mosquito bloodmeal. The second line consists of a constitutive machinery of hemolymph proteins, equivalent to vertebrate complement. Its activation leads to a dramatic reduction of parasite numbers or complete control of the infection and transmission blockade. These findings opened new avenues towards understanding the mosquito/parasite interactions and identifying novel strategies to control malaria transmission.

M27 Genetic control of mosquito populations to combat malaria: modelling approach

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Vector borne diseases remain a very heavy burden in large parts of the world. Thanks to advances in genetic engineering, the genetic control of the vector populations may become a new tool to combat these diseases. Amongst the different genetic drive mechanisms that have been considered to that purpose are the Homing Endonuclease Genes (HEGs). These site-specific selfish genes exploit the recombinational repair system of the cell to copy themselves into a determinate DNA sequence and could be used to knock-out genes in a target species. To reduce the transmission of malaria, one can aim at depleting the mosquito populations or at making them refractory to the *Plasmodium*. Population depletion can be achieved by reducing individual fitness, but also by biasing the sex-ratio towards males, and HEG-based constructs may be engineered for such purposes. Prior to any practical application, it is necessary to study these strategies, and to determine the most efficient and safest ones. Using population genetics model we first studied the conditions for spread of such HEG-constructs and thereby investigated the efficiency and reliability of these control strategies depending on properties of the genetic constructs and associated fitness costs. We then built a model combining population dynamics and genetics, which enabled us to evaluate the impact of these strategies on mosquito densities and on the prevalence of the disease in humans.

M28 Modelling the impacts of climate change on malaria transmission

Paul E. Parham & Edwin Michael
Grantham Institute for Climate Change, Imperial College London, London SW7 2AZ

Climate change remains one of the biggest environmental threats to human health over the coming century, with the effects on changing temporal and spatial patterns of malaria of particular interest. Yet despite the considerable sensitivity of malaria transmission to changes in environmental variables, there is still substantial debate as to the exact role that climate plays in driving malaria epidemics. We illustrate the power of integrated process-based transmission models with explicit links to climate, and embedded within fluctuating environments, as a tool to investigate important aspects of disease transmission, including the roles of biological, environmental and socioeconomic factors driving changes in malaria distribution over time.

Although shifts in the mean values of climatic variables will influence changing patterns of disease, this work additionally emphasises the need to consider temporal variability in climatic factors. The results show that malaria transmission is sensitive to variability in such factors on daily, seasonal, inter-annual and decadal timescales, and that such variability may strongly affect disease emergence, persistence and extinction. We also demonstrate the role of uncertainty in large-scale climate model output on the predictions of such frameworks, and discuss the associated implications for malaria control, elimination and mitigation.

M29 * Impact of anaemia on the feeding and fitness of the malaria vector *An. gambiae* s.s.

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Anaemia is a common health problem affecting women and children in developing countries. This condition is characterized by a reduction in red blood density, primarily resulting from malnutrition and/or infectious diseases such as malaria. As red blood cells are the most important source of protein for mosquitoes, any reductions due to anaemia could significantly impede the subsequent fecundity and survival of mosquitoes who feed on affected hosts. Here laboratory experiments were conducted to assess the impact of variation in human red blood cell density consistent with severe anaemia on the fitness of the African malaria vector *An. gambiae* s.s. In 3 experiments, human blood of either normal red cell density (50% Packed Cell Volume [PCV]) or that typical of a severely anaemic patient (15% PCV) were fed to groups of *An. gambiae* s.s. females using a membrane feeder. In all experiments, the mass of blood protein that mosquitoes obtained from feeding was significantly lower from anaemic than normal blood ($p \leq 0.014$ in all cases, LS mean=2.550-11.240 ug of haematin). However despite this reduction in protein intake (indexed by haematin excretion), mosquitoes that fed on anaemic blood went to produce a similar number of eggs than the controls ($p \geq .05$ in all experiments). An explanation for these results could be that mosquitoes convert anaemic blood into egg resources more efficiently than normal blood; with the numbers of eggs produced being significantly influenced by both haemoglobin intake ($F_{1, 124}=19.636$, $p \leq 0.001$) and blood treatment ($F_{1, 124}=22.327$, $p \leq 0.001$). These results suggest that contact with anaemic hosts does not reduce the fitness of malaria vectors, and indicates that mosquitoes may exploit resources for reproduction more efficiently from anaemic than normal hosts.

Session 8A - Malaria: Control and drug discovery (Chair: Colin Sutherland)

M30 Invited Speaker
The Malaria Parasite Mitochondrion: Bioenergetics and Drug Development

Giancarlo Biagini
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To date, the role of the malaria parasite mitochondrion in energy metabolism and subsequent parasite development has remained somewhat elusive. There is limited information regarding the respiratory components present during parasite development and their co-operative interactions in response to environmental fluctuations is not understood. Here, we report our working hypothesis regarding the cellular and molecular bioenergetics of the parasite's mitochondrion. Further, we report the development of a drug discovery programme, generating small molecule inhibitors against the atypical respiratory enzyme, type II NADH:quinone oxidoreductase.

M31 New alleles of *pfmdr1* and other markers of *P. falciparum* resistance in the post-chloroquine era in Africa

Colin Sutherland, Khalid Beshir, Nahla Gadalla, Mary Oguike & Rachel Hallett
London School of Hygiene & Tropical Medicine, WC1E 7HT, UK

In collaboration with partners in many African institutions, we are performing detailed sequence analysis of a series of genes encoding established markers of drug resistance. We present evidence that, since the adoption of ACT-based treatment policies throughout the region, new

forms of many of these genes have become apparent. The possible origins of these alleles, and the potential contribution of new parasite genotypes to the evolution of resistance to ACTs, will be discussed.

M32 Development and Evaluation of a Novel Magneto-Optical Test (MOT) for the Diagnosis of Malaria

Henk Schallig[1], Petra Mens[1], Raphael Matelon[2], Luke Savage[2], Lesley Wears[2], James Beddow[3], Martin Cox[3], John Heptinstall[3] & Dave Newman[2]

[1]Koninklijk Instituut voor de Tropen/Royal Tropical Institute, Parasitology Unit, Meibergdreef 39, 1105 AZ Amsterdam, The Netherlands; [2]School of Engineering, Computing and Mathematics, University of Exeter, Exeter EX4 4QF, UK; [3]Faculty of Health and Life Sciences, Coventry University, Coventry CV1 5FB, UK

The objective of the work was to develop a magneto-optic technology-n=based test (MOT) for rapid malaria diagnosis based on haemozoin detection, the waste product of *Plasmodium*, which is produced in a form that under the action of an applied magnetic field gives rise to an induced optical dichroism characteristic of the haemozoin concentration. The analytical performance of the device was evaluated in the laboratory.

The validity of the MOT device was assessed on measurements on live parasitized erythrocytes obtained from a *Plasmodium in vitro* culture. In a small clinical trial, stored blood samples from confirmed malaria patients, cases of undifferentiated fever, rheumatic-associated disease or heamoglobinpathies, were tested for *Plasmodium* infection with RDT and MOT test.

The detection limit of the device, as measured using artificial haemozoin (synthesised β-haematin) 0.01µg/ml, corresponding to around 10 parasitized red blood cells/µl. Blind testing of the MOT device revealed that there was an good correlation between MOT testing, RDT results and clinical confirmation. The ease with which the system detected a *P. ovale* infection, known for its low parasiteamia, is particularly encouraging.

M33 Who transmits malaria?

Petra Schneider

Centre for Immunity, Infection and Evolution, School of Biological Sciences, Ashworth Laboratories, University of Edinburgh, Edinburgh EH9 3JT, UK

'Who transmits malaria?' A big question with a deceptively simple answer: 'people infected with *Plasmodium* parasites that can infect mosquitoes'. But how well do we really know the human infectious reservoirs for *Plasmodium falciparum*? It is often assumed that adults, asymptomatic infections and sub-microscopic gametocytes contribute little to transmission. With the application of molecular gametocyte detection, it is becoming increasingly clear that these assumptions are wrong and therefore malaria control is often based on incomplete information.

I will discuss why current assumptions about human infectious reservoirs are misleading, what this means for malaria control and for the evolutionary trajectories of parasites. I will outline future studies required to facilitate successful long-term malaria control, which requires an understanding of factors that determine infectiousness and, therefore, identification and monitoring of human infectious reservoirs. With little knowledge about the infectious reservoirs for *Plasmodium falciparum*, the selection pressures that interventions pose on malaria parasites will be difficult to estimate and the long-term impact of interventions on disease and transmission will be difficult to predict.

Column B – Ecology

TUESDAY 30th MARCH

Session 1B - Parasite and Host Life history traits (Chair: Mike Begon)

S34 Invited Speaker
Parasite life history traits and environmental interactions
Mark Viney
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The life history traits of parasites are affected by their environment, which therefore includes the within-host environment. Parasites also vary in these traits (and thus in how they interact with their environment) which can be selected for, sometimes inadvertently. Parasite life-history traits can have important effects on hosts. I will especially consider the life history traits of parasitic nematodes and show the remarkable degree to which these important aspects of parasite biology are intimately linked to host biology.

S35 Sex ratio and morphological polymorphism in an isolated, endemic *Teladorsagia circumcincta* population
Barbara H. Craig, Jill G. Pilkington & Josephine M. Pemberton
Institute of Evolutionary Biology, School of Biological Sciences, University of Edinburgh, West Mains Road, Edinburgh, Scotland.

Teladorsagia circumcincta is a polygamous nematode that exhibits morphological polymorphism. Sex ratio is typically female-biased and the male nematodes occur in association with the genetically similar, minor morphotypes *T. davtiani* and *T. trifurcata*. In experimental infections, sex ratio and the proportion of minor male morphs observed have been shown to be influenced by both host and nematode related factors. As similar investigations from natural systems are rare, this study examined whether sex ratio and minor male morph frequency were associated with host age and sex and nematode infra-population size in the isolated Soay sheep population on St. Kilda. Generally, the intensity of *Teladorsagia* nematodes increased with host age until the age of two years before decreasing. In a year when abundance of nematodes was generally higher, nematode sex ratio was negatively associated with host age and tended to be negatively associated with nematode intensity. Within the male nematode subpopulation *T. circumcincta* always predominated, followed by *T. davtiani* and then *T. trifurcata,* with little variation in the relative proportions between hosts. The presence of each minor morph was primarily associated with the intensity of male *T. circumcincta* and in those hosts where all three male morphs were detected, intensity of each minor morph was most associated with intensity of *Teladorsagia* females.

S36* Consequences of parasitism for survivorship and fecundity in the cockroach host
Joanna Rumsey, Jo Cable & Jo Lello
School of Biosciences, Cardiff University, Cardiff, CF10 3AX, UK.

Parasitic infections can deplete host energy stores and alter host trade-offs between survival and reproduction. Understanding the alteration in these host life history traits is essential if the host population dynamics are to be explained. In this present study, the survivorship and fecundity of adult German cockroaches (*Blattella germanica*) was measured in uninfected individuals and cockroaches infected with a protozoan gut parasite, *Gregarina blattarum*. Late stage juveniles were removed from colonies and isolated until completion of their final moult. Newly moulted adults were then paired and monitored for lifetime fecundity and longevity. All nymphs were removed and the cohorts reared until adulthood. Results show effects on both host survivorship and fecundity under different conditions. Such individual life history trade-offs are likely to have significant effects on host population dynamics, and this will be explored in future work.

S37 Modes of egg production and larval development as 'organisers' of life-history in parasitoid wasps and plant-parasitic Lepidoptera

Mark Jervis & Peter Ferns
School of Biosciences, Cardiff University, Cardiff, CF10 3AX, UK.

In attempting to make sense of the marked diversity in life-histories found among members of each of two ecologically important parasitic insect groups (the parasitoid Hymenoptera and the plant-parasitic Lepidoptera), we have sought to identify an organising trait - one that has a pervasive influence, determining and explaining variation in many other life-history variables. Our research has identified mode of lifetime egg maturation as an 'organiser'. We are currently exploring the possibility that, in parasitoid wasps, there is a link between egg maturation strategy and another, previously identified 'organiser' - mode of larval development. Establishing a connection between the two strategies could provide a unified conceptual framework for understanding the evolution and diversity of life-history strategies in such insects.

S38 Stress, drugs and the evolution of reproductive restraint in malaria parasites

Sarah E. Reece, Eltayeb Ali, Petra Schneider & Hamza A. Babiker
Centre for Immunity, Infection and Evolution, School of Biological Sciences, University of Edinburgh, EH9 3JT, UK.

Life history theory predicts that sexually reproducing organisms must resolve resource allocation trade-offs between growth/survival versus reproduction, and current versus future reproduction. Malaria parasites replicate asexually in their vertebrate hosts, but must reproduce sexually to infect their vectors and transmit to new hosts. As different parasite stages are required for these functions, the division of resources between these life-history components is a fundamental evolutionary problem. We test how parasites resolve the trade-off between in-host replication and between-host transmission when exposed to anti-malarial drugs. We treated multiple drug-sensitive and drug-resistant field isolates of the human malaria *Plasmodium falciparum* with low doses of drugs *in vitro*. Previous studies have shown that parasites increase investment in sexual stages when exposed to stressful environmental conditions, such as drugs. However, we demonstrate the opposite can occur as drug-sensitive parasites facultatively decreased investment in sexual stages in drug-treated cultures. Furthermore, drug-resistant parasites did not adjust their investment when treated, suggesting that parasites respond to changes in their proliferation rather the presence of drugs. In contrast to previous studies, we tested parasites from a region where chronic infections contribute significantly to transmission and anti-malarial treatment is common. We hypothesise that parasite ecology shapes whether in-host survival over between-host transmission are adaptively prioritised when exposed to drug-treatment.

Session 2B - Host-Parasite Co-evolution (Chair: Phil Harris)

S39 Invited Speaker

Evolutionary arms races between *Drosophila* and its pathogens

Frank Jiggins, Mike Magwire & Lena Wilfert
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Evolutionary arms races can result in recurrent selective sweeps through host and parasite populations. These selective sweeps may be occurring at a very high rate, as we have estimated that 60% amino acid substitutions in *Drosophila* immunity genes have been fixed by natural selection. In natural populations, we have found several major-effect polymorphisms controlling resistance to viruses in *Drosophila,* and at least one of these is matched by polymorphisms that overcome this resistance in viral populations. These polymorphisms are under strong selection, with resistance sweeping through the fly populations and counter-adaptations sweeping through the virus populations. We conclude that arms races can drive rapid evolution in both insects and their pathogens.

S40 * Geographic mosaic of the Red Queen: more sex in coevolutionary hotspots

Kayla C. King[1], Lynda F. Delph[1], Jukka Jokela[2] & Curtis M. Lively[1]

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Under the Red Queen Hypothesis, coevolving parasites reduce the reproductive advantage of asexual reproduction by adapting to common clonal genotypes. In addition, the Geographic Mosaic Theory of coevolution proposes that structured populations of interacting species can produce selection mosaics manifested as coevolutionary "hotspots" and "coldspots". We tested whether a habitat-specific cline in the frequency of sexual reproduction in a freshwater snail could be explained by the existence of hotspots/coldspots for coevolving parasites. We found that parasites are locally adapted to the shallow-water margins of lakes where sex is more common and not adapted to deeper habitats where sex is rare. The results are consistent with the Geographic Mosaic Theory and the Red Queen Hypothesis in that sex is associated with coevolutionary hotspots for virulent parasites.

S41 Decoupled coevolutionary cycles under two-step infection genetics

Andy Fenton[a], Janis Antonovics[b], Michael A. Brockhurst[a] & J. Antonovics[b]

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Most host-parasite systems involve multiple steps of the infection process at which host resistance can evolve. However, despite the ubiquity of multi-step infection processes, their consequences for host-parasite coevolutionary dynamics have yet to be fully explored; standard coevolutionary models typically assume a single-stage infection process (e.g. the well-studied gene-for-gene or matching-allele models). We present a novel coevolutionary model with a two-step infection process which accounts for different gene recognition mechanisms underlying each step of the infection process. This model predicts unexpected and novel patterns of coevolution. Most significantly, we show that multi-step infection can often lead to unique decoupled coevolutionary cycles, where coevolving parasites and hosts undergo gene frequency fluctuations across different regions of their genomes. Therefore, attempts to detect co-evolutionary dynamics that focus on functionally coupled genetic systems may fail to detect coevolutionary responses. Our findings may therefore help to explain puzzling patterns of imbalanced levels of polymorphism in functionally linked host and parasite genes. Overall, we argue that multistep infection processes are common, resulting in important coevolutionary dynamics not considered by current models. Accounting for such multistep processes will greatly enhance our understanding of the coevolutionary process occurring within many host-parasite systems.

S42 Antagonistic coevolution drives extremely rapid and divergent molecular evolution

Steve Paterson[1], Tom Vogwill[1], Angus Buckling[2], Andrew Spiers[3], Nick Thomson[4], Neil Hall[1] & Michael Brockhurst[1]

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The Red Queen Hypothesis proposes that coevolution of hosts and parasites should drive molecular evolution through continual natural selection for adaptation and counter-adaptation. Whilst the divergence observed at some host resistance and parasite infectivity genes is consistent with this, the long time periods typically required to study coevolution have so far prevented any direct empirical test. Here we used experimental populations of the bacterium *Pseudomonas fluorescens* SBW25 and its viral parasite, phage Φ2 to show that the rate of molecular evolution in the phage was far higher when both bacterium and phage coevolved with each other than when phage evolved against a constant host genotype. Coevolution also resulted in far greater genetic divergence between replicate populations, which was correlated with the range of hosts that coevolved phage were able to infect. Consistent with this, the most rapidly

evolving phage genes under coevolution were those involved in host infection. These results demonstrate, at both the genomic and phenotypic level, that antagonistic coevolution is a cause of rapid and divergent evolution and is likely to be a major driver of evolutionary change within species.

S43 Virulence evolution and competition

Peter Staves & Rob Knell
School of Biological Sciences, Queen Mary University of London, UK.

Theoretical models of the evolution of virulence predict that competition between parasites should select for higher virulence. While this idea makes intuitive sense, empirical data to support it are rare and equivocal. We investigated the relationship between fitness and virulence during both inter- and intra-specific competition for a fungal parasite of insects, *Metarhizium anisopliae*. Contrary to theoretical expectations, competition favoured parasite strains with either a lower or a higher virulence depending on the competitor: when in interspecific competition with an entomopathogenic nematode, *Steinernema feltiae*, less virulent strains of the fungus were more successful, but when competing against conspecific fungi, more virulent strains were better competitors. We suggest that in this case the nature of competition (direct via toxin production when competing against the nematode, indirect via exploitation of the host when competing against conspecific fungal strains) is determining the relationship between virulence and competitive ability.

Session 3B - Parasite Transmission (Chair: Jo Cable). This session marks the retirement of Prof Richard Tinsley from the Chair of Zoology at Bristol and his contributions to the BSP as Council Member, Hon. Meetings Secretary and Hon General Secretary.

S44 Invited Speaker

Transmission characteristics of *Protopolystoma xenopodis* (Monogenea) in a population of African *Xenopus laevis* isolated in Wales for over 40 years

R.C. Tinsley
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The urinary bladder monogenean *Protopolystoma xenopodis* has persisted for over 40 years in Wales in an introduced population of the 'lab animal' *Xenopus laevis* (originating in South Africa). Predictably, low temperatures exert a major constraint on life cycle dynamics: host invasion is limited to about 10 weeks/ year and time to patency may exceed 1 year (even 2 years). Lab experiments show that host x parasite genetic interactions have an additional major influence on rates of development, reproduction and survival. A 10 year field study of individually-marked *Xenopus* (n=600) demonstrates control of the parasite population by powerful acquired immunity: following an initial primary infection, most adults remain free of patent infection. This restricts reproducing parasites to juvenile hosts and to c. 15% of adults that experience periodic lapses in protection. Longitudinal studies, based on repeated recaptures of the same host individuals, show that egg output responsible for transmission is maintained in each host age class typically for 1-2 years before development of immunity that can then protect for over 10 years. Experimental challenge infection of wild-caught adults confirms effectiveness of this protective immunity. Despite these constraints, the parasite has persisted, dependent on host population recruitment and on the fraction of adult hosts with less effective immunity.

S45* Getting out of the host: Transmission strategies in protozoan parasites

Laura C. Pollitt & Sarah E. Reece
Institutes of Evolution, Immunology and Infection Research, University of Edinburgh, EH9 3JT, UK

Both malaria and trypanosome parasites produce specialised stages which are pre-adapted to transmission to the vector and are unable to replicate within the vertebrate host. These parasites must therefore balance their allocation of resources between within-host replication and between-host transmission in order to maximise their fitness. We have demonstrated that malaria parasites

plastically alter their resource allocation strategies in line with changes in genetic diversity of infections and the availability of red blood cell resources, according to the predictions of evolutionary theory. This includes diverting investment away from between-host transmission when experiencing competition to maximise their ability to compete for host resources. These findings reveal that an evolutionary-ecology approach, developed to explain the biology of multi-cellular organisms, can usefully be used to understand the trade-offs parasites face and how, why and when they vary their behaviours. This allows fine-tuning of existing evolutionary frameworks to formulate predictions for parasite behaviour under certain conditions as well as providing novel tests of the generality of the evolutionary theory. We describe how this approach has helped us to understand trade-offs in malaria parasites and the next challenge is applying it to other parasites experiencing similar life history trade-offs, such as trypanosomes, to predict their investment strategies for within-host replication and between-host transmission and the resulting implications for virulence.

S46* To boldly go where no fish has gone before: impact of host personality on parasite transmission

Loys Richards[1], Raphael Girardin[1], Cock van Oosterhout[2] & Jo Cable[1]
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Fish reduce predation risk and increase foraging success by forming shoals as group members gain benefits (shared vigilance, increased mating probability, improved hydrodynamic efficiency) compared to solitary individuals. However, a potential disadvantage of this behaviour is increased parasite transmission. Early work on social organisation within groups focused on interactions between pairs of individuals, but more recently the effects of network interactions and individual variation in behaviour on group structure have been considered. Also 'animal personalities' have been increasingly documented in many organisms, with evidence suggesting a wide taxonomic distribution for one aspect of personality, the shy-bold continuum. In the current study, parasitized familiar bold or shy focal guppies (*Poecilia reticulata*), infected with the directly transmitted ectoparasitic worm, *Gyrodactylus turnbulli*, were introduced into shoals of uninfected bold or shy female conspecifics. With regard to shoaling behaviour, shy fish formed larger and tighter shoals for longer periods compared to bold fish. Shy-Bold status of infected focal fish also had a significant effect on the average shoal size of both bold and shy conspecifics. For parasite transmission, results will be discussed in terms of rate and extent of parasite population growth and transfer between shy-bold hosts. This is the first study to examine the effect of boldness-shyness on transmission of a fish parasite.

S47 Do parasites fit into Lindeman's classic biomass pyramid pattern of food webs?

Alexander D. Hernandez[1] & Michael V.K. Sukhdeo[2]
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Biomass pyramids (Lindeman, 1942) are a classic concept in ecology that argues that life can be organized into relatively simple trophic levels where trophic biomass is limited by thermodynamic principles that restrict energy transfer across levels. The pyramidal pattern represents energy flow in communities across all trophic levels, but a glaring omission is parasite biomass. Parasitism is one of the most ubiquitous feeding strategies in nature yet it is unclear how parasites fit into the energetic biomass picture of food web organization. We investigated 24 food chains with trophically transmitted helminth parasites from a stream ecosystem using empirical measures of weight, as well as estimates of biomass calculated from length and width measures of individual parasites. Pyramidal biomass patterns emerged in food chains containing the most abundant host species, and these were also the hosts that were infected most frequently. Differences in pyramidal shape can be partly explained by discrepancies between bio-volume estimates and actual measures of parasite mass. Nonetheless, it is clear that parasite-host associations seem to fit into biomass patterns that are consistent with thermodynamic principles, which restrict energy flow (biomass) between trophic levels.

S48 *Heligmosomoides polygyrus* and *H. bakeri*: host shifts, host dispersal and co-evolution

P.D. Harris[1] & J. Behnke[2]
[1.] National Centre for Biosystematics, University of Oslo, Norway, and [2]. School of Biology, University of Nottingham, UK

Heligmosomoides is well known as a model GI nematode of laboratory mice, and as a common parasite of woodmice, genus *Apodemus*, throughout Europe. The relationship between these two forms is less clear. We have suggested previously that *Heligmosomoides* from laboratory mice is a distinct species, *H. bakeri*, divergent by some 3% at ITS and 8% at COI from the species present in woodmice, *H. polygyrus*. However, the relationship between these two species was unknown, with a strong possibility that *H. bakeri* was more closely related to North American heligmosomatids than to *H. polygyrus*. It is now clear that *H. bakeri* is more or less identical to forms from the house mouse in Corsica (previously known as *H. polygyrus corsicus*), and also to worms from *Apodemus* collected in Turkey by Nieberding (2006, *J. Biogeography **33**, 1212-1222*), but despite the common ancestral host, *H. bakeri* and *H. polygyrus* are predicted to be unable to interbreed based on ITS-2 structure. It appears that divergence from *H. polygyrus* took place in parasites of *Apodemus* before the host shift to *Mus*; *H. bakeri* subsequently spread with *Mus* from the fertile crescent as a result of human activity. This represents an excellent system in which to examine the molecular changes accompanying host specialisation following a host shift.

Session 4B - Parasite community ecology (Chair: Andy Fenton)

S49 Invited Speaker

Patterns and processes of microparasite coinfection in a wildlife host

Mike Begon[1], Sandra Telfer[2], Richard Birtles[1], Xavier Lambin[2] & Steve Paterson[1]
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Studies of parasites and infection typically focus on a single parasite species, even though most hosts most of the time are concomitantly infected with several. Patterns of coinfection may arise because the hosts themselves are intrinsically or temporarily unusually susceptible to all or to particular parasites; or parasites may interact with one another, either positively or negatively. However, such patterns, and the processes underlying them, have been investigated only rarely in natural populations, especially so amongst aggregates of different microparasite species. Here, we elaborate, and seek to account for, patterns of coinfection of cowpox virus, *Babesia microti*, *Anaplasma phagocytophilum* and *Bartonella* spp. in natural populations of field voles. We find that these represent a network of strong associations, both positive and negative. Patterns remain when variations in host susceptibility are accounted for, suggesting that they are mostly generated by interactions between the pathogens themselves rather than by factors associated with exposure risk. The temporal order of infection can be critical in determining the effect of coinfection on susceptibility, and in general, effects are short-lived, depending on current infection status, rather than previous infections. Our results highlight the caution that should be exercised when following the standard practice of studying single species of parasite in isolation.

S50 Co-infection causes variation in transmission

Sandra Lass[1], Sarah E. Perkins[1], Juilee Thakar[1,2,] & Peter J. Hudson[1]
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A dominant feature of parasite-host interactions is the large variation in the response of the host to infection which leads to variation in their ability to transmit. A few hosts become responsible for much of the transmission and the epidemiology is driven by a minority of hosts. The question is: Why is there so much variation in transmission between hosts? Here, we investigate the role of co-infection in causing such variation. During the lifetime of any host, they are exposed to a wide diversity of parasites such that at any one time the response to an infection is determined in part by previous infections and in part by their ability to modulate current infections. This variation may

help explain a large component of the variation in infectiousness between hosts. We postulate that co-infections influence transmission in individual hosts and this effect is mediated by the host's immune response. We show strong interactions between the respiratory pathogen *Bordetella bronchiseptica* and the gut helminth *Heligmosomoides polygyrus* in mice. Co-infection increases the number of transmission stages and prolongs the period of shedding in the helminth. Our study demonstrates the fundamental importance of co-infections as one driving factor of variation in transmission between hosts.

S51 Resolving the *Wolbachia* paradox

Andrew Fenton[1], Karyn Johnson[2], Jeremy Brownlie[3] & Greg Hurst[1]
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Wolbachia represent the most common symbiotic partner of arthropods, present in more than 30% of species. Examination of the phylogenetic history of host and bacteria indicate occasional lateral transfer events drive the incidence of *Wolbachia*. However, lateral transfer when achieved in the laboratory meets with mixed success; where hosts are distantly related, the infection commonly fails to propagate or show phenotype. Simple models of the population biology of *Wolbachia* indicate these infections will not spread, creating a paradox – the infection is common due to lateral transfer, but the strains transmitted following lateral transfer are apparently too poor to spread. It has recently been discovered that *Wolbachia* can induce anti-viral resistance in its host. The effects of this resistance on the ability of *Wolbachia* to propagate is examined in a model exploring the joint population biology of host, *Wolbachia* and virus. Natural enemy resistance appears to resolve the *Wolbachia* paradox, allowing poorly adapted strains to invade which, it is conjectured, then evolve improved transmission and phenotype in their new host species.

S52 Do worms promote or prevent malaria? Using meta-analysis to assess the evidence in mice and men

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Across much of its range, *Plasmodium falciparum* is co-endemic with several helminth species, and human co-infection with worms and malaria parasites is common. The question of whether co-infecting helminths and malaria parasites interact has attracted much attention in recent years, and is important in the context of intervention strategies for diseases caused by both types of parasite. The literature now contains numerous empirical studies investigating whether worms affect malarial disease, both in human populations and also in murine lab models, yet firm conclusions have yet to emerge. Here we report the results of two parallel meta-analyses (covering the human and mouse literature respectively) in which we quantitatively assess existing evidence on this question. Specifically, we address (1) whether studies suggest antagonistic or synergistic effects of helminth infection on malarial disease and (2) which factors explain variation in effect size among studies, including biological factors such as host age, helminth species, and the outcome measure used, as well as methodological factors such as experimental design.

S53 Individual based measure of interactive/isolationist degree of infracommunities: towards an assessment of the role of intrinsic and extrinsic factors

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Analysis of the determinants of parasite community structure is a key topic in parasite ecology, with a growing focus on the influence of interaction between co-occurring species. The idea that communities may be something more than the result of the current assemblage and may reflect the evolutionary process, has led to the proposal of the interactive / isolationist classification of parasite communities (i.e. structured by their interspecific or intraspecific interactions respectively). However, communities are allocated, mainly through qualitative assessment, to one or other of

these two extremes, thereby missing the continuum that occurs in nature. Attempts to quantify the degree of isolationism / interactivity have been addressed mainly at a host population level.
Parasite interactions occur within individual hosts and variation in infection intensity may lead to individual host communities being differentially defined as isolationist / interactive. Analysis at a population level may miss the role of intrinsic and extrinsic factors which lead to these differences. We developed a measure of within host contact for each individual parasite and apply this measure to mountain ruminant parasite communities, which are characterized by high seasonal, age and sexual variability. Mean parasite interaction showed high variability mainly related to temporal effects, with a lesser influence played by host factors.

WEDNESDAY 31ST MARCH

Session 5B - Wildlife Disease and Invasions (Chair: Sarah Perkins)

S54 Invited Speaker
Managing a most unusual parasite: Tasmanian devil facial tumour
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The largest surviving marsupial carnivore, the Tasmanian devil, *Sarcophilus harrisii*, is threatened with extinction by a cancer in which the tumour cells themselves are the infectious agent. The tumour can be considered a clonally reproducing parasitic mammalian cell line. Analysis of extensive field data shows that transmission is frequency, rather than density dependent, which means that this species-specific parasite is capable of causing the extinction of its host. Since the disease was first observed in 1996, the total Tasmanian devil population has declined by more than 60%. Where the disease is well established, continuing population decline of up to 95% has been observed and prevalence remains over 50%. Management actions being investigated include isolating uninfected animals, disease suppression by removal of all animals showing clinical signs, and attempting to identify genotypes that may show some resistance. A disease suppression trial on a semi-isolated peninsula has so far failed to halt population decline or to show any decrease in the rate of transition from healthy to infected status. Tumour cells from one animal are thought to be able to infect another because of very low MHC diversity in the devil population. However, the disease is now spreading to the north-west of Tasmania where MHC diversity is higher. Prevalence is increasing more slowly in this area than in previously infected locations and age structure remains similar to uninfected populations. Whether this indicates that some of the north-western genotypes are resistant remains unclear and the outcome of coevolutionary forces on the host parasite interaction is also uncertain.

S55* Patterns of stable isotope signatures and malaria infections in *Ficedula* flycatchers
Katarzyna Kulma[1], Marcel Klaassen[2] & Anna Qvarnström[1]
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The character of host-parasite interactions as well as the direction where such co-evolution may lead depends on the spatio-temporal ecological context. The ecological circumstances influence, for example, the abundance and virulence of parasites. Moreover, different ecological circumstances may set different costs and thus shape different trade-offs and life-history strategies. Pied (*Ficedula hypoleuca*) and collared flycatchers (*F. albicolis*) are closely related migratory passerines that occupy different wintering sites in Africa and follow different migration routes. They breed in different parts of Europe, but their distributions overlap in two contact zones. One of these zone is the Baltic islands (Öland and Gotland) where two flycatcher species compete fiercely over nest-sites and food resources. They experience seasonal changes in both relative fitness and in malaria infection patterns. To investigate whether malaria infected and non-infected

birds had occupied different areas on their wintering grounds in Africa, we determined the prevalence and types of malaria blood parasites using a PCR protocol. Next, we tested for differences in feather stable isotope signatures (δ13C, δ15N). Such signatures reflect individual's diet and environmental conditions at the wintering location during feather replacement. I compare these results with breeding performance data and discuss possible consequences for host species.

S56* Molecular phylogenetics of *Thelohania muelleri* like parasites infecting gammarid amphipods

Toby J Wilkinson[1], Martin Kamler[2] & Joseph E Ironside[1]
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Thelohania muelleri is a well described Microsporidian found infecting the musculature of gammarid amphipods. It produces a distinctive pathology, consisting of white masses of spores visible in the abdomen of the host, which is commonly used as the diagnostic feature when identifying the infection. Difficulty in obtaining molecular data has resulted in the species only previously being characterised ultrastructurally. 16S rDNA sequence was acquired from individuals of *Gammarus lacustris*, *G. duebeni* and *G. fossarum* all showing the typical pathology associated with *Thelohania muelleri*. Phylogenetic analysis by Bayesian Inference suggests that the species is actually a member of the genus *Dictyocoela*. A formal description of *Dictyocoela* has yet to be published. However, previous studies associate *Dictyocoela duebenum* with light infections of the gonad and ectodermal tissues, with no mention of the gross muscle pathology associated with *T. muelleri*. Therefore to obtain a true full description, infection in the musculature must also be investigated. It is possible that if *Thelohania muelleri* is in fact a *Dictyocoela* parasite that other morphologically similar species such as *T. hereditaria* should also be transferred to the genus.

S57* Spatial distribution of *Pseudamphistomum truncatum* and *Metorchis albidus* in England and Wales using ArcMap GIS

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Otter (*Lutra lutra*) populations in the UK have greatly recovered following a severe decline during the 1960s, however further investigation of their parasitic fauna is important and may help prevent future population crashes. As road-killed otters from England and Wales are routinely collected for post mortem by the Cardiff University Otter Project, 477 gall bladders were screened for parasites. Two digenean species were recovered from the bile and bile ducts, *Pseudamphistomum truncatum* and *Metorchis albidus.* Both species are thought to have only recently been introduced to the UK and there is no record for either prior to 2000. The spatial spread of these parasites was examined using a modified Ripley's K statistic, K[i.](r), with Ripley's isotropic edge correction to assess for clustering. *P. truncatum* infections cluster and are over-represented in the Southwest of England and South Wales whereas *Metorchis albidus* infections appear randomly distributed. At present, there is no detectable increase in prevalence with year for either parasite. The pathological damage these parasites cause to the biliary system makes it necessary to monitor their distribution to protect both wild and domestic piscivores against these generalist pathogens. The presence of these parasites in the UK now provides an ideal opportunity to understand how a pathogen can spread across a novel habitat.

S58 Parasites alter the predatory interactions between native and invading species.
Alison M. Dunn, Jaimie T.A. Dick, Michael Armstrong, Hazel C. Clarke, Keith D. Farnsworth, Melanie J. Hatcher, Neal Haddaway, Ruth Wilcox & Rachel Heptonstall
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Invasive species can have profound impacts on the communities that they invade and these effects may be mediated by parasitism. Here, we investigate the influence of parasitism on the predatory interactions between native and invasive species in two ongoing UK invasions. *Gammarus pulex* is an invasive amphipod that competes with and excludes the native *G. duebeni*. Surprisingly, we found that *G. pulex* individuals infected with the acanthocephalan parasite *Echinorynchus truttae* consumed significantly *more* prey than did uninfected individuals. The animals displayed Type II functional responses (FRs), with the FR for parasitized animals rising more steeply and with a higher asymptote compared with unparasitised individuals. *G. pulex* has been found to alter the wider community structure in freshwaters that it invades through its predatory impact. The increase in the predatory response of infected hosts may enhance the impact of this invader on the recipient community.
Parasitism may also increase the impact of invasion by the American signal crayfish, which is driving the native white clawed crayfish extinct throughout Britain. Native crayfish infected by the microsporidian parasite *Thelohania contejeani* showed a lower FR than did uninfected native or invasive species as well as a change in diet. These changes reduce the ability of the white clawed crayfish to compete with the invasive signal crayfish and may increase the rate of extinction.

Session 6B - Population dynamics and theoretical ecology (Chair: Jo Lello)

S59 Invited Speaker
Modelling Dengue: Epidemic Patterns and Persistence through Vector Transmission
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Dengue virus, the causative agent of dengue fever and dengue hemorrhagic fever, is widespread throughout tropical and subtropical regions. The virus exists as four distinct serotypes, all of which have co-circulated in Bangkok for several decades with epidemic outbreaks occurring every 8-10 years. Using an epidemic model with stochastic seasonal forcing showing 8-10 year epidemic oscillations, we demonstrate that moderate cross-protective immunity gives rise to persistent out-of-phase oscillations similar to those observed in the data, but that strong or weak cross-protection or cross-enhancement only produces in-phase patterns. In many regions dengue incidence fluctuates seasonally with few if any infections reported in unfavourable periods. It has been hypothesized that vertical transmission within the mosquito population allows the virus to persist at these times. A review of the literature shows that vertical infection efficiencies are between 1 - 4%. Using a mathematical model we argue that at these infection rates vertical transmission is not an important factor for long term virus persistence.

S60 Geographical variations in elimination criteria for global helminth control programs
Manoj Gambhir & Edwin Michael
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Neglected tropical disease control relies on a variety of lines of attack: mass drug administration, vector control and improvements in sanitation are a few. These approaches achieve different levels of impact on the diseases they are aimed against, depending on host-parasite ecologies for each of disease. In the case of indirectly transmitted infections, such as lymphatic filariasis and schistosomiasis, these ecologies can be complicated, involving the infection dynamics of several life-stages of the parasite within different hosts.
We investigate the consequences of parasite population dynamics on the control helminth infections. We construct mathematical models of the relevant worm population ecologies, and investigate the effects of density dependent mechanisms on 1) the threshold vector biting rate,

below which infection cannot be sustained; 2) the breakpoint parasite density, which implies the existence of a minimum parasite population below which extinction will result; 3) the rate at which the parasite level will change when it is perturbed from its initial level.
Fitting the models to available human and, in some cases, vector data - while quantifying residual uncertainty in the models - shows that parameters relating to the density dependences vary over geographical areas and that these parameters influence estimates of parameters such as R_0. We extract general principles from these results to highlight the importance of considering complex systems dynamics in the design of effective helminth control and elimination programs.

S61 Epidemic malaria and warmer temperatures in recent decades in an East African highland

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Malaria is a complex vector-borne disease and a major public health burden in endemic regions around the tropic. Non-endemic regions have shown pronounced patterns of increase in incidence and re-emergence in the past three decades. Despite extensive knowledge accumulated for almost a century on the biology of both the parasite and the mosquito vector, the reasons for these patterns of exacerbation are not well understood. Climate change, human migrations, and drug resistance are different hypotheses but evaluating these mechanisms from time series data remains elusive. In this work, we focus on the recent past and on the temporal population dynamics of the disease, to address whether warmer temperatures have already increased the incidence of epidemic malaria in an East African highland. Our analyzes rely on a monthly time series of confirmed cases from 1970 to 2003 in the Kericho region of Kenya and on an epidemiological model for the population dynamics of the disease that includes both the human host and the mosquito vector. Our findings suggest that climate change has already played a role in the exacerbation of malaria in this region. The relative effect of other potential factors acting either in addition to or synergistically to warmer temperatures remains to be carefully evaluated.

S62 Integrating theory and experiment to understand variation in malaria parasites

Nicole Mideo[1,2], Andrew Read[3], Troy Day[4] & Sarah Reece[1]
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Understanding interactions between parasites, host resources and other within-host 'ecological' factors is important because they ultimately shape disease outcomes of interest (e.g. virulence). Malaria parasites are known to differ in how they use host resources, for example the four human *Plasmodium* species invade different age ranges of host red blood cells (RBCs). Such differences in host exploitation traits give rise to different levels of disease severity and are predicted to determine the outcome of competitive interactions between species and strains. Using a model malaria system (*P. chabaudi* infections in mice) we first used a theoretical approach to describe within-host infection dynamics and predict differences in exploitation traits, including the parasite invasion rates of target RBCs of different age and the number of progeny parasites produced per infected cell, between two strains and, second, we used experiments to measure these traits and test our predictions. We find some support for our model predictions, and discover that within-host ecology matters, suggesting that malaria parasites adaptively vary their host exploitation strategies in an adaptive way in response to changes in the within-host environment.

S63* Density-dependent mortality of the human host in onchocerciasis: relationships between microfilarial load and excess mortality

Martin Walker[1], Mark P. Little[1], Karen S. Wagner[2], Edoh S. Alley[3] & María-Gloria Basáñez[1]
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Background. The parasite *Onchocerca volvulus* has, until recently, been regarded as the cause of a chronic yet non-fatal condition. New analyses, however, have indicated that in addition to blindness, the parasite can also be directly associated with human mortality. Such analyses also suggested that the relationship between microfilarial load and excess mortality might be density dependent. Determining the functional form of such relationship would contribute to quantify the population impact of mass microfilaricidal treatment.
Methodology/Principal Findings: Data from the Onchocerciasis Control Programme in West Africa (OCP) collected between 1975 and 2002 were used to determine functional relationships between microfilarial load and excess mortality of the human host. The goodness-of-fit of three candidate functional forms were explored and a saturating exponential type function was deemed to be statistically the best fit.
Conclusions/Significance: Incorporation of a functional relationship between microfilarial load and excess human mortality into mathematical models for onchocerciasis transmission and control will have important implications for our understanding of the parasite's population biology and of the projected benefits of control programmes for this disease in both human and economic terms.

Session 7B - Environmental and Social influence on Parasite Epidemiology (Chair: Mark Booth)

S64 Invited Speaker
Epidemiological and evolutionary effects of intensive farming

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Intensive farming creates conditions for parasite transmission that is drastically different from what parasites experience in wild host populations. These conditions will certainly change epidemiological parameters, but could also alter selection on parasite life history traits. The most obvious difference between hosts living under natural conditions, as compared to farmed hosts, is a significant increase in population density. It has been shown that host population density is positively correlated with both parasite abundance and species richness. Moreover, in situations where parasites experience a rapid increase in the number of available hosts, transmission opportunities will be changed, which in turn could select for new combinations of parasite life history traits. This is of particular concern, since life history traits of parasites could be linked to virulence.
The global rapid increase in fish farming during the last decades could be considered a large-scaled experiment providing an opportunity to study the effects of artificially increased host density on parasite epidemiology and evolution. Using salmon farming as an example, this talk will review the emergence of parasites and pathogens following a dramatic ecological change in the marine environment, and also discuss possible evolutionary implications.

S65* Estimating the Global Burden of Disease of the Neglected Tropical Disease Onchocerciasis

Simon O'Hanlon,[1] Luc Coffeng,[2] Wilma Stolk,[2] Sake de Vlas,[2] Sébastien Pion[3] & María-Gloria Basáñez[1]
1 *Department of Infectious Disease Epidemiology, Imperial College London, W2 1PG, UK. 2 Erasmus Medical Centre, Rotterdam, The Netherlands.* 3 *IRD Montpellier, France*

Background. The Global Burden of Disease (GBD) study aims to quantify the global and regional effects of diseases, injuries and risk factors on population health using the established metric of

the DALY (Disability Adjusted Life Years). This project represents a considerable challenge to groups focusing on neglected tropical parasitic diseases like onchocerciasis. Aside from the perceived unsuitability of the DALY to measuring the burden of chronic parasitic infections of the poor, many disease cases go unreported due to unsatisfactory monitoring and registration of morbidity within these populations.
Methods/Results. We present results from a systematic review of available literature on onchocercal disease and analysis of monitoring & evaluations data from large-scale control programmes to try to re-estimate the GBD due to onchocerciasis, quantifying relationships between infection prevalence, intensity, and morbidity in order to quantify the number of DALYs lost to onchocerciasis.
Conclusions/Significance. We have updated the disease model to include morbidities associated with onchocercal infection not included in previous estimates. Non-linear relationships between parasitological indicators and various morbidities (e.g. blindness) highlight the importance of accurately accounting for people at risk (with high infection intensities) in high prevalence areas so as to reduce the chances of underestimating disease burden.

S66* Lymphatic Filariasis mapping using Bayesian spatial techniques
Hannah Slater & Edwin Michael
Department of Infectious Disease Epidemiology, Faculty of Medicine, Imperial College London

Lymphatic Filariasis (LF) is a vector-borne infectious disease endemic in the tropics, including sub-Saharan Africa, where an estimated 50 million people are infected. It is thought to present the second largest health burden of any disease worldwide. Accurate modelling of geographic distribution of the disease at a region level is crucial to guiding the planning of control programmes. We attempt to map the prevalence of LF infection across Africa using a Bayesian generalised spatial linear model in conjunction with community-level infection data obtained from the published literature. We use the package 'geoRglm' implemented in 'R' to create a disease prevalence distribution model with spatially correlated random effects. The model parameters are estimated using Markov chain Monte Carlo techniques. We use the model to explore (1), the relationship between LF infection prevalence and the environmental variables, (2) assess the biologically plausibility of the estimated functional relationships, and (3) relate this to climate dependency in LF transmission dynamics. The prevalence map is also used to estimate the total number of people infected with LF in Africa. Finally, we use future climate predictions to investigate the impact global warming could have on future LF spatial distribution, prevalence and burden.

S67* Environmental conditions and senescence in parasite resistance of a wild mammal
Adam D. Hayward, Alastair J. Wilson, Jill G. Pilkington, Josephine M. Pemberton & Loeske E.B. Kruuk
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Despite increasing evidence for a decline in immune performance with advancing age in natural populations, it remains unclear how this translates to changes in actual parasite burdens. Moreover, in populations where individuals may experience heterogeneous and even adverse environmental conditions, there is the potential for large variation in individual life-history trajectories. We present analysis of gastrointestinal helminth faecal egg count (FEC) data collected over 20 years from a free-living population of Soay sheep, with the aim of investigating the impact of ageing and environmental conditions on parasite resistance. We show that individuals experience an increase in FEC with increasing age; however, simple 'chronological' age, measured in years, fails to fully explain changes in FEC. 'Environmental age', a measure of cumulative environmental conditions experienced over the whole life-history, predicts an additional increase in FEC. Finally, we show that these factors interact, such that individuals that have experienced adverse and stressful conditions show an accelerated age-specific increase in FEC when compared to individuals that have experienced favourable conditions. Chronological age is thus associated with a decline in parasite resistance, but heterogeneity in experience of

environmental conditions and stressors seems an important source of variation in changes in parasite resistance across the host life-history.

S68 Effects of temporal environmental variation on host-parasite dynamics in the *Paramecium caudatum - Holospora undulata* system

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The environment is rarely constant and organisms are exposed to temporal and spatial variation that will impact life-histories and inter-species relations. It is important to understand how such variation affects epidemiological dynamics in host-parasite systems. We explored effects of temporal variation in temperature on experimental populations of the ciliate *Paramecium caudatum* and its bacterial parasite *Holospora undulata*. Infected and uninfected populations of 2 *P. caudatum* genotypes (K8 and VEN) were created and 4 constant temperature treatments (26, 28, 30 and 32ºC) compared to 4 variable treatments with the same mean temperatures. Variable temperature treatments were achieved by alternating populations between permissive (23°C) and restrictive (35°C) conditions daily over 30 days. Variable conditions caused greater declines in host populations at higher temperatures. This effect was disproportionate for host clone VEN, especially for infected populations. Variable conditions were also detrimental for the parasite causing greater declines in levels of infection. This was much worse for parasites infecting host clone K8 with declines observed in all variable conditions with mean temperatures greater than 28ºC. These results highlight how variable conditions can impact persistence of both host and parasite populations. They also demonstrate that sign and rates of population decline can depend on host genotype and parasite infection.

Session 8B - Ecology meets Immunology (Chair: Mark Viney)

S69 Invited Speaker

The use of predator-prey-like models to assess the action of the immune system on parasite infections

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So-called `embedded' models are more and more used to assess the interaction between epidemiology and immunology. Embedded models suppose that epidemiological parameters such as disease-induced mortality (virulence), transmission and/or recovery result from an explicitly given model for the dynamics of parasite and immune system within an infected host. This approach has been useful to explain the emergence of virulence/transmission trade-offs that underlie most models for virulence evolution. Extensions of this approach are necessary to understand the potential for interaction between different parasite strains within the same host: often they will feel each other's presence only via their effect of the immune system. The approach also has highlighted the importance of a few neglected questions: modellers typically make the assumption that infections are chronic or acute depending on the disease they want to model but this aspect should actually be an outcome predicted by theory. In particular, embedded models tend to predict chronic infections: the mechanisms that permit an immune system to eradicate an infection are poorly understood. Many embedded models are inspired by models for predator-prey interactions but it is worthwhile to point out that models for parasite-immune system interactions be interesting for ecologists.

S70 Acellular Pertussis Vaccination Facilitates *Bordetella parapertussis* Infection in a Rodent Model of Bordetella Infection

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Center for Infectious Disease Dynamics, Department of Biology, The Pennsylvania State University, University Park, PA 16802, USA. [#]Currently at: *The Department of Animal and Plant Sciences, University of Sheffield, Western Bank, Sheffield S10 2TN, UK*.

Despite over fifty years of population-wide vaccination, whooping cough is re-emerging in highly vaccinated populations. Although *Bordetella pertussis* is regarded as the major causative agent of human whooping cough, *B. parapertussis* infections are not infrequent. The widely-used acellular whooping cough vaccines (aP) are composed exclusively of *B. pertussis* antigens, which hold little or no efficacy against *B. parapertussis*. Using a rodent model of infection, we show that aP vaccination helped to clear *B. pertussis* infection from the lower respiratory tract (LRT), but resulted in a c.40-fold increase in *B. parapertussis* lung colony-forming units (CFUs). We show that such vaccine-mediated facilitation of *B. parapertussis* infection did not arise as a result of competitive release. Rather, aP vaccination, by reducing lung inflammatory responses (measured by cytokine responsiveness in the lung) and lung neutrophil recruitment, delayed *B. parapertussis* clearance. Our data raise the possibility that aP vaccination can create hosts that are more susceptible to *B. parapertussis* infection.

S71* Why do antibodies induced by malaria and helminths cross-react?

Karen J. Fairlie-Clarke, Tracey J. Lamb[‡], Jean Langhorne[§], Judi E. Allen & Andrea L. Graham[#]
Institutes of Evolution, Immunology and Infection Research, Edinburgh University EH9 3JT, UK. [#]Department of Ecology and Evolutionary Biology, Princeton University, Princeton, NJ 08544 USA. [‡]School of Biological Sciences, Reading University RG6 6AJ, UK. [§]Division of Parasitology, National Institute for Medical Research NW7 1AA, UK

Cross-reactive antibodies that recognise antigens from distinct pathogens were observed in a model of malaria-helminth co-infection. To determine the relative strength of cross-reactive versus antigen-specific responses, in co-infected mice, we used ELISA to calculate antibody titre from a serial dilution of serum. We further examined whether cross-reactive responses were targeted toward carbohydrate or protein moieties by treating the parasite antigens with periodate, thus disrupting carbohydrate epitopes by oxidising carbohydrates to aldehydes. Periodate treatment affected both antigen-specific and cross-reactive responses.
We are interested in exploring the possibility that limitations in the immune system's ability to discriminate antigens result in cross-reactive responses. For example, if the antigenic distance between two parasites is small the immune system may not perceive them as different and cross-reactivity would result. Maintaining some degree of polyclonality may confer a broader spectrum of protection raising the interesting question of whether production of cross-reactive antibodies might be the optimal response for a host faced with infection by an unpredictable range of parasites.

S72 Post-genomic strategies allow the analysis of immunological signalling networks in wild animals: a case study on immunodynamics in the field vole, *Microtus agrestis*

Joseph Jackson[1], Steve Paterson[1], Richard Birtles[2], Jan Bradley[3] & Mike Begon[1]
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A revolutionary advance in ecological immunology is that postgenomic technologies now allow molecular mediators defined in laboratory models to be measured at the mRNA level in field studies of naturally-occurring species. Here we demonstrate the application of such an approach to generate meaningful immunological profiles for wild mammals. We sampled a field vole population across a full annual cycle and developed a battery of cellular assays in which functionally different pro- and anti-inflammatory signalling responses (transcription factor and cytokine mRNAs) were activated and quantified by Q-PCR. Temporal trends and infection status accounted for a significant proportion of the observed variation in immunological expression. Macroparasites were significantly positively associated with regulatory activity, whilst

microparasites and some macroparasites were associated with an apparently counter-regulated Th1–Th2 axis (cestodes low, microparasites high, Th1 expression low, Th2 expression high). There were highly significant annual and non-annual temporal immunological trends and systematic differences in the immunological status of animals occurred between equivalent points in the annual cycle (Winter 2008 and 2009). Pinpointing the causes and consequences of such non-seasonal temporal variability may help identify underlying environmental drivers of individual fitness and demographic fluctuation.

S73* Immunogenetics, selection and parasite resistance in a natural population of field voles (*Microtus agrestis*)

Andrew Turner, Mike Begon, Joe Jackson & Steve Paterson
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Most of the research on the role of host genetic variation on parasite resistance has been on humans, livestock or laboratory organisms. However, the majority of such studies focus on well-nourished individuals that are under limited environmental stresses, often infected with only a single experimental pathogen at a time, and are therefore not ecologically valid. The relatively few studies that have concentrated on natural populations have largely focussed solely on the genetics of the major histocompatibility complex; there is therefore a need to expand research away from the MHC to other immune genes and away from laboratory to natural populations. Our work has addressed both of these issues by examining the genetic diversity of cytokine genes, key regulators of the immune response, within a well-studied natural population of field voles (*Microtus agrestis*) in Kielder Forest, UK. We used population genetic methods to identify a signature of natural selection acting on several of these genes and then demonstrated that this genetic diversity can lead to phenotypic effects, such as variation in gene expression levels and parasite resistance between individuals. We conclude that immunogenetic diversity in the vole population is widespread and that host-parasite interactions provide a possible mechanism for its maintenance.

Column C - General Parasitology

Session 1C - Helminth Immunology (Chair: Joe Jackson)

S74 *Fasciola hepatica*: investigation of triclabendazole (TCBZ) resistance in field cases of fasciolosis using histopathological and immunocytochemical methods to identify apoptosis in the reproductive structures of TCBZ-sensitive flukes.

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Certain well-defined histological changes are recognisable in the reproductive structures of TCBZ-sensitive flukes following recent TCBZ treatment of the host. Such changes are not seen in TCBZ-resistant flukes from TCBZ-treated hosts. Hence histopathological methods can be used to investigate TCBZ resistance status in field cases of fasciolosis. Amongst the changes seen in TCBZ-sensitive flukes exposed to metabolites of TCBZ in treated sheep, the development of apoptotic-like bodies in testes, vitelline follicles and ovary predominates. The cells mainly affected are those undergoing mitosis or meiosis. The occurrence of apoptosis in this situation has been verified by the use of a commercially available kit for immunocytochemical localization of apoptotic cell death. The method, which relies on recognition of DNA cleavage fragments generated by endonuclease following a trigger event, could improve sensitivity and facilitate quantification of histopathological analysis in the investigation of TCBZ resistance.

S75* Proteomic and immunological comparative studies of different trematode species

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Schistosoma haematobium and *Schistosoma bovis* are blood trematodes that cause diseases in human and ruminant hosts, respectively. *Echinostoma caproni* is an intestinal trematode that mainly affects mammals and birds and is used as a model to study helminthiasis. Here, we compare protein expression between these 3 species using 2D-Differential In gel Electrophoresis (DIGE) and study the cross-reactivity of antigens from *S. haematobium* and *E. caproni*. Sera from *S. haematobium* and *E. caproni* infected hamsters were used to screen crude extracts of *S. haematobium* and *E. caproni* by western blotting. Our results show that there is differential protein expression between the three species. Furthremore we demonstrated that as expected, the antigen recognition profiles differed between sera from animals infected with *S. hameatobium* v.s. those infected with *E. caproni*. Interestingly, we also demostrated that there were some commonly recognised antigens. The relevance of our findings of both differentially expressed proteins and common antigens are discussed in relation to phylogenetic studies, diagnostic tests and vaccine development.

S76* Investigation into the mechanisms underlying the slow development of human anti-schistosome immunity

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Field studies suggest that protective immunity against schistosomes takes many years to develop. During chronic infection, humans are exposed to several different schistosome lifecycle stages. One hypothesis for the slow development of protective immunity is that exposure to dying (long-lived) adult worms is needed to stimulate a protective response. Another hypothesis is that a threshold level of antigen must be experienced before a protective response is initiated.
Mathematical models were used to investigate the importance of different parasite lifecycle stages in stimulating antibody responses, and to assess the impact of an antigen threshold. Model outputs were compared with *Schistosoma haematobium* field data from Zimbabwe. Results from deterministic models describing mean levels of infection and antibody in homogeneously exposed populations indicated that protective antibody responses stimulated by dying adult worms can reproduce age-infection and age-antibody profiles consistent with field data, but that other life-stages could also be the principal source of protective antigen. Inclusion of an antigen threshold enhanced the model's ability to generate observed infection and antibody profiles. Stochastic individual-based models, which permit heterogeneous exposure, allow us to determine whether these mechanisms can additionally explain observed infection and antibody distributions.
Identifying the mechanisms underlying the development of protective immunity is important for understanding how mass chemotherapy programmes may impact the development of natural immunity, with consequent effects on infection.

S77 Imbalance of regulatory and activated T cells in human *Schistosoma haematobium* infections

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Acquired immunity against helminths is characterised by a complex interplay between Th1 and Th2 immune responses, and is often manifest only with increasing age. Data from experimental models suggest that immunity is also influenced by regulatory T cells (Treg), but as yet studies on Treg in human schistosome infections are limited. We therefore characterized regulatory and

activated T cell (Tact) populations in Zimbabweans (aged 8-60 years) exposed to *Schistosoma haematobium* parasites. Activated T cells were classified as $CD4^{+}CD25^{+}FOXP3^{-}$ while Treg were defined as $CD4^{+(dim)}CD25^{+(high)}FOXP3^{+}CD127^{low}$. While the proportion of Treg within the total $CD4^{+}$ T cell population did not change with age, the proportion of Tact increased significantly, resulting in reduced ratios of regulatory to activated cells with age. Moreover, there was a significant positive correlation between Treg:Tact ratio and infection intensity. The strongest correlation occurred in the youngest age groups in whom infection was rising and peaking, but the association was lost in the older age group with declining infection levels. This significant change in the correlation between the Treg:Tact ratio and infection intensity with host age coincides with the development of acquired resistance to infection/re-infection. This study in a human population supports a paradigm derived from experimental models in which the balance between effector (Th1/Th2/Th17) and regulatory responses determines resistance or susceptibility to helminth infection/re-infection.

S78 Targeting of host lipoproteins by the parasitic worm *Schistosoma mansoni*

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Schistosomes have developed multiple strategies to evade and resist the immune response of the mammalian host, allowing them to reside for many years in the blood vessels. Much research has focused on immune modulatory proteins excreted by schistosome eggs such as IPSE/alpha-1 or Omega-1, and on the Th2 skewed-immune response typically found in helminth infections.
We here describe a completely different mechanism of immune modulation where host lipoproteins serve as transporters of schistosome antigens. Like many schistosome proteins, these antigens are glycosylated with schistosome specific glycoproteins. As a result of transfer of schistosome antigens to host lipoproteins, circulating antibodies against schistosome antigens will indirectly bind to these lipoproteins. We have demonstrated the presence of IgG on low-density lipoprotein particles from serum from infected individuals, whereas no antibodies were found on lipoproteins of healthy controls. Subsequently, these antibody-opsonised lipoproteins are phagocytosed by immune cells carrying an Fc-receptor. Indeed, accumulation of lipids within several types of blood derived immune cells occurred in infected individuals only. *In vitro*, this lipid accumulation was associated with apoptosis and reduced viability of neutrophils. The consequences of these lipoprotein binding antibodies for immune cells and for the anti-helminth host response will be discussed.

S79 Eosinophil degranulation against adult *Onchocerca ochengi* during macrofilaricidal chemotherapy is dependent on depletion of *Wolbachia*

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Adult filariae of *Onchocerca volvulus* in humans, and its close relative *O. ochengi* in cattle, reside in collagenous nodules for a decade or more. The basis of the symbiotic relationship between filariae and their *Wolbachia* endosymbionts is thought to be metabolic, but a role for *Wolbachia* in defence against immune attack has received little attention. Neutrophils are attracted to *Wolbachia* but they are replaced by eosinophils following antibiotic chemotherapy, which degranulate on the worm cuticle. However, it is not clear whether the eosinophils are involved in killing the filariae or if they are attracted secondarily to dying worms. In this study, cattle infected with *O. ochengi* were treated with macrofilaricidal regimens of oxytetracycline or melarsomine. In contrast to oxytetracycline, melarsomine had no significant effect on the viability of *Wolbachia*. Eosinophil infiltration and degranulation increased significantly only in the oxytetracycline group; whereas nodular gene expression of the chemokine interleukin-8 was lowest in this group. Moreover, in the early time-points, intense eosinophil degranulation was associated with worm vitality, not degeneration. These data are consistent with a role for eosinophils in antibiotic-mediated killing, and suggest that *Wolbachia* prevents a local eosinophilia by recruiting neutrophils.

Session 2C - Disease Control (Chair: Rupert Quinnell)

S80 Invited Speaker

Paradigm lost: How parasite control may alter pattern and process in human helminthiases

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Most of our knowledge of pattern and process in helminth population biology is based on the steady state of endemic equilibrium that characterises helminth infections prior to control perturbations. This shapes the way we think about age profiles of infection, distributions of parasites among hosts, and mechanisms regulating parasite populations. Many large-scale (chemotherapy-based) programmes are now in place which aim at controlling infection, reducing morbidity, and eliminating infection where deemed possible. Depending on whether anthelmintics are mass administered or targeted at particular age- or occupational groups, age-infection profiles will be modified; immune responses will be affected; prevalence vs. intensity relationships will change (underlying parasite distributions may become more aggregated); density-dependent regulatory processes will relax, and the contribution to transmission and morbidity of different groups in the host population will shift (as infection intensity decreases and per capita parasite population rates increase or decrease depending on negative or positive density dependence). Additionally, we need to monitor for changes in drug efficacy and develop novel assays for detection of active infection and exposure as the sensitivity of parasitological indicators decreases, programmes approach transmission breakpoints, and tools are required to ascertain when to stop. We need to understand how the parasite population biology paradigm is changing if we aim at supporting parasite control efforts effectively.

S81 Porcine parasites in Northern Ireland: incidence, distribution and correlation with management and control strategies

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Endoparasitic worms inflict a huge economic burden on pig production through reduced growth, poor feed conversion efficiencies and higher medication costs. As yet, anthelmintic resistance has not been reported in swine herds in the UK or Ireland. However, benzimidazole and levamisole resistant nematodes have been identified from herds in Germany concurring with the situation in other nematode parasites of sheep and cattle where multi-drug resistance is rife. The aim of this study is to investigate the prevalence and distribution of helminth parasites in pig units across Northern Ireland (N. I.), and to correlate these findings with questionnaire-derived husbandry-management and worm control practices, and milk spot incidence at abattoir. So far, 1260 faecal samples from 42 large indoor farms (1000+ pigs) have been surveyed. 87% of all units examined were positive for one or more nematode species. Of the 7 endoparasite species identified, *Ascaris suum* and *Strongyloides ransomi* were the most common and found in > 75% of establishments. Other species identified included: *Hyostrongylus rubidis*, *Oesophagostumum dentatum*, *Metastrongylus apri*, *Trichuris suis* and *Fasciola hepatica*. These findings indicate that despite good husbandry practice, high-levels of worms are commonplace on most pig units in N.I. in large enough numbers to warrant concern.

S82* Detection of *Echinococcus granulosus* in farm dogs in South Powys, Wales using coproELISA

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Echinococcus granulosus is a dog tapeworm that causes the zoonotic disease, cystic echinococcosis. Canine echinococcosis appears to have re-emerged in Powys, Wales following a control programme in the 1980s (Buishi et al., 2005). The Office of the Chief Veterinary Officer and

the Department for Public Health and Health Professions in the Welsh Assembly Government jointly funded a pilot dog worming campaign as a preventative public health measure. To evaluate the impact and efficiency of a short-term supervised dog dosing scheme, collection of faecal samples on farm visits and worming of dogs with praziquantel commenced in South Powys in May 2008. In Year 1, approximately 1500 canid faecal samples were collected from registered farms and delivered to the University of Salford to be tested. In total 1351 faecal samples were tested by coproELISA, of these 609 samples were collected at baseline and were found to have a coproantigen prevalence of 10.8%. A total of 742 samples tested after 3 treatments gave a coproantigen prevalence of 0.7%. The study will continue to compare the coproantigen prevalence over one year after cessation of dosing.

S83* A novel nicotinic acetylcholine receptor subunit of parasitic nematodes

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Nicotinic acetylcholine receptors (nAChRs) are significant drug targets for parasitic nematodes, and several sub-types of these receptors are present on body wall muscle. We recently identified a novel nAChR subunit gene, *acr-26*, from *Ascaris suum*. A survey of parasitic nematodes, using a combination of sequence data and primary material, demonstrates that *acr-26* is conserved in several parasitic species from clades III and V, including *Brugia malayi, Haemonchus contortus* and *Dirofilaria immitis*. To date however, no evidence has been found of *acr-26* in any free-living or plant parasitic nematode. A specific antibody against the Asu-ACR-26 subunit was produced; immunocytochemistry on native tissue showed that the subunit was expressed in the head muscle of *A. suum* but not body wall muscle. Sequence similarities with other nAChRs and computer modelling predicted that ACR-26 was capable of forming a homomeric channel. *In vitro* expression of ACR-26 in the *Xenopus* oocyte expression system confirmed this prediction and showed that the receptor responded to both acetylcholine and nicotine, but was not sensitive to levamisole. The pharmacology and function of this new receptor are being investigated further.

S84 Vaccination of rats against fasciolosis by a multivalent vaccine of stage-specific cathepsin proteases induces significant protection

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Fasciola hepatica produces cathepsin B and cathepsin L in their excretory-secretory material. These proteases are proposed to be key virulence factors for parasite infection and are targets for vaccination. Cathepsin B isoforms are predominantly released in the juvenile life cycle stage while different cathepsin L isoforms are released throughout the cycle. Three proteases (cathepsin L5, cathepsin L1g and cathepsin B1) were expressed using cDNAs isolated from adult, metacercariae and juvenile flukes, respectively. Each was used singly or in combination to vaccinate rats that were challenged with *F. hepatica* metercercariae. All vaccinated groups yielded significantly fewer and smaller flukes than the control group, suggesting vaccination retarded liver fluke development. Maximal protection of 83% was seen in the group vaccinated with a combination of an adult (cathepsin L5) and a juvenile (cathepsin B1) protease This study confirms that juvenile-derived proteases are potentially important vaccine candidates and that combination protease vaccines may have superior efficacy against fasciolosis.

Session 3C - RNA interference in Parasitic Nematodes (Chair: Angela Mousley)

S85 Invited Speaker
Developing an alternative RNA interference protocol to study druggable targets in parasitic nematodes
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Diseases caused by parasitic nematodes perpetuate socioeconomic instability in developing countries by inflicting crippling morbidity and significant mortality. One reason for the persistence of these diseases is an inadequate portfolio of effective drugs; new, more effective compounds are needed. A major obstacle to their development is the lack of available tools to validate potential novel drug targets in parasitic nematodes. RNA Interference (RNAi) is a tool that allows researchers to suppress genes of interest in experimental organisms or tissues and has become standard for target validation in drug development but a reliable and reproducible protocol for parasitic nematodes has yet to be established. Here we describe an innovative RNAi strategy to study gene function and validate drug targets in parasitic nematodes. Our approach uses the filarial nematode *Brugia malayi* as a model and targets developmental stages of this parasite whilst still in the mosquito host. We can profoundly suppress expression of a cathepsin-L-like gene using both short interfering RNA and long double stranded RNA injected directly into infected mosquitoes. RT-qPCR confirms suppression is specific and profound, resulting in an 83% decrease in transcript abundance. Cathepsin L-like suppression elicits aberrant worm phenotypes with motility defects and reduced transmission potential. Finally we present evidence that other genes are susceptible to this approach.

S86 An eye on roundworm RNAi
Jonathan J Dalzell[1], Paul McVeigh[1], Neil Warnock[1], Makedonka Mitreva[2], David M Bird[3], Pierre Abad[4], Angela Mousley[1], Nikki J Marks[1] & Aaron G Maule[1]
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Since completion of the *Caenorhabditis elegans* genome sequence the generation of genomic and transcriptomic datasets for nematode parasites has evolved relatively slowly. More recent advances in power sequencing have fuelled a significant expansion in transcriptomic datasets and publication of the first genome sequences for nematode parasites. An additional stimulus has been the discovery of RNA interference (RNAi) and its potential to allow gene function (or, drug target validation) studies in organisms previously refractory to reverse genetic manipulation. Aiming to decipher the functional context of these sequence data, parasitologists have attempted to adapt RNAi for use in parasitic nematodes. While progress has been significant in plant parasites, gene silencing in animal-parasitic nematodes has floundered under the difficulties associated with triggering robust transcript knockdown. It seems likely that this variation is due to inter-phylum differences and / or deficiencies in RNAi pathway components. To test this we used 77 *C. elegans* RNAi pathway proteins as query sequences in BLAST trawls of nematode-derived genomic and transcriptomic resources. Although preliminary screens indicate that most proteins are reasonably well conserved, notable omissions include proteins responsible for RNAi uptake and spreading.

S87 Investigating the efficiency of RNA interference in the clade III nematode parasite *Ascaris suum*
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RNA interference (RNAi) provides a reverse genetics platform which facilitates investigations into gene function, providing opportunities for drug target identification and validation. In plant parasitic

nematodes (PPNs) RNAi can be readily achieved upon delivery of double stranded (ds)RNA by simple soaking methodologies. However, despite initially encouraging results from the scientific community working on animal parasitic nematodes (APNs), recent experimentation has revealed that the RNAi response is not robust across all APN species. Here we report efforts to achieve RNAi in the clade III parasitic nematode, *Ascaris suum*. Our approach includes the examination of 9 gene transcript targets whose selection was based on transcript abundance, localisation (neuronal, gut, reproductive or global tissue expression) and previous success in other APN-RNAi experiments. DsRNAs were delivered to the infective stage of *A. suum* (L3) and changes in transcript levels post-RNAi were assessed using quantitative real time PCR (qPCR); a range of experimental parameters were varied in efforts to optimise silencing including dsRNA concentration, delivery method and exposure time. Our results indicate variable susceptibility of the targets; no trend was observed between transcript abundance or localisation and susceptibility. These data are supplemented by ongoing EST/genomic analyses of core RNAi pathway proteins in *A. suum*.

S88 In vitro and in vivo RNAi of *Haemonchus contortus H11*

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RNAi has been applied very successfully in *C. elegans* to study gene function. In parasitic nematodes RNAi has proven to be less effective. We have carried out a detailed RNAi study in the sheep gastrointestinal nematode *Haemonchus contortus* to examine why some genes seem to be more susceptible to RNAi silencing than other genes. We have obtained specific and reproducible silencing for several genes including the *H. contortus* vaccine candidate *H11*. Silencing of *H11* transcript can be achieved following soaking of L3 infective stage larvae in dsRNA for 24 hours. Larvae are viable after this treatment with no detrimental effects observed in vitro. To examine any in vivo effects of *H11* silencing, sheep were infected with larvae pre-treated with dsRNA to *H11* or to a control *C. elegans* gene. At 28 days post-infection, there were significant decreases in faecal egg counts (FEC) and worm burdens in sheep infected with *H11* dsRNA treated larvae compared to the control group (at least 50% reductions). This is the first demonstration of an in vivo effect following gene silencing in an animal parasitic nematode.

S89* RNAi reveals a role for *Mi-che-3*, *Mi-osm-3* and *Mi-flp-21* in the plant host finding response of the root knot nematode *Meloidogyne incognita*

Johnathan J. Dalzell[1], Paul McVeigh[1], Rachel Kerr[1], Colin C. Fleming[2] & Aaron G. Maule[1]
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The host-finding response of plant parasitic nematodes (PPNs) centres on the identification and localisation of chemotactic factors which elicit from the host plant. This study reveals that putative *Meloidogyne incognita* orthologues of *Caenorhabditis elegans* cillial motor proteins, *Mi-che-3* and *Mi-osm-3*, perform functionally comparable roles in the amphids, the main nematode chemosensory organs. We demonstrate that transcript knockdown by RNAi can recapitulate the aberrant response to chemotactic gradients characteristic of the respective *C. elegans* mutants. Our sensory assays indicate that knockdown of either transcript using short interfering (si)RNAs, inhibits the normal attraction and repulsion responses of *M. incognita* J2s. In addition, we find that the neuropeptide encoding *Mi-flp-21*, which is involved in the social feeding phenotype of *C. elegans*, plays a role in modulating sensory perception in *M. incognita*. In *C. elegans*, *flp-1* is also known to be involved in coordinating osmotic avoidance responses; however, we find that *M. incognita Mi-flp-1* silenced worms display a reduced motility potential, which we could not easily divorce from a specific chemosensory inhibition. This work demonstrates that RNAi can recapitulate null-phenotypes of functionally conserved amphid proteins in the root knot nematode *M. incognita*, and further reveals a role for neuropeptides in coordinated sensory perception.

Session 4C - Helminth Neurobiology / RNAi (Chair: Aaron Maule)

S90 The use of siRNAs to probe cathepsin L function in *Fasciola hepatica*

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Fasciola hepatica is a trematode of both economic and medical importance. With control relying heavy on triclabendazole, resistance is well established and highlights the need for novel control options. *F. hepatica* cathepsin L (FheCL) is a key virulence protein found in all life cycle stages and plays essential roles in migration, feeding and parasite survival. Within the genus *Fasciola* there are at least eighteen sub-types of FheCL which show remarkable sequence conservation. Structural and bioinformatic studies along with the identification of enzyme specificity have allowed the division of multiple cathepsin Ls into sub-categories designated clades; these appear to have distinct functional roles. Through the exploitation of RNA interference (RNAi) in *F. hepatica* newly excysted juveniles (NEJs) and adults, FheCL transcript knockdown has been triggered by both promiscuous and clade-selective small interfering RNAs (siRNA). Promiscuous siRNAs have been designed to silence multiple clades simultaneously, whereas the selective siRNAs are clade specific. Adults have been exposed to promiscuous siRNAs and dsRNAs. Real time PCR (qPCR) has revealed variable transcript knockdown. Also, selective siRNAs for clades 3 and 4 have been tested in NEJs. In addition to qPCR, the impact of silencing on phenotype has been examined. The preliminary data highlight variation in silencing susceptibilities and efficiencies and demonstrate the need to optimize individual siRNAs prior to functional assessments.

S91* Tissue-specific sensitivity to RNA interference in the plant parasitic nematode, *Globodera pallida*

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Potato cyst nematodes, for example *Globodera pallida,* have been estimated to cause ~£300 million damage in the potato crop yield in Europe alone. Until recently, carbamate and organophosphate-based nematicides have been used to control plant parasitic nematodes, but have been withdrawn due to environmental concerns. One potential alternative method of control is based on RNA interference (RNAi), which utilises double-stranded (ds)RNA or short interfering (si)RNA to trigger the degradation of parasite mRNA transcripts during plant infection. In order to exploit this phenomenon, dsRNA/siRNAs must be expressed *in planta* so that effective silencing of specific, essential parasite genes (e.g. parasitism, motor, chemosensory genes) can occur. In an effort to investigate if the RNAi-susceptibility of potato cyst nematode gene transcripts is tissue-dependent, we set out to knockdown *G. pallida* genes with distinct tissue-specific expression patterns. Initially, genes expressed exclusively in neuronal cells of the head ganglia (*Gp-ace-2* encoding acetylcholinesterase) or in the subventral oesophageal gland cells (*Gp-cell-1* and *Gp-cm-1* encoding B-1, 4 endoglucanase [cellulase] and chorismate mutase, respectively). Here, siRNAs have been used to knockdown mRNA transcripts, measured using real-time qPCR and revealing variability in RNAi sensitivity in different tissue types. The end goal will be to breed plants expressing nematode-specific dsRNA or siRNAs *in planta* which will be resistant to parasitic nematode infestation.

S92 Using *Caenorhabditis elegans* to study drug targets from parasitic nematodes

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Glutamate-gated chloride channels (GluCls) are targets of the macrocyclic lactone anthelmintics. *C. elegans* with mutations in three of their GluCl subunit genes (*avr-14*; *avr-15*; *glc-1*) have high levels of resistance to ivermectin. Our aim is to assess whether ivermectin sensitivity in this strain can be restored by expressing GluCl subunit cDNA from *Haemonchus contortus*, a parasitic

nematode from the same clade as *C. elegans*. We have used particle bombardment to create transgenic *C. elegans* lines, and have measured the rescue using "thrashing" assays in ivermectin solution. The triple mutant containing *Hco-avr-14B* cDNA behind a *Cel-avr-14* promoter showed a 93% reduction in the thrashing rate (5.5±2.7 thrashes/min) compared to the control triple mutant strain (76.2±6.1 thrashes/min) in $1x10^{-6}$ M ivermectin. Dose response curves for ivermectin were comparable between the *Hco-avr-14B* strain and an *avr-15*; *glc-1* mutant, indicating that the *H. contortus* cDNA rescued the *avr-14* component of the drug resistance. In contrast, the triple mutant containing *Hco-avr-14A* cDNA did not show any rescue of ivermectin sensitivity (70.0±7.5 thrashes/min). Our data using *C. elegans* support previous *in vitro* experiments indicating that the *Hco-avr-14B* subunit may be important for the anthelmintic effects of ivermectin. Furthermore, we show that *C. elegans* could be an important model for studying drug resistance mechanisms in the future.

S93 A PAL for *Schistosoma mansoni* PHM

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The majority of biologically active neuropeptides display a C-terminal amide (NH_2), generated by the sequential action of two enzymes, peptidylglycine alpha-hydroxylating monooxygenase (PHM) and peptidylglycine alpha- amidating lyase (PAL). In vertebrates, PHM and PAL are expressed as separate domains of the bifunctional protein peptidylglycine alpha-amidating monooxygenase (PAM), while a number of invertebrates display various different arrangements of monofunctional enzymes. Previous work has characterised *Schistosoma mansoni* PHM, suggesting that SmPHM and SmPAL are expressed as separate monofunctional proteins. In this study biochemical methods and post-genomic tools have been used in an attempt to validate SmPAL and SmPHM candidature as drug targets. *In situ* hybridization demonstrated that in adult schistosomes, SmPAL mRNA (*Sm-pal-1*) is expressed in the central nervous system, consistent with its role in the amidation of neuropeptides in *S. mansoni*. Heterologous expression showed that SmPAL is a catalytically active, efficiently secreted amidating enzyme, with functional characteristics analogous to other eukaryotic amidating enzymes. Unfortunately, RNA interference (RNAi) of *Sm-phm-1* and *Sm-pal-1* was inconsistent and did not associate with any observable aberrant phenotype. Nevertheless, the fundamental role of SmPAL in neuropeptide maturation, and structural differences from the host enzyme, make it appealing as a drug target candidate.

S94 Neuronal RNAi in *Schistosoma mansoni*

Paul McVeigh[1], Louise Atkinson[1], Michael J. Kimber[2], Nikki J. Marks[1], Tim A. Day[2] & Aaron G. Maule[1]

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Schistosomes are amongst the few human-parasitic helminths in which RNA interference (RNAi) has shown much promise as a reverse genetics tool. Published studies in *S. mansoni* have largely used RNAi to silence constitutively-expressed genes within schistosome surface tissues - targets readily accessible to exogenously introduced double-stranded RNA (dsRNA), even in the absence of functional dsRNA amplification and spreading mechanisms (the existence of which are currently unknown). Here we aimed to investigate whether RNAi could be triggered in deeper tissues, by performing a small-scale RNAi screen of six neurone-specific gene transcripts identified from the *S. mansoni* genome (prohormone convertase PC2; choline acetyltransferase; peptidylglycine-□-hydroxylating monooxygenase, PHM; the synaptic fusion proteins synaptobrevin and synaptotagmin; and the neuropeptide-encoding gene, *Sm-npp-1*). Using electroporation-mediated delivery of 250 bp dsRNA to *in-vitro* schistosomules, and assaying subsequent transcript knockdown by semi-quantitative real-time PCR, we achieved specific transcript knockdown with all of our targets, although the degree of knockdown varied markedly between genes. Despite target transcript knockdown typically exceeding 55%, we have not yet observed aberrant phenotypes in

any of our experiments. Although these experiments are ongoing, our conclusion at this stage is that our target genes are refractory to phenotypic analysis, due either to functional redundancy, or insufficient ablation of transcript level.

S95 Voltage-operated calcium currents in *Schistosoma mansoni* are enhanced by FMRFamide-like peptides (FLPs)

Ekaterina Novozhilova[1], Michael J Kimber[1], Hai Qian[1], Alan P Robertson[1], Paul McVeigh[2], Mostafa Zamanian[1], Aaron G Maule[2] & Tim A Day[1*]
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The neuromuscular system of schistosomes is fertile ground for therapeutic intervention, yet the details of physiological events involved in neuromuscular function remain largely unknown. Short amidated neuropeptides, FMRFamide-like peptides (FLPs), are distributed abundantly throughout the nervous system of every flatworm examined and they produce potent myoexcitation. Our goal here was to determine the mechanism by which FLPs elicit contractions of schistosome muscle fibers. Contraction studies showed that the FLP YIRFamide contracts the muscle fibers through a mechanism that requires Ca^{2+} influx through sarcolemmal voltage operated Ca^{2+} channels (VOCCs), as the contractions are inhibited by classical VOCC blockers nicardipine, verapamil and methoxyverapamil. Whole-cell patch-clamp experiments revealed that inward currents through VOCCs are significantly and reversibly enhanced by the application of 1 μM YIRFamide; the sustained inward currents were increased to 190% of controls and the peak currents were increased to 180%. In order to examine the biochemical link between the FLP receptor and the VOCCs, protein kinase C inhibitors calphostin C, RO 31-8220 and chelerythrine were tested and all produced concentration dependent block of the contractions elicited by 1 μM YIRFamide. Taken together, the data show that FLPs elicit contractions by enhancing Ca^{2+} influx through VOCC currents using a PKC-dependent pathway.

WEDNESDAY 31ST MARCH

Session 5C - Schistosomes I (Chair: Joanne Webster)

S96 Invited Speaker

Contrasting reservoirs or transmission patterns for *Schistosoma japonicum* between marshland and hilly regions of China

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Schistosoma japonicum remains highly endemic in China and has recently re-emerged in previously controlled regions. To investigate the potential role for any animals other than humans and bovines in the transmission, longitudinal investigation of *S. japonicum* infection was performed across two contrasting geographical regions/settings: marshland versus hilly regions throughout 2006-2007, followed by chronobiological trials of cercarial emergence and population genetic analyses of parasites. The highest prevalence and infection intensity were observed in rodents in the hilly region and in the cattle in the marshland. A late afternoon shedding pattern was observed in the hilly and a morning-afternoon dual shedding pattern within the marshland. Characterisation of the parasite population genetic diversity also indicated cattle to be the main definitive host reservoir species in the marshland, which was further confirmed by sibling relationship analyses. In the hilly region, however, in addition to the role of rodents as the main reservoirs to maintain the disease, dogs, with their higher mobility, may also play a significant role in *S. japonicum*

transmission. The implications of these results, in terms of parasite strain sub-structuring and targeted disease control, were discussed.

S97 New insights into the epidemiology and interactions of *Schistosoma haematobium* group species in Senegal

Bonnie Webster, Tine Huyse, Katja Polman, Russell Stothard, Mohmoudane Seye, Oumar Diaw & David Rollinson

Natural History Museum, Cromwell Road, London SW7 5BD. Katholieke Universiteit Leuven, Laboratory of Aquatic Ecology, Ch. Deberiotstraat 32, B-3000 Leuven, Belgium. Institute of Tropical Medicine, Department of Parasitology, Nationalestraat 155, B-2000 Antwerpen, Belgium. Institut Sénégalais de Recherches Agricoles, Isra route des Hydrocarbures, Bel Air 3120 Dakar Sénégal.

Schistosome flukes cause significant disease in humans and ruminants in tropical and sub-tropical regions. The two host-life cycle with a sexual stage within the mammalian host facilitates inter species interactions. Hybridization between schistosome species can occur but in most cases host specificity and ecology are thought to maintain species barriers. Here we report on the emergence of new hybrid strains of schistosomes found in Senegalese children and cattle resulting from introgressive hybridization between ruminant and human parasites. This situation has arisen due to the increasing close contact of livestock and people potentially brought about by water development projects and the spread of appropriate intermediate snail hosts. Our findings have come to light due to optimized sampling and genotyping techniques of individual schistosome larval stages enabling multi loci molecular analyses of parasites directly from the field. Gene exchange following hybridization can lead to phenotypic innovations that can ultimately lead to changes in disease epidemiology. Understanding the biology and interactions of these dioecious parasites is essential for developing strategies for schistosomiasis control in West Africa.

S98* IgE and IgG4 responses in human *Schistosoma haematobium* and atopy

Nadine Rujeni[1], Norman Nausch[1], Claire Bourke[1], Nicholas Midzi[2], David Taylor[3], Takafira Mduluza[4] & Francisca Mutapi[1]

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Emphasis has been given to the existence of a strong regulatory network in response to high and prolonged exposure to helminth parasites. We investigated the influence of*. S. haematobium infection* on atopy, aiming to understand the relation at an immunological level in Zimbabweans (0.5-80 years old) living in a *S. haematobium* endemic area. Total IgE, specific IgE and IgG4 to *S. haematobium* and house-dust mite were measured by ELISA and atopic reactivity to common allergens assessed by skin prick test. The prevalence of atopic reactions was 18.1% and was significantly lower in *S. haematobium*-infected people compared to uninfected participants. Furthermore, there was a significant negative correlation between infection intensity and the allergen specific IgE. These results indicate that skin sensitivity to common aeroallergens may be suppressed in *S. haematobium* infected people. Interestingly, no correlation was observed between the skin prick results and the allergen-specific IgE suggesting that regulation might occur at the effector interface of immune responses.

S99* The *Schistosoma mansoni* cercarial elastase: investigating its potential as a vaccine for schistosomiasis

Marwa El Faham, Richard S. McIntosh, Jianguo Shi, Mike J. Doenhoff, Jon Sayers[#] & Richard J. Pleass
Institute of Genetics and School of Biology, University of Nottingham, UK. [#]School of Medicine & Biomedical Sciences, University of Sheffield.

A vaccine against schistosomiasis remains an unfulfilled goal. A concept we are investigating is to target immune responses at poorly immunogenic molecules during normal infection. Such antigens might be the "Achilles' heel" of the organism and the *Schistosoma mansoni* cercarial elastase (Sm-CE) is one such molecule. Antibodies which neutralize this enzyme might abort the infection at an early stage and our hypothesis is that such antibodies may be induced if Sm-CE is in an inactivated, but properly-folded form. To test this concept, we have generated an inactive His-tagged recombinant Sm-CE (rSmCE-His). Immunization of Balb/C mice with rSm-CE-His on aluminum hydroxide induced high levels of specific anti-Sm-CE IgG, IgG1, IgG2a and IgG2b within two weeks of the first immunization. We are currently trying to express the His-tagged recombinant protein in a correct (native) secondary structure.We have produced a rSm-CE protein fused to the Fc region of the mouse IgG2a, a form which shows proteolytic activity. Attempts will be made to inactivate the enzymatic activity of the rSm-CE-Fc by site-directed mutagenesis of the active site serine (S218). Experiments are underway to test whether either of the recombinant products, rSmCE-His and rSm-CE-Fc, induce protective immunity against schistosome challenge in mice.

S100 Development and validation of a quantitative, high-throughput, fluorescent-based bioassay to detect *Schistosoma* viability

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Human schistosomiasis is currently reliant on the use of one drug (Praziquantal) for its global control. Resistance to this drug has the potential to remove our ability to treat this neglected tropical disease and so lends urgency to the search for novel drugs and drug targets.
Current methods for detecting schistosome viability rely on qualitative microscopic criteria, which require an understanding of parasite morphology, and most importantly, can be subjectively interpreted. These methods are unsuitable for high-throughput functional genomics- or drug based- screens currently required by the schistosome community to accelerate discovery of novel chemotherapeutics. Here we present a quantitative microtiter-based method for objectively detecting schistosomula viability that takes advantage of the differential uptake of fluorophores by living organisms. We have validated this high-throughput system using a known inhibitor of thioredoxin glutathione reductase (TGR) and a range of small compounds with previously-described or suggested anti-schistosomal activities.
The described method is sensitive (200 parasites/well can be screened), does not require extensive *a priori* knowledge of schistosome biology, is relevant to both industrial (384-well capacity) and academic (96-well capacity) settings and can be adapted to meet a variety of end user needs. Further refinement of the assay to increase its speed and applicability across other parasitic metazoans will be discussed.

Session 6C - Schistosomes II (Chair: Mike Doenhoff)

S101* *Schistosoma mansoni* Annexin B2: characterization, immunolocalization and function studies

Cibele A. Tararam[1], Leonardo P. Farias[1], Bogar Omar Araújo-Montoya[1], Henrique K. Rofatto[1], Alan Wilson[2], Patrick Skelly[3] & Luciana C.C. Leite[1]
[1]Biotechnology Center, Instituto Butantan, Brazil; [2]Department of Biology, University of York, UK; [3]Department of Biomedical Sciences, TUFTS University, USA.

Schistosoma mansoni is the predominant parasite responsible for schistosomiasis, which affects more than 200 million people worldwide. We here describe the *S. mansoni* Annexin B2, previously identified in the tegument by proteomic studies, and as an up-regulated gene in the schistosomulum stage by microarray data. Annexins are a large family of Ca^{+2}-dependent phospholipid-binding proteins implicated in important biological processes. *In silico* analysis predicts a conserved core containing four repeat domains of ANX and a variable N-terminal region. Real-time RT-PCR and Western blot analysis determined that Annexin B2 is significantly up-regulated in the transition from free-living cercaria to schistosomulum and adult worm parasitic stages. Immunolocalization experiments identified the Annexin B2 in the tegument of schistosomula and adult worms. When fractions of the tegument of parasites were incubated in the presence and absence of calcium, Annexin B2 is found to be able to bind to the membranes in a calcium-dependent manner. Finally, treatment of schistosomula with siRNAs targeting the Annexin B2 gene resulted in potent suppression (> 80%), analyzed by real time RT-PCR. However, no difference in the size and *in vitro* viability of the treated parasites was observed. Annexin B2 continues to be investigated as a potential target for immune intervention.

S102 Investigation of Venom Allergen Like Proteins (VALs) from *Schistosoma mansoni* as vaccine candidates

Leonardo P. Farias, Dunia Rodrigues, Vinicus Cunna, Cibele A. Tararam, Patrícia A. Miyasato, Toshie Kawano & Luciana C. C. Leite
Centro de Biotecnologia, Instituto Butantan, 05503-900 São Paulo, SP, Brazil.

Mining the database generated from *S. mansoni* transcriptome using Gene Ontology categorization, potentially surface-exposed or exported proteins with similarity to venom allergen proteins (SmVALs) were identified. The expression profile of this class of molecules was recently well characterized by RT-PCR by the group of Karl Hoffmann. Based on the transcriptome, microarrys, real time and proteomics data available for these molecules, we selected four members (SmVALs 4, 5, 7 and 26) to be investigated as potential vaccine candidates. The protein expression profile across the life cycle was determined by Western blot and strongly correlated with the mRNA levels. Additionally, we demonstrated that the native proteins, SmVAL4 and 5, are N-glycosylated. Preliminary immunization and challenge assays revealed that SmVAL5 induced a 40% worm burden reduction. To gain insight into the allergenic potential of these proteins, we explore the murine model for asthma, and found that SmVAL-4 caused eosinophil (45.9%) and macrophage (37.5%) recruitment into the lungs of BALB/c mice after sensitization and challenge. Ours results suggest the potential of this class of molecules as vaccine candidates, but also highlights that the allergenic effects of some of these molecules should be considered in the design of a schistosomiasis vaccine. Financial support: FAPESP, CNPq, Fundação Butantan.

S103* Whole Blood Cytokine Responses in Urinary Schistosomiasis: from pathway immunology to human disease

Claire Bourke & Francisca Mutapi
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BACKGROUND: Urinary schistosomiasis is caused by chronic *Schistosoma haematobium* infection. Murine models have identified cytokine secretion patterns associated with resistance to helminthiases, but these paradigms are yet to be tested in human *S.haematobium* infection. Here

we present a comprehensive profile of the natural human whole blood cytokine response to *S.haematobium*.

MATERIALS & METHODS: Whole blood was collected from 255 Zimbabweans (aged 0.5–84 years) inhabiting an *S.haematobium*-endemic region who had never been treated with anti-helminthics. Blood was cultured with crude (cercariae, egg and adult worm homogenates) and purified (GST and Sh13) parasite antigen preparations for 48 hours. Culture supernatants were harvested and IFNγ, TNFα, IL-2, IL-4, IL-5, IL-6, IL-8, IL-9, IL-10, IL-12, IL-13, IL-17A, IL-21, IL-23 and IL-33 were measured via ELISA. *S.haematobium* infection was quantified by urine filtration. *Schistosoma mansoni*, soil-transmitted helminth and malaria positive cases were excluded. Multivariate data was analysed by ANOVA and uncorrelated variables were grouped using principal components analysis (PCA).

RESULTS/CONCLUSIONS: *S.haematobium* prevalence was 51.44% and mean infection intensity was 28.5 eggs/10ml urine and both peaked at age 11-14 years. Cytokines showed distinct patterns according to antigen stimulation and host age. Cytokine profiles by age also differed according to schistosome infection status. PCA showed that 15 cytokines directed against 5 parasite antigens clustered into groups corresponding to CD4+ T cell phenotypes characterised in murine pathway immunology.

S104* The Effects of *Schistosoma mansoni* Haemozoin on Macrophage Activation in a Changing Cytokine Milieu

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Adult schistosomes detoxify free heme, liberated during haemoglobin digestion, via its crystallization into haemozoin, a mechanism shared with *Plasmodium* parasites. *Schistosoma* haemozoin, regurgitated into the host circulation, accumulates in the liver and is phagocytosed by macrophages in the egg-induced granuloma. As a body of evidence from work with *Plasmodium* haemozoin suggests that it has immunomodulatory capacities, the effects of *S.mansoni* haemozoin on macrophage responses to immune stimuli were investigated.

Haemozoin, purified from adult worms, was introduced into RAW264.7 macrophage cultures, alongside classically or alternatively activating stimuli. Macrophages were also 'switched' from classical to alternative stimulation, mimicking the Th1 to Th2 shift in immune response observed in schistosome infections concomitant with the appearance of eggs and haemozoin in the liver. Measures of arginine metabolism and real-time PCR gene expression profiling were used to assess macrophage activation status.

Cells 'switched' in the presence of haemozoin fail to develop a normal alternatively activated phenotype. Instead, they display a 'mixed' phenotype in which both Arginase-1 activity and Nitric Oxide production occur. Expression of characteristic alternatively activated transcripts is also perturbed; *Arg1* and *Ym1* are expressed normally but *Fizz1* expression is significantly suppressed, explaining previous observations that macrophages are not a major source of *Fizz1* in the granuloma, and suggesting that *S.mansoni* haemozoin subtly manipulates the immune responsiveness and functional capabilities of macrophages.

S105 Observed Reductions in *Schistosoma mansoni* Transmission from Large-scale Administration of Praziquantel in Uganda: a Mathematical Modelling Study

French,M.D.[1,2], Churcher,T.S.[2] , Gambhir,M.[2], Fenwick,A.[1], Webster,J.P. [1,2] Basáñez,M.-G.[2]
1. Schistosomiasis Control Initiative, Imperial College London.
2. Department of Infectious Disease Epidemiology, Imperial College London.

Background: To date schistosomiasis control programmes based on chemotherapy have been aimed at controlling morbidity in treated individuals rather than at suppressing transmission. Here, a mathematical modelling approach estimated reductions in the rate of *Schistosoma mansoni* reinfection following annual mass drug administration (MDA) with praziquantel in Uganda over four years (2003-2006), with the aim of quantifying the benefits of MDA in reducing community transmission.

Methods: Age-structured models were fitted to a longitudinal cohort followed up across four successive rounds of annual treatment (n= 1,764). Infection and immunity processes were combined in order to estimate a composite force of infection (*FOI*).
Results: MDA achieved significant and substantial reductions in *FOI* following one round of treatment in areas of low baseline infection intensity, and following two rounds in areas with high and medium intensities. In all areas, *FOI* remained suppressed following a third round of treatment.
Conclusions: This is one of the first attempts to monitor a large-scale MDA schistosomiasis control programme for reductions in *FOI*. The results show that the morbidity control programme is having a significant ancillary impact on reducing transmission, with health benefits to those who are untreated. The results have implications for evaluating the cost-effectiveness of control programmes and monitoring and evaluation approaches in general.

S106 Systemic antibody and cytokine levels differ with infection status and age in people exposed to *S. haematobium*
Thomas Milner[1], Liam Reilly[1], Norman Nausch[1], Nicholas Midzi[2], Takafira Mduluza[3,], Rick Maizels[1] & Francisca Mutapi [1*]
[1]Institute for Immunology & Infection Research, University of Edinburgh, Ashworth Laboratories, King's Buildings, West Mains Rd, Edinburgh, EH9 3JT, UK. [2]National Institutes for Health Research, Box CY 570, Causeway, Harare, Zimbabwe. [3]Department of Biochemistry, University of Zimbabwe, P.O. Box 167, Mount Pleasant, Harare, Zimbabwe

Experimental schistosome infections induce strong parasite-specificTh2 responses. This study determined if human schistosome infections were associated with Th2 systemic responses. Levels of anti- *Schistosoma haematobium* antibodies and plasma cytokines were measured in 227 Zimbabweans (6-60 years old) resident in a schistosome-endemic area were related to schistosome infection status. IL-4, IL-13 and IL-21 were detected with high frequency in all participants. Schistosome egg-positive people had a mixed systemic environment with more people having detectable IL-2 and IL-23 than egg-negative people. Furthermore, they had significantly higher levels of parasite-specific antibodies, IFN-γ and IL-2 than egg-negative people who had significantly higher levels of IL-10 and some parasite-specific antibodies. The systemic environment in the egg-negative people varied with schistosome exposure history. Older, life-long residents had significantly higher amounts of most parasite-specific antibodies, IL-4 and IL-5, than younger egg-negative people. A mixed systemic environment occurs in people carrying a patent schistosome infection while a modified Th2-like systemic environment occurs in putatively resistant individuals with a longer history of exposure to schistosome infection.

THURSDAY 1ST APRIL

Session 7C - Veterinary Parasitology (**Chair: Oliver Sparagano)**

S107 Invited Speaker
An evolutionary perspective on gastrointestinal nematodes of sheep
Michael Stear, Louise Matthews & Darran Singleton
Faculty of Veterinary Medicine, University of Glasgow, G61 1QH, UK

The pattern of faecal egg counts was observed in monthly samples from cohorts of 200 lambs on a single farm over the course of 5 years. Patterns varied among seasons but the pooled data rose to a peak in July then fell with a small rise in October. Mathematical modelling of the immune responses controlling fecundity and establishment provided a close fit to the data and suggested that the host parasite interaction was remarkably robust to perturbations. One plausible explanation for this robustness is that nematodes and their ancestors have been infecting sheep and their ancestors for tens of millions of years. Both host and parasite appear co-adapted. Features such as the periparturient rise in nematode egg counts are obviously beneficial to the parasite but may also benefit the host by providing a low level of controlled infection during a period when lambs are largely suckling. There is little evidence for the widespread belief that

coevolution is driven by the parasite. Indeed, molecular analyses of protein sequences indicate that some parasite antigens are structurally similar across diverse nematode parasites and suggest relatively slow parasite evolution. The immune response was very variable among hosts and under strong genetic control suggesting that improved immune responses may not be under strong directional selection. Enhanced immunity could exacerbate the pathology; alternatively, the model suggests a feedback loop where enhanced immunity diminishes parasite egg production which leads to decreased infection and decreased immunity. Enhanced immunity early in the season could increase infection later on. Finally, the late season rise appears to be due to intestinal species which have adapted to host immunity by ensuring that their eggs are produced late in the season and have an increased chance of surviving to infect the next generation of lambs.

S108 Reduced efficacy of ivermectin-pour-on on English cattle farms

Stafford, K.A., Morgan, E.R. & Coles, G.C.
Department of Clinical Veterinary Science, University of Bristol, Langford House, Bristol BS40 5DU, UK.

The first report of ivermectin resistance in cattle in the northern hemisphere was with *Cooperia* on a Somerset farm 11 years ago. In 2008 we tested 16 farms at the time of treatment and 14 days later using samples submitted by farmers. Initial counts were performed using the McMaster technique and second counts with FLOTAC accurate to 1 epg. Ivermectin failed to fully control infections on 10/12 of these farms and moxidectin on 2/3 farms. On 4 farms egg counts rose after treatment and *Ostertagia* was found on 3 farms following treatment. In 2009 30 farms were tested by FLOTAC. 12 farms delayed treatment as their egg counts were very low thereby avoiding treatment throughout the season. Ivermectin was fully effective on 2/11 farms whilst moxidectin failed on the two farms where it was used. On one farm moxidectin and levamisole injection were not fully effective, the first report of dual resistance on a UK cattle farm. Before macrocyclic lactones are used for treatment of nematodes in cattle sensitivity tests are required and the activity of alternative anthelmintics should be established. Strategies to slow the development and spread of anthelmintic resistant nematodes in cattle need to be introduced. Work funded by EBLEX (2008) and Defra (2009).

S109* Effects of mixed grazing on nutrition and growth of grazing goats infected by gastrointestinal nematodes

S. d'Alexis[1], M. Mahieu[1], M. Boval[1] & F. Jackson[2]
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[2]Moredun Research Institute, Pentlands Science Park, Bush Loan, Penicuik, Midlothian, EH26 0PZ, Scotland.

Infection by gastrointestinal nematodes (GIN) is a major pathology in small ruminants at pasture in the tropics, which is the most common farming system there. The frequent use of anthelmintics has led to the emergence of multiple resistances to these drugs. Alternative approaches should be used to reduce the development of resistance, such as the use of grazing systems mixing small ruminants and cattle, which is known to reduce the GIN infection rate and increase meat production.
An experimental design was carried out with 6 goats per treatment (repeated 5 times on 2 paddocks) associated or not with cattle and experimentally infected with 6000 larvae of *Haemonchus contortus.* Goats infected and mixed with cattle (n=2) had higher Organic Matter Intake, digestible Organic Matter intake and average daily gain (61.7 gOMI/kgP $^{0.75}$, 55.0 gdOMI/kgP $^{0.75}$, and 35 g/d) than goats infected but reared alone (43.5 gOMI/kgP $^{0.75}$, 38.8 gdOMI/kgP $^{0.75}$ and 24 g/d, $P<0.0001$).
The association of goats and cattle improves the nutrition of goats and consequently increases their bodyweight and helps them to be more resilient to GIN. Mixed grazing methods are of major interest given that the GIN infection risk will probably be amplified with global warming.

S110 Using lectins to identify "hidden antigens" in the liver fluke, *Fasciola hepatica*

Heather McAllister, Alasdair Nisbet, Dave Knox, Susan Ekoja & Philip Skuce
Moredun Research Institute, Pentlands Science Park, Bush Loan, Edinburgh, EH26 0PZ, UK

Fasciola hepatica is responsible for substantial economic losses within the agricultural sector and is of major animal welfare concern. With the emergence of flukicide resistance and increasing levels of incidence, vaccination would be an attractive alternative control strategy. Hidden antigens have proved successful in providing protection against infection with certain blood feeding parasite species, most notably the gastrointestinal nematode, *Haemonchus contortus* and the cattle tick *Rhipicephalus (Boophilus) microplus*. This approach has not been evaluated to date in *F.hepatica*, which is also known to ingest blood. As a preliminary step, lectins have been valuable tools for identifying and separating such antigens from protein-rich parasite extracts. Lectins are carbohydrate-binding proteins of non-immune origin and have specificity for terminal or sub-terminal carbohydrate residues. In this study, sections of adult fluke have initially been screened with a panel of fluoresceinated lectins to identify those that specifically bind to the gastrodermis. A select number were then used to enrich putative gut-specific proteins, which were then characterised using a number of enzymatic assays. Preliminary work identified lectins which could potentially be used to extract gut-specific proteins. Further, these proteins exhibited intense enzymatic activity against a number of different substrates.

S111 Molecular detection of haemopathogen infections of dogs, cats and horses using the reverse line blot hybridization assay

Karla Georges[1], Chuckwudozie Ezeokoli[1], & Olivier Sparagano[2]
[1] School of Veterinary Medicine, University of the West Indies, Trinidad. [2] School of Agriculture, Food & Rural Development, Newcastle University, NE1 7RU, UK

This study describes the simultaneous molecular detection of arthropod transmitted haemopathogens of dogs, cats and horses a reverse line blot hybridization assay (RLB). A combination of previously published oligonucleotide probes and new probes designed in this study were used for detecting haemopathogens. The results of RLB were compared to a quantitative real-time PCR (qPCR) for the feline haemoplasmas
Members of the *Anaplasma/Ehrlichia* genera (*Anaplasma platys*, *Anaplasma phagocytophilum* and *Ehrlichia canis*), *Babesia/Theileria* genera (*Babesia canis vogeli*, *Babesia caballi, Theileria equi*) and the feline haemoplasmas (C*andidatus* Mycoplasma haemominutum (CMhm) and *Mycoplasma haemofelis* (Mhf)) were simultaneously detected using the RLB. *E. canis* (49/348, 14.1%) and *B. caballi* (8/94, 8.3%) were most frequently detected in dogs and horses respectively and CMhm most frequently detected in cats (26.3 % by 48/152 qPCR). Mixed infections of *Anaplasma/Ehrlichia* and *Babesia/Theileria* DNA were observed in only 5/ 348 dogs (1.4%). The agreement between the qPCR and the RLB assays for an overall positive feline haemoplasma result and a positive result for CMhm was strong: kappa = 0.662, and 0.872 respectively with specificity and positive predictive values of 100%.
The RLB assay is a less expensive option for improving the diagnostic capacity for veterinary laboratories in developing nations by providing molecular diagnostic technology for detecting commonly occurring arthropod- borne haemopathogens and can be developed further and applied to detect agents of economic and public health importance.

Session 8C - Aquatic Parasitology (Chair: Iain Barber)
Sponsored by the Environment Agency.

S112 Invited Speaker
Host-parasite interactions in variable environments
Otto Seppälä
EAWAG, Department of Aquatic Ecology (ECO), and ETH-Zürich, Institute of Integrative Biology (IBZ), Überlandstrasse 133, PO Box 611, 8600, Dübendorf, Switzerland

Environmental variation has been suggested to play a major role in ecology and evolution of host-parasite interactions. This is because environmental conditions often modify host immune function and parasite infectivity. For example, unfavourable environmental conditions may increase the susceptibility of hosts to infections and predispose natural populations to disease outbreaks. Furthermore, if these effects interact with host genetics, environmental heterogeneity may maintain genetic variation in pathogen resistance by favouring alternative host genotypes over time and/or space. Here I present how the availability of resources (food) for a freshwater snail *Lymnaea stagnalis* affects host immune function and susceptibility to infection by a trematode parasite *Echinoparyphium aconiatum*. Food limitation reduces snail immune defence, but it does not predispose snails to infection in this system. Instead, food limited snails are the least susceptible to the infection. These findings suggest that environmental stress for hosts can reduce parasite transmission success, although it reduces host immune defence in general. Furthermore, both snail immune function and susceptibility to infection vary among snail families indicating genetic variation for parasite resistance. This variation, however, is modified by food availability (G × E interaction) suggesting that environmental variation may maintain genetic variation in resistance in this system.

S113 Parasites as agents of selection in perch (*Perca fluviatilits*)? MHC variability and parasite host dynamics
Jasminca Behrmann-Godel, Daniela Harrer & Claus Oppelt
Limnological Institute, University of Konstanz, Germany

Parasites influence the fitness of individuals and thus can be seen as major agents for the evolution of defence mechanisms. Local differences in the parasite community may lead to a geographic mosaic of adaptations in the host population. Molecular genetic analysis revealed that even in small lakes perch populations can be subdivided by so called “cryptic barriers to gene flow”. We analysed the local parasite community of perch at 10 different sampling locations within one large prealpine lake. 19 metazoan parasite species were found and the parasite community differed significantly between several sampling locations. In addition we investigated MHC genes of perch. MHC class II genes present parasite-derived antigens to immune cells and induce the adaptive immune response. As such they are perfect candidates for adaptive traits in parasite host coevolution. High allele variability was found for perch with up to 8 MHC class II loci and 41 alleles. Similar to the parasite community analysis, significant differences in the MHC allele patterns were found between many of the sampling locations. Our investigations can be seen as a first indication for an ongoing local coevolution between perch and their parasites and future work will show if this may be a selective mechanism that causes barriers to gene flow in this species.

S114* Growth, metabolism and ultrastructure of *Spironucleus vortens*
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Spironucleus species are anaerobic, flagellated protozoa, which can be either parasitic or commensal and represent a significant problem in aquaculture. Despite their importance, they have been little investigated, and a better understanding of the biology of these parasites is essential for effective disease management. Using *Spironucleus vortens*, a parasite of cichlids, we investigated the growth, metabolism, ultrastructure and control of this model organism. *S. vortens* proved to be a non-fastidious organism, and grew to high densities (2.6×10^6 cells. ml^{-1}) with a very short doubling time (1.8 h), but demonstrated an unusual biphasic pattern of growth. Despite

being categorized as anaerobic, the organism exhibited both an unusually high tolerance and affinity for O_2. Metabolic investigations demonstrated that the organism could use glucose, but that the compound was not its preferred substrate. The organism was also found to contain large pools of endogenous substrates, exhibited high proteolytic activity and was capable of rapid phagocytosis of non-soluble particles. Although *Spironucleus* species were described as lacking hydrogenosomes, *S. vortens* produced H_2 at a very high rate, and ultrastructural and enzymatic studies revealed that despite previous reports, the organism possessed hydrogenosome-like, redox-active organelles. Besides hydrogenosomes, mitosome-like organelles were also detected; such a combination is currently unique in the eukaryotic kingdom.

S115* Sex and hybridization in gyrodactylid monogeneans

Bettina Schelkle[1], Patricia Faria[1], Cock van Oosterhout[2], Mireille Johnson[1] & Jo Cable[1]
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Hybridization between divergent populations leads to homogenization of gene pools, introgression, rapid adaptation and diversification via reinforcement. In parasites, hybridization might be a major driver of speciation, particularly in specious groups such as the gyrodactylids. These ubiquitous ectoparasites of teleost fish are thought to reproduce asexually, parthenogenetically and sexually, with host switching being a key mechanism of speciation. Here, three strains of *Gyrodactylus turnbulli* (*Gt3, Gt1, Gt8*), differing in their inbreeding status (isolated for 12, 8 & 1 year, respectively) were cross bred on guppies (*Poecilia reticulata*). Microsatellite analysis identified hybrid worms confirming the occurrence of sexual reproduction in *G. turnbulli*. At a population level, mixed strains exhibited hybrid vigour. This was reflected in a higher parasite burden over time, increased maximum parasite burden and a longer duration of infection. The results provide further insight into the evolutionary history of gyrodactylids, but also have important implications for the emergence of new diseases.

S116 *Anisakis simplex* associated with 'red vent syndrome' in wild adult Atlantic salmon *Salmo salar* in Great Britain

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Wild Atlantic salmon *Salmo salar* L. with swollen, bleeding vents have been observed returning to rivers across England, Wales and Scotland. Low numbers of salmon with this condition, now termed Red Vent Syndrome, have been recorded since 2005, but reports increased in 2006 and 2007 and continue to be recorded in returning fish. Third stage larvae of the nematode *Anisakis simplex* were observed in the tissue surrounding the vent. This location in the fish host appears to be novel for the parasite. Histological investigation of the vent tissue revealed gross lesions with an inflammatory response associated with encapsulated and non-encapsulated nematode worms in the connective tissue and skeletal muscle.
Prevalence of red vent syndrome and intensity of *A. simplex* infection in rivers throughout Britain are presented, as well as the distribution of nematodes within red vent affected and unaffected fish. Preliminary results of molecular analysis suggest that nematodes located in the vent and body cavity are *A. simplex* sensu stricto. The implications of this condition in wild salmon populations are discussed along with possible causes. It is hypothesised that fluctuations in the marine environment may be an important influence on these infections.

TUESDAY 30th MARCH

TRYPANOSOMIASIS/LEISHMANIASIS

Session 1D - Trypanosomatid Systems Biology (Chair: Mike Barratt)

T/L117 Invited Speaker
Trypanosome Systems Biology
Rainer Breitling
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Trypanosomes have recently emerged as promising model systems for Systems Biology. Knowledge of the complete genome sequence, the availability of a large diversity of molecular and genetic tools, and the large amount of enzyme kinetic information make trypanosomes particularly attractive for comprehensive quantitative modeling. This talk describes the current state of the field and focuses on remaining bottlenecks and the way forward towards establishing a "Silicon Trypanosome".

T/L118 Integrated spectroscopic time-trajectory assessment of *Trypanosoma brucei brucei* in the mouse
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Management of Human African Trypanosomiasis is still far away from optimal and needs improvement on all three fronts, disease prevention, diagnostic and treatment. A previously conducted ^{1}H nuclear magnetic resonance (NMR) spectroscopy-based metabolic profiling approach in the *Trypanosoma brucei brucei*-mouse model has shown potential use in identifying early candidate biomarkers, whereas time trajectory analysis built on principal component analysis delivered additional insight in the temporal progress of disease. Here we extended the range of metabolic profiling tools and assessed a subset of the urine samples by UPLC-MS, in order to maximise and co-analyse the metabolic information on the disease progress and to evaluate a strategy for assessing the quality of a MS-run. The MS-derived time trajectory analysis showed, in contrast to the temporal changes in the ^{1}H NMR data set, a statistically significant separation of day 28 from all other late-infection time-points, including d14, 21 and 33, which might be due to the higher sensitivity and larger range of metabolites which MS can recover, compared to NMR and hence represent a highly complementary tool to the NMR approach, which can efficiently pre-screen large data sets.

T/L119 Relevance of 2,4-dienoyl-coa reductase of *Leishmania* for virulence
Daniel Paape,*[1,2,] Christoph Lippuner[2], Toni Aebischer[1,2,3]
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A novel method was developed which combined classical purification with fluorescent activated cell sorting of transgenic *Leishmania* parasites for the enrichment of intracellular *Leishmania* spp. This approach allowed for the first time the purification of amastigotes from their intracellular habitat for direct proteome analysis. The proteomic dataset contained many to date tested vaccine candidates, hence it is a useful resource for selecting new candidates. Further the dataset revealed that amastigotes express more proteins predicted to be involved in the catabolism of fatty

acids. This confirmed earlier findings by others and led us to hypothesise that proteins of this subset are virulence factors. A candidate 2,4-dienoyl-coa reductase (DECR) was chosen for further investigation. For the analysis of its contribution to virulence and pathogenicity, *decr* deficient parasites were generated. Infection of BALB/c mice revealed that compared to wild type parasites, those devoid of *decr* were significantly less virulent. In summary, a novel virulence factor in *Leishmania* was identified, which could be an appropriate target for the development of novel anti-parasitic drugs.

T/L120 * The *Trypanosoma brucei* RNA-binding protein RBP10 is required for maintenance of glycolytic energy metabolism in the bloodstream form

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The energy metabolism of *Trypanosoma brucei* shows strong developmental regulation. In the bloodsteam form (BS), ATP is generated by glycolysis. In the procyclic form (PC), additional pathways are present. *T. brucei* RBP10 is a cytoplasmic RNA-binding protein containing one RNA recognition motif. RBP10 is found only in BS trypanosomes. RNAi targeting RBP10 was lethal in BS, but had no effect in PC. In BS *RBP10* RNAi, 100 mRNAs were significantly decreased; strikingly, the most strongly affected transcripts included many encoding glycolytic enzymes such as PGKC and proteins involved in glucose metabolism, e.g. the hexose transporter THT1. VSG mRNA was decreased, while EP procyclin mRNA increased. It is unclear which of these effects are a direct result of RBP10 depletion and which are secondary due to metabolic disturbance. Many developmentally-regulated mRNAs were not affected and the results were not just due to growth arrest.
Overexpression of myc-RBP10 in PC caused a growth defect and up-regulation of endogenous *RBP10* mRNA and RBP10 protein. *PGKC* and *THT1* mRNAs increased, while PC-specific *THT2* and *PGKB* mRNA decreased. So far, the results suggest that RBP10 may be a key regulator for expression of proteins required for bloodstream-form energy metabolism. Currently I am investigating whether RBP10 is able to bind to its potential targets directly by purifying bound mRNA and subsequent RT-PCR.

Session 2D - Cell Biology I (Chair: Tansy Hammarton)

T/L121 Invited Speaker

A novel phosphatase cascade regulates differentiation in trypanosomes via a glycosomal signaling pathway

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In the mammalian bloodstream, trypanosomes are held poised for transmission by the activity of a tyrosine phosphatase, *Tb*PTP1 (Szoor et al., J Cell Biol. 2006). This prevents differentiation of the transmissible 'stumpy-forms' until entry into the tsetse fly, whereupon *Tb*PTP1 is inactivated and major changes in parasite physiology are initiated to allow colonization of the arthropod vector. Using a substrate-trapping approach we have identified the downstream step in this developmental signaling pathway as a DxDxT phosphatase, *Tb*PIP39, which is activated upon tyrosine-phosphorylation and hence negatively regulated by *Tb*PTP1. Interestingly, *Tb*PIP39 promotes the activity of *Tb*PTP1 in vitro, thereby reinforcing its own repression, this being alleviated by the trypanosome differentiation triggers citrate and cis-aconitate, generating a potentially bistable regulatory switch. Supporting a role in signal transduction, *Tb*PIP39 becomes rapidly tyrosine phosphorylated during differentiation and RNAi-mediated transcript ablation in stumpy-forms inhibits parasite development. During differentiation *Tb*PIP39 localises in glycosomes, peroxisome-like organelles that compartmentalize the trypanosome glycolytic reactions among other enzymatic activities. Our results invoke a phosphatase-signaling cascade in which the developmental signal is trafficked to a unique metabolic organelle in the parasite,

glycosomes. This is the first characterized environmental signaling pathway directly targeted to a peroxisome-like organelle in any eukaryotic cell.

T/L122 The RNA helicase DHH1 is central to the correct expression of many developmentally regulated mRNAs in trypanosomes

Susanne Kramer[1], Rafael Queiroz[2,3], Louise Ellis[1], Jörg D. Hoheisel[3], Christine Clayton[2] and Mark Carrington[1*]
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In trypanosomes, the predominant mechanisms of regulation of gene expression are post-transcriptional. The DEAD-box RNA helicase DHH1 was identified in a screen for genes necessary for the instability of the *GPI-PLC* mRNA in insect-stage trypanosomes. Expression of an ATPase deficient dhh1 mutant caused a rapid growth arrest associated with a decrease in polysomes, an increase in P-bodies and a slight decrease in average mRNA levels. However, the effect of dhh1 mutant expression on both turnover and translational repression of mRNAs was selective. While there was little effect on the stability of constitutive mRNAs, the control of a large cohort of developmentally regulated mRNAs was reversed; many mRNAs normally down-regulated in insect-stage trypanosomes were stabilized and many mRNAs normally up-regulated decreased in level. One stabilised mRNA, *ISG75*, was characterised further. Despite the overall decrease in polysomes, the proportion of the *ISG75* mRNA in polysomes was unchanged and the result was ISG75 protein accumulation. Our data show that specific mRNAs can escape DHH1-mediated translational repression. In trypanosomes, DHH1 has a selective role in determining the levels of developmentally regulated mRNAs.

T/L123 Transcripts and Cofactors of a Post-Transcriptional Regulator Complex

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The genome of *Trypanosoma brucei* is regulated almost entirely at the post-transcriptional level. The *Tb*ZFP3 mRNA–protein complex (*Tb*ZFP3mRNP) is a long-sought trans-regulator of differential Procyclin surface protein expression in trypanosomes. To investigate other targets of the *Tb*ZFP3mRNP, specific RNA-precipitation coupled with Solexa analysis of selected transcripts has been used, this revealing significant enrichment of several transcript classes. Interestingly, *in silico* analysis of the 3'UTRs of these transcripts demonstrates the presence of significantly enriched motifs that are shared with selected *procyclin* isoforms. Combined with an ongoing analysis of protein components of the *Tb*ZFP3mRNP, our data are dissecting the pathway of gene expression control mediated by a novel group of RNA binding proteins, for the first time linking specific mRNA targets to the gene expression machinery in trypanosomes. We demonstrate (i) that ectopic overexpression of *Tb*ZFP3 but not the cofactor *Tb*ZFP2 regulates Procyclin in an isoform-specific manner, as evidenced by mass spectrometric analysis of the Procyclin expression signature in the transgenic cell lines, (ii) that both *Tb*ZFP2 and *Tb*ZFP3 colocalize with P-bodies in response to serum starvation, (iii) that potential transcript targets specifically associate with TbZFP3 and (iv) that additional isolated cofactors demonstrate functional relevance in *procyclin* transcript stabilization.

T/L124 TAO is imported into mitochondria via unique pathway in the bloodstream form of *Trypanosoma brucei*

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Trypanosome alternative oxidase (TAO), an essential respiratory protein in the bloodstream form (BF) of *T. brucei*, needs to be imported into mitochondria for its function. TAO possesses a predicted N-terminal mitochondrial targeting sequences (MTS) consisting of multiple positively charged residues. In the procyclic form (PF) TAO import is membrane potential (ψ)-dependent and

the preprotein is processed after import as expected. In contrast to the PF, TAO import is ψ-independent and processing occurs at different position in the BF. Deletion of the first ten amino acids did not affect the import of TAO in mitochondria from either form, whereas deletion of the first twenty amino acids inhibited import in mitochondria of the PF only. Unlike the full-length protein, the first ten amino acid deletion mutant of TAO depends on ψ for import in mitochondria of the BF, suggesting that length of and basic residues within the MTS of TAO play important roles for ψ requirement. Import of TAO is specifically inhibited in mitochondria depleted of TbTob55, an outer membrane β-barrel protein. In addition, depletion of TbTim17 differentially affects the import of TAO into mitochondria from PF and BF. Together, it indicates that TAO is imported via different mechanisms in two developmental forms of *T. brucei*.
This project is supported by NIH Grants 1SC1GM081146 and 1F31AI083011

T/L125 The assembly of iron-sulfur clusters in *Trypanosoma brucei*
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Iron-sulfur (Fe-S) clusters are ancient and ubiquitous cofactors of proteins that are involved in a variety of biological functions. In the procyclic and bloodstream stage of *T. brucei*, we have down-regulated via RNAi several evolutionary conserved components of the Fe-S cluster assembly pathway, such as cysteine desulfurase IscS, metallochaperones IscU, Nfu1, Nfu2 and Nfu3 , frataxin, ferredoxin, Isa1 and Isd11. As expected, all of them are essential for the survival of procyclics, but surprisingly, many of these proteins are either non-essential or undetectable in the bloodstream stage. Moreover, our data indicate that while the bulk of Fe-S clusters is synthesized in the mitochondrion of procyclics (same as in all other eukaryotes), in the bloodforms the synthesis appears to be transferred to the cytosol. This and other peculiarities of the Fe-S cluster assembly in these excavates will be discussed.

Session 3D - Cell Biology II (Chair: Keith Matthews)

T/L 126* The regulation of the sub-cellular localisation of GPI-PLC
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The GPI-specific phospholipase C (GPI-PLC) catalyses the release of the VSG molecules from the plasma membrane of bloodstream form trypanosomes by hydrolysing the phosphoglycerol bond in the GPI anchor. The VSG is not shed at a significant rate so how is the activity of the GPI-PLC regulated? One favoured mechanism is isolation from the VSG substrate by regulated sub-cellular localisation. The GPI-PLC is predominantly on the flagellum membrane; a three-cysteine motif has been shown to be palmitoylated in *Xenopus* oocytes and mutation of all three residues affected the accessibility of GPI-PLC to its VSG substrate. Here, the relationship between subcellular localisation and access to the VSG substrate has been investigated through expression of GPI-PLC mutants in a null background. With all cysteines mutated to serines, GPI-PLC is cytoplasmic but retains wild type activity. The localisation of the GPI-PLC to the flagellum appears to be essential for access to the VSG on hypotonic lysis. The first cysteine in the motif was found to be sufficient but not necessary for flagella localisation. In addition when the first cysteine and either the second or third cysteine was mutated to serine the GPI-PLC was localised in the endosomal system suggesting that the correct pattern of modifications is required for successful trafficking of GPI-PLC.

T/L 127* The N-terminus of phosphodiesterase TbrPDEB1 of *T. brucei* contains the flagellar targeting information

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The phosphodiesterases (PDEs) TbrPDEB1 and TbrPDEB2 are essential enzymes of T. brucei, and their downregulation by RNAi leads to rapid cell lysis in cultute, and to elimination of the parasites in the mouse model. They are closely similar enzymes that share about 80 % sequence identity. Nevertheless, their subcellular localization is strikingly different: TbrBPDEB1 is mostly confined to the flagellum, where it is stably associated with the flagellar cytoskeleton. In contrast, TbrPDEB2 is mostly in the cell body, where it behaves as a Triton-soluble protein. However, TbrPDEB2 can form heterodimers with TbrPDEB1 and thus can be passively conducted to the flagellum and be stably integrated into its cytoskeleton. We have determined that the N-terminal 70 amino acids of TbrPDEB1, but not of TbrPDEB2, contain the information required to stably integrate the protein into the flagellar cytoskeleton. Mutagenesis experiments should further delimit the signals required for the subcellular localization of TbrPDEB1.

T/L128* AIR9 is a cytoskeleton-associated protein required for organelle positioning and cytokinesis in *Trypanosoma brucei*

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Division of Infection & Immunity and Wellcome Trust Centre for Molecular Parasitology, University of Glasgow, Glasgow, UK

Cell cycle regulation in *Trypanosoma brucei* is unusual, and differs between life cycle stages. Completion of mitosis is required for cytokinesis in bloodstream, but not procyclic parasites. Notably the flagellum plays a prominent role in cytokinesis in both life cycle stages. One protein identified in the flagellar proteome is a homologue of a plant cytokinesis regulator, AIR9, whose roles in *Arabidopsis thaliana* include positioning the cell wall. Examination of cell lines expressing GFPtyAIR9 from the endogenous locus showed this protein to be associated with the trypanosome cytoskeleton. Downregulation of AIR9 showed this protein to be essential causing a growth defect in procyclic and bloodstream parasites. Phenotypic examination revealed AIR9 depletion resulted in malpositioning of the nucleus and kinetoplast, causing the generation of abnormal daughter cells following cleavage furrow ingression. Additionally, in bloodstream parasites, defects in cytokinesis abscission were evident prior to organelle positioning defects, implying that AIR9 also directly contributes to cytokinesis in this life cycle stage.

T/L129 *Trypanosoma brucei* Rab28 mediates late endocytic processes including sensitivity to trypanosome lytic factor

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Endocytosis in African trypanosomes is a vital process for the acquisition of nutrients and also for interaction with the host acquired and innate immune systems. Specifically, immunoglobulins and other immune effectors that recognize or bind to the parasite surface are removed by an efficient endocytosis, proteolysis and recycling mechanism that maintains the variant surface glycoprotein density at the plasma membrane. However, there are additional pathways present that are also important for maintaining the trypanosome surface composition and for control of additional endocytic activities. We examined the functions of Rab28, a small GTPase that is uncharacterized in any organism. Firstly we localized Rab28 to endosomal regions of the parasite, but determined that these did not correspond to any structure previosuly defined by a trypanosome Rab; rather Rab28 colocalized with trypanosome Vps23, an ESCRT complex component. Using RNAi, we find that Rab28 has important roles in co-ordinating maintenance of the Golgi complex, which fragments in Rab28 knockdown cells, and in maintaining the expression levels of retromer and ESCRT factors. These data suggest a direct interaction between Rab28 and late endocytic events, as well as Golgi - endosome membrane trafficking pathways. Significantly, Rab28 expression is

required for full sensitivity to trypanosome lytic factor (TLF) indicating that TLF requires passage through the MVB for full activity.

T/L130 Functional characterisation and drug target validation of a mitotic Kinesin-13 in *Trypanosoma brucei*

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Kinesin-13 family proteins are microtubule-depolymerizing motorproteins that are involved in length regulation of microtubule-based cellular structures. We have characterised one of the six members of the kinesin-13 family in *T. brucei*, termed TbKif45. Biochemical analysis shows TbKif45 as an ATP-dependent, microtubule-depolymerizing kinesin. In situ, Tbkif45 colocalises with the intranuclear mitotic spindle. Protein depletion by RNAi leads to abnormally formed nuclei, extended and distorted mitotic spindles. Overexpression blocks mitotic progression due to the inability to form a functional spindle. Both protein depletion and overexpression are lethal to procyclic and bloodstream-form parasites. Morerover, RNAi-mediated protein depletion in infected mice completely protects from an infection. The availability of an enzyme assay suitable for high throughput screening, the validation in an animal model and transferable experience from the ongoing development of kinesin-directed anti-cancer drugs make TbKif45, and likely other motorproteins, potential drug targets against sleeping sickness.

T/L131 Regulation of NDR kinase activity in *Trypanosoma brucei*

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NDR (nuclear DBF2-related) kinases regulate cell cycle processes, growth and development in organisms from yeast to humans. Their activity is regulated by autophosphorylation and through phosphorylation by upstream kinases, and also for some family members, by the binding of a MOB partner protein. *Trypanosoma brucei* has two NDR kinases, PK50 and PK53, both of which we have shown are essential in the bloodstream stage of the parasite. They may function at different stages of cell division since depletion of PK50 inhibits cytokinesis initiation whereas a lack of PK53 stalls cells during furrow ingression. We have purified recombinant PK50 and PK53 proteins, which unusually, are able to phosphorylate generic kinase substrates *in vitro* in the absence of upstream kinases and MOB proteins. Multiple (auto)phosphorylation sites have been identified on these recombinant kinases using mass spectrometry. These include conserved regulatory phosphorylation sites as well as trypanosome-specific phosphosites. The significance of these phosphosites for catalytic activity of PK50 and PK53 *in vitro* as well as the *in vivo* effects of expression of the mutated proteins will be discussed.

Session 4D - Nuclear and Chromatin Structure (Chair: Mark Field)

T/L132 Invited Speaker:
Nuclear architecture and chromatin interactions underlying *VSG* epigenetic regulation in *Trypanosoma brucei*
Jean-Mathieu Bart, David Landeira, Diana López-Farfán, Daria Van Tyne & Miguel Navarro
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Increasing interest in nuclear compartments and the dynamic chromatin interactions underlying transcriptional regulation has raised interest in *Trypanosoma brucei* as a model system. Infective bloodstream form trypanosomes undergo antigenic variation, displaying different types of VSGs on the surface whose genes are transcribed by the highly compartmentalized RNA polymerase I in a mutually exclusive manner. *In vivo* GFP tagging of chromosomes applied to trypanosomes allowed us to analyse the position in the nucleus of a specific DNA locus, such as the active *VSG* expression site (ES). This approach permitted us to interrogate whether the *VSG*-ES interacts with particular nuclear structures as well as the dynamics of such interactions upon differentiation or cell cycle progression. Upon differentiation to the insect stage, down-regulation of active *VSG*-ES transcription is coordinated with temporal association with the nuclear periphery. Analysis of active *VSG*-ES sister chromatid dynamics during the cell cycle showed a delay in the separation of sisters during mitosis. The cohesin complex is important in preserving this delay and the *VSG*-ES active state, as cohesin knockdown led to an increase in *VSG*-ES transcriptional switching. Thus, in addition to the role of this complex in holding sister chromatids together, cohesin is involved in the epigenetic regulation of the *VSG*-ES. Recent results suggest that cohesin facilitates a chromatin insulator activity which defines the active *VSG*-ES chromatin domain.

T/L133* Genome-wide mapping of Orc1/Cdc6 DNA binding sites and replication origins in Trypanosoma brucei
Calvin Tiengwe[1], Lucio Marcello[1], Catarina Gadelha[2], Stephen D Bell[2], Dave Barry[1] & Richard McCulloch[1]
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The machinery of DNA replication initiation is well-conserved among characterised eukaryotes. The origin recognition complex (Orc1-Orc6; ORC), Cdc6 and Cdt1 are recruited sequentially to replication origins, and then load the replicative helicase complex (MCM2-7). In *T. brucei* and related kinetoplastids bioinformatic searches identify only a single protein, related to Orc1 and Cdc6, from the eukaryotic ORC complex, perhaps suggesting that origin designation is related in mechanism to that seen in archaea, which display a similarly streamlined machinery. Using RNA interference (RNAi) we demonstrate that knockdown of *TbOrc1/Cdc6* inhibits nuclear DNA synthesis, as revealed by cell cycle analysis and BrdU incorporation. We have developed a near-tiled oligonucleotide array (NimbleGen) for *T. brucei* and performed chromatin immunoprecipitation with functional, epitope-tagged TbOrc1/Cdc6 to map binding sites along the megabase chromosomes in the genome. Approximately 60 % of the mapped TbORC1/Cdc6 binding sites are located within the core of chromosomes, while the others are more clustered and present in subtelomeric *VSG* arrays, associated with sub-telomeric elements and in *VSG* expression sites. These results identify a global set of putative replication origins in *T. brucei* and perhaps suggest novel functions for TbOrc1/Cdc6 in chromosomal architecture and dynamics.

T/L134 NUP-1, a large coiled-coil protein in *Trypanosoma brucei*, performs analogous functions to lamins of Metazoa

Kelly N. DuBois[1], Johanna Buisson[2], Michal Swiderski[3], Phillipe Bastein[2], David Barry[3], Michael P. Rout[4] and Mark C. Field[1]

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The nucleus is separated from the cytosol by the nuclear envelope, which serves both to maintain the unique compositions of each compartment and acts as a framework for additional functions. In metazoan cells the coiled-coil lamin family proteins are now recognized as major players in maintenance of nuclear structure and organization of chromatin at the nuclear periphery; laminopathies such as progeria lead to destabilized nuclei and inappropriate gene expression. Lamins are restricted to metazoans, but there must be analogous structures maintaining nuclear organization in other lineages. NUP-1 is a large (~400kDa) coiled coil protein in Trypanosoma brucei associated with fibrils at the inner face of the nuclear envelope. Here, using a combination of RNAi, live cell imaging and transcriptome probing we have investigated NUP-1 function. We demonstrate that NUP-1 forms an immobile cage around the nuclear matrix, is required for nuclear structural integrity and also serves to repress genes located at the nuclear periphery. Further, knockdown leads to a classic nuclear pore clustering phenotype. All of these data suggest that NUP-1 fulfills highly similar functions to metazoan lamins.

T/L135 Elongator protein 3b regulates ribosomal RNA transcription in trypanosomes

Sam Alsford and David Horn

London School of Hygiene & Tropical Medicine, Keppel Street, London, WC1E 7HT, UK

The Elp3 histone acetyltransferase is the catalytic component of Elongator, a complex associated with elongating RNA polymerase II in yeast and human cells; it also has roles in exocytosis, tRNA modification, tubulin acetylation and DNA demethylation. Unlike other eukaryotes, Trypanosomatids possess two Elp3orthologues, with distinct patterns of nuclear localisation. GFP-ELP3a occupies nuclear territories distinct from RNA polymerase subunits. GFP-ELP3b localises to the nucleolus, the site of ribosomal RNA (*rRNA*) transcription, but not to the expression site body, the other RNA polymerase I compartment in *T. brucei* and the site of variant surface glycoprotein (*VSG*) transcription. Both ELP3 proteins are dispensable; however, null *elp3b* trypanosomes display a growth defect and reduced sensitivity to the transcription elongation inhibitor, 6-azauracil, suggesting that ELP3b has an unexpected negative impact on transcript elongation. Following prolonged growth, these null strains display adaptation to the growth defect. Consistent with negative control of transcription elongation, ELP3b depleted strains display a relative increase in nascent *rRNA* promoter-distal transcripts. Null *elp3b* cells display decreased nascent and steady-state *rRNA* transcripts and increased sensitivity to the translation inhibitor, G418, indicating that the molecular basis of adaptation was decreased *rRNA* transcription initiation. Our results are consistent with a role for ELP3b in regulating ribosome biogenesis and indicate that the transcription regulatory roles of the Elp3 proteins extend to RNA polymerase I. ELP3b is the first regulator shown to discriminate between *rRNA* and *VSG* mRNA synthesis.

T/L136 Centromere-associated topoisomerase activity in bloodstream form *Trypanosoma brucei.*

John M. Kelly, Samson O. Obado, Christopher Bot, Vanina E. Alvarez*, Martin C. Taylor and Maria C. Echeverry

Department of Infectious and Tropical Diseases, London School of Hygiene and Tropical Medicine, London WC1E 7HT, UK; *IIB, Universidad Nacional de General San Martin, Buenos Aires 1430, Argentina

Topoisomerase-II accumulates at centromeres during prometaphase, where it resolves the DNA catenations that represent the last link physical between sister chromatids. Previously, using approaches including etoposide-mediated topoisomerase-II cleavage, we mapped centromeric domains in trypanosomes, organisms in which chromosome segregation is poorly understood. In

bloodstream form *Trypanosoma brucei*, RNAi-mediated depletion of topoisomerase-IIα, but not topoisomerase-IIb results in the abolition of centromere-localised activity and is lethal. Both phenotypes can be rescued by expression of the corresponding enzyme from *Trypanosoma cruzi.* Therefore, processes which govern centromere-specific topoisomerase-II accumulation/activation have been conserved within trypanosomes, despite their long evolutionary divergence and differences in centromeric DNA organisation. The variable carboxyl terminal region of topoisomerase-II has a major role in regulating biological function. We generated *T. brucei* lines expressing *T. cruzi* topoisomerase-II truncated at the carboxyl terminus and found that a region necessary for nuclear localisation could be delineated to 6 residues. In other organisms, SUMOylation of topoisomerase-II is required for regulated chromosome segregation. Using tandem affinity purification and antibodies raised against trypanosome SUMO, we demonstrate that the *T. brucei* enzyme is SUMOylated. However, this modification is not required for centromere-specific cleavage activity.

WEDNESDAY 31ST MARCH

Session 5D - Chemotherapy (Chair: Simon Croft)

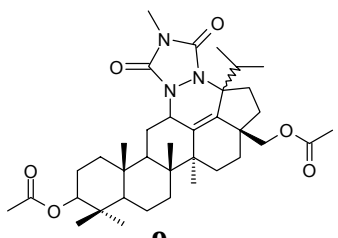
T/L137 Invited Speaker
Targeting the *Leishmania* kinome for the development of novel anti-parasitic strategies
Gerald Späth[1] on behalf of the members of the LEISHDRUG consortium[2]
[1]G5 Virulence Parasitaire, Institut Pasteur, CNRS URA 2581, Paris, France; [2]www.leishdrug.org

The LEISHDRUG consortium uses an interdisciplinary approach to exploit *Leishmania* signaling for anti-parasitic drug development. The consortium is based on three clusters with each two interactive scientific work packages that follow the major stages of the drug development process, including identification of hit compounds and targets, hit-to-lead validation and lead characterization. We apply two innovative drug screening concepts. First, we use a phenotype-based strategy that relies on visual high-content screening to discover compounds capable to kill intracellular amastigotes without deteriorating the host cell. Second, we apply a target-based strategy that exploits proteomics approaches, in combination with chemical libraries and cell-penetrating peptides, to interfere with signaling events essential for parasite survival. We identified the *L. major* MAP kinase LmaMPK10 as promising drug target, validated by the amastigote-specific activity of this protein kinase, and its structural as well as regulatory differences to mammalian orthologs. In addition, quantitative phosphoproteomic analysis identified stage-specific phosphorylation of *L. donovani* heat shock proteins as being an important factor in post-translational control of the parasite heat shock response. Mutagenesis analysis uncovered two phosphorylation sites in the co-chaperone STI-1 that were essential for *L. donovani* viability. Together our data demonstrate that *Leishmania* protein phosphorylation represents a powerful traget for drug development via inhibition of protein kinase activities and quenching of substrate phosphorylation.

T/L138 Anti-leishmanial activity of betulinic acid derivatives in *Leishmania (L.) braziliensis.* Comparison of statistical methods to evaluate the IC50 of best candidates
Wilmer Alcazar-Guerra[3], Sami Alakurtti[1,2], Jarí Yli-Kauhaluoma[1], Alicia Ponte-Sucre[3]. [1]Division of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Helsinki, [2]Technical Research Centre of Finland, Espoo, Finland, and [3]Laboratorio de Fisiología Molecular, IME, Universidad Central de Venezuela.

Leishmaniasis is a public health problem in tropical and subtropical world areas. Moreover, the increasing incidence of Leishmania-HIV co-infection, the appearance of drug-resistant Leishmania strains and the unsatisfactory features of antileishmanial treatment, stress the importance to develop inexpensive, effective and rapid new therapies. Betulin (lup-20(29)-ene-3b,28-diol) is an abundant naturally occurring triterpene found in bushes and trees, being the principal extract of the bark of birch trees [1]. Terpenoids belonging to the betulinic acid derivatives are active against parasites, among them, *L. donovani and L. tropica* (IC50=14.6 μM) [2]. The IC50 indicates the

efficacy and potency of antiprotozoal agents. However, the methodologies and criteria used to assess the dose-effect relationship and describe the IC50 are heterogeneous [3]. Herein we evaluated the in vitro antileishmanial activity of 30 betulinic acid derivatives against *L. braziliensis* calculating the IC50 by the methods: Interpolation, Probit analysis, Logit analysis, Trimmed Spearman-Karber and Litchfield-Wilcoxon method. Our results demonstrate that 6 out of 30 betulinic acid analogues tested are active against promastigotes after a 3-day incubation period, with IC50 lower than 10 mM and 1 out of 30 with an IC50 lower than 1 mM (11), with consistent results within the methods applied.



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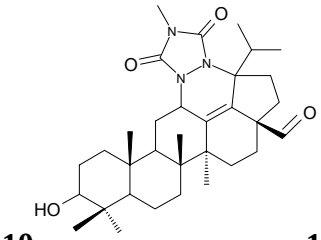

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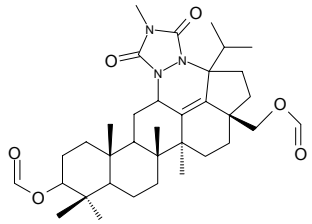

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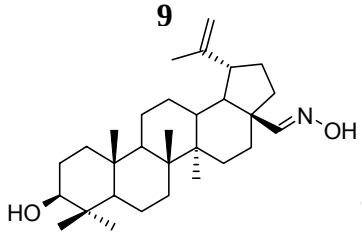

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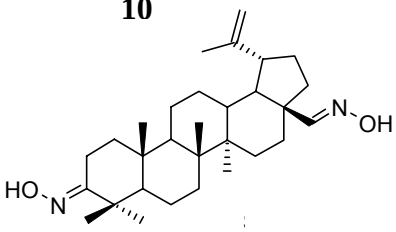

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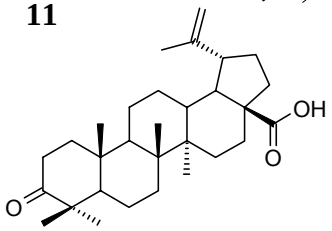

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T/L139 Dissecting the essentiality of the bifunctional trypanothione synthetase-amidase in *Trypanosoma brucei* using chemical and genetic methods

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Division of Biological Chemistry and Drug Discovery, Wellcome Trust Biocentre, College of Life Sciences, University of Dundee, Dundee, DD1 5EH, Scotland.

The bifunctional trypanothione synthetase-amidase (TRYS) comprises two structurally distinct catalytic domains for synthesis and hydrolysis of trypanothione. This unique dithiol plays a pivotal role in thiol-redox homeostasis and in defence against chemical and oxidative stress in trypanosomatids. A tetracycline-dependent conditional double knockout of TRYS (cDKO) was generated in bloodstream *Trypanosoma brucei*. Culture of cDKO parasites without tetracycline-induction resulted in loss of trypanothione and accumulation of glutathione, followed by growth inhibition and cell lysis after 6 days. In the absence of inducer, cDKO cells were unable to infect mice, confirming that this enzyme is essential for virulence *in vivo* as well as *in vitro*. To establish whether both enzymatic functions were essential, an amidase-dead mutant cDKO line was generated. In the presence of inducer, this line showed decreased growth *in vitro* and decreased virulence *in vivo*, indicating that the amidase function is not absolutely required for viability. The druggability of TRYS was assessed using a potent small molecule inhibitor developed in our laboratory. Growth inhibition correlated in rank order cDKO, single KO, wild-type and over-expressing lines and produced the predicted biochemical phenotype. The synthetase function of TRYS is thus unequivocally validated as a drug target by both chemical and genetic methods.

T/L140 Unraveling the trypanocidal mechanism of nifurtimox

Belinda Hall & Shane Wilkinson
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Nifurtimox treatment of African sleeping sickness and Chagas disease continues despite issues relating to toxicity and emerging resistance. Moreover, its mode of action is unclear. For over thirty years it was believed that this drug worked by causing oxidative stress in the parasite via generation of nitroradicals and superoxide anions, a reaction mediated by type-II nitroreductases. However, this has been called into question by the identification of a bacterial-like, type-I nitroreductase (NTR) located to the trypanosome mitochondrion. Functional studies have shown that altering the expression level of NTR in *T.brucei* and *T.cruzi* affects parasite susceptibility to nifurtimox. Here, we confirm that the *T.cruzi* and *T.brucei* NTRs are of the type-I class: both are oxygen-insensitive, FMN-binding and NADH-dependent, transferring reducing equivalents to nifurtimox via a ping pong-like mechanism. LC/MS analysis of the reduction products generated under both aerobic and anaerobic conditions yielded a single major peak with an m/z corresponding to a molecular weight of 255. This size, in conjunction with its absorbance spectrum, suggests that the product is an open chain nitrile. After purification this compound was shown to kill *T. brucei* BSF in a dose-dependent manner giving an IC_{50} of ~5μM, independent of NTR expression levels. Taken with previous studies analyzing the trypanosomal oxidative defense systems, our data shows that nifurtimox mediated parasite killing occurs primarily through formation of the open chain nitrile form in the mitochondrion rather than via production of nitroradicals/superoxide anions.

T/L141 Cyclic nucleotide phosphodiesterases of kinetoplastids: new targets for drug development?

Thomas Seebeck[1,4] , GeertJan Sterk[2,4], Hermann Tenor[3,4] & Stefan Kunz[1]
[1]Institute of Cell Biology, University of Bern, CH-3012 BERN, Switzerland; [2]Mercachem, 6503 GE NIJMEGEN, The Netherlands; [3]Nycomed Pharma, D-78467 Konstanz, FRG; [4]the TI Pharma Consortium T4-302.

Target finding for trypanocidal drugs has traditionally concentrated on 'exotic' enzymes essential for the parasite, but absent in mammalian hosts. However, translating results generated by this approach into successful drug development projects has often proven difficult.
On the other hand, medicinal chemistry has made enormous progress in designing compounds that inhibit enzymes in a highly organ-specific or isoform-specific manner. This calls for a re-evaluation of how to select parasite drug targets. Similarity of an essential parasite enzyme to a pharmacologically well studied human homologue might indeed represent a significant benefit over "exotic" parasite-specific targets. It will allow to fully use the expertise and technology already established for developing compounds that are active against the human homologues.
We explore this concept using cyclic nucleotide specific phosphodiesterases (PDEs) of kinetoplastids as a test case. Human PDEs are important drug targets for a number of clinical conditions. The kinetoplastid genomes contain four PDE families whose catalytic domains are very similar to those of their human homologues. Inibition of PDE activity, or genetic deletion of the respective genes leads to rapid death of T. brucei. The development of parasite-specific PDE inhibitors might represent a novel approach to kinetoplastidicidal drugs.

Session 6D - Cell Death and Autophagy (Chair: Nicolas Fasel)

T/L142 Invited Speaker
Cell death mechanisms in protozoan parasites
Rudolf Koopmann, Thosten Barth & Michael Duszenko
Interfaculty Institute for Biochemistry, University of Tubingen, Tubingen, Germany

Mammalian parasites survive the host's hostile environment, because they have evolved effective means to escape the immune response. However, uncontrolled cell growth carries the risk to overgrow the host, leading to its premature death and rendering infection epidemiologically irrelevant. Thus, from the viewpoint of a parasite, a balanced population density would be ideal that ensures both, unharmed survival within the host and a high chance for uptake by the transmitting vector. The detection of typical apoptosis markers in diverse protozoan parasites are indicative for an active contribution of these cells to control its population density, but raised a debate about the evolution of self destruction in single cell organisms. Although this problem seems to be solved by the concept of clonal selection and stage conversion, the subdivision of cell death in accidental necrosis on the one hand and programmed cell death on the other hand, seems to be oversimplified. Thus in 2009 the nomenclature commission of Cell Death and Differentiation suggested dividing cell death in all organisms in three categories only: necrosis, apoptosis and autophagy related cell death. This concept reflects the cognition that in any case an affected cell takes genetically encoded measures to cope with harassments from intracellular or extracellular stimuli. All three cell death mechanisms have been observed and described in protozoan parasites. Consequences of these mechanisms on the struggle for survival will be summarized and discussed with a main focus on kinetoplastid parasites.

T/L143 Autophagy in *Leishmania*
Roderick A. Williams,[1] Jeremy C. Mottram[2] and Graham H. Coombs[1]
[1]Strathclyde Institute of Pharmacy and Biomedical Sciences, University of Strathclyde, The John Arbuthnott Building, 27 Taylor Street, Glasgow G4 0NR, UK; [2]Division of Infection & Immunity and Wellcome Centre for Molecular Parasitology, Glasgow Biomedical Research Centre, University of Glasgow, Glasgow G12 8TA, UK

Macroautophagy is a catabolic process involved in the development of organisms. The main molecular players involved, and identified in higher eukaryotes, are from two conjugation pathways: ATG8 lipidation and ATG12-ATG5 conjugation. Reported analyses on the genomes of trypanosomatids suggested a secondary loss of the ATG12-ATG5 conjugation pathway. However, we identified genes potentially encoding the pathway's components and have now used various complementary approaches to validate the existence of this conjugation pathway in *Leishmania.* The ATG8 lipidation pathway has been confirmed in *Leishmania*, with ATG8-GFP being used as a molecular marker for monitoring autophagy. The pathway involves ATG4, for which there are two genes. We have generated *ATG4*-deficient mutant lines to assess the roles of the individual ATG4s in the parasite's development and ability to withstand stress. The findings show that they have complementary but distinct functions. Potential for targeting *ATG* genes as a means of interfering with the parasite's autophagic pathway and development will be discussed.

T/L144 Neuropeptides kill trypanosomatids by targeting intracellular compartments and inducing autophagic-like cell death
Jenny Campos-Salinas, Mario Delgado, Marta Caro and Elena Gonzalez-Rey.
Department Cell Biology and Immunology.
Institute of Parasitology and Biomedicine Lopez –Neyra, CSIC, Granada, Spain.

Protozoan parasites cause a spectrum of diseases in several millions of people world-wide. Available treatments are ineffective, toxic, and susceptible to resistance by these parasites. Here we show that various endogenous neuropeptides act as potent antiparasitic agents. Neuropeptides exerted their trypanolytic activity through an unusual mechanism that involves peptide uptake by the parasite, disruption of lysosome integrity and cytosolic accumulation of glycolytic enzymes. This promotes an energetic metabolism failure that initiates an autophagic-like

cell death. Neuropeptides-based treatment improved clinical signs in a chronic model of trypanosomiasis and leishmaniasis by reducing the parasite burden in various target organs. Of physiological importance is the fact that hosts respond to parasites infection producing neuropeptides as part of their natural innate defense. From a therapeutic point of view, targeting of intracellular compartments by neuropeptides suppose a new promising strategy for the treatment of these diseases.

T/L145* Processing of metacaspase into a cytoplasmic catalytic domain mediating cell death in *Leishmania major*

Habib Zalila[a], Iveth J. González[a,1], Maria B. Delgado[a], Chantal Desponds[a], Jeremy C. Mottram[b], Nicolas Fasel[a,]
[a] University of Lausanne, Epalinges, Switzerland; [b] University of Glasgow, Glasgow, UK

Metacaspases are cysteine peptidases that could play a role similar to caspases in the cell death program of plants, fungi and protozoa. The human protozoan parasite *Leishmania major* expresses a single metacaspase (LmjMCA). In this study, we investigated the processing sites important for the maturation of LmjMCA catalytic domain, the cellular localization of LmjMCA polypeptides, and the functional role of the catalytic domain in the cell death pathway of *Leishmania* parasites. Although LmjMCA polypeptide precursor form harbors a functional mitochondrial localization signal (MLS), we determined that LmjMCA polypeptides are mainly localized in the cytoplasm due to an amino-acid sequence downstream of the MLS, which impaired its transport into the mitochondrion. In stress conditions, such as exposure to heat shock, hydrogen-peroxide (H_2O_2) or anti-Leishmania drugs, LmjMCA precursor forms were extensively processed into soluble forms containing the catalytic domain. We showed that this domain was sufficient to enhance sensitivity of parasites to H_2O_2 by disrupting the mitochondrion. These data provide experimental evidence of the importance of the activity of the catalytic domain in disrupting mitochondria and of LmjMCA processing into an active catalytic domain which could be relevant in the design of new drugs to fight leishmaniasis and likely other protozoan parasite diseases.

T/L146 Contribution of cathepsin B in the cell death pathway of *Leishmania* parasites

Amal Kuendig, Haroun Zangger, Chantal Desponds, Iveth Gonzalez, Habib Zalila, Cédric Schaff, Jeremy C. Mottram[#] and Nicolas Fasel
University of Lausanne, Switzerland and [#]University of Glasgow, UK

In the human protozoan parasite *Leishmania*, drugs as well as oxidative stress induce phenotypic markers of cell death (CD) similar to those markers described in higher eukaryotes. In several studies, CD in *Leishmania* and lower eukaryotes was detected by using caspase substrates or inhibitors. However, most of the lower eukaryotes do not encode caspase(s) but for metacaspase (MCA). We have been interested to determine which enzyme was capturing the z-VAD substrate. We identified lysosomal cathepsin B-like enzyme (LmjCPC) as binding to z-VAD-fmk when CD is induced and confirmed the specific interaction of z-VAD-fmk to CPC by showing that z-VAD binding is absent in an *L. mexicana* strain in which the CPC gene was deleted. We also showed that parasites exposed to various stress conditions undergo lysosomal membrane permeabilisation (LMP) resulting in CPC release in a soluble fraction. Finally, we confirmed the role of CPC in the CD pathway by showing that when exposed to an anti-leishmania drug such as miltefosine or H2O2, CPC knock-out parasites survive better than wild type parasites. Thus, we propose a model in which a similar CD pathway is turned on even when different CD inducers are used and that this pathway implicate LMP and CPC release.

Session 7D - Biochemistry (Chair: Michael Ginger)

T/L147 Invited Speaker

Sugar-kinases and anomerization of sugar-phosphates in trypanosomes

Paul Michels, Morena Magnani, Nadine Möbius, Shreedhara Gupta, Ana Caceres & Artur Cordeirode

Duve Institute, Brussels, Belgium

Trypanosomatids possess a hexokinase (HK) located in their glycosomes. In addition, a glucokinase (GlcK) was found in the glycosomes of *Trypanosoma cruzi* and *Leishmania* species, but not in *Trypanosoma brucei*. The crystal structure of TcGlcK with bound glucose showed that the sugar was present in its beta-anomeric form; this preference was confirmed in activity assays. In contrast, all known HK-glucose structures possess alpha-glucose, in line with results from kinetic studies that HKs have a preference for this latter anomer. The enzymes following the sugar-kinases in the glycolytic and pentosephosphate pathways, PGI and G6PDH, are highly specific for respectively the alpha- and beta-form of the glucose 6-phosphate. The presence of both a HK and GlcK in glycosomes could be related to these different specificities. In *T. brucei*, where GlcK is absent, a glucose-6-phosphate-1-epimerase (G6PE) would be required for the anomerization. Indeed, a homologue of yeast G6PE, with a glycosome-targeting signal, was identified in the TriTryp databases. The activity was confirmed with recombinant TbG6PE. Knocking down its expression in bloodstream-form trypanosomes has no effect on their growth in regular HMI-9 medium. However, the cells are susceptible to oxidative stress in a non-reducing medium, very similar to results obtained for trypanosomes in which G6PDH has been depleted. These results suggest a role for TbG6PE in making glucose 6-phosphate available for the pentosephosphate pathway and NADPH production.

T/L148 Distinctive biochemistry in the mitochondrial intermembrane space of trypanosomatids

[1]James W. A. Allen, [2]Vilmos Fülöp, [1]Stuart J. Ferguson and [3]Michael L. Ginger

[1]Department of Biochemistry, Oxford University, UK; [2]Department of Biological Sciences, Warwick University, UK; [3]School of Health and Medicine, Lancaster University, UK.

C-type cytochromes are essential proteins for respiration in most organisms. They contain heme, covalently bound to the polypeptide in a post-translational modification that requires dedicated accessory proteins. Surprisingly, the four known heme-attachment systems are all absent from trypanosomatids, and trypanosome *c*-type cytochromes are unique – the heme is attached to a single cysteine residue (an XXXCH motif), rather than to two cysteines (CXXCH) as in all other organisms. This is persuasive evidence that biogenesis of trypanosome *c*-type cytochromes requires a novel, probably unique, heme-attachment apparatus. However, the overall structure of trypanosome cytochrome *c* is remarkably similar to that of typical mitochondrial cytochrome *c* raising questions about why the single cysteine heme attachment has evolved. Disulfide bond oxidizing and reducing proteins are essential for protein import and folding in the mitochondrial intermembrane space (IMS) of yeast. Cysteine-rich proteins enter the IMS and are oxidized to form intramolecular disulfide bonds by Mia40. Mia40 is reoxidized by the sulfhydryl oxidase Erv1, which in turn passes electrons to cytochrome *c*. However, trypanosomes have no homologue of Mia40 and do not always have a cytochrome-dependent respiratory chain. Together, our observations suggest that the disulfide bond-dependent redox environment of the trypanosomatid mitochondrial IMS may be distinct from that in other eukaryotes.

T/L149* On the role of glutaredoxins in *Trypanosoma brucei,* an organism that lacks the classical glutathione/glutathione reductase system

Sevgi Ceylan , Vera Seidel,R. Luise Krauth-Siegel
Biochemie-Zentrum der Universität Heidelberg, Im Neuenheimer Feld 504, 69210 Heidelberg, Germany

Trypanosoma brucei, the causative agent of African sleeping sickness, has a unique thiol metabolism where the GSH/glutathione reductase couple is replaced by a trypanothione/trypanothione reductase system. The parasite encodes two glutaredoxins (Grx), small dithiol proteins usually involved in cellular redox functions.
Grx1 has the classical CPYC active-site but is more similar to that of human Grx2 (40 % identity) than human Grx1 (31%). The protein contains an additional cysteine in the C-terminal part that is conserved in human Grx1. Grx2 has a CQFC active-site, and is only distantly related with the parasite Grx1 and other glutaredoxins.
The *T. brucei* genes were cloned and overexpressed in *E. coli*. In the classical HEDS (hydroxyethyl-disulfide)-assay, Grx1 and Grx2 possess 30 % and 3 %, respectively, of the activity of *E. coli* Grx1. Both proteins catalyze the deglutathionylation of glutathionylated proteins with Grx1 being more efficient than Grx2. *T. brucei* Grx2 – but not Grx1 – reduces protein disulfides.
The disulfide forms of the proteins are reduced by glutathione or trypanothione, whereby the reduction of Grx1 by trypanothione is four orders of magnitude faster than by glutathione.
Grx1 and Grx2 are expressed in procyclic and blood-stream parasites. No differences were observed for the levels in different growth phases.
Down-regulation of Grx2 - but not of Grx1 - by RNA interference resulted in impaired proliferation of procyclic cells.

T/L150* Functional genomics of amino acid permeases from *Leishmania donovani*: The story of proline transport

Ehud Inbar[1], Doreen Shliselberg[1], Marianne Suter-Grotemeyer[2], Doris Rentsch[2] and Dan Zilberstein[1]
[1]Faculty of Biology, Technion-Israel Institute of Technology, Haifa 32000, Israel and [2] Institute of Plant Sciences, University of Bern, 3013 Bern, Switzerland

We have identified and functionally characterized a proline transporter (*Ld*AAP24, LinJ.10.0760) in the plasma membrane of *Leishmania donovani* promastigotes. Heterologous expression in *Saccharomyces cerevisiae* mutants indicated that LdAAP24 has low affinity, high capacity and low specificity to proline. Polyclonal antibodies that were raised against the hydrophilic N-terminus localized LdAAP24 to the plasma membrane and flagella pocket. It is exclusively expressed in promastigotes and both LdAAP24 mRNA and protein disappeared 2.5 hours after differentiation of promastigotes to amastigotes initiated. Further analyses indicated that LdAAP24 is an unstable protein with a high turnover rate. Deleting *LdAAP24* from its chromosome revealed a second, high affinity and low capacity proline transporter. While LdAAP24 resembles the previously described cation-dependent system A, the second transporter is the cation-independent system B. ∆LdAAP24 lost most of its cellular pool of proline and about half that of glutamate and alanine. In addition, glutamate transport in mutant cells was about half that of WT. Surprisingly, mutant cells tripled cellular pool of arginine even though its uptake remained unchanged. Finally, we identified and demonstrated proline transport activity of LdAAP24 orthologues in *Trypanosoma cruzi* and *T. brucei* (TbAAP24 and TcAAP24, respectively). The results indicate that LdAAP24 plays an important role in regulating amino acid metabolism in Leishmania. This study facilitates the first molecular characterization of proline transport in parasitic protozoa.

T/L151* *Trypanosoma brucei* metacaspases – surprises from the fourth family member

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The metacaspases (MCAs) are a family of caspase-family cysteine peptidases found in plants, fungi and protozoa, but absent from mammals. Three of the five MCAs found in *T. brucei* (MCA2, 3 and 5) contain the conserved catalytic cysteine and histidine residues found in the caspase active site. Biochemical characterisation of recombinant MCA2 has provided evidence of arginine/lysine specific cysteine peptidase activity, which appears to be distinct from the aspartic acid specific activity of the caspases (Moss *et al*., 2007 FEBS Lett. 581(29):5635-9). Functional analysis of the three metacaspases in bloodstream form *T. brucei* showed that sequential gene knockout was possible, but rapid RNAi downregulation of all three resulted in a cytokinesis defect and ultimately cell death (Helms *et al*., 2006 JCS 119:1105-17).
To develop our understanding of the MCAs of *T. brucei,* a study into the function of MCA4 has been undertaken. This unusual MCA contains an active site substitution, where the cysteine residue is replaced by a serine. RNAi and genetic knockout were used to investigate the role of MCA4 in the *T. brucei* lifecycle and the significance of the active site substitution on enzymatic activity has been studied using recombinant protein in biochemical assays.

Session 8D - Mixed Session II (Chair: Graham Coombs)

T/L152* *Leishmania infantum* IZT3 is an inducible zinc transporter

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The ZIP family (ZRT/IRT-like Protein) comprises proteins involved in the transport of divalent metals in several organisms. *Leishmania infantum* has three putative ZIP members. One of these genes, IZT3 (Iron/Zinc Transporter #3), was found to be involved in the acquisition of zinc. By functional complementation of yeast, it was the only *L. infantum* ZIP gene able to rescue the growth of yeast deficient in zinc acquisition systems. In *L. infantum*, IZT3 expression is induced in the presence of the metal chelator EDTA and decreased by the addition of small amounts of zinc (1µM). Copper and cadmium also induce IZT3 expression, probably due to competition with zinc.
Regulation of IZT3 levels may be attained, primarily, by mRNA stability. IZT3 mRNA levels are increased when the parasites are grown in low-zinc medium and decreased in zinc-rich medium. However, due to localization of IZT3 in the cellular membrane and in the flagellar pocket of promastigotes, regulation of its surface levels may also take place by endocytosis. Together, these data suggest that IZT3 is a high-affinity zinc transporter, the first identified in *Leishmania*.

T/L153 TbMLP1: A putative iron transporter in *Trypanosoma brucei*

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Iron is an essential nutrient in all trypanosomatids analysed. Bloodstream form *T. brucei* derive iron from the host carrier transferrin. Transferrin is endocytosed after binding to the ESAG 6/7 heterodimer in the flagellar pocket. Iron is subsequently released from transferrin in the late endosome/lysosome compartment. However the pathway by which iron is then transported into the cytosol from the endosomal system has not been resolved.
Here, we report the identification of an orthologue of the human endosomal iron-release channel Mucolipin 1, which may perform this role in *T. brucei*. TbMLP1 (Mucolipin-like protein 1) is a seven transmembrane domain protein confined to a single vesicular cellular compartment located between the nucleus and the kinetoplast. RNA interference mediated knockdown of the *TbMLP1* gene resulted in a slight growth retardation which only became apparent between 3 and 6 days

after induction. However, when exposed to the iron chelator deferroxamine the induced cells have a severe growth defect in comparison to the uninduced controls. The TbMLP1 gene has proved to be refractory to deletionsuggesting some level of expression is necessary in both bloodstream form and procyclic cells. Current work is focussed on generating a conditional knockout cell line and further characterising the biological function of TbMLP1.

T/L154* Biophysical characterization of the *Leishmania donovani* peroxin 14 and its role in glycosomal protein import

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Leishmania, and other kinetoplastids, compartmentalizes glycolysis and other vital metabolic pathways in the glycosome, a subcellular organelle that is evolutionary related to peroxisomes. Previous studies have indicated that the impairments in the targeting of proteins to the glycosome can result in a lethal phenotype for these parasites. Consequently the glycosome biogenesis machinery consists of an attractive therapeutic target. A key component of the glycosomal protein import machinery is the *L. donovani* PEX14 (LdPEX14), a membrane associated protein required for translocation of proteins across the glycosomal membrane. Quaternary structure analysis of LdPEX14 revealed that this protein forms a large oligomeric complex. Domain mapping showed that elimination of a hydrophobic region and a coiled-coil motif were necessary to disrupt oligomer formation. Moreover, calorimetry, intrinsic fluorescence, circular dichroism and analytical ultracentrifugation experiments showed that binding of LdPEX5 caused a dramatic conformational change in the LdPEX14 complex that was accompanied by a reorganization of a hydrophobic segment in common to PEX14 proteins. Furthermore, protein-membrane interaction studies using liposomes that mimic the glycosomal membrane composition, showed that LdPEX14 was capable of binding to these lipid bilayers and to recruit the PTS1 receptor LdPEX5 to the liposomes. These studies will allow a more fundamental understanding of the glycosome biogenesis machinery and its interaction with the glycosomal membrane.

T/L155* Cyclosporin A treatment of *Leishmania donovani* reveals stage-specific functions of cyclophilins in parasite differentiation, proliferation, and viability

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Cyclosporin A (CsA) has important anti-microbial activity against parasites of the genus *Leishmania,* proposing CsA-binding cyclophilins (CyPs) as potential drug targets. Sequence analysis identified 17 *Leishmania* CyPs with highly conserved functional residues implicated in PPIase function and CsA binding. CsA treatment of promastigotes resulted in a dose-dependent inhibition of cell proliferation and induced morphologically changes reminiscent to developing amastigotes, suggesting a role for parasite CyPs in *Leishmania* growth and differentiation. In contrast to promastigotes, CsA was highly toxic to amastigotes at 10 □M, revealing for the first time a direct lethal effect of CsA on the pathogenic mammalian stage, independent from host CyPs. We showed that CsA-mediated cell death is temperature dependent and thus like due to inhibition of CyP chaperone functions relevant for parasite thermotolerance. Structural modeling and enrichment of CsA-binding proteins from parasite extracts by FPLC indeed identified the co-chaperone LmaCyP40 as a major inhibitor target. Our data allow important new insight into the function of the *Leishmania* CyP protein family in differentiation, growth, and intracellular survival, and define this class of molecules as important drug targets.

T/L156 The trypanosomatid sphingolipid synthases: common enzymes with divergent functions

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Sphingolipids are important components of eukaryotic membranes involved in a diverse array of pivotal functions. In the Eukaryota the biosynthetic pathway for the formation of these lipid species is largely conserved. However, several pathogenic fungi and protozoa synthesize inositol phosphorylceramide (IPC) as the primary phosphosphingolipid rather than sphingomyelin (SM) like mammals. This process is catalyzed by IPC synthase, a target for anti-fungals encoded by the AUR1 gene. Recently, functional orthologues of AUR1p have been identified in the pathogenic trypanosomatids. The parasite enzymes are closely related to each other but bare little similarity to yeast AUR1p, accounting for the fact that they lay unidentified in the genome databases for so long. However, despite similarity within the trypanosomatid proteins at the primary sequence level they demonstrate surprising diversity of function. Our recent work, focused on comparative *in vitro* analyses of these enzymes, has revealed that those from the intra-cellular *Leishmania* spp. and *Trypanosoma cruzi* parasites function as conventional IPC synthases, catalysing the transfer of phosphoinositol from phosphatidylinositol to ceramide. In contrast, the *Trypanosoma brucei* sphingolipid synthase functions as a novel dual function enzyme, driving the formation of both IPC and SM. As such it behaves as an amalgam of the distant yeast and mammalian orthologues. These contrasting functions will be discussed in terms of inhibition, lifestyle and evolution.

T/L157 *In vitro* activity and host cell dependence of anti-leishmanial drugs

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Biological evaluation of anti-leishmanial compounds *in vitro* relies on models that mimic the environment encountered by the clinical relevant amastigote stage of *Leishmania*.
Here we present the results of a direct comparative analysis of the anti-leishmanial activity of amphotericin B, miltefosine, sodium stibogluconate and paromomycin sulphate against *L. donovani* in different host cells / macrophages. Host cells assessed include mouse peritoneal exudate macrophages (PEMs), mouse bone marrow derived macrophages (BMMΦs), human monocyte derived macrophages and the human monocytic THP-1 cell line. The course of infection in the different macrophage populations was monitored over the same period as drug activity was evaluated. The outcome of this study will be presented and its implications in drug discovery and development discussed.

Session 2E - Comparative Genomics (Chair: Christiane Hertz-Fowlerl)

T/L158 Invited Speaker

i-seq profiling in trypanosomes: next-generation sequencing for genome-scale RNA interference studies

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We have combined >10x genome-scale RNA interference libraries with massive-parallel, next-generation sequencing to provide 'fitness scores' associated with the knock-down of each gene in African trypanosomes. This interference-sequencing (i-seq) approach reveals >2000 genes associated with loss-of-fitness. Applied to differentiated libraries (bloodstream and insect-stage),

the approach highlights i. Core essential functions; for e.g. putative regulators of mRNA abundance are over-represented likely reflecting low-level transcriptional control. ii. Developmental stage-specific differences in energy metabolism and in flagellum, nuclear and trans-membrane channel proteomes. iii. Proteins involved in differentiation; mitochondrial proteins are over-represented in the category. We further exploit the i-seq data-set to identify putative regulators of the multi-functional RNA polymerase I in *T. brucei*; in the bloodstream parasite, pol I drives monoallelic transcription of Variant Surface Glycoprotein (*VSG*) genes in an extranucleolar compartment, a process that underlies antigenic variation and persistence of disease. These studies reveal a chromatin chaperone (CAF-1) and a histone deacetylase (DAC3) required for *VSG* expression-site silencing.

T/L159 Genome sequencing project has begun on two plant trypanosomatids (*"Phytomonas"*)

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Some trypanosomatids are responsible for wilt in tropical crops, such as hartrot disease in the coconut palm. They have a major economic impact in Latin America and the Caribbean. In the latex vessels of some plants, other trypanosomatids appear to be "symbiont-like", without any negative effect on their host, and others multiply in fruits and seeds. The latter usually belong to the genera Crithidia, Herpetomonas and Leptomonas, formerly known as "lower trypanosomatids". Only one arbitrary genus name has been proposed as yet for all these trypanosomatids living in plants, in different tissues, with different consequences: "Phytomonas". After the Trytrip project and the recent discovery of lower trypanosomatids in immuno-suppressed patients, it became increasingly interesting to compare genome sequences outside Trypanosoma and Leishmania in order to better understand the molecular evolutionary relationships within the protozoan order Kinetoplastida.
In 2008, the French National Research Agency (ANR) decided to sponsor a project on the sequencing of two plant trypanosomatids, one -Hart 1, group H - responsible for a disease of coconut (hartrot) in Latin America, and non-pathogenic one –EM1, group D - from Euphorbia in France.
Sequencing of the isolates was performed using data from three different technologies (454 Titanium, Illumina GAIIx and Sanger). Assemblies obtained by Newbler (Roche) showed high continuity. We also obtained cDNA sequences using 454 Titanium to help annotation. The assemblies were of sufficient continuity to start an automatic annotation phase, using procedures that involve cDNA, matches to protein data, and de novo gene finding.

T/L160 Aneuploidy and mosaicism in Leishmania strains as adaptative means to changing host environments

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Using FISH analysis of individual cells, we have shown that *Leishmania* strains are aneuploid and display a mosaic structure. This is generated by a high rate of asymmetric chromosomal allotments in mitotic cells. We discuss here of how this aneuploidy could be set up and why it may be tolerated by this parasite. This phenomenon allows an extraordinary potential for genetic differentiation and, as such, may have a relevant impact upon the genetic structuration of *Leishmania* strains. Thus, the alternating transition from disomy to monosomy (and vice-versa) for a given chromosome will ineluctably lead to a large majority of homozygous cells. We present a very simple model showing that this would happen even if the rates of asymmetric chromosomal allotments were low. Remarkably, however, this maintains a strong genetic heterogeneity of the strain population, which may then appear as overall heterozygous, although it is constituted of homozygous cells. The addition of this phenomenon to that of automixy (or endogamy) will also be discussed. The original genetic structure that follows from these data may conjugate the

evolutionary advantages of the haploid and diploid states, and confer to the parasite powerful means to adapt to multiple and changing environments.
* Both authors contributed equally to this work

T/L161 Whole comparative genome sequencing reveals several levels of genomic diversity among Leishmania donovani strains in Indian subcontinent.
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Leishmania donovani (*L. donovani*) of the Indian subcontinent causes visceral leishmaniasis, a disease characterized by high clinical polymorphism. This contrasts with the low genomic heterogeneity found so far in this parasite population. New sequencing technologies now offer the possibility to characterize the whole genome of multiple strains and better assess its impact on phenotypic diversity. In the frame of the GeMInI project (the Genome and Metabolome Integrated Initiative), we apply this concept to *L. donovani*, using drug resistance as paradigm of phenotypic diversity.
After construction of a *de novo* reference sequence of *L. donovani*, genomic diversity was assessed among strains derived from 20 Nepalese clinical isolates. Three types of genomic diversity elements were identified: (i) single nucleotide polymorphisms (SNPs), (ii) copy number variations (CNVs) and (iii) chromosome number. Coding SNPs were correlated with observed clinical phenotypes and previously observed population structure. Several large scale CNVs were also identified. Most prominent genomic diversity was found at the level of chromosome number and ploidy which appeared to be strain-specific. We are currently examining the functional impact of these findings and in the near future plan to integrate genomic diversity results with metabolomic diversity elements identified in parallel in our GeMInI study.

T/L162 Functional characterization of cyclopropane fatty acid synthetase in *Leishmania*
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Cyclopropane fatty acid synthetase (CFAS) catalyses the cyclopropanation of unsaturated fatty acids, a reaction that involves transfer of a methylene group from *S*-adenosyl-*L*-methionine donor to a carbon-carbon double bond within a fatty acyl chain. Cyclopropanated fatty acids have previously been described in a range of organisms but their physiological role remains unknown. CFAS enzymes are common in pathogenic bacterial species but rare in non-pathogenic ones. In eukaryotes, *CFAS* is present in fungi and plants but absent in mammalian species.
Comparative genome analysis in the genus *Leishmania* identified a single *CFAS* gene in some species but not in *Leishmania major*. We have characterized CFAS in *L. infantum* and shown that the gene is expressed and functional in generating cyclopropanated lipids. Although these modified lipids are absent in wildtype *L. major*, they are detected in *L. major* transgenic cell lines expressing the *L. infantum CFAS* gene, indicating the presence of a common substrate. In *L. infantum*, CFAS is membrane-associated and located in the ER. *In vivo* studies using CFAS mutant parasites suggest that the enzyme is essential for *L. infantum* survival and replication in the mammalian host, although its expression needs to be tightly regulated for optimal production of cyclopropanated fatty acids. Overall, our data implicate cyclopropane modification as a factor in *Leishmania* pathogenesis.

Session 5E - Immunology (Chair: Jim Alexander)

T/L163 Invited Speaker

Neutrophil-derived CCL3 is essential for the rapid recruitment of dendritic cells to the site of *Leishmania* inoculation in resistant mice

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Neutrophils are rapidly and massively recruited to sites of infection. We will present a new role in the early recruitment of dendritic cells (DCs) in response to *Leishmania major* inoculation. *L. major* induced the abundant production of CCL3 by neutrophils from *L. major*-resistant (C57BL/6) but not –susceptible (BALB/c) mice. The presence of CCL3 induced chemotaxis of immature DCs, an effect markedly impaired once CCL3 was depleted from neutrophil supernatants. One day post *L. major* inoculation in the ear dermis, DC recruitment was markedly decreased in mice depleted of neutrophils prior to infection. Pharmacological or genetic inhibition of CCL3 resulted in a significant decrease in DC recruitment at the site of parasite inoculation one day post infection, while no defect of neutrophil migration was noticed. The decrease was corrected by the transfer of C57BL/6 neutrophils at the time of infection. The early release of CCL3 by neutrophils was further shown to have an impact on the development of a protective immune response. Altogether, we identified an essential role for neutrophil-secreted CCL3 in the first wave of DC recruitment to the site of infection with *L. major* and the development of a protective immune response.

T/L164 Invited Speaker

B cell dysfunction in African trypanosomiasis

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African trypanosomes cause human trypanosomiasis and Nagana in cattle. The surface coat of a *T. brucei* parasite consists of 10^7 identical densely packed Variant Surface Glycoprotein (VSG) molecules, which enable it to escape the host's B-cell response. While VSG-specific clearance of trypanosomes is mediated by antibodies, the parasite can activate an endogenous phospholipase C that results in the release of soluble (s)VSG, a strong pro-inflammatory molecule.

We aim at understanding the mechanisms and consequences of trypanosomiasis associated inflammation, including the destruction of various B-cell compartments and B-cell memory. Our recent work has shown that during infection a premature egression of B cell precursor cells out of the bone marrow occurs due to the loss of essential CXCL12 retention signals. This, in part, is compensated by extramedullary B lymphopoiesis in the spleen, evidenced by increases in all precursor B cell populations. Final B-cell maturation is however hampered during infection by apoptosis of mature B-cells, most likely due to sVSG-induced inflammation. We hypothesise that early on in infection, NK and NKT cell activation regulates the balance between inflammatory and anti-inflammatory responses later on in infection. Indeed, using a PLC-KO trypanosome for infection has shown that by preventing the release of sVSG, both inflammation and B-cell compartment destruction are limited. The effect of infection-associated B-cell dysfunction in the context of vaccination efforts is currently under investigation.

T/L165 Modulation of dendritic cell antigen presentation by *Leishmania mexicana*

Owain R. Millington[1,2], Elmarie Myburgh[3], Muhannad Sweash[2], Helen McGachy[2], James Brewer[3], Graham Coombs[2], Paul Garside[3], Robin Plevin[2], Jeremy Mottram[3], James Alexander[2]
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Leishmania mexiciana causes a chronic, non-healing infection associated with an ability to subvert host immunity. Previous studies have identified *L. mexicana* cysteine peptidases (CP) as important virulence factors, which may facilitate immune evasion by skewing the immune response towards a Th2 phenotype and/or inhibiting Th1 generation. Importantly, this modulation may be associated with alteration of the processing and/or presentation of parasite derived antigens. Whilst much research has focused on the host macrophage, dendritic cells (DCs) are critical for induction of primary immune responses. We therefore developed a model to allow us to track parasites, monitor antigen presentation and assess the induction of parasite specific adaptive immune responses. We found that DCs readily phagocytose fluorescent *L. mexicana* parasites, but presentation of parasite-derived antigen could not be detected. This was independent of CPB expression, and was also irrespective of the localization of antigen expression. As a consequence, T cell responses to parasite antigen were not primed in vitro or in vivo. These data demonstrate the ability of *L. mexicana* to circumvent the antigen processing/presentation pathways and suggest an important mechanism independent of CPB that mediates immune evasion and allows the parasite to establish chronic infection.

T/L166 Complement 3 deficiency influence lesion progression and immune response during *Leishmania major* infection in C57BL/6 mice

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Complement and Fc receptors (FcR) are known to play an important role in *Leishmania* invasion of mammalian host cells in vitro and cell recruitment to sites of infection. In this work, infection of complement 3 deficient mice ($C3^{-/-}$) with *L. major* resulted in an enhanced immune response and earlier healing of leishmanial lesions compared to wild type mice. This enhancement was abolished when FcR γ chain was also lacking. Infection of C3/FcR γ chain/ FcγRIIb triple knockout mice with *L. major* resulted in a further increased resistance compared to wild type and $C3^{-/-}$ mice, while lesion progression in FcγRIIb, FcR γ chain/ FcγRIIb and FcR γ chain deficient mice were not different from wild type mice. Comparable levels of parasite loads were detected in all strains 2 weeks post-infection. However, a significant reduction in the parasite loads was observed 4 weeks post infection in both C3 and triple knockout mice. This reduction was accompanied with higher IgG2a levels in the serum of the triple knockout mice but not in C3 -/- mice. These results suggest that different FcR play contradictory roles in modulating the immune response to *L. major* infection in the absence of complement 3 in vivo.

T/L167 Interleukin-13 reduces hyperalgesia and the level of Interleukin-1β in BALB/c mice infected with *Leishmania major* and upregulates the level of Interleukin-6

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The anti-inflammatory cytokines interleukin-10 (IL-10) and interleukin-13 (IL-13) were shown to be hypoalgesic in some models such as rats exposed to UV rays. In addition, IL-10 was also shown to reduce hyperalgesia in high dose of *Leishmania major*-induced inflammation in BALB/c mice accompanied by a significant decrease in the levels of interleukin-1β (IL-1β) in the paws of infected mice, while no effect on the levels of IL-6 was observed. In this study, we injected BALB/c mice with a high dose of *Leishmania major* and treated them with IL-13 (15 ng/animal) for 2 consecutive weeks (except during the weekends) and hyperalgesia was assessed using thermal pain tests. Furthermore, the levels of IL-1β and IL-6 were also assessed at different post-infection

days. Our results show that IL-13 is more efficient in reducing hyperalgesia than IL-10 and that it can reduce the levels of IL-1β during the whole experiment (21 days). Interestingly, our data suggest that IL-13 leads to the upregulation of the levels IL-6 which initially seems to have no direct role in the induced hyperalgesia.

Session 6E - Mixed Session I (Chair: Karen Grant)

T/L168 Dyskinetoplastic trypanosomes: how to live without mitochondrial DNA

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Although the organization of mitochondrial DNA into a structure called kinetoplast is the defining feature of the *Kinetoplastida*, there are cases where kinetoplast DNA (kDNA) has been lost, giving rise to dyskinetoplastic forms. Examples are the naturally occurring (sub)species *Trypanosoma brucei evansi* and *T. b. equiperdum*. Since replication and expression of kDNA are normally essential processes in *T. brucei*, the dyskinetoplastic forms must have developed mechanisms to compensate for the essential mitochondrial gene product(s).
We are currently testing the hypothesis that mutations in the nuclearly encoded gamma subunit of the ATP synthase complex can surpass the requirement for a mitochondrial genome in *T. brucei*. Evidence will be presented suggesting that a single point mutation in this subunit is indeed necessary and sufficient to permit survival of bloodstream *T. brucei* in the absence of kDNA.

T/L169 Characterization of the Trypanosome Orthologue of DIP13, a Protein Implicated in Sjögren's Syndrome

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TbDIP13 is the *T. brucei* orthologue of a *Chlamydomonas* basal body protein implicated in flagellum biosynthesis. The human orthologue is NA14 (Nuclear Autoantigen for Sjögren's syndrome of 14 kDa), which is specifically implicated in the highly prevalent autoimmune disease Sjögren's syndrome. However, orthologues of this protein are not present in the common eukaryotic model systems *S. cerevisiae*, *C. elegans* and *Drosophila* and the biological function of NA14 remains unknown. TbDIP13 overexpression has a novel phenotype in *T. brucei* BSF which has been characterised using confocal and scanning immuno-electron microscopy. The endogenous protein is highly stable, localising to the cytoskeleton. Comparative proteomics have revealed 10 candidate binding partners of TbDIP13, including a centrin and a C2-domain protein, which are currently being validated. As a small protein (14 kDa) with over 70% predicted coiled-coil content and no other discernible motifs or domains, DIP13 could act as a mobile coiled-coil unit operating as a molecular switch for other molecules, or, in stable oligomer conformation, as a scaffold *e.g.* for the assembly of complexes. While previous studies on human cells showed that NA14 is essential for cell cycle progression, a viable double knockout line has been produced in *T. brucei* BSF. Comprehensive analysis of this cell line is now in progress.

T/L170 Assessing 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 important in infection dynamics and contributes to ordered antigenic variation. The study of the differentiation between these forms, however, has been hindered by the lack molecular markers for the stumpy life-stage.
PAD1 is a recently identified stumpy-specific surface protein. The identification of this molecule allows investigation of stumpy-formation, parasite infection-dynamics and gene expression control in the transmissible form of the life-cycle. To monitor stumpy formation *in vitro,* reporter cell lines

coupling the chloramphenicol acetyltransferase (CAT) reporter gene to the *PAD1* 3'UTR have been created in pleomorphic cells (i.e. cells capable of stumpy formation). These cells showed increased CAT expression upon differentiation to the stumpy life-stage, confirming appropriate reporter gene regulation. In a monomorphic line (normally unable to generate stumpy forms), treatment with compounds reported to induce stumpy formation *in vitro* also generated an increase in CAT expression. Hence, these cell lines enable compounds able to induce stumpy formation to be investigated.
The expression of PAD1 has also been used to monitor stumpy formation *in vivo* by a quantitative RT-PCR assay. These data will allow understanding of the kinetics of stumpy formation *in vivo* and a modelling of the slender/stumpy dynamic during a chronic infection.

T/L171 Blocking synthesis of the FACT subunit Spt16 causes cell cycle specific derepression of the ES-promotor in *Trypanosoma brucei*

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The surface of *Trypanosoma brucei* is covered with a dense coat of Variant Surface Glycoprotein (VSG), which is encoded by one out of more than 1,000 VSG genes. The active VSG is transcribed from one of about 15 telomeric VSG expression sites (ES). The monoallelic expression of ESs appears to be controlled at the level of transcription, although the mechanism responsible is still an enigma.
Spt16 is a subunit of the FACT (FAcilitates Chromatin Transcription) complex. In other eukaryotes, this histone remodelling complex is involved in various processes like replication, transcription and DNA-repair. Blocking synthesis of the *T. brucei* Spt16-homologue results in cells stalling after DNA replication but before chromosome segregation. This results in an accumulation of 2K1N cells with 4n DNA content. Specific to this G2-phase block is also a derepression of telomeric VSG-expression sites, both in bloodstream and procyclic forms. Interestingly, elevated levels of transcription are only observed around the ES-promoter, but do not proceed to the telomere end. These data indicate that the FACT complex plays a role in chromosome segregation as well as ES silencing in *Trypanosoma brucei*, probably by influencing chromatin structure.

T/L172 Evolutionary reconstruction of the retromer complex and its function in *Trypanosoma brucei*

V. Lila Koumandou*, Emily Herman[$], Mary Klute[$], Ricardo Nunez Miguel**, Joel B. Dacks[$] & Mark C. Field*
*Departments of Pathology and **Biochemistry, University of Cambridge, Tennis Court Road, Cambridge, UK; [$]Department of Cell Biology, University of Alberta, Edmonton, Alberta, Canada

Intracellular trafficking and protein sorting are closely controlled by specific protein complexes. The retromer complex is involved in endosomal sorting, particularly retrograde traffic from the endosome/lysosome to the Golgi complex. Retromer, identified initially in *Saccharomyces cerevisiae*, has since been characterised in considerable detail in yeast and mammals. Here, comparative genomics and phylogenetic reconstruction of retromer across all six eukaryotic supergroups were used to probe the early evolution of retromer and its function(s) across all eukaryotes. Our results indicate that the retromer complex is a universal, and ancient, feature of eukaryotic cells. However, the cargo of the retromer shows considerable variability between lineages. *In silico* analysis was combined with functional characterisation of retromer in the divergent eukaryote *Trypanosoma brucei*, the causative agent of African sleeping sickness. Our earlier transcriptome analysis[1] identified the retromer complex as important for developmental control of membrane trafficking, with a putative role in pathogenesis of the parasite. Our results indicate that the endocytic localisation of retromer is conserved in kinetoplastids, although its precise role in the cell remains elusive because of the lack of the classical cargo molecules described in yeast and mammals.
[1] Koumandou *et al.*, 2008 BMC Genomics 9:298

T/L173 Ubiquitin-mediated mechanisms for turnover of invariant surface glycoproteins in *Trypanosoma brucei*

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The invariant surface glycoprotein (ISG) 65 and ISG75 families are trans-membrane domain (TMD) anchored proteins exclusively expressed in the bloodstream form of Trypanosoma brucei. ISG65 and ISG75 possess highly conserved cytoplasmic lysines and are potential sites for ubiquitylation, which can act as an endocytosis and/or protein degradation signal. Previously we demonstrated that internalisation and degradation of ISG65 depends on post-translational ubiquitylation of site-specific cytoplasmic lysines. Using a reporter protein, BiPNHA, containing the TMD and cytoplasmic domain of ISG75, we now show that this mechanism extends to ISG75. Furthermore, ISG75 lysine null mutant not only abolished ubiquitylation but also led to near-complete inhibition of protein turnover and stabilisation on the cell surface. To identify E3 ubiquitin ligases involved in ISG regulation, we used the sequences of Rsp5/NEDD4 and c-Cbl, E3 ligases involved in the down-regulation of surface TMD proteins in higher eukaryotes, to interrogate the T. brucei genome. Absence of orthologues prompted further in silico analysis of the ubiquitylation machinery, which revealed that while the E1 and E2 class proteins are well conserved across the eukaryotes, the E3 system in T. brucei is highly divergent. In summary, ubiquitin-mediated endocytosis is a general mechanism of TMD protein internalisation and turnover in T. brucei.

THURSDAY 1ST APRIL

Session 7E - Molecular Epidemiology and Population Genetics (Chair: J-C Dujardin)

T/L174 Invited Speaker

Epidemiological and population genetic studies in the *L. donovani* complex

Gabriele Schönian, Katrin Kuhls, Ahmad Amro, Mohammad Z. Alam, Tesfaye Gelanew
Institute of Microbiology and Hygiene, Charité University Medicine Berlin

Analysis of length polymorphisms of microsatellite sequences has become an important tool for population and genetic studies for *Leishmania* species. Highly discriminatory multilocus microsatellite typing (MLMT) based on variable numbers of repeats in 14-15 microsatellite markers has been applied to 229 strains of *L. donovani* from East Africa and the Indian subcontinent and 398 of *L. infantum* from the Mediterranean and South America. MLMT can be applied directly on biological materials without cultivation of the parasite.
Analysis of MLMT data showed hierarchical population structures in different foci. Six main genetically distinct populations were detected in the *L. donovani* complex according to place and time of strain isolation, two populations comprising *L. infantum* MON-1 and non-MON-1 strains, respectively; two populations of *L. donovani* from Sudan and Ethiopia; one of *L. donovani* MON-2 from India; and one consisting of strains of *L. donovani* from Kenya and India. MLMT allowed further differentiation of strains belonging to the predominating zymodeme of *L. infantum*, MON-1. The MON-1 strains from southwest Europe and South America were clearly separated from those of eastern and southern Mediterranean provenience and both populations were further subdivided. The detection of hybrid profiles, of mosaic and heterozygous genotypes, of high inbreeding within and gene flow between the populations pointed to at least occasional recombination events.

T/L175 Diversity within *Trypanosoma (Duttonella) vivax* revealed by Fluorescent Fragment Length Barcoding, FFLB

Emily Adams[1,2], Patrick Hamilton[3], Adriana Rodrigues[4], Imna Malele[5], Vincent Delespaux[6], Marta Teixeira[4], Wendy Gibson[1]
[1]University of Bristol, UK; [2]Royal Tropical Institute, Netherlands; [3]University of Exeter, UK; [4]University of Sao Paulo, Brazil; [5]Tsetse and Trypanosomiasis Research Institute, Tanzania; [6]Institute of Tropical Medicine, Belgium

Tsetse-transmitted trypanosomes pose a substantial threat to livestock, but their full diversity is not known. DNA samples from infected proboscides of *Glossina pallidipes* and *G. swynnertoni* collected in Tanzania were identified using FFLB, which discriminates species by size polymorphisms in multiple regions of the ribosomal RNA locus. FFLB identified 61.9% of proboscis infections: 2 FFLB profiles were similar but not identical to reference West African *T. (D.) vivax*, while 5 other profiles belonged to known trypanosome species. Phylogenetic analysis of the glycosomal glyceraldehyde phosphate dehydrogenase (gGAPDH) gene revealed that the Tanzanian *T. (D.) vivax* samples fell into 2 distinct groups, both outside the main clade of African and South American *T. (D.) vivax*. These new *T. (D.) vivax* genotypes were common and widespread in tsetse in Tanzania. In trypanosome phylogenetic trees, subgenus *Duttonella* now forms a major clade with 3 subclades, demonstrating higher levels of diversity within the subgenus than previously realised. Investigation of mammalian host range and pathogenicity will reveal the importance of these new *T. (D.) vivax* genotypes for the epidemiology and control of animal trypanosomiasis in East Africa.

T/L176 Re-assessment of role of domestic animals in the epidemiology of kala-azar in Nepal

Narayan Raj Bhattarai[1,2,5], Albert Picado[3], Gert Van der Auwera[2], Suman Rijal[1], Niko Speybroeck[2,4], Basudha Khanal[1], Bart Ostyn[2],Clive Davies[3], Marc Coosemans[2,5], Marleen Boelaert[2], Jean-Claude Dujardin[2,5]
[1]B.P. Koirala Institute of Health Sciences, Dharan, Nepal; [2]Institute of Tropical Medicine, Antwerpen, Belgium; [3]London School of Hygiene and Tropical Medicine, London, UK; [4]Institute of Health and Society, Université Catholique de Louvain, Belgium; [5]Department of Biomedical Sciences, University of Antwerp, Belgium

In the Indian subcontinent visceral leishmaniasis (VL) is considered an anthroponosis, although reasons for its persistence in inter-epidemic periods are debated. Within the frame of a bednet intervention study (www.kalanet.org), we mapped *Leishmania* infections among healthy humans and animals in an active VL transmission focus in Nepal. Samples were collected over a 4-months period from humans, goats, cows and buffaloes in a peri-urban ward of Dharan (Terai region). *Leishmania* infections were assessed by PCR. We found infections among humans (6.1%), cows (5%), buffaloes (4%) and goats (16%). Data were geo-referenced and entered into a Geographical Information System. The bivariate K-function results indicate a spatial clustering of human and domestic animal PCR-positives. The classification tree analysis determined that among several risk factors possibly involved in PCR-positivity among humans, goat PCR-positivity ranked as the first one. These results were confirmed by a parallel serological study. Though our data do not necessarily mean that goats constitute a reservoir host of *L. donovani*, these observations warrant further investigation of their possible role in VL transmission.
Financial support: European Union, INCO-DEV KALANET project (EU contract no 015374)

T/L177* Is vector-parasite interaction a determining factor for the population structure of *L. donovani* in East Africa?

Tesfaye Gelanew[1], Katrin Kuhls[1], Asrat Hailu[2], Gabriele Schoenian[1]
[1]Institut Institute of Microbiology and Hygiene, Charité University Medicine Berlin, Germany; [2]Faculty of Medicine, Addis Ababa University

In the Horn of Africa, two phlebotomine sand fly species have been implicated to transmit parasites of the *L. donovani* complex: *Phlebotomus orientalis* in northwest Ethiopia (NWE) and Sudan, and *P. martini* in southwest Ethiopia (SWE) and Kenya. It remains to be established

whether the differences in biology and ecology of these sand fly vectors may have consequences for the population structure of the *L. donovani* parasites they harbor and transmit. We investigated 64 strains of *L. donovani* newly isolated from VL cases in the two main foci in Ethiopia, NWE and SWE, by using 14 highly polymorphic microsatellite markers and compared the microsatellite profiles obtained to those of *L. donovani* strains from Sudan, Kenya and India. Multilocus microsatellite based population genetic analysis placed strains from SWE and Kenya (n=31) in one population and strains from NWE and Sudan (n=66) in another population. The two genetically separated populations corresponded to the areas where the two different sand fly species are prevalent. High inbreeding was found in strains isolated in SWE and Kenya. Whether the two genetically distinct populations in Ethiopia truly reflect different parasite-vector associations needs further investigations which should involve more strains from the focus in South Omo where the two vectors overlap.

T/L178* The effectiveness of a targeted re-treatment intervention in reducing the prevalence of trypanosomiasis in cattle in Uganda.

Louise Hamill[1], Richard Selby[1], Christine Amongi Acup[1,2], Beatrix Von Wissman[1], Kim Picozzi[1], Sue Welburn[1]
[1]Centre for Infectious Diseases, The University of Edinburgh, Edinburgh, UK; [2]Makere University, Kampala, Uganda

The “Stamp Out Sleeping Sickness (SOS)” programme was launched in 2006, and aims to halt northward spread of *Trypanosoma brucei rhodesiense* in Uganda by mass trypanocidal treatment of the cattle reservoir. Phase 1 targeted the most northerly of the newly affected districts. Post-treatment monitoring revealed a cluster of villages in which *T. b. rhodesiense* remained present in the cattle reservoir. The villages were located close to one another and within parishes that continued to report human sleeping sickness cases, indicating transmission may not have been properly interrupted. Subsequently, re-treatment of this high risk area was undertaken.
This work assesses the impact of the SOS re-treatment intervention on the prevalence of *T. vivax*, *T. b. brucei* and *T. b. rhodesiense* by analyzing cattle blood samples from 20 villages within the re-treatment area. Samples were taken immediately before and six months after re-treatment. Samples were then subjected to PCR based methods for the detection of parasite DNA.
The results of this analysis show the re-treatment programme was successful in reducing the overall prevalence of trypanosomiasis in the targeted area. A significant drop in trypanosome prevalence was observed between the baseline and six month samples, both overall and in each individual species detected.

Session 8E - Sex in the Vector and in the Field (Chair: Wendy Gibson)

T/L179 Invited Speaker
Demonstration of a Leishmania Sexual Cycle in the Sand Fly Vector

[1]David Sacks, [2]Natalia Akopyants, [1]Nicola Kimblin, [1]Phillip Lawyer, [1]Dia-eldin Elnaiem, and [2]Stephen Beverley.
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Based on the strong linkage disequilibrium displayed by Leishmania species, these parasites are argued to be essentially clonal. This notion must be reconciled, however, with the accumulating examples of naturally occurring hybrid strains, which appear to share phenotypic and genotypic markers from two perceived species, providing circumstantial evidence for sexual recombination. Clearly, the clonality vs sexuality debate would be far better informed if Leishmania were actually shown to be capable of genetic exchange. By co-infecting sand flies with two parental lines of Leishmania bearing distinct drug resistant markers, we have explored the possibility of genetic exchange occurring during the growth and development of extracellular stage *L. major* promastigotes in the midgut of a natural sand fly vector species, *Phlebotomus duboscqi*. We provide the first conclusive evidence that the invertebrate stages of Leishmania are fully capable of a sexual cycle, generating both diploid and triploid progeny bearing hybrid genotypes that

strongly suggest meiotic division and uniparental inheritance of maxicircle kDNA. Fluorescent reporter genes have also been used to screen for genetic hybrids, including backcross hybrids generated between F1 progeny and each parental line. The frequency, timing, and anatomical location of hybrid formation within the midgut, and most importantly, the existence of a haploid, gamete stage are currently being explored.

T/L180 Visualizing meiosis in trypanosomes

Lori Peacock[1,2], Reuben Sharma[3], Vanessa Ferris[1,2], Mick Bailey[2], Mark Carrington[3] and Wendy Gibson[1]
[1]Biological Sciences, University of Bristol, BS8 1UG, UK; [2]Clinical Veterinary Science, University of Bristol, BS40 7DU, UK; [3]Biochemistry, University of Cambridge, CB2 1GA, UK

Trypanosoma brucei undergoes genetic exchange in the salivary glands of the tsetse vector by an unknown mechanism. The process undoubtedly involves meiosis and fusion, but details are lacking. Investigation is constrained by the difficulties of working with trypanosomes within the vector and the rarity/transience of putative intermediates. However, several key meiosis genes such as SPO11, MND1, DMC1 and HOP1 have been identified in the trypanosome genome by sequence homology. These genes were tagged with a gene for yellow fluorescent protein (YFP) so that any trypanosomes expressing the proteins could be identified by fluorescence microscopy in situ within dissected tsetse organs. YFP fluorescence was observed solely in trypanosomes from the salivary glands, not from the midgut or proventriculus, and importantly was localized to the nucleus, consistent with the various roles of these proteins in binding to chromosomes during meiosis. The unequivocal identification of trypanosomes undergoing meiosis will allow a full description of these cell types and allow us to study their interactions.

T/L181 Origins of natural *Trypanosoma cruzi* hybrid lineages

Michael Lewis[1], Martin Llewellyn[1], Matthew Yeo[1], Nidia Acosta[2], Michael Gaunt[1], Michael Miles[1]
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The single celled eukaryote *Trypanosoma cruzi* is a mammalian parasite transmitted by dozens of species of triatomine bug in the Americas. It is also the cause of Chagas disease in infected humans. *T. cruzi* generally maintains itself by asexual binary fission and appears to have a clonal population structure. However, at least two of the six major circulating genetic lineages, TcV (TcIId) and TcVI (TcIIe), are hybrid and are frequently isolated from humans. Nevertheless, a prevalent view is that hybridisation events in *T. cruzi* were evolutionarily ancient and that active recombination is of little or no epidemiological importance. We analysed the genotypes of a large panel of *T. cruzi* strains for markers representing three distinct evolutionary rates: nuclear *GPI* sequences, mitochondrial *COII-ND1* sequences and 28 polymorphic microsatellite loci. Using Maximum Likelihood and Bayesian phylogenetic approaches we dated key evolutionary splits in the *T. cruzi* clade including the emergence of the hybrid lineages TcV and TcVI, which we estimated to have surprisingly recent common ancestors, dated to within the last 60,000 years. The clearly distinguishable microsatellite genotypes of TcV and TcVI were highly heterozygous and almost totally lacking in intra-lineage diversity. Overall, we infer two independent hybridisation events and a recent, rapid spread into domestic transmission cycles concomitant with, or as a result of, disruption of natural transmission cycles by human activities.

T/L182 *Trypanosoma cruzi* population genetics: progress and new perspectives from multilocus microsatellite typing (MLMT)

Martin S Llewellyn, Michael D Lewis, Sofia Ocana, Jamie Costales, Matthew Yeo, Louisa Messenger, Michael W Gaunt, Mario Grijalva & Michael A Miles
The London School of Hygiene and Tropical Medicine, Keppel Street, London, WC1E 7HT

Chagas disease is a major cause of death in many Latin American countries. The etiological agent, *Trypanosoma cruzi*, is ancient and diverse, with six major genotypes reported, now reclassified by international consensus as TcI-TcVI. Our understanding of the genetics and evolution of this organism has improved in recent years with the sequencing of a reference genome, discovery of an *in vitro* mechanism for genetic recombination and identification of

naturally occurring hybrids. However, the dynamics of *T. cruzi* at the population (within DTU) level, including the frequency and mechanism of recombination, remain unclear. We have developed a versatile MLMT system for *T. cruzi* that allows unprecedented insight into the population genetics of this pathogen in both sylvatic and domestic transmission settings. We describe current work aimed at using MLMT as a tool to address community level epidemiological questions, genetic recombination, multiclonality and parasite infrapopulation structure.

POSTER TITLES

MALARIA

P1 * Multiple Targets of Antimalarial Bis-Cations and Delayed Parasite Death
Archana Kaniti**,** Paul Stocks, Patrick Bray, Steve Ward & Henri Vial
P2 Effect of artemisinin derivatives on endothelial cells: role of hypoxia
Sarah D'Alessandro, Nicoletta Basilico, Yolanda Corbett, Sara Finaurini, Marilena Scaltrito & Donatella Taramelli
P3 A Novel Drug for Uncomplicated Malaria: Targeted High Throughput Screening (HTS) against the type II NADH:Quinone oxidoreductase (PfNDH2) of *Plasmodium falciparum*
Giancarlo A Biagini, Alasdair Hill, Alison Mbekeani, Alison Shone, Gemma Nixon, Paul Stocks, Peter Gibbons, Richard Amewu, W David Hong, Victoria Barton, Chandra Pidathala, James Chadwick, Louise Le Pensee, Ashley Warman, Raman Sharma, Nick Fisher, Neil G. Berry, Paul M. O'Neill & Steve A. Ward
P4 Malaria in mothers and young children in Uganda
Martha Betson, Mary Oguike, Jose Figueiredo, Narcis Kabatereine, Colin Sutherland & J.Russell Stothard
P5 Post-adhesion signaling by malaria infected red blood cell bound human endothelial cell
Kwannan Nantavisai, Parnpen Viriyavejakul, Giancarlo Biagini & Stephen Ward
P6 Molecular & Biochemical Characterisation of Atovaquone Resistance in the Malaria Parasite *Plasmodium falciparum* (TM90-C2B)
Abd Majid. Roslaini, Nick Fisher, Ashley Warman, Hilary Ranson, Stephen A Ward & Giancarlo A Biagini
P7 Antiparasitic activity and ADME-Tox profile of new 4- aminoquinoline derivatives Chiara Rusconi, Anna Sparatore, Nicoletta Basilico, Sergio Romeo, Nadia Vaiana, Manolo Casagrande, Luca Rizzi, Valerio Tazzari, Livia Vivas, Paola Verducci, Daniela Jabes and Donatella Taramelli
P8 Potential Role of Proteases in Endothelial Disruption in Severe Malaria
Steven L Ruscoe, David Eardley, Monique F Stins, Derek L Mattey & Srabasti J Chakravorty
P9* Anti-plasmodial and anti-inflammatory activity of medicinal plants used in Burkina Faso against malaria
Denise P. Ilboudo, Silvia Parapini, Nicoletta Basilico, Mario Dell'Agli, Richard Sawadogo, Jacques Simpore, Jean-Baptiste Nikiema, Enrica Bosisio & Donatella Taramelli
P10 Binding of endothelin-1 to the lipid moiety of haemozoin and *P. falciparum* parasitised red blood cells
Nicoletta Basilico, Silvia Parapini, Sarah D'Alessandro, Yolanda Corbett, Fausta Omodeo-Salè, Piero Olliaro & Donatella Taramelli

P11* Bioinformatic and proteomic analysis of *Plasmodium falciparum* succinate dehydrogenase
Thomas Antoine, Nicholas Fisher, Giancarlo Bialagini & Stephen Ward
P12* Heterogeneity in malaria exposure and vaccine response: implications for the interpretation of vaccine efficacy trials
Michael T White, Jamie T Griffin, Chris J Drakeley & Azra C Ghani
P13 Erythrocyte molecules in *Plasmodium falciparum* rosette formation
Clare Fennell, Ashfaq Ghumra & Alex Rowe
P14 MALACTRES: African-European Research Consortium dedicated to ACT drug-resistance and diagnostics
Henk Schallig (Royal Tropical Institute, Meibergdreef 39, 1105 AZ Amsterdam, The Netherlands) on behalf of the MALACTRES consortium
P15* Screening synthetic derivatives of buchtienine for antimalarial properties
Monnery D, Harper A, Allin S & Horrrocks P
P16 Erythrocyte Shape change prevents *Plasmodium falciparum* (Pf) invasion
Johnson N. Boampong, Sumie Manno, Ichiro Koshino & Yuichi Takakuwa
P17 Developing Novel Chemical Probes to Identify Cytochrome P450s Associated with Pyrethroid Resistance in Malaria Vectors
Hanafy M. Ismail, David W. Hong, Paul M. O`Neill, Janet Hemingway & Mark J.I. Paine
P18 Density dependence within the malarial mosquito and its possible impact on the success of transmission blocking interventions
Thomas S. Churcher, Emma J. Dawes, Robert E. Sinden, George Christophides, Jacob Koella & María-Gloria Basáñez
P19 In-Check™ platform: For Fast Detection of Malaria Species and Resistant Variants
Joseph Mugasa & Lisa Ranford-Cartwright
P20 Improvement of Rapid Diagnostics for Malaria: *In vitro* detection and stability of novel antigen targets
J.H. Kattenberg, I. Versteeg, P.F. Mens & H.D.F.H. Schallig

ECOLOGY AND GENERAL PARASITOLOGY POSTER TITLES

P21 Definition of liver fluke population groups by DNA polymorphisms
D. Teofanova, G. Radoslavov, P. Hristov, S. Walker, A. Trudgett, V. Kantzoura, G. Theodoropoulos & I. Bankov
P22 New metalloprotein of parasitic nematodes from the genus *Trichinella*
Petar Hristov, Georgi Radoslavov, Rositca Jordanova, Denica Teofanova, Eva Liebau & Ilia Bankov
P23 Naming Genes in the Parasitic Nematodes
Robin N. Beech, Joseph A. Dent, Cédric Neveu & Adrian J. Wolstenholme
P24 Parasitological collection at the Institute of Experimental Pathology and Parasitology, Bulgarian Academy of Sciences
Vladov I, Mizinska - Boevska I, Nedeva I, Radev V & Arnaudova E
P25 A state-dependent modelling framework for exploring the interactions between macroparasites and their intermediate hosts
Sean Rands
P26* An *in silico* pipeline for identification of molecular mimicry candidates from parasites
Philipp Ludin, Daniel Nilsson & Pascal Mäser
P27 Helminths and microparasites - a novel interaction
Lizeth Lacharme-Lora, Vyv Salisbury & Sarah E. Perkins
P28* Sero- prevalence of *Toxoplasma gondii* among butchers in Khartoum, Sudan
F. Abayzeed, T. Saber, R. Siddig & K. Mohamed
P29* Modelling the co-infection of malaria and lymphatic filariasis
Hannah Slater, Manoj Gambhir, Paul E. Parham & Edwin Michael
P30 Factors influencing rabbit parasite communities
Brian Boag, Isabella Cattadori, Alex Hermandez, Lisa Murphy & Joanne Lello
P31 Toxic metal accumulation in the hare-*Passalurus ambifuus* system from Bulgaria
V. Nanev, M Anisimova & M. Gabrashanska
P32* Is there any link between *Blastocystis* and irritable bowel syndrome (IBS)? Mohammed Alfellani & Graham Clark
P33 TORCH pathogen in high-risk Qatari patients
Marawan Abu-Madi

P34 Screening for *Echinococcus multilocularis* in red foxes from England
Irene A. Zimmer, Jane Learmount, Ruth Grant, Chris Conyers, Colin P. Morgan, Valerie Boughtflower, Elizabeth Lunn, Graham C. Smith, Barbara H. Craig & Mike A. Taylor
P35 Species identification of *Trichinella* isolates from Bulgaria
Svetlozara Petkova & Rositsa Milcheva
P36* Co-parasitism of rhinonyssid mites (Parasitiformes: Gamasina, Rhinonyssidae) in the nasal cavities of birds
O.M Butenko, M.K. Stanjukovich & I.Dimov
P37 Temporal and spatial variations in the ectoparasite community of the vole *Myodes glareoli*
A. Paziewska, P.D. Harris, L. Zwolinska & E. Sinski
P38 Saltatory dispersal of the troglotrematid digenean *Collyricloides massanae*
P.D. Harris, A. Paziewska, L. Zwolinska & E. Sinski
P39 Etiopathological aspects of protostrongylid infections in red deer
M. Panayotova-Pencheva & M. Alexandrov
P40* Studies on the life cycle and transmission of the trematode *Plagiorchis muris* at Malham Tarn, Yorkshire
Kellyanne Boyce, Helen Bradshaw, Mike Rogan, Adrian Pickles & Philip Craig
P41 *Lymnaea stagnalis* – intermediate host of some trematodes long the Danube River in Bulgaria
Hrusanov DV, Radev V, Nanev V, Manov K, Kanchev & P. Dimitrov
P42 Seroprevalence and zoonotic potential *of Neospora* species infection in Jordanian women with miscarriage
Mahmoud N. Abo-Shehada, Raida Khalil & Marwan Abu-Halaweh
P43* Investigating the effects of parasites on the immune system of the field vole, *Microtus agrestis*, in its natural environment
Amy J. Hall, Joseph A. Jackson, Catriona Ralli, Malgorzaka Zawadzka, Ann Lowe, Steve Paterson, Richard Birtles, Xavier Lambin, Michael Begon & Jan E. Bradley
P44* Correlation of Regulatory T cells with resistance and susceptibility to *Teladorsagia circumcincta* infection in lambs
Virginia M.Venturina, Anton G. Gossner, David W. Taylor & John Hopkins
P45 Research on the protective properties of haemocyanin from *Helix vulgaris (HvH)* and its derivates against infection with *Trichinella spiralis*
Liliya Yossifova, Ivan Iliev, Elena Gardeva, Pavlina Dolashka-Angelova, Vesela Moshtanska, Lyudmila Velkova & Siya Zacharieva

P46 Study on the immune properties of *Rapana venosa* hemocyanin and its application as experimental anti-parasitic vaccine

I. Iliev, L. Yosifova, E. Gardeva, P. Dolashka – Angelova & S. Zacharieva

P47 Evasion, suppression and anticipation of the host's immune responses: can filarial nematodes be defeated by a vaccine?

Simon A. Babayan, Andrew F. Read, Odile Bain & Judith E. Allen

P48 Complete absence of the GPI biosynthetic pathway in *Trichomonas vaginalis*

Yuk-Chien Liu, Jeremy C. Mottram & Alvaro Acosta-Serrano

P49* Characterization og gene family encoding EG95 protein in Echinococcus granulosus **from G6/G7 genotypes**

Cristian Alvarez, Charles Gauci & Marshall Lightowlers

P50* Simple visual discrimination of a new diagnostic for sleeping sickness

Sally Wastling, Kim Picozzi & Sue Welburn

P51* Purine Transport in *Trichomonas vaginalis*

Manal J. Natto, Vishnu Karra, Wassem Ahmed, Neils B. Quashie & Harry P. de Koning

P52* Mapping risk foci for endemic sheep scab

H. Rose, J. Learmount, M. Taylor & R. Wall

P53* Nucleic acid amplification tests for *Trypanosoma congolense*: LAMP (loop- mediated isothermal amplification) versus PCR

Mathieu Vanhove, Sally Wastling, Louise Hamill, Kim Picozzi & Sue Welburn

P54 *Angiostrongylus costaricensis* egg antigen for the immunodiagnosis of abdominal angiostrongyliasis

Paolo Mesén-Ramírez, Elizabeth Abrahams-Sandí, Katherine Fernández-Quesada & Pedro Morera

P55* Garlic: a potential cure for hole-in-the-head disease in fish?

Catrin Williams, Coralie Millet, Jo Cable, Mike Coogan, David Lloyd & David Williams

P56* Uptake of Quantum-dots: a comparison of feeding by *Spironucleus vortens* and *Giardia intestinalis*

Coralie Millet, Anthony Hayes, Dan Matthews, Huw Summers, Jo Cable & David Lloyd

P57 Short interfering RNA-mediated knockdown of drosha and pasha in undifferentiated *Meloidogyne incognita* eggs leads to embryonic lethality

Johnathan J. Dalzell, Neil D. Warnock, Michael A. Stevenson, Angela Mousley, Colin C. Fleming, Aaron G. Maule

P58 Variation in parasite fitness of *Schistosoma haematobium* genotypes in Mali

C. M. Gower, A. Gabrielli, M. Sacko, R. Dembelé, R. Golan, A. Emery, D. Rollinson & J.P. Webster

P59 Recombinant SmNPP-5 induces antibodies that partially inhibit the enzymatic activity but fail to prevent the infection with *Schistosoma mansoni*
Henrique K. Rofatto, Leonardo P. Farias, Cibele A. Tararam, Bogar Omar Araujo Montoya, R. Alan Wilson & Luciana C.C. Leite
P60* Detection of DNA-Methylation in *Schistosoma manoni*
Kathrin K. Geyer, Carlos M. Rodriguez, Michael J. Wilkinson & Karl F. Hoffmann
P61 Release of Apoptosis inducing factor is one of the early events of focal apoptosis in skeletal muscle cell due to trichinellosis in mice
R. Milcheva, S. Petkova, Z. Hurniková, P. Janega & P. Babál
P62* Development of a diagnostic test for active sheep scab infestation based on biomarkers
Beth Wells
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P93 Infection of host macrophages with Leishmania involves a new ABCG transporter implicated in phosphatidylserine exposure

Jenny Campos-Salinas, David León-Guerrero, Santiago Castanys, José M. Pérez-Victoria & Francisco Gamarro

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P103* Characterisation of unusual ATG8-like proteins in *Leishmania major*

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P104 The autophagic response is recruited and required by *Trypanosoma cruzi* GP82-mediated cell invasion

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P108* Global climate changeability: Pervasiveness of visceral leishmaniasis by sand-fly

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P109* Exploiting a luciferase reporter system for rapid *Trypanosoma cruzi* amastigote drug screening

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P110* Structure-activity relationship of the High Affinity Pentamidine Transporter in *Trypanosoma brucei*

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P111* Complex melarsoprol: a possible oral therapy for Human African trypanosomiasis

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P112 Bisphosphonium salt analogues against *Trypanosoma brucei brucei* bloodstream form

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P113 Synthesis and antiprotozoal activity of *N*-alkoxy derivatives as possible prodrugs of bis(2-aminolimidazolinium) lead compounds

Carlos H. Ríos Martínez, Lidia Nieto, Ainhoa Mascaraque, Marcel Kaiser, Reto Brun & Christophe Dardonville

P114 African Animal Trypanosomiasis: are multidrug resistance extrusion systems involved in drug resistance?

Vincent Delespaux, Hervé Vitouley, Tanguy Marcotty, Niko Speybroeck, Dirk Berkvens, Krisna Roy, Stanny Geerts & Peter Van den Bossche

P115 Molecular analysis of pentamidine transporters in *Trypanosoma brucei*

Jane C Munday; Ibrahim Teka; Richard JS Burchmore; Michael P Barrett & Harry P de Koning.

P116 Assessment of choline-derived analogs as new anti-kinetoplastid lead compounds

Hasan M. S. Ibrahim, Mohammed I. Al-Salabi, Neils B. Quashie, Abdulsalam A. M. Alkhaldi, Roger Escale, Terry K. Smith, Henri J. Vial & Harry P. de Koning

P117 New bisphosphonium salt derivatives are potent antiprotozoal agents: in vitro activity against *Trypanosoma brucei*, *Trypanosoma cruzi*, *Leishmania donovani*, and *Plasmodium falciparum*

Christophe Dardonville, Eddysson Flores Pérez, Alan Healy, Carlos H. Ríos Martínez, Marcel Kaiser,Reto Brun, Abdulsalam M. Alkhaldi & Harry de Koning

P118 Parasitic loads in tissues of *Trypanosoma cruzi*-infected mice treated with AmBisome

Sabrina Cencig, Nicolas Coltel, Carine Truyens & Yves Carlier

P119 New Anti-malarial drug therapy? Efficacy of Pyronaridine-Artesunate Combination *in vivo*

Hollie Lander, Simon Croft & Livia Vivas

P120 RNA editing as a drug target in trypanosomes: identification of inhibitors of the essential enzyme REL1 by virtual screening

Laurence Hall, Jacob D. Durrant, Rommie Amaro & Achim Schnaufer

P121 Population genetic structure of Central African *Trypanosoma brucei gambiense* isolates using microsatellite DNA markers

Gustave Simo, Flobert Njiokou, Christopher Tume, Smiths Lueong, Thierry De Meeûs, Gerard Cuny &Tazoacha Asonganyi

P122 Patterns of population genomic variation in *Leishmania donovani*

Tim Downing, Hideo Immamura, Saskia Decuypere, Christiane Hertz-Fowler, Jean-Claude Dujardin & Matt Berriman

P123* Functional analysis of species-specific genes that may contribute to *Leishmania* tropism

Yerim Her, Samuel O. Oyola, Michael R. Hodgkinson & Deborah F. Smith

P125 The Genome of *Leishmania mexicana*

Matthew B Rogers, Jim D Hilley, Daniel P Depledge, Kathy Seeger, David Harris, Jeremy C Mottram, Deborah F Smith, Christiane Hertz-Fowler & Matthew Berriman

P126* The novel AT-hook protein ETR1 is involved in transcriptional control in *Trypanosoma brucei*

Mani Shankar Narayanan, Manish Kushwaha, Alexander Fullbrook, Klaus Ersfeld, Tara Stanne & Gloria Rudenko

P127 GPEET procyclin regulation: a nucleolar affair?

Gabriela Schumann, Patricia Araujo & Isabel Roditi

P128 HAT3 and SIR2rp1 control RAD51-dependent DNA repair in African trypanosomes

Lucy Glover & David Horn

P129 Visualizing mating in trypanosomes: what are they getting up to in the fly?

Lori Peacock, Jack Sunter, Vanessa Ferris, Mick Bailey, Mark Carrington & Wendy Gibson

P130* Standardisation of NASBA method by using 18S rRNA gene for identification of *Leishmania major* parasite

Shahnaz Shirbazuo, Abdolhossein Dalimi, Fatemeh Ghaffarifar, Mehdi Furozandeh Moghadam

SPRING MEETING POSTER ABSTRACTS

MALARIA

P1 * Multiple Targets of Antimalarial Bis-Cations and Delayed Parasite Death

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The Vial group in Montpellier has developed several series of bis-cations with potent antimalarial activity, including quaternary ammonium molecules, alkyl amidines and guanidines. All these compounds have been shown to inhibit the synthesis of phosphatidyl choline in the parasite. This prevents the synthesis of new membranes and kills the parasite. In Liverpool, we have developed a related range of compounds, based on the bis-benzyl amidine structure. Interestingly, these compounds appear to work in a very different way to the bis-cations developed by the Vial group. The bis-benzyl amidines act more like chloroquine: ie by binding to heme and interfering with the production of hemozoin crystals in the parasite.
We have undertaken a study of the heme-binding properties of bis-quarternary ammonium compounds and alkylamidines from the Vial group. The strength of the interaction with heme and the ability of these compounds to inhibit the production of hemozoin crystals were measured. These results are compared to results obtained with the Liverpool compounds and indicate that the Vial compounds have a dual mode of action, hitting both hemozoin crystallization and phosphatidyl choline synthesis. As the membrane synthesis is inhibited at much lower concentrations than heme crystallization we propose that heme binding functions as a drug reservoir mechanism to drive and maintain drug uptake at very high levels in the parasite, possibly explaining the “delayed death” effects of these drugs.

P2 Effect of artemisinin derivatives on endothelial cells: role of hypoxia

Sarah D’Alessandro, Nicoletta Basilico, Yolanda Corbett, Sara Finaurini, Marilena Scaltrito, Donatella Taramelli
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Artemisinin derivatives are the most effective and safe antimalarial drugs available. They possess anti-angiogenic and antitumor activity as well. We reported that the inhibition of growth and differentiation of endothelial cells (EC) depends on the artemisinin derivative used (D’Alessandro 2007). We extended here these observations by evaluating the role of hypoxia in the response of EC to artemisinins.
Hypoxia plays a crucial role both in malaria and tumours and is a potent signal for EC itself. In severe malaria, microvascular occlusion by infected red blood cells generates a hypoxic microenvironment. In cancer, the growth of tumour mass causes a failure in the oxygen and nutrients supply and generates hypoxia.
We found that dihydroartemisinin (DHA), the active metabolite of most artemisinins, causes a time and dose-dependent inhibition of EC growth which is influenced by the oxygen tension: at low doses (those found in plasma of malaria patients) DHA is more active in hypoxia, whereas at high doses (those proposed as antiangiogenic and antitumor) it is more effective in normoxia. At high doses DHA induces EC apoptosis and affects the cell cycle via oxidative stress. At low doses cytostasis seems to be present since neither apoptosis nor oxidative stress were detected. The implication of these findings for therapeutic regimens with artemisinins is under discussion.
Financial support by EU18834AntiMal is acknowledged

P3 A Novel Drug for Uncomplicated Malaria: Targeted High Throughput Screening (HTS) against the type II NADH:Quinone oxidoreductase (PfNDH2) of *Plasmodium falciparum*.

Giancarlo A Biagini[1], Alasdair Hill[1], Alison Mbekeani[1], Alison Shone[1], Gemma Nixon[1], Paul Stocks[1], Peter Gibbons[2], Richard Amewu[2], W David Hong[2], Victoria Barton[2], Chandra Pidathala[2], James Chadwick[2], Louise Le Pensee[2], Ashley Warman[1], Raman Sharma[2], Nick Fisher[1], Neil G. Berry[2], Paul M. O'Neill[2], Steve A. Ward[1].
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PfNDH2 represents a metabolic choke point in the respiratory chain of *Plasmodium falciparum* mitochondria and is the focus of a drug discovery programme towards the development of a novel therapy for uncomplicated malaria. Here we describe a miniaturised assay for recombinant PfNDH2 with robust assay performance measures that has been utilised for the high throughput screening (HTS) of small molecule inhibitors. The objectives of the HTS were to (i) Increase the number of selective PfNDH2 inhibitors and (ii) to expand the number of inhibitor chemotypes. At the time of screening, only one proof of concept molecule, 1-hydroxy-2-dodecyl-4-(1H)quinolone (HDQ), was known to have PfNDH2 inhibitory activity. This molecule was used to initiate a primary similarity-based screen of 1000 compounds from a compound collection of 750,000 compounds (curated by Biofocus-DPI). A range of chemoinformatics methods and filters were applied to the hits from this initial phase in order to perform a hit expansion screen on a further ~16,000 compounds. The chemoinformatic strategy allowed us to cover ~16% diversity whilst screening just ~2% of the compound collection. The HTS resulted in a hit rate of 0.29% and 150 compounds were progressed for potency against PfNDH2. Of these compounds, 50 were considered active with IC50s ranging from 100 nM to 40 µM. Currently seven distinct chemotypes are being progressed from hit to lead using traditional synthetic medicinal chemistry strategies.

P4 Malaria in mothers and young children in Uganda

Martha Betson*, Mary Oguike[#], Jose Figueiredo*, Narcis Kabatereine[$], Colin Sutherland[#] and J.Russell Stothard*
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Malaria continues to be a serious public health problem, particularly in sub-Saharan Africa. As part of a cohort study investigating intestinal schistosomiasis and malaria in lakeshore communities in Uganda, around 1850 mothers and young children were screened for malaria using the Paracheck rapid diagnostic test and blood films. In addition, real-time PCR analysis was carried out on blood spots to assess levels of *Plasmodium falciparum* infection and to determine whether there were any *P. malariae* or *P. ovale* infections present.
The overall prevalence of malaria infection was high in children (77.4% by paracheck; 72.4% by blood film) and lower in mothers (21.8% by paracheck; 24.3% by blood film). *P. falciparum* was responsible for the majority of malaria infections. However, *P. malariae* and *P. ovale* infections were also present. A novel real time PCR assay designed to distinguish between classic and variant *P. ovale* indicated that both forms were present in the same communities.
Analysis of the relative contribution of mixed- and single-species infections to the malaria burden in these lakeside communities will be presented as well as observations on epidemiological interactions with intestinal schistosomiasis. The distribution of different *Plasmodium* species among households will also be considered.

P5 Post-adhesion signaling by malaria infected red blood cell bound human endothelial cell

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Cerebral malaria (CM) is one of the most serious complications of *Plasmodium falciparum* infection. It is characterized by the sequestration of parasitised red blood cells (PRBCs) in cerebral microvascular beds which is thought to cause compromising of the blood-brain-barrier (BBB) integrity. It has been proposed that breakdown of blood-brain-barrier may result from parasite-induced apoptosis of endothelial cells. However, apoptosis of cells can occur via several paths, such as extrinsic (death receptor) pathway, intrinsic (mitochondrial) pathway, or ER stress pathway, and the precise apoptosis pathways that underlie this event have not been verified yet. To investigate the post-signaling events that occur after malaria-infected red cells cytoadhere to endothelial cells, various apoptotic inhibitors were selected to use in the experiments. The results using caspase-8 and caspase-9 specific inhibitors showed the involvement of both extrinsic and intrinsic pathways. Measurement of attached endothelial cell mitochondria membrane potential using JC-1 staining showed the loss of mitochondria membrane potential of attached cell which confirmed the involvement of intrinsic pathway in apoptosis of those cells. Furthermore, apoptosis of endothelial cell attached to infected red cell also relate to the transcription factor NF-κB and calcium signaling pathway, as shown by use of specific inhibitors of transcription factor NF-κB and intracellular calcium chelator.

P6 Molecular & Biochemical Characterisation of Atovaquone Resistance in the Malaria Parasite *Plasmodium falciparum* (TM90-C2B)

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Atovaquone is an anti-malarial that when used in combination with proguanil (Malarone™) is widely used for the curative and prohylactic treament of malaria. Atovaquone, a 2-hydroxynaphthoquinone, is a competitive inhibitor of the quinol oxidation (Q_o) site of the mitochondrial cytochrome bc_1 complex. Inhibition of this enzyme results in the collapse of the mitochondrial membrane potential and subsequent parasite death. Although parasite resistance to atovaquone has been associated with mutations in the bc_1 Q_o, to date no studies have been performed to characterize the resistance phenotype in the parasite. DNA sequencing of cytochrome *b* from the atovaquone resistant (IC_{50} 12μM) *P. falciparum* isolate TM90-C2B revealed the exchange of tyrosine for serine at codon 268 (Y268S), a Q_o-site mutation that has been previously observed in other atovaquone-resistant strains. Enzymological characterisation of TM90-C2B bc_1 complex (steady-state decylubiquinol:cytochrome oxidoreductase assay) showed that enzyme turnover was approximately 50% of the atovaquone-sensitive strain (3D7, IC_{50} 0.8 nM), with a threefold increase in K_m for decylubiquinol to 18 □M. Use of multiplex PCR to compare the profile gene expression between 3D7 and TM90-C2B reveal that a number of energy metabolism and genes associated with redox control are upregulated in the resistant parasite. These data indicate that the Y268S mutation might be expected to confer a fitness penalty upon the parasite and that this cost is mitigated by the upregulation of key components of energy and redox metabolism.

P7 Antiparasitic activity and ADME-Tox profile of new 4- aminoquinoline derivatives
Chiara Rusconi[1], Anna Sparatore[1], Nicoletta Basilico[2] Sergio Romeo[1], Nadia Vaiana[1], Manolo Casagrande[1], Luca Rizzi[1], Valerio Tazzari[1], Livia Vivas[3], Paola Verducci[4], Daniela Jabes[5] and Donatella Taramelli[2]
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To overcome parasite resistance and increase metabolic stability, the lateral chain of 4-amminoquinoline has been modified by the addition of a quinolizidinyl or pyrrolizidinyl moiety. Two of these compounds (rAM1 and MG3) are highly effective *in vitro* against multi drug resistant strains of *P. falciparum* and no toxic against mammalian cells. (Sparatore A. *et al.* 2005, 2008). They all inhibit beta-haematin formation suggesting a mechanism of action similar to CQ. *In vivo*, both inhibit parasitemia with ED50 of 5-10 mg/kg, per os against *P. berghei* or *P. chaboudi ,* a dose that is very similar to that of CQ. By comparing the synthetic process, the pharmacokinetic parameters when given orally to the rat, the in vitro and in vivo metabolism and toxicity, MG3 performed slightly better than rAM1. Therefore, MG3 can be considered a promising candidate for an effective antimalarial agent suitable for artemisinin-based combination therapy (ACT).
The ANTIMAL –EU18834 support is acknowledged.

P8 Potential Role of Proteases in Endothelial Disruption in Severe Malaria
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Severe malaria (SM) is associated with interactions between *Plasmodium falciparum*-infected red blood cells (IRBC) and endothelial cells (EC) within microvasculature resulting in endothelial activation and loss of endothelial integrity.
Supernatants from co-cultures with laboratory parasite line ItG and human microvascular EC lines from skin (HDMEC; Promocell) and brain (HBMEC, gift from Monique Stins) were analysed. Using the endothelial electric cell-substrate impedance sensing (ECIS) technology, we demonstrated time dependent reduction in the HBMEC monolayer resistance (integrity), by 15% within 15 hours; in response to IRBC/EC co-culture supernatants. The supernatants were analysed for proteases released from EC in response to IRBC, as candidate molecules responsible for loss of endothelial integrity; including Zinc-dependent matrix metalloproteinases (MMP) -2 and -9 and disintegrin and metalloproteinases with thrombospondin motifs (ADAMTS) -1 and -4. These proteases have been implicated in multiple sclerosis, where brain endothelial integrity is disrupted.
MMP2 and MMP9 were constitutive in HBEC (894 pg/ml) and HDMEC (11 pg/ml). IRBC induced upregulation of MMP2 and MMP9 in HBEC (1236 pg/ml) and HDMEC (208 pg/ml), respectively; while. ADAMTS4 was induced in HDMEC. Immunofluorescence studies showed IRBC-induced reduction of the tight junction protein, vinculin in EC monolayers. These studies suggest a potential role for proteases in the loss of endothelial barrier integrity in SM.

P9* Anti-plasmodial and anti-inflammatory activity of medicinal plants used in Burkina Faso against malaria
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Plasmodium falciparum malaria is the most common cause of death in Burkina Faso. The persistence of drug resistance makes the disease difficult to control. The reliability of indigenous herbal drugs may be helpful. In Burkina Faso, the decoctions of *Canthium henriquesianum* Schum, *Gardenia sokotensis* Hutch. and *Vernonia colorata* Willd. are used to treat malaria. The

study objective was to evaluate the antiplasmodial properties of these plant extracts and to check the relevance of their use. Decoctions of aerial parts were prepared and screened for antiplasmodial activity against both chloroquine-sensitive (D10) and resistant (W2) strains of *P. falciparum.* The aqueous extract from *C. henriquesianum* was the most active with IC_{50} of 103±44.2 and 66.8±21.6 µg/ml on D10 and W2, respectively. The ethyl acetate extract was even more potent with IC_{50} 24.0±7.4 µg/ml. No toxicity was observed against mammalian cells, suggesting a good therapeutic index. The decoction of *C. henriquesianum* contains hydrolysable tannins and no alkaloids. Extracts of *C. henriquesianum* also inhibited the production of IL-1β, but not of TNFα, by human monocytes, thus confirming its traditional use as antipyretic. Attempts to identify the active principle for antiplasmodial and anti-inflammatory activities are ongoing.
Financial support by EU18834AntiMal is acknowledged

P10 Binding of endothelin-1 to the lipid moiety of haemozoin and *P. falciparum* parasitised red blood cells

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Plasmodium falciparum infection may evolve into severe disease if untreated or inadequately treated, causing an estimated one million deaths annually. The severe forms of malaria are characterized by the release of inflammatory cytokines and the cytoadherence of parasitized red blood cells (pRBC) to the vascular endothelium. This results in the sequestration of pRBCs in various organs causing micro-circulatory obstruction and subsequent tissue hypoxia. Endothelin1 (ET-1) is a 21 amino acid peptide produced by the vascular endothelium under hypoxia, that acts locally as regulator of vascular tone and inflammation. The role of ET-1 in falciparum malaria is still controversial and largely unknown, although tissue hypoxia is frequent as a result of the sequestration of pRBC to the microvasculature. In the present work, we show that both synthetic and endothelial-derived ET-1 are removed by pRBC and native haemozoin (malaria pigment), but not by normal RBC, delipidized haemozoin or synthetic beta-haematin. The effect is selective for ET-1, but not for its precursor, bigET-1, and not due to a direct binding to ET-A or ET-B receptors. These findings may help understand the consequences of parasite sequestration in severe malaria. The financial support of AntiMal EU 188134 is acknowledged

P11* Bioinformatic and proteomic analysis of *Plasmodium falciparum* succinate dehydrogenase

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Mitochondrial succinate dehydrogenase (succinate:quinone oxidoreductase, Complex II) is key component of both the respiratory electron transfer chain and the tricarboxylic acid cycle, and presents itself as a potentially attractive antimalarial chemotherapeutic target. Eukaryotic Complex II is an integral membrane protein, consisting of four subunits. Despite quantifiable succinate:quinone oxidase activity in *P. falciparum* membrane preparations (which we demonstrate here for the first time), the identity of two Complex II subunits (SDH3 and SDH4) remains obscure in the human malaria parasite genome sequence data. By using a bioinformatic analysis based on a structural filtering of all genes from PlasmoDB database, we identified candidates for SDH3 and SDH4. Despite the poor cross-species sequence conservation of SDH3 and SDH4, our proteins are predicted to exhibit convincing structural and functional homology with the elucidated crystal structures of Complex II.
To examine the validity of the SDH3 and SDH4 candidates, we present a proteomic analysis of *P. falciparum* mitochondrial membrane fractions based on immunocapture with a cross-species antibody against Complex II followed by Liquid Chromatography Mass Spectrometry (LC-MS).

P12* Heterogeneity in malaria exposure and vaccine response: implications for the interpretation of vaccine efficacy trials

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Background: Phase III trials of the malaria vaccine, RTS,S, are now underway across multiple sites of varying transmission intensity in Africa. Heterogeneity in exposure, vaccine response and waning of efficacy may bias estimates of vaccine efficacy.
Methods: We use theoretical arguments to identify the expected effects of a) heterogeneity in exposure to infectious bites; and b) heterogeneity in individual's response to the vaccine.
Results: Heterogeneity in exposure and vaccine response leads to a smaller proportion of trial participants becoming infected than one would expect in a homogeneous setting. This causes estimates of vaccine efficacy from clinical trials to be underestimated if transmission heterogeneity is ignored, and overestimated if heterogeneity in vaccine response is ignored.
Conclusions: Failure to account for heterogeneities in exposure and response, and waning of efficacy in clinical trials can lead to biased estimates of malaria vaccine efficacy. Appropriate methods to reduce these biases need to be used to ensure accurate interpretation and comparability between trial sites of results from the upcoming Phase III clinical trials of RTS,S.

P13 Erythrocyte molecules in *Plasmodium falciparum* rosette formation

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Infection with *P. falciparum* is responsible for the majority of malaria morbidity and mortality in sub-Saharan Africa. Although the mechanisms of pathogenesis are not well characterised, the ability of *P. falciparum* infected erythrocytes to adhere to host cells and tissues is clearly critical. In particular, rosetting (the binding of uninfected erythrocytes to *P. falciparum* infected erythrocytes) is the only parasite phenotype consistently associated with severe malaria in sub-Saharan Africa. Rosette formation depends on interactions between parasite ligands on the surface of infected cells and human receptor molecules on the surface of uninfected erythrocytes. To date, a single parasite protein PfEMP1 is the only parasite-derived molecule confirmed to contribute to this interaction. In contrast, current evidence implicates a number of erythrocyte molecules: complement receptor 1 (CR1), heparan-sulfate-like molecules, and the A or B blood group antigens (reviewed, Rowe et al. 2009), however these interactions are poorly or incompletely characterised. This project aims to uncover the erythrocyte molecules responsible for rosetting. We present preliminary data using antibodies against PfEMP1 to investigate its binding partners on uninfected erythrocytes.

P14 MALACTRES: African-European Research Consortium dedicated to ACT drug-resistance and diagnostics

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A research consortium comprising Centre Muraz (Burkina Faso), Kilimanjaro Christian Medical Centre (Tanzania), Tropical Diseases Research Group (Benin City, Nigeria), Wageningen University (The Netherlands), London School for Hygiene & Tropical Medicine (UK), Institute for Tropical Medicine (Antwerp Belgium) and Royal Tropical Institute (Amsterdam, The Netherlands) aims to assess specific genetic markers for ACT resistance and development of innovative malaria diagnostics.
Research will be conducted towards:

- Development/evaluation of low-tech molecular diagnostic tests and tools able to demonstrate presence of mutations conferring drug resistance in *Plasmodium*;

- Identification of resistance genes associated with increased transmission success of *Plasmodium* after ACT treatment in clinical trials with endpoints at gametocyte or infected mosquito level;
- Performing ACT treatment trials with parasitological and clinical efficacy endpoints, including *in vitro/vivo* resistant determination test, and measure abundance of parasites carrying candidate markers

Novel simple molecular tests have been developed and ACT treatment trials are ongoing. The project moves knowledge of ACT resistance forward in 2 complementary ways: 1) MALACTRES will use its unprecedented access to DNA/RNA from parasites isolated from ACT-treated individuals, and from mosquitoes fed on blood from ACT-treated individuals, to identify and validate genetic markers for selective changes induced by ACT; 2) The project will result in development and validation of simple tests in new formats for new and existing resistance markers. More information see: www.malactres.eu

P15* Screening synthetic derivatives of buchtienine for antimalarial properties.

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Parasitic diseases, such as malaria, pose significant health and socioeconomic problems to Developing Nations. A massive challenge to the treatment of these diseases is the narrow drug discovery pipeline to replace current front-line therapies that are failing due to widespread drug resistance. In recent years there has been a substantial investment in screening of medicinal plant metabolites for antiparasitic properties that have yielded successes such as the artemisinin class of antimalarials. One limitation of this approach, however, has been in securing a sustainable source of these metabolites, often present in low yields, for medicinal chemistry and screening.
Buchtienine, a metabolite derived from a Malaysian medicinal plant, is already known to possess activity against leishmania parasites with our preliminary data indicating a similar activity for early synthetic intermediates of Buchtienine against malaria parasites. Here we will present our preliminary lead generation data revealing key aspects of the structure activity relationship between the 3D shape, enantiomeric series and substitution of the indole backbone of these derivatives against growth of *P. falciparum* intraerythrocytic parasites.

P16 Erythrocyte Shape change prevents *Plasmodium falciparum* (Pf) invasion

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Normal erythrocytes have biconcave discoid shape that represents large surface area with higher cell surface to volume ratio than that of spherical shape. This appears to allow membrane internalization required for *Plasmodium falciparum* (Pf) invasion into erythrocytes. Indeed, abnormal erythrocytes shape with decreased surface area to volume ratio such as hereditary spherocytosis limits invasion of the parasite. In the present study, using several agents to induce erythrocyte shape changes, we examined whether echinocytic shape changes with membrane projections in opposite direction to membrane internalization and/or stomatocytic shape change with decreased surface area to volume ratio that would be required for internalization, prevents Pf invasion. Having microscopically, confirmed echinocytic and/or stomatocytic shape changes and also measured extensibility using ektacytometer of the treated cells, subsequent Pf invasion assay was performed and parasitaemia determined. Both sodium fluoride (NaF) and Phospholipase A^2 (PLA_2) induced echinocytic shape change whereas Phospholipase D (PLD), Sphingomyelinase (SMase) and Chlorpromazine (CPZ) caused stomatocytic shape change with decreased extensibility of erythrocytes. In both situations, Pf invasion was prevented, indicating that biconcave discoid shape of normal erythrocytes with high surface to volume ration is required for membrane internalization when Pf invades into erythrocytes.

P17 Developing Novel Chemical Probes to Identify Cytochrome P450s Associated with Pyrethroid Resistance in Malaria Vectors

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Background: The use of insecticide treated materials (ITMs) is a major preventive tool in the global fight against malaria. Synthetic pyrethroids are the only class of insecticides suitable for ITMs and resistance to this class of insecticide may impair malaria control.
Aims, Methodology and Principal Findings: Cytochrome P450-mediated insecticide metabolism is a major cause of pyrethroid resistance. The present study aims to develop novel selective P450 inhibitors based on a pyrethroid scaffold to label and identify P450s associated with pyrethroid resistance mechanism in mosquitoes. A number of permethrin and deltamethrin mimetic activity based probes (ABPs) were synthesized and these were screened against Anopheles gambiae pyrethroid metabolisers (CYP6P3, CYP6M2) and non-metabolisers (CYP6Z2) to identify probes capable of selective labeling. In vitro labeling studies indicate the ability of these probes to label recombinant P450s in an activity-based manner with variable selectivity and specificity to CYP6P3, CYP6M2 and CYP6Z2.
Significance: The combination of genetic studies and pyrethroid mimetic probes will enable us to selectively identify and profile P450-pyrethroid interactions. This will improve our molecular understanding of how P450s interact and degrade pyrethroids, and ultimately aid the synthesis and development of new insecticides and formulations for vector control.

P18 Density dependence within the malarial mosquito and its possible impact on the success of transmission blocking interventions

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The population dynamics of the malaria parasite within the mosquito may influence the success of transmission blocking interventions. The progression of *Plasmodium berghei* through *Anopheles stephensi* and the survival of the vector have both been shown to depend on the number of parasites ingested by the mosquito. The complex interaction between these different density-dependent processes could cause a partially effective intervention to increase parasite transmission. An individual-based stochastic mathematical model is used to investigate how transmission blocking interventions which target different parasite life-stages within the mosquito may influence transmission intensity. Interventions which reduce ookinete density beneath a threshold level are likely to have auxiliary benefits as they utilise density-dependent processes which restrict sporogonic development at low parasite densities. However, under certain scenarios control measures could increase transmission by increasing the number of bites made by a mosquito whilst failing to sufficiently reduce its infectivity. A greater understanding of the underlying biology of the malaria parasite within the mosquito is required to fully evaluate the effectiveness of transmission blocking interventions. If parasite-induced vector mortality does influence the population dynamics of *Plasmodium* in malaria endemic regions then studies must quantify the variability and duration of its effectiveness to ensure benefits of control measures are not overestimated.

P19 In-Check™ platform: For Fast Detection of Malaria Species and Resistant Variants

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Malaria continues to be a major cause of morbidity and mortality worldwide. This has been partly attributed to the resistance of *Plasmodium falciparum* to commonly used antimalarials. Malaria control relies mainly on early diagnosis for prompt and effective treatment of infected individuals. Microscopy remains the gold standard tool for malaria diagnosis, but needs skilled manpower and is tedious and timeconsuming. Currently, there are no tools for diagnosis of drug resistance in

patients other than relying on reports of failure of curative treatments. Available molecular methods are laborious and time-consuming preventing timely and appropriate decisions on clinical management. Here we describe a collaborative project for the development of a new lab-on-chip based platform (In-check™) for molecular diagnosis of malaria species and drug resistant variants. The In-Check™ Platform is an integrated system combining a fast PCR and microarray based diagnostic test using the Lab-On-Chip format. Detection of the five human malaria-causing *Plasmodium* species (*Plasmodium falciparum*, *Plasmodium vivax*, *Plasmodium ovale, Plasmodium knowlesi* and *Plasmodium malariae*) is performed using PCR based on 18s rRNA followed by species-specific probe on the microarray. In drug resistance detection, specific PCR targeting polymorphic regions of the gene PfCRT, PfDHFR, PfDHPS, PfCTYB that confer resistance to Chloroquine, Sulphadoxine – Pyrimethamine and Malarone were amplified followed by hybridisation with specific SNP probes on the microarray. The development of the In-Check™ Platform for diagnosis of malaria and drug resistance will be discussed. This work is supported by European commission FP7 (TM REST)

P20 Improvement of Rapid Diagnostics for Malaria: *In vitro* detection and stability of novel antigen targets

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Monoclonal antibodies for new target malaria antigens are being developed for the improvement of rapid diagnostic tests (RDTs) in a collaborative effort with the Foundation for Innovative New Diagnostics (FIND). Although current RDT targets perform quite well, there is still a need for the identification of novel targets and more thermostable reagents. Tests detecting HRP2 are generally less expensive, more stable and have a lower detection threshold than pLDH tests, but have their limitations as well: they detect only *Plasmodium falciparum,* the antigen persists in the blood after parasite death and there can be false-negatives due to antigenic variation.
Three antigens have been identified based on literature searches; Glutamate Rich Protein (GLURP) (*Pf* only), Dihydrofolate Reductase-Thymidylate Synthase (DHFR-TS) and Heme Detoxification Protein (HDP) (both all human species). Recombinant antigens were produced and used to immunise mice, from which antibody producing cells were subsequently isolated. These antibodies were screened for specificity against *P. falciparum* and *P. vivax* and 30 are being used for the development of lateral flow immunochromatographic devices.
To select the most optimal antibody couples, we looked at the *in vitro* stability of the antigens in different strains of cultured *P.falciparum* upon addition of antimalarials. For this purpose we used a sandwich-ELISA with the antibody couples, and the results were compared to a commercial ELISA for the detection of HRPII.

P21 Definition of liver fluke population groups by DNA polymorphisms

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Fasciolosis is a major problem for human, veterinary medicine and agriculture because of the huge losses it causes and antihelminthic drugs resistance. The aim of our investigation is to define the polymorphisms of liver flukes' DNA from different countries and regions of Eastern Europe and how specific are they for each population. These assays are part of an international 6th FW grant for worldwide study of *Fasciola hepatica.* Molecular and genetic methods for that analysis have been used. Variations in mitochondrial DNA (mtDNA) and ribosomal DNA markers (28S RNA gene) have been analyzed to determine species' phylogeny, geographic occurrence of genetic variations and demographic history of populations. In liver fluke three potentially most variable and informative regions in mtDNA for sequence assay have been found. They have been analysed for the presence of SNP (single nucleotide polymorphism) sites. Data sets have been created and phylogenetic trees have been built from different liver fluke populations. According to that separate clades have been formed. Our future plans are to extend geographic areas and to apply additional methods for analysis.

P22 New metalloprotein of parasitic nematodes from the genus *Trichinella*

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Differences between host and parasite biochemistry present possible targets for chemotherapy and are the object of intensive research in parasitology. In parasitic nematodes, specific metabolic pathways are modified to meet their actual physicochemical environment, the host organism. There is much interest in molecules produced by *Trichinella* that modulate host functions. A major focus has been on excretory-secretory (ES) products obtained from larvae and various proteins have been described that are antigenic determinants, secretory products with immunogenic properties or surface proteins. A new type 45kDa PCHT (Poly-Cys and His-Tailed Protein) from *T. spiralis* larvae has been isolated, purified and whole sequenced. Studies on the secondary structure by circular dichroism showed that its organization was predominantly β-structural. The exact molecular mass has been shown by mass spectroscopy and tryptic digestion. The protein contains two homology domains with strong conservative cystein residue positions and a poly-His tail. The Cys and His residues could be involved in the heavy metal binding properties of the protein. The newly sequenced fragments did not show homology with any known sequence (with the exception of the ESTs of *Trichuris muris* and *T. vulpis*).These results could be used for species diagnostics and could also have practical application as antigens and in DNA diagnostics for trichinelosis and trichuriosis that are anthropozoonosis diseases.

P23 Naming Genes in The Parasitic Nematodes

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A well chosen gene name can concisely convey a wealth of relevant biological information. A consistent nomenclature within and between nematodes adds transparency that can have a real impact on the advancement of our understanding of gene function. Currently, genes in parasitic nematodes are named *ad hoc*. We believe that research on parasitic nematodes would benefit from adherence to an agreed genetic nomenclature, based on the *Caenorhabditis elegans*

standard. We use the ligand-gated ion channel superfamily, which contains many genes involved in anthelmintic drug action and resistance, to illustrate our proposals. Comparing this gene family between species reveals examples of gene duplications and possibly gene loss, leading to considerable differences between nematode species; we propose a method for dealing with this diversity while retaining useful biological information on the relationships between genes in the different species. The flood of genome sequence data means now is the perfect time to reach a consensus on nomenclature standards for the next 30 years, and so there will be a Gene Naming Workshop at ICOPA XII in Melbourne in August 2010. We encourage all members of the parasitology community to contribute to this discussion, even if they are not attending ICOPA, and will describe mechanisms to facilitate this.

P24 Parasitological collection at the Institute of Experimental Pathology and Parasitology, Bulgarian Academy of Sciences

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The parasitological collection in our institute contains a range of parasites isolated from a large number of hosts from across Bulgaria. This collection derives from the work of many Bulgarian scientists over the last 70 years. To date the catalogue includes 25,000 exhibits: 20,000 microscope slides and 5,000 macroscopic preparations. The former consists of 468 helminth species from 272 host species, the oldest preparation collected in 1934 – a nematode from a wild cat. The material includes the major taxonomic groups of helminths: Trematoda, Monogenea, Cestoda, Nematoda and Acanthocephala isolated from mammals, birds, fish, amphibians and plants (http://www.iepp.bas.bg/collection.html).
The IEPP parasite collection represents a valuable source of material for morphological and molecular descriptions of new taxa, especially cryptic species. Over the past five years, re-cataloging of the IEPP collection has begun, as well as creation of a website. This will allow wider access to this important source of material for both Bulgarian and foreign scientists.

P25 A state-dependent modelling framework for exploring the interactions between macroparasites and their intermediate hosts

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The foraging behaviour of animals has been extensively studied by behavioural ecologists, and a suite of modelling techniques have been developed to explore the decision-making processes made by foragers. More recently, these techniques have been expanded to consider the decisions made by individuals when they interact with others, looking at the role of the individual in social foraging situations. The relationship between a macroparasite and its intermediate host makes similar assumptions (with obvious differences) to the relationship between two animals foraging socially, where both parasite and host experience changes in their reserves in response to the 'foraging' behaviour shown by both individuals (where the host gains energy from foraging in the external environment, whilst the parasite gains energy, to the detriment of the host, from 'foraging' on the host's own stored resources). I describe a framework exploring the decision-making processes made by hosts and parasites (where a 'decision' is defined as a life-history choice made by an individual about how much resource to harvest over a short period of time), using a state-dependent dynamic programming framework. As well as being a useful tool for exploring general questions about life-history evolution, these models can be used to explore more targeted questions about specific systems where the biology of the hosts, parasites, and their environment are well characterised.

P26* An *in silico* pipeline for identification of molecular mimicry candidates from parasites

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Molecular mimicry is a common strategy among pathogenic microorganisms for camouflage, cytoadherence, or manipulation of host signaling. With genome data from host and parasite, we can investigate molecular mimicry of linear amino acid epitopes by comparative genomics. A genome-wide *in silico* survey with parasite proteins for mimicry candidates to the host or vector was performed. All predicted parasite proteins were broken down into overlapping fragments, each of which was screened for close hits in the human proteome. Control searches were carried out against unrelated, free-living eukaryotes to eliminate the generally conserved proteins, and with randomized versions of the parasite proteins to estimate statistical expectancy. Perl scripts were developed to run the intensive computations and process the results. Several interesting candidates were found, such as an amino acid stretch identical to the multifunctional, immunosuppressive protein vitronectin in several *P. falciparum* PfEMP1 variants, or a *B. malayi* fragment that matched with the immunosuppressive CKS-17 motif of the human HERV-H_2q24.3 provirus ancestral Env polyprotein. The results were made available to the scientific community by means of a searchable online database for molecular mimicry candidate proteins in pathogens.

P27 Helminths and microparasites - a novel interaction

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Faecal-oral transmitted pathogens, including common food-borne pathogens, such as *Salmonella,* can survive for long periods of time in the environment. Whilst in the soil these bacterial pathogens often come in contact and are ingested by free-living microbivorous helminths (both parasitic and non-parasitic). We investigated the hypothesis that free-living helminths play a role in microparasite persistence and transmission. First, using the model system of the free-living non-parasitic helminth *Caenorhabditis elegans* we found *Salmonella* bacteria that were ingested by this helminth had increased survival under harsh environmental conditions, such as low pH, chlorination and UV irradiation. Secondly we found that ingestion of both free-living and parasitic helminths that had fed on *Salmonella* could cause systemic infection by *Salmonella* in vertebrate hosts. Finally, to determine how pervasive the association of pathogens with helminths was we carried out a pilot study using parasitic helminths of sheep and cattle finding evidence that they contained both human and animal pathogens. We conclude that helminths can provide a protective micro-environment for microparasites of importance for human and animal health and may play an important role in the persistence and transmission of microparasites in the environment.

P28* Sero- prevalence of *Toxoplasma gondii* among butchers in Khartoum, Sudan

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Toxoplasmosis is an infection caused by the parasite *Toxoplasma gondii.* Human infection occurs through the ingestion of cysts in raw or undercooked meat, ingestion of mature oocysts in food or water contaminated with cat feces, or through transplacental passage of the parasite from the mother to the foetus, with acute infection of the latter. The present study was designed to investigate the sero-prevalence rate of toxoplasmosis in butchers in Khartoum State, Sudan. A total of 160 blood samples were collected from butchers. Sera were separated by centrifugation after allowing the blood samples to clot overnight at 4°C and kept in labeled containers at -20°C. Latex agglutination test (LAT) was used to screen the sera. A questionnaire was distributed

amongst the butchers to study different aspects related to the infection with *Toxoplasma gondii*. Eighty one butchers (i.e. 50.6 %) were tested positive for toxoplasmosis. Eating raw meat, drinking raw milk and contact with cats at working areas were found to be the significant cause of infection (P=0.017, P= 0.000 and 0.008, respectively).

P29* Modelling the co-infection of malaria and lymphatic filariasis

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Malaria and lymphatic filariasis (LF) are vector-borne infections transmitted by the same mosquito genus *Anopheles* and together give rise to the most severe public health and economic burdens in Africa. The co-incidence of these infections has led to calls for endemic countries to adopt integrated intervention strategies targeting both infections simultaneously. It is also thought that changes, both short term and long term, could alter the epidemiologies of these infections, and thus investigations of co-transmission dynamics must in addition take into account different climate scenarios when making predictions.
Here, we illustrate an integrated framework for the combined modelling of malaria and LF, which includes climate-based effects in host and parasite population dynamics. This model is used to investigate the impact of different disease intervention strategies, such as Mass Drug Administration (MDA) and vector control, on the transmission of both infections. We also assess the role that climate plays in malaria and LF transmission by looking at how global climate change may affect relative infection prevalences under conditions of co-transmission. Early results show that treating LF by MDA can lead to increased malaria prevalence, highlighting both the possible detrimental impact that a given control measure targeted at one infection may have on an important co-infection, and the importance that considering integrated approaches will play in mutual parasite control under such circumstances.

P30 Factors influencing rabbit parasite communities

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Care must be taken when comparing parasite communities in rabbits to identify and quantify the impact of the different factors responsible for parasite community structure e.g. no cestodes are recorded from rabbits from Australasia probably due to introduced rabbits lacking these species and tapeworms are also not present in rabbits in intensive agricultural areas due to the lack of orbatid mites. Myxomatosis which has been shown to have a significant impact on some parasites is absent form New Zealand. It is only once factors like these above have been taken into consideration that other more fundamental factors which control parasite communities can be investigated. Research into the impact of climate and other intrinsic factors can best be undertaken by long term intensive sampling at a single study site. Over 5000 rabbits collected over a 34 year period monitoring a range of diseases and environmental factors have identified a rise in temperature to be related to increased *Graphidium strigusum* numbers while immunity may play an important role in influencing the interactions between species.

P31 Toxic metal accumulation in the hare-*Passalurus ambifuus* system from Bulgaria

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Environmental pollution with toxic metals is a dangerous problem that is recognized worldwide. Among possible biomonitoring species, hares (*Lepus europeaus* Pallas, 1778) have been widely used due to their wide distribution and the most abundant herbivorous animals in Bulgaria. Because the hares are frequently infected with endoparasites it is necessary to follow their possible influence on the metal levels in the host tissues. The aim was to assess the toxic metal accumulation in the system hare - *Passalurus ambiguus* (Nematoda) in various ecological regions. Zinc, iron, copper, manganese, lead and cadmium were determined in kidney, liver,

musculature and *P. ambiguous,* by atomic absorption spectroscopy. Differences in host tissue concentrations (infected and uninfected) and parasites were done with Student t-tests. The data were evaluated using the PRISM 4.0.Variation in the metal concentrations among tissues in the infected hares indicated differences in the degree of their accumulation between host (uninfected and infected) and the nematode. The similar metal status of the helminths and their hosts demonstrated that they were integrated, dynamic systems reflecting ecosystem conditions. The combined results for metals in the host and the parasites gave more reliable and detailed information for the environmental contaminants. The model hares - *P. ambiguus* is a promising bioindication system to evaluate some toxic metals exposure in terrestrial habitats in field conditions.

P32* Is there any link between *Blastocystis* and irritable bowel syndrome (IBS)?
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Blastocystis is an obligate, anaerobic, protistan parasite found in the intestinal tract of humans and other animals. *Blastocystis* is highly prevalent in Irritable Bowel Syndrome (IBS) patients but it is unclear whether it causes the disease. Some of the uncertainty may be due to the fact that *Blastocystis* is genetically very diverse. We have investigated the possibility of a link between IBS and the genetic subtype(s) of *Blastocystis* present. A total of 268 *Blastocystis* isolates were used in this study, 160 from patients in IBS clinics and 108 from random samples submitted by doctors to the Diagnostic Parasitology Laboratory of LSHTM. Subtypes were identified by 'barcoding'. Eight different subtypes were identified and subtype 3 had the highest frequency overall. However, in IBS samples subtype 4 was the most common (38%), nearly twice as prevalent as in the random samples (21%). Analyses show that the distribution of subtypes is significantly different between the two sample groups, mostly due to the subtype 4 difference as the other subtype prevalences are quite similar. The result suggests that subtype 4 of *Blastocystis* may be linked to some cases of IBS-like symptoms.

P33 TORCH pathogen in high-risk Qatari patients
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Background: Testing of patients who are deemed to be at high risk for TORCH pathogens is important so that appropriate and specific treatment can be initiated, especially for pregnant women, their foetuses, and neonates. Association of other TORCH pathogens with *Toxoplasma gondii* infection may be related to socio-economic factors, cultural background, hygiene practices, or living conditions.
Methods: The study included 1857 patients tested for TORCH pathogens between 2005 and 2008. Logistic regression was used to evaluate factors associated with *T. gondii* seropositivity.
Results: Among 823 women of child-bearing age, 35.1% and 5.2% tested positive for *T. gondii* IgG and IgM, respectively. Three infants ≤6 months old (0.8% of 353) were congenitally infected. Factors associated with *T. gondii* IgG seropositivity included older age, East Mediterranean or African nationality, positive CMV and HSV-1 serostatus, and negative rubella IgG results. Seroprevalence of *T. gondii* IgM declined significantly between 2005 and 2008.
Conclusions: Sixty-five percent of women of child-bearing age were at risk of toxoplasmosis and should take precautions to prevent transmission during pregnancy. Although the incidence of congenital toxoplasmosis in Qatar is low, serology fails to detect many neonatal infections**.** Population-based studies of newborns would help to more accurately estimate incidence of congenital toxoplasmosis. The decreasing prevalence of IgM antibodies between 2005 and 2008 suggested that exposure to *T. gondii* in the food chain or from environmental sources declined over this period in Qatar.

P34 Screening for *Echinococcus multilocularis* in red foxes from England

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Echinococcus multilocularis, the causative agent of human alveolar echinococcosis, has not yet been reported in the UK. Consequentially, to avoid this parasite being imported from highly endemic regions on the continent, pets travelling under the Pet Travel Scheme (PETS) are currently required to be treated with praziquantel prior to entry into the UK. This is a derogation from the EU requirements which is due to expire in June 2010 or, if an extension is granted, at the end of 2011. In this context, a study is currently being carried out to investigate the prevalence of *E. multilocularis* in red foxes, the main definite host in the sylvatic cycle. Archived fox faecal samples, which have been collected from locations throughout England, are being used for this work. *E. multilocularis/granulosus* eggs will be isolated from the faecal samples using a combination of flotation in zinc chloride solution followed by sequential sieving through nylon filters. This will be followed by egg-DNA isolation and PCR.

P35 Species identification of *Trichinella* isolates from Bulgaria

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Trichinellosis is the most common helminthosis resulting from consumption of thermally untreated meat from domestic pigs, horses and game and constitutes a part of the nutritional chain of human. It is well known in medicine since the middle of the 18th century but its importance enhanced in the last two decades when circulation of at least 8 *Trichinella* species was found in nature. Each of them was characterized according to its biology, geographical distribution and wide variety of hosts. Trichinellosis is one of the most common helminthozoonoses in Bulgaria with important medico- sanitary impact. For the period 2002-2006, 40 outbreaks and 45 sporadic cases were officially registered in the country. A complex study was designed, comprising morphometric analyses, cross-breeding, histology, infectivity and PCR, to determine which of the already known *Trichinella* species are infectious for humans and animals in Bulgaria. Thus, 35 *Trichinella* isolates were obtained from various wild and different animals and three *Trichinella* species were identified: *T. spiralis*, *T. britovi* and *T. pseudospiralis*. All three species established in Bulgaria are pathogenic to wild and domestic animals, as well as to humans.

P36* Co-parasitism of rhinonyssid mites (Parasitiformes: Gamasina, Rhinonyssidae) in the nasal cavities of birds

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Rhinonyssidae mites (Gamasina, Rhinonyssidae) are endoparasites of birds that inhabit sites such as the nasal cavity, trachea and lungs. Co-parasitism is fairly common among the rhinonyssidae, where two or three species infect the same host. Such mites, specific for that particular bird species, belong to different genera. Most cases of co-parasitism have been noticed between species in the genus *Sternostoma* Berlese et Trouessart, 1889, with representatives of the *Larinyssus* Strandtmann, 1948 and *Rhinonyssus* Trouessart 1894 genera. Less common is co-parasitism among mites belonging to close species (*Ptilonyssus* Berlese et Trouessart, 1889 and *Neonyssus* Hirst, 1921; *Ptilonyssus* and *Passeronyssus* Fain, 1960) as well as among mites belonging to different species of the same genus.
Up to four different rhinonyssid mite species co-occur on single host species from the Charadriiformes, Columbiformes and Passeriformes. There are three known rhynonyssid mite species in Anseriformes and Gruiformes, while only two mite species have been found in Podicipediformes, Ciconiiformes, Srigiformes and Coraciiformes. A single species of rhinonyssids occurs in Falconiformes, Galliformes, Cuculiformes, Caprimulgiformes, Apodiformes and

Piciformes. Co-parasitism of different rhinonyssid species and genera highlights the complex interactions of mite infections in birds.
Specimens used in the current study are derived from the large rhinonyssid mite collections of the Zoological Institute of Russian Academy of Science (St. Petersburg) and Okskii State Biosphere Reserve (Ryazan region). The research has been supported partly by the grant from the Russian Fund of Fundamental Research (RFFI), Grants N 09-04-00390.

P37 Temporal and spatial variations in the ectoparasite community of the vole *Myodes glareoli*

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The bank vole *Myodes glareolus* supports a large and diverse community of arthropod ectoparasites, which in forests in Mazury, Poland, consists of 2 species of ticks, 1 louse, 9 flea species, 7 gamasid mites, 4 fur mites and one trombiculid mite. During monthly sampling from 2007 to 2009, an overall seasonal pattern was noted for some species, but superimposed on this was a less predictable temporal pattern; thus the louse *Hoplopleura edentula* and the fur mite *Listrophorus* were not seen prior to autumn 2007, but then increased dramatically to remain abundant until the end of the study. More significantly, the abundance of these ectoparasites varied across three trapping lines which, at their closest point, were less than 200 m apart. The mean abundance of *Listrophorus* varied by 5X between the 3 different lines, while the mean abundance of *Radfordia clethrionomydis* (another fur mite) varied by 2X. These differences across such small distances suggest that these small ectoparasites with limited powers of dispersal cycle within particular parts of the continuous vole population in a manner which varies from year to year but which, in any part of the forest, is essentially unpredictable.

P38 Saltatory dispersal of the troglotrematid digenean *Collyricloides massanae*

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Collyriclum faba is a moderately well known, if rare, renicolid digenean living in epidermal cysts of passerine birds in Central Europe. *Collyricloides massanae* is a morphologically almost identical renicolid from intestinal cysts of small rodents in mediterranean Europe. During field work in Mazury, NE Poland, we recovered several examples of *C. massanae* from intestinal cysts of the bank vole, *Myodes glareolus*, 1800 km to the north of its previous range in rodents. Examination of these specimens suggests that the differences between *C. faba* and *C. massanae* are trivial, inconsistent and potentially due to the different site of development in birds and rodents. There is a strong possibility that *C. faba* and *C. massanae* represent the same species, and that saltatory dispersal of these digeneans between rodent populations takes place via bird migrations. Parasites in birds are full of eggs despite their ectopic location; although the mollusc intermediate host is unknown, the life cycle must be capable of completion in NE Poland since the rodent hosts are unable to migrate. Further work requires molecular analysis of *C. faba* from birds, but we suggest that this may represent a novel dispersal strategy for an otherwise sedentary digenean.

P39 Etiopathological aspects of protostrongylid infections in red deer

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Faecal samples and lungs of red deer inhabited the State Game-Breeding Station Vitinya in Bulgaria (Balkan Mountain) were studied. A larvoscopic examination of the faeces and helminthological necropsies, pathoanatomical and histological examinations of the lungs were performed. The overall infection extensity with protostrongylids was 84.7%. Two small lungworm species were registered - *Elaphostrongylus cervi* and *Varestrongylus sagittatus.* Three types of macroscopic changes were established in the lungs parasitized by those helminths. They were

classified as follows: 1. Gray, dark-red to black stained portions on the lung surface varying in shape and size; 2. Brown-black nodes clearly differentiated from the surrounding tissue; 3. Small, hard subserous nodules. Together with the presence of different stages in the development of both parasite species the most frequently microscopic changes were desquamatous bronchitis, alveolitis associated with accumulations of alveolar macrophages and eosinophilic granulocytes, haemorrhages in the alveoli and the alveolar septa, development of parasitic granulomas in the interstitium as well as a peribronchial, perilobular, disseminated and intralobular hyperplasia of the lymphoid tissue. These alterations depended in the first place on the parasite species found in the inflammatory areas.

P40* Studies on the life cycle and transmission of the trematode *Plagiorchis muris* at Malham Tarn, Yorkshire

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Plagiorchis muris is an intestinal trematode found in wild rodents from several countries. Recently, the first recorded occurrence in the UK was reported in the wood mouse *Apodemus sylvaticus* at Malham Tarn, North Yorkshire (Rogan *et al*., 2006). The trematode has been recorded each September for a 13 year period, with a mean prevalence of 18.4%. The lifecycle of this trematode is not well understood and the reasons for its occurrence in this location within the UK remain unclear. Aquatic snails are the first intermediate host and a total of 25 species of freshwater snail have been recorded within the Malham area. Aquatic insect larvae are the second intermediate host and infected dragonflies, mosquitoes and chironimids have been most frequently reported. To define the lifecycle of *P. muris* at Malham Tarn, species specific molecular primers able to identify adult and larval stages are currently being designed. Primers will be used to identify primary and secondary intermediate hosts sampled from the tarn and surrounding water bodies by PCR amplification of the mitochondrial cytochrome oxidase C gene and ribosomal ITS regions of *P. muris.* Additionally, *A. sylvaticus* will be sampled seasonally from three distinct locations within the area to determine prevalence and intensity rates and dietary analysis will be performed to identify insect remains.

P41 *Lymnaea stagnalis* – intermediate host of some trematodes long the Danube River in Bulgaria

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Lymnaea stagnalis is known as an intermediate host for trematodes causing ailments in domestic and wild animals as well in the human. It is wide spread and quite common fresh-water snail species in Bulgaria. A total 1280 specimens were collected for establishing of the species composition and the degree of infection in *L. stagnalis* from natural biotopes along the Danube River valley. In 116 (9.06 percent) of the investigated snails an infection with larvae of trematodes were established. They were morphologically investigated and defined as developmental stages of trematodes belonging to the following genera *Sanguinicola*, *Trichobilharzia*, *Diplostomum* and *Opisthioglyphe*. The results shown that *L. stagnalis* snail species is an important biotic factor in the epidemiology of trematodoses of vertebrates humans in the investigated biotopes.

P42 Seroprevalence and zoonotic potential *of Neospora* species infection in Jordanian women with miscarriage

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Serological detection of *Neospora* spp. was carried out on 445 Jordanian women with miscarriage, using the indirect fluorescent antibody test and *N. caninum* antigen. The type of hospital, age, cat and dog contact, raw meat and wild plant consumptions, number of miscarriages and stillbirths, were tested as risk factors for the seroprevalence using univariable and multivariable logistic regression analyses. At cutoff titers of 1:200 and 1:400 the seroprevalences of *Neospora* were 25% and 6% for IgG and 12% and 7% for IgM, respectively. *Neospora*-IgG-seropositivity was associated with having a dog in the household or in the immediate environment with odds ratios of 2.6 and 4.1 respectively, and had stillbirth with an odds ratio of 0.1. *Neospora*-IgM-seropositivity was associated with women with miscarriage visiting a private hospital. The current results provide serological and epidemiological evidence for the emerging zoonotic potential of *Neospora* species infection to be categorized as a level one zoonosis.

P43* Investigating the effects of parasites on the immune system of the field vole, *Microtus agrestis*, in its natural environment

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Much of our understanding of immunology is based on studies of rodents reared in artificial conditions, free of stress and pathogens. As the vertebrate immune system has evolved in a stressful environment with exposure to infections, additional insight into immune responses is likely to be gained from the study of natural populations. We are studying the field vole, *Microtus agrestis*, in its natural environment in Kielder Forest, Northumberland. Over 2 years, voles have been repeatedly captured and assessed for the presence of parasites. The immunological state of individual animals is being monitored by the expression of cytokines and transcription factors in longitudinal samples of peripheral blood. This data will be correlated with the presence and absence of parasites to investigate the effect of parasites on the immune system and to establish their role in immunoregulation. This will provide a greater understanding of the 'hygiene hypothesis' which suggests that the development of allergies in modern humans is the result of higher standards of hygiene and reduced exposure to infection.

P44* Correlation of Regulatory T cells with resistance and susceptibility to *Teladorsagia circumcincta* infection in lambs

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Teladorsagia circumcincta is a common and economically important abomasal nematode of sheep, which shows genetically-linked heterogeneity to infection. Female lambs from parents with variability in resistance to *T. circumcincta* were trickle infected with L3 larvae for 3 months. A range of parameters were measured throughout the period and at post-mortem (13 weeks), which resulted in the identification of 57 lambs with a range of susceptibilities to infection. Faecal egg count was positively correlated with worm fecundity and worm length and inversely correlated with IgA anti-*T. circumcincta* antibody levels and body weight (Beraldi *et al.* 2008. *Int. J. Parasitol. 38; 1567-77*).

This project is investigating the role of the immune response in *T. circumcincta* resistance. T cell subsets are being quantified by immunohistology including the visualization of T_{reg} cells by anti-Foxp3/CD25; and we have cloned and sequenced transcripts for a range of sheep regulatory cytokines associated with T_H1, T_H2, T_H17 and T_{reg} functions; including IFNγ, TGFβ, IL-4, IL-10, IL-17E, IL-21, IL-23A, and IL-35 (EBI3). qRT-PCR assays have been developed for each of these sheep cytokines, and for sheep Foxp3, to quantify expression levels in the abomasal mucosae and gastric lymph node. Analyses of these data will be presented and correlated with relevant phenotypic parameters to help clarify the immunological basis for variation in susceptibility to *T. circumcincta* infection.

P45 Research on the protective properties of haemocyanin from *Helix vulgaris (HvH)* and its derivates against infection with *Trichinella spiralis*

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We studied the effects of application of hemocyanins, derived from *Helix vulgaris* (garden snail) on experimental infection with *Trichinella spiralis* in laboratory animals. For this purpose we used white Wistar rats (age 30 days, weight 120g), which were immunized with hemocyanin alone or conjugated with total larval antigen according to immunization protocol. Rats immunized 20 days p.i. exhibited very low inhibition of muscle larval burden. Animals immunized once with the conjugate showed a significant reduction of larval burden up to 89%. After therapeutic application with the conjugate (single immunization, p.i.), pathomorphological alterations in Trichinella larvae were observed – i.e. a progressive destruction of capsule wall and resorption, which allowed the penetration of inflammatory cells. The matrix was reduced and altered and the capsule was infiltrated with cellular debris. This process ends in total destruction of larvae and their entire lysis. Our results showed low protective effects but strong therapeutic potential of of HvH and its conjugates. Results obtained elucidate some function of hemocyanins as good carrier molecules and activators of the immune system and define their properties as feasible preparations in the therapy of parasitic zoonoses.

P46 Study on the immune properties of *Rapana venosa* hemocyanin and its application as experimental anti-parasitic vaccine

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Hemocyanins are respiratory glycoproteins with quaternary structure, localized in hemolymph of Mollusca and Arthropoda. This one from *Rapana venosa* (RvH) was found to share common antigen determinants with the parasitic nematode *Trichinella spiralis*.
In general, our studies are focused on RvH properties as a vaccine against *T. spiralis.* ELISA assay detected cross-reaction of rabbit-anti-RvH hyperimmune serum with crude extract glycoproteins from infectious *T. spiralis* larvae. Western blot analysis showed a broad spectrum of *Trichinella* antigens reactive to the anti-rabbit-serum. The most prominent signal showed products with relative molecular weight of 45-50 kDa. Fluorescent immunohistochemistry on *T. spiralis* tissue sections demonstrated affinity of antibodies from rabbit-anti-RvH hyperimmune serum to structures located within the stychosome or situated on the nematode cuticle. These antigen structures of *Trichinella* are probably glycosylated since further chemical deglycosylation of the infectious larvae diminished the rabbit-anti-RvH antibodies reactivity.
The hemocyanin of *Rapana venosa* seems to be very useful as a molecular carrier of *Trichinella* antigen determinants in design of a vaccine against trichinellosis. Our further work will be focused on detailed sequence of these common oligosaccharide moieties.

P47 Evasion, suppression and anticipation of the host's immune responses: can filarial nematodes be defeated by a vaccine?

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Filarial nematodes can cause debilitating pathologies in humans such as elephantiasis and river blindness. Given the occurrence of natural immunity to infection and of the experimental elicitation of partial immunity, it is in principle possible to create a vaccine that would augment and complement current strategies. Yet, what prospect is there for a vaccine given the adaptations that allow filariae to establish chronic infections?
Anti-filarial immunity requires the presence of interleukin-5 driven eosinophils. However, when cutaneous eosinophils are already present, the filariae accelerate their larval development, mature earlier and produce more offspring. Moreover, their development is further accelerated by, but not dependent on, adaptive immunity. This suggests that filariae use eosinophils as an environmental predictor of their longevity, and consequently adjust their developmental schedule in order to maximise their chances of transmitting offspring. This raises the possibility that the filariae's developmental plasticity could negate the expected benefits of vaccination. We thus vaccinated mice with DNA plasmids that encode genetically modified parasite proteins to undermine parasite-driven immune suppression while reducing the presence of developmental cues.

P48 Complete absence of the GPI biosynthetic pathway in *Trichomonas vaginalis*

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Glycosylphosphatidyl inositols (GPIs) are considered ubiquitous in eukaryotic organisms and are particularly abundant in parasitic protozoa, where they are essential for parasite infectivity, survival and pathogenesis. *Trichomonas vaginalis* expresses high amounts of lipophosphoglycans (TvLPG), which are a virulence factors involved in adhesion and cytotoxicity. TvLPG has been reported to be anchored to the parasite membrane via a GPI. However, none of the conserved genes involved in GPI synthesis are present in the genome of *T. vaginalis*. In this work, using a combination of bioinformatics, cell-free system experiments, metabolic incorporation of radiolabelled sugars, and mass spectrometry, we demonstrate that *T. vaginalis* is unable to make GPI molecules. We also show evidence that TvLPG is anchored to the parasite membrane by a novel type of PI-glycan. Rhamnose, which is a major constituent of TvLPG., is not found in humans, so enzymes of the rhamnose biosynthetic pathway are potential drug targets. rmlD, the fourth enzyme of the rhamnose biosynthetic pathway has been expressed, purified and characterized. Efforts to generate rmlD deficient parasites will be discussed. *T. vaginalis* represents the first example of a eukaryotic cell lacking the entire GPI pathway and, consequently, it is the first example of a eukaryotic pathogen not using GPI-glycoconjugates as virulent factors.

P49* Characterization og gene family encoding EG95 protein in *Echinococcus granulosus* from G6/G7 genotypes

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A highly effective vaccine has been developed against *E. granulosus* infection in intermediary hosts which is based on a protein expressed by the EG95 cDNA. *E. granulosus* shows a great intra specific variability; at the present there are ten different strains or genotypes (G1-G10). Currently our knowledge about the extent to which the EG95 antigen varies between different

genotypes of *E. granulosus* is incomplete. Knowledge of the extent to which the ***eg***95 gene family varies in different strains of *E. granulosus* would allow predictions to be made about the likely efficacy of the EG95 vaccine against the genotype studied. Restriction patterns observed in Southern blots of G1 and G6/G7 *E. granulosus* genomic DNA probed with the eg95 cDNA differ substantially, suggesting that variability occurs in the gene eg95 gene family between the different strains. Genomic DNA fragments have been cloned into a lambda vector and clones containing these fragments screened for hybridization with the EG95 cDNA. Current work involves the sequencing of the cloned eg95 genes and the design of gene specific PCR primers which will allow rapid identification of the EG95 predicted protein in different isolates of G6/7 genotype parasites.

P50* Simple visual discrimination of a new diagnostic for sleeping sickness

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Loop-mediated isothermal amplification (LAMP) is a DNA amplification technique whose advantages over traditional PCR have put it at the forefront of the search for innovative new diagnostics for infectious diseases. Simple visual discrimination of the test result is critical in developing a straightforward diagnostic. Several simple endpoint detection methods have been developed for LAMP to enable immediate read out of the result. These methods vary in cost, technology and additional processing stages.
Here, 4 such methods are investigated: turbidity; Quant-iT PicoGreen; calcein and $MnCl_2$ and hydroxynaphthol blue, with a specific focus on their use for detecting *Trypanosoma brucei* s.l. parasites. First, the sensitivity, specificity and relative ease of each method was evaluated, with published LAMP assays for these parasites, using serially diluted DNA samples. Second, human patient blood samples were assayed with a novel LAMP assay, and many observers were asked to read the output. In this way we considered sample variation in both cases and readers. This is critical when attempting to generalise the performance of any subjective diagnostic system, but, to our knowledge, has not been addressed in the LAMP literature to date.

P51* Purine Transport in *Trichomonas vaginalis*

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Trichomoniasis, caused by an infection with *Trichomonas vaginalis*, is one of the most common non-viral sexually transmitted diseases in the world. The disease affects over 170 million people in the world annually. Recently the parasite has developed resistance to most of the available drugs, especially metronidazole. There is a need therefore for novel anti-trichomoniasis drug discovery. In the light of this, purine salvage pathways in the parasite are a good drug target, because the parasite is incapable of purine synthesis *de novo* as it lacks the required enzymes and has to salvage preformed nucleoside and/or nucleobase from the host milieu. Knowledge of the number, selectivity and kinetic parameters of purine/ pyrimidine transporters expressed in *T. vaginalis* would facilitate a rational purine/pyrimidine-based chemotherapy of trichomoniasis. Purine transport in the parasite was therefore investigated using the rapid oil stop technique. We demonstrated the presence of at least four purine transporters in the parasite which can be classified as either high or low affinity. A K_m value of 6.5±0.7μM and efficiency of uptake of 1.7pmol $(10^7 \text{ cells})^{-1}$μM was obtained for adenosine. Overall, the findings from this study are comparable to that reported for purine transport in other protozoans, which show high affinity for most nucleosides (cytidine, guanosine and uridine, thymdine, adenine) but low affinity for nucleobases (hypoxanthine and inosine).

P52* Mapping risk foci for endemic sheep scab

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Psoroptic mange in sheep, resulting from infestation by the astigmatid mite *Psoroptes ovis*, is increasingly prevalent in Europe and other parts of the world. As a step towards improved national control, regional or local scab management programmes that target high-risk areas and aim to maintain the number of outbreaks below an acceptable level may be an effective initial use of time and resource. To facilitate such a management approach, scab outbreak farms were identified using a questionnaire survey of sheep farmers, the data from which were used to build a national scab risk model for Great Britain. Modelling the distribution of the reported scab outbreaks identified height above sea level, temperature and rainfall as significant predictors of the probability of an outbreak, superimposed on an underlying pattern of sheep abundance. It is argued that scab management programmes directed at these foci have the potential to allow a more targeted approach to scab control and significantly reduce the prevalence of scab in the UK and other European countries.

P53* Nucleic acid amplification tests for *Trypanosoma congolense*: LAMP (loop- mediated isothermal amplification) versus PCR

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Loop-mediated isothermal amplification offers several advantages over traditional PCR. It takes one third of the time and can be performed in a simple water bath. LAMP reagents do not require a rigorous cold chain for storage and the end result can be determined by simple visual methods such as turbidity in the reaction tube, or colour change with hydroxynaphthol blue. Three *T. congolense* specific LAMP primer sets have been published. Here we compare these LAMP assays to the usual PCR reaction for *T. congolense* savannah. This comparison is made using both well characterised control DNAs and host blood samples stored on Whatman FTA cards. Since this is the typical format in which field samples are typically collected, we are also able to assess the utility of both of these methods for research and epidemiological surveillance. We also ask: does LAMP have potential as a new pen-side diagnostic for animal trypanosomiasis?

P54 *Angiostrongylus costaricensis* egg antigen for the immunodiagnosis of abdominal angiostrongyliasis

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Angiostrongylus costaricensis is the aetiological agent of human abdominal angiostrongyliasis, a zoonotic parasitic disease reported from the United States to Argentina, with a widespread occurrence of the nematode throughout Central and South America. This study assesses the performance of *A. costaricensis* egg as antigen in an enzyme-linked immunosorbent assay (ELISA), for the determination of parasite-specific IgG1 antibodies. The specificity and the sensitivity of the method were 87% and 90.5%, respectively. Through this test it was possible to demonstrate a sharp and early decline in IgG1 antibody in serum samples taken from patients with histopathological diagnosis of abdominal angiostrongyliasis at different time points after surgical treatment. The present work demonstrated the usefulness of the egg antigen in the development of a specific diagnostic test for abdominal angiostrongylosis.

P55* Garlic: a potential cure for hole-in-the-head disease in fish?

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The flagellated protozoan *Spironucleus vortens* is a renowned parasite of economic importance in ornamental fish aquaculture. It commonly infects members of the Cichlidae family, such as *Pterophyllum scalare* and *Symphysodon discus*, the trade of which are valued at an estimated US$7000 million per year. Systematic infection leads to characteristic symptoms of 'hole-in-the-head' disease, often resulting in fish mortality. The 5-nitroimidazole drug metronidazole is the current drug of choice used to treat spironucleosis. In recent years, however, there has been controversy surrounding its prescription due to its adverse effects on the environment, including antibiotic resistance and carcinogenicity, prompting its ban from use in outdoor farming of food fish. Here, we evaluate the antiparasitic effect of *Allium sativum* (garlic) whole extract and isolated compounds (ajoene, allicin, dithiins and thiosulfinates) on the growth and gas metabolism of *S. vortens* as a potential natural alternative to metronidazole in the treatment of hole-in-the-head disease in fish.

P56* Uptake of Quantum-dots: a comparison of feeding by *Spironucleus vortens* and *Giardia intestinalis*

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Pathogenicity, infection and persistence of parasites are dependent on their nutritional status: mechanisms of feeding are an important aspect of these processes. We used confocal laser scanning microscopy of endocytosis to compare uptake processes in three protistan flagellates by fluorescence (405nm excitation-585nm emission) of streptavidin-coated Quantum-dots (10-15nm dia. Invitrogen). These markers, approximately the size of single protein molecules, show extremely bright emission, and unlike organic fluorophores are not photobleached even after extended excitation. In the fish parasite, *S. vortens*, large clusters of dots appeared within 2 s in flagellar pockets (as confirmed by electron microscopy): then they appeared in numerous small vacuoles, before becoming concentrated in to fewer, larger vacuoles after 1 min. Then, intracellular dot numbers declined as they were egested, perhaps as their coating (streptavidin ligated to an amphiphilic polymer surrounding a ZnS sheath) was digested, thereby exposing the toxic semiconductor core material (which contains Cd Selenide). In contrast, for *G. intestinalis* the process of nutrient uptake appears to be completely different, as we were unable to observe any internalisation of Quantum-dots, even after extended (up to 1.5 h) exposure times.

P57 Short interfering RNA-mediated knockdown of drosha and pasha in undifferentiated *Meloidogyne incognita* eggs leads to embryonic lethality

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Micro (mi)RNAs play a pivotal role in the developmental regulation of plants and animals. We reasoned that disruption of normal heterochronic activity in differentiating *Meloidogyne incognita* eggs may lead to irregular development, lethality, and by extension, a novel target for parasite control. On silencing the nuclear RNase III enzyme drosha, a critical effector of miRNA maturation in animals, we found a significant inhibition of normal development and hatching in siRNA-soaked *M. incognita* eggs. Developing juveniles presented with highly irregular tissue patterning within the egg, and there was no observable phenotypic recovery following removal of the environmental siRNA. Aberrant phenotypes were exacerbated over time, and drosha knockdown proved embryonically lethal. Subsequently, we identified and silenced the drosha cofactor pasha, revealing a comparable inhibition of normal embryonic development within the eggs to that of drosha-silenced eggs, eventually leading to embryonic lethality. On silencing the cytosolic RNase III enzyme dicer, we found an unexpected and substantial up-regulation of dicer

transcript abundance, which did not impact on egg differentiation or hatching rates. Silencing of the individual transcripts in hatched J2s was significantly less successful, and resulted in temporary phenotypic aberration of the J2s, which recovered within 24 h to normal movement and posture on washing out the siRNA. We propose that drosha, pasha and their ancillary factors may represent excellent targets for novel nematicides and/or *in planta* controls aimed at *M. incognita*, and potentially other parasitic nematodes, through disruption of miRNA-directed developmental pathways.

P58 Variation in parasite fitness of *Schistosoma haematobium* genotypes in Mali

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Schistosoma haematobium is responsible for the largest number of human infections with schistosomiasis, but is poorly studied in relation to *S. mansoni.* Variations in the fitness of individual genotypes are an important factor in likely evolutionary changes in parasite populations under selection by large-scale drug treatment. Here we report the development of novel microsatellite assays and their use to genotype the parasite populations of 47 children from 2 schools in the Ségou region of Mali, prior to the introduction of a national schistosomiasis control program. There was only limited evidence of population subdivision between individual children or between the two schools suggesting that few barriers to gene flow exist in this population. Older children and boys harboured more diverse infections, as measured by the number of unique adult genotypes present inferred using sib-ship analyses, but there was no difference in the average diversity of their larval populations. Individual parasite genotypes had variable reproductive success, and parasite fitness was reduced in older children. Rare alleles were more commonly found in the most heavily infected children. This data will serve as a baseline against which to measure the effect of treatment on parasite population genetics.

P59 Recombinant SmNPP-5 induces antibodies that partially inhibit the enzymatic activity but fail to prevent the infection with *Schistosoma mansoni*

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Recent proteomic characterization of the *S. mansoni* tegument, the major parasite-host interface and a source of potential antigens, identified a putative nucleotide pyrophosphatase/ phosphosdiesterase (NPP) 5, as a plasma membrane-associated surface-exposed protein, but enzyme activity had been detected long ago. NPPs are ubiquitous membrane-associated or secreted ecto-enzymes that require divalent cations and alkaline pH; they act by regulating the metabolism of extracellular nucleotides, and consequently they have a role in purinergic signaling which affects diverse biological processes as platelet aggregation, apoptosis, cell proliferation, differentiation and motility. We here verify its potential as a vaccine candidate; we cloned the gene, heterologous express it in *E. coli* and purified it by nickel affinity chromatography. The dialyzed protein was used to immunize mice targeting antibodies production; immunized animals were also challenged with cercariae. The antibodies were used to confirm the protein localization by immunoblotting and immunolocalization; we also demonstrated that they partially inhibit the enzymatic activity in ex vivo live adults worm (~60%). Forty five days after cercarian challenge the immunized animals were perfused and did not reduce worm burden in spite of induce a specific immune response; the animals presented higher titles of IgG1 than IgG2a before and after the challenge, low levels of IFN-g and high levels of Il-10 and Il-5 were also detected. Financial support: FAPESP, USP.

P60* Detection of DNA-Methylation in *Schistosoma manoni*

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As expected for a complex metazoan parasite, schistosomes exhibit a high degree of gene regulation. Large-scale transcriptome studies have shown stage-specific, as well as gender-specific expression profiles of some genes. DNA-methylation-mediated gene silencing as a means of transcriptional control has evolved in higher eukaryotic organisms. While recent studies have established that schistosomes post-translationally modify histones and express small regulatory RNAs, no compelling evidence exists that demonstrates this parasite's genome is methylated. However, the sequencing of the schistosome genome revealed the presence of key proteins involved in the DNA-methylation machinery - a putative Dnmt2-like protein as well as a Methyl-Binding-Domain Protein (MBD). Here, using methylation sensitive amplification polymorphism (MSAP) and methylated genome capture methodologies; we provide preliminary evidence that the genome of this blood fluke is indeed methylated. Specifically, cytosine methylation is detected in both, free-living and parasitic life stages as well as in both sexes. Mapping of potentially methylated genomic fragments to the recently assembled *S. mansoni* genome links intragenic and intergenic DNA methylation to the important roles of repetitive element silencing and gene expression control. Collectively these observations will contribute to a greater understanding of Platyhelminthes developmental biology, assist in the evolutionary reinterpretation of DNA methylation across the Metazoa and perhaps, provide a cautionary note for transgene expression studies within the parasite.

P61 Release of Apoptosis inducing factor is one of the early events of focal apoptosis in skeletal muscle cell due to trichinellosis in mice

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Unlike the majority of intracellular parasites, *Trichinella* occupies the host muscle cell without killing it, which results in a symbiotic structure called a nurse-cell. The molecular mechanisms responsible for this unique relationship are likely to involve pathways of apoptosis. The possible role of apoptosis inducing factor (AIF) in myocyte dedifferentiation after occupation by *T. spiralis* was investigated in mice. Its relationship with other apoptosis-related factors such as Bax, Bcl-2 and caspase-3, was evaluated by immunohistochemistry. In the context of low Bax and caspase-3 expression and strong AIF release in the sarcoplasm and translocation to the nucleus at the very early stage of infection, we suppose that AIF-mediated and caspase and Bax independent signaling is involved in the apoptosis activation in the area of *Trichinella* occupation. In the time course of nurse cell formation Bax, Bcl-2 and caspase-3 migrate into the enlarged nuclei but in the end of encapsulation of *Trichinella*, caspase-3 and AIF disappear. It seems that up-regulation of certain factors of apoptosis may be implicated in the mechanisms of dedifferentiation of the occupied muscle cell rather than in the processes leading to its death.

P62* Development of a diagnostic test for active sheep scab infestation based on biomarkers

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Sheep scab is a highly contagious ectoparasitic disease caused by the mite, *Psoroptes ovis.* The disease causes an intensely pruritic lesion with severe dermatitis, and is a major welfare and production issue in the national flock. Current treatments based on chemical intervention are unsustainable due to concerns over residues in meat, their effect on the environment and operator health. Early diagnostic tests are therefore crucial for future disease control. Recent microarray studies have identified over 600 host genes differentially expressed in circulating leukocytes following *P. ovis* infestation. Many of these genes encode for proteins known to be involved in inflammatory responses in other diseases and are highly conserved, forming a list of

potential biomarkers. Further advantages of these proteins are that they will give an early diagnosis of disease and will also indicate the current disease status of the animal. To date potential biomarkers have been filtered and ranked and are now being evaluated, with the most promising undergoing validation using samples from experimental trials and the field. Finally the possibility of combining the biomarker diagnostic with an antibody test currently being developed at Moredun will be investigated, to give a full profile of infestation and disease status of individual animals in a flock.

P63 RNA-interference gene knockdown in the poultry red mite, *Dermanysus gallinae*: studies on histamine-releasing factor and cathepsin-D

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The poultry red mite, *Dermanyssus gallinae*, is a parasite of fowl, cage birds and wild birds. In the UK, the mite is the most economically deleterious ectoparasite of laying hens where control and production losses are estimated at €130M p.a. *D. gallinae* control is typically by use of synthetic acaricides. Continued use of these chemicals is hampered by issues including development of resistance, residue and other environmental effects. There is a need for environmentally-friendly control methods such as vaccination.
Vaccine development necessitates identifying a putative critical/vital antigen and verifying the efficacy of "attacking" such a target. We investigated if it is possible to utilize gene-knockdown approaches to assess the likely success of pursuing certain targets, thereby, greatly expediting vaccine development programmes. Histamine-releasing factor and a proteolytic enzyme were the candidate genes/protein chosen and were targeted by double-stranded RNA-interference. The small size of the mite prevents micro-injection of dsRNA being practical. Alternative methods of dsRNA administration were assessed such as immersion of mite tissues and whole mites and feeding. Our results towards these aims are presented.

P64 Effect of induced coccidiosis in three hybrid strains of industrial broiler chicken

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Coccidiosis is one of the major menaces for poultry industry throughout the world. Chemotherapy is the primary practiced approach to combat coccidiosis in commercial poultry operations but the development of resistance against various strains has created problem in this regard. Secondly, the drug residue in foods going into the human food chain has already become a problem and issue of proactive concern at international level. It is therefore crucial that alternatives to chemotherapeutic approaches be developed to eliminate or at least minimize coccidiosis and/or its economic impact. In the present study, effect of induced coocidiosis was investigated in three strains of broiler chickens: Hubbard, Starboro and ArbourAcr (N = 300 x 3). Percent mortality, oocyst per gram of droppings, humoral and cellular responses after induced coocidiosis were determined. Humoral response was detected by indirect haemagglutination (IHA). IHA antibody titres at Day 15 post-infection was maximum in Hubbard strain (GMT 955.37) followed by Starboro (831.74) and ArborAcr (90.50). Cell mediated immunity was assessed by measuring amplitude of toe-web swelling after injection of PHA-P in comparison with the control. Maximum swelling was recorded in Hubbard (9.76mm) followed by Starboro (7.76) and ArborAcr (8.06) 72 h post injection. Post-infection mortality was maximum in Starboro (43.07%) followed by Hubbard (38.4 %) and ArborAcr (36%). From the results of the present study it was concluded that Hubbard strain is resistant to *Eimeria* infection as compared to Starboro and ArbourAcr strains.

P65 Characterization og gene family encoding EG95 protein in *Echinococcus granulosus* from G6/G7 genotypes

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A highly effective vaccine has been developed against *E. granulosus* infection in intermediary hosts which is based on a protein expressed by the EG95 cDNA. *E. granulosus* shows a great intra specific variability; at the present there are ten different strains or genotypes (G1-G10). Currently our knowledge about the extent to which the EG95 antigen varies between different genotypes of *E. granulosus* is incomplete. Knowledge of the extent to which the *eg*95 gene family varies in different strains of *E. granulosus* would allow predictions to be made about the likely efficacy of the EG95 vaccine against the genotype studied. Restriction patterns observed in Southern blots of G1 and G6/G7 *E. granulosus* genomic DNA probed with the eg95 cDNA differ substantially, suggesting that variability occurs in the gene eg95 gene family between the different strains. Genomic DNA fragments have been cloned into a lambda vector and clones containing these fragments screened for hybridization with the EG95 cDNA. Current work involves the sequencing of the cloned eg95 genes and the design of gene specific PCR primers which will allow rapid identification of the EG95 predicted protein in different isolates of G6/7 genotype parasites.

P66 *Fasciola hepatica*: characterisation of the surface carbohydrates of the miracidia

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The *Fasciola hepatica* miracidia were examined by ruthenium red staining and by binding of lectin-peroxidase and lectin-gold conjugates in attempt to demonstrate the glycocalyx and to identify the carbohydrate epitopes. Over cilia, ciliated plates and intercellular ridges, the distinct patterns of glycocalyx were revealed. Using peroxidase labelled lectins, the binding of *Canavalia ensiformis* (Con A) and *Triticum vulgaris* (WGA) were found on the larvae. The results for *Lens culinaris* (LCA), *Glycine max* (SBA) and *Ulex europaeus* (UEA-I) were negative. Further detailization by gold labeled Con A and WGA revealed that binding sites for Con A are located on the whole larval surface whereas for WGA they except on the ridges.
In attempt to clarify the participation of carbohydrate-lectin interactions in the parasite-snail host relationships, we examined the effect of exogenous lectins, which specifically bind to the miracidial surface on *in vitro* miracidium-to-sporocyst transformation. Clear differences of number of received sporocysts in presence or absence of lectins were found. This indicates that interactions between miracidial surface carbohydrates and snail lectins may initiate this process on entering of the miracidia into snail host.

P67* Prevalence of hydatid cysts in slaughtered animals in Sirte, Libya

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The aim of the present study was to determine the prevalence of cysts echinococcosis infection among herbivorous animals in abattoir of Sirte, Libya. The liver, lungs and other organs of indigenous sheep (n=3794), goats (8123), camels (739) and cattle (113), slaughtered for human consumption between July 2004 and May 2005, were examined for the presence of hydatid cysts. Overall, 3.3% of these animals had hydatid cysts, with a prevalence of 4.9% in sheep, 2.4% in goats, 2.7% in camels and 15% in cattle. The level of infection was significantly higher in cattle probably as (i) they tend to graze on plains which are more exposed to *Echinococcus granulosus* eggs voided by infected dogs in the field and/or (ii) cattle are usually slaughtered at older age increasing their exposure to infection. The current dataset of cystic echinococcosis prevalence was compared with previously recorded data from Libya and other Arab countries.

P68 Some (worms) like it hot! Elevated water temperature increases parasite growth in the stickleback-*Schistocephalus* system

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Anthropogenically altered thermal regimes can have important consequences for the biology of aquatic organisms, and they potentially affect the interactions between hosts and parasites. However, few experimental studies have tested the specific hypothesis that elevated temperatures alter infection phenotypes in fish. In this study we exposed lab-bred three-spined sticklebacks *Gasterosteus aculeatus* to infective stages of the pseudophyllidean cestode *Schistocephalus solidus* and reared them under two temperatures (15 and 20°C), during which fish were fed *ad libitum* for a period of 8 weeks. Postmortem analysis revealed that experimentally infected fish reared under the 20°C treatment harbored plerocercoids that were significantly larger – both absolutely and in relation to the fish host – than those that were reared under the 15°C treatment. Further work is now required to identify the mechanisms underlying the temperature effects. Both the infectivity of *Schistocephalus* to bird hosts and their reproductive output as adults is strongly linked to plerocercoid size. Our results therefore suggest that, under elevated temperatures, the total input of infective stages into aquatic environments is likely to increase dramatically, with consequences for the fitness of individuals and the potential survival of stickleback populations.

P69 Safety in numbers? The impact of acanthocephalan infection on aggregation behaviour in amphipods

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The amphipod *Gammarus pulex* has recently been found to show an increased inclination towards aggregation in the presence of fish predator cues. Here we investigate the impact of parasitism on this newly proposed anti-predator strategy. *G. pulex* that are the intermediate hosts of acanthocephalan parasites have previously been found to have altered phototactic and geotactic responses. Here we test the hypothesis that acanthocephalan infection will also reduce the tendency of *G. pulex* to aggregate in the presence of predator cues. Such parasite induced changes in aggregation behaviour are likely to increase the vulnerability of *G. pulex* to predation by the definitive host.

P70* Environmental Influences on the prevalence and distribution of parasites associated with wild caught ornamental Freshwater Catfish in Trinidad, West Indies

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A total of 581 catfish (52 *Ancistrus* sp, 470 *Hypostomus robinii* and 59 *Corydoras aeneus*) from 27 sites on ten river drainages in Trinidad were collected and screened for parasites over a 15 month period. Water quality parameters were also measured at the time of collection or from samples collected and analyzed in the laboratory. The most common parasite found was leeches (7.3%), followed by unknown encysted digean eggs (5.7%) and freshwater mussel glochidia (5.0%). *Corydoras aeneus* had the highest association with parasites (5.5%) followed by *Hypostomus robinii* (4.1%) and *Ancistrus* sp. (1.9%). Generally, parasites were widely distributed among the sites with the exception of *Argulus* sp., which was found only in localized region of the Caroni drainage. The prevalence of leeches was inversely related to nutrient levels whereas *Argulus* sp. was positively related to high nutrient levels. Size as a factor affecting prevalence of parasites would be discussed as well.

P71 Species of *Gyrodactylus* from Grayling (*Thymallus thymallus*) and brown trout (*Salmo trutta*) from the Danube basin in Austria

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Three species of *Gyrodactylus* on grayling and brown trout from 15 Austrian rivers were characterised using sequencing of rDNA ITS-1 and ITS-2; from grayling 32 parasites were identified as *G. thymalli*/*G. salaris* (65.3%), 11 as *G. truttae* (22.5%), and six as *G. teuchis* (12.2%). From brown trout, three parasites were identified as *G. thymalli*/*G. salaris* (7%), 25 as *G. truttae* (60.5%), and 13 as *G. teuchis* (32.5%). Trout infections frequently exceeded 30 *Gyrodactylus* specimens per fish, whereas there was a maximum of three parasites on individual grayling. Sequencing of a 745 bp-long fragment of mitochondrial cytochrome oxidase subunit 1 of the *G. thymalli*/*G. salaris* revealed 10 new haplotypes that grouped into at least two well supported clades when compared to all previously known mitochondrial haplotypes of this species. Of the 4 different analytical approaches used (Neighbor-joining, Maximum parsimony, Maximum likelihood, and Bayesian inference) none resolved the internal nodes of the reconstructed phylogeny. There is therefore no evidence for the hypothesis that the Danube basin served as a glacial refugium for Northern European *G. thymalli*/*salaris*.

P72 *Ergasilus sieboldi* – mortalities and management

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Ergasilus sieboldi (von Nordmann, 1832) is a pathogenic crustacean parasite that infects the gills of freshwater fish. It is one of many parasites that have been introduced to England and Wales with the international trade in fish, and has been associated with disease throughout many parts of the world. It is well recognised that infections can reduce the respiratory, osmoregulatory and haemopoietic capacity of their hosts, leading to reduced growth rates, condition loss, respiratory failure and death. However, there is very little information on its impacts within freshwater fisheries.

The Environment Agency has an important role in maintaining and improving fisheries, and investigating fish mortalities where disease outbreaks have occurred. Two case studies are presented to highlight the effects of *E. sieboldi* in stillwater fisheries. The first investigated disease in rainbow trout, *Oncorhynchus mykiss*, in an extensive, well-managed reservoir in southern England. The second involved large scale losses of bream, *Abramis brama*, and tench, *Tinca tinca*, from a small, intensively stocked fishery in north west England. Impacts at the host level included severe gill damage, reduced growth, condition loss and mortality. Fishery level impacts involved loss of stock, a reduction in fishery performance, reduced angler satisfaction and economic loss. The importance of good fishery management in preventing the introduction of this parasite and reducing subsequent disease impacts is emphasised.

P73 A new genus and two new species of Phyllobothridae (Cestoda: Tetraphyllidea) from the carcharhinid shark *Carcharhinus* cf. *dussumieri* in the Persian Gulf

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New collections of tapeworms from the carcharhinid shark species *Carcharhinus* cf. *dussumieri* (Whitecheek Shark) from the Persian Gulf have yielded one new genus and species of tetraphyllidean tapeworm (Phyllobothriidae), *Doliobothrium haselii* n. gen. n. sp. Histological sections and scanning electron microscopy confirm that this new genus differs from all other Phyllobothriid genera in its possession of Bothridia that lack apical suckers and are tubular in form, bearing proximal and distal apertures. Furthermore two new species belonging to the genus

Paraorygmatobothrium. (Phyllobothriidae) were found. Both new species resemble the subset of *Paraorygmatobothrium* species. In combination, their shorter lengths, and possession of fewer testes and smaller bothridial apical suckers distinguish both *Paraorygmatobothrium mobedii* n. sp. and *Paraorygmatobothrium sinuspersicense* n. sp. from all but 2 of their congeners. They conspicuously differ from *P. exiguum*, in that the spinitriches on their distal surfaces are not arranged on "bumps" and from *P. filiforme* in their possession of fewer proglottids. The two new species differ from one another in cirrus sac shape, testes shape and arrangement, vitelline follicle arrangement (i.e., 2 vs. 4-6 follicles in each lateral band). In addition, statistically significant differences were seen in terminal proglottid length, ovary length, and apical sucker diameter. This is the first description of tapeworms from elasmobranchs from the Persian Gulf.

P74 Epizoic copepods of ghost shrimp, *Neocalichirus indicus* (Crustacea: Decapoda: Callianassidae) from the Persian Gulf and Gulf of Oman

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In the course of study on ghost shrimps of Iran from 2006-2010, specimens of *Neocalichirus indicus* were mostly found to be infested with clausiid copepods. The ghost shrimps were collected from 40 locations along the intertidal coasts of the Persian Gulf and Gulf of Oman. The results from the current study indicate that there are two species of clausiids in the region. The copepods were mostly found on the carapace and large chela. Based on previous studies only two species namely, *Clausidium senegalense* and *C. saldanhae* were described from the West Indian Ocean. In the current study, one species was observed only at Qeshm Island and the other congeneric species was found as a dominant species in other localities. Both species are new to science. Here, the interspecific variations of the clausiid copepods of the region are discussed.

P75* Role of the flavoenzyme lipoamide dehydrogenase in African trypanosomes

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In nearly all eukaryotes lipoamide dehydrogenase [*dihydrolipoamide* + NAD^+ ↔ *lipoamide* + *NADH* + H^+] is present as a subunit of the mitochondrial α-ketoacid dehydrogenase multienzyme complexes and of the glycine cleavage complex. As a component of these complexes, lipoamide dehydrogenase plays an important role in the production of acetyl-CoA and NADH, the tricarboxylic acid cycle, the degradation of branched chain amino acids and the break down of glycine generating methylenetetrahydrofolate for the subsequent synthesis of thymidylate.
Trypanosoma brucei encodes a single copy gene for lipoamide dehydrogenase. The gene has been cloned and overexpressed in *E. coli*. Western blot analyses with the polyclonal antiserum against the recombinant protein yielded a protein concentration of 1-3 and 25-30 µM in bloodstream and procyclic cells, respectively. Immunofluorescence microscopy revealed a mitochondrial localisation in both parasite forms. RNA interference against lipoamide dehydrogenase resulted in a proliferation phenotype for both forms of the parasite. In the case of bloodstream parasites, growth could be restored by the thymidine present in the medium, suggesting that lipoamide dehydrogenase could play an essential role as part of the glycine cleavage complex. A lipoamide dehydrogenase knock out bloodstream cell line was generated in the presence of thymidine. Further experiments are in progress to elucidate the distinct roles of this FAD-disulfide oxidoreductase in the two life stages of African trypanosomes.

P76* Distinct function of the glutathione peroxidase-type enzymes in african trypanosomes

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Trypanosoma brucei, the causative agent of African sleeping sickness, has a unique system for hydroperoxide detoxification. The parasites lack catalase and glutathione peroxidases but have 2-Cys peroxiredoxins and cysteine-homologues of classical peroxidases, that obtain their reducing equivalents from trypanothione, in a reaction mediated by the small dithiol protein tryparedoxin. Despite similar in vitro substrate specificities towards small, artificial hydroperoxides and overlapping cellular localization, both peroxidases proved to be essential for the parasites.
To elucidate the distinct functions of the proteins, the rate constants for the reduction of different hydroperoxides, ranging from small hydrophilic to bulky hydrophobic compounds, such as the hydroperoxides of arachidonic acid, were determined. We could show that the glutathione peroxidase-type enzyme is highly efficient in reducing lipophilic hydroperoxides. In contrast the peroxiredoxin prefers small hydrophilic substrates such as hydrogen peroxide and is rapidly and irreversibly inactivated by the hydrophobic hydroperoxides. Comparing the expression levels of the proteins in different growth phases by Western blot analysis revealed, that both enzymes are constitutively expressed. Bloodstream parasites that overexpress the glutathione peroxidase-type enzyme twofold, showed a significantly decreased sensitivity against exogenous hydrophobic hydroperoxide whereas the sensitivity against hydrogen peroxide was not affected. These results are consistent with the in vitro data and suggest that the two types of tryparedoxin peroxidases are essential because of their distinct substrate specificity.

P77* Characterization of *Leishmania donovani* Thiol Dependent Reductase 1 knockout

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Leishmania donovani Thiol Dependent Reductase 1 (TDR1) is an interesting parasite-specific enzyme that presents some similarities with human omega-GST, as demonstrated previously by its thiol transferase and dehydroascorbate reductase activities (Denton *et al*., 2004). This enzyme

also catalyzes *in vitro* the conversion of pentavalent into trivalent antimonial, an ability that could be important in the mechanism of action of pentavalent antimonials against *Leishmania*. However, the role of TDR1 *in vivo* remains unclear. In order to provide insight into the function of TDR1, attempts were made to delete the encoding genes by homologous gene replacement. Attempts to get the null mutant after two rounds of gene replacement failed to generate a null mutant. This could have been explained by the gene being essential, at least in the promastigote stage. Nevertheless, we investigated the possibility that chromosome 33, on which the gene resides, is triploid. We report here the disruption of three copies of the gene in *L. donovani* by successive rounds of gene replacement and gene rescue with a plasmid construct containing TDR1. This provides definitive evidence for the plasticity of *Leishmania* genome in terms of ploidy. Phenotypic analysis of the null mutants is ongoing, the data to date will be presented.

P78* Investigating fucosylation in *Trypanosoma brucei*

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Glycoconjugates play a very important role in the survival and infectivity of *Trypanosoma brucei*. GDP-fucose has previously been detected in the sugar nucleotides pool of *T. brucei* [1], and its *de novo* synthesis has been shown to be essential for parasite cell growth [2]. Here we describe the characterization of *T. brucei* fucosyltransferase (*Tb*FT), the enzyme responsible for the transfer of fucose to the growing glycan chains. *Tb*FT shows homology to a1,2-fucosyltransferases (CAZy GT11 family) and characterization of the reaction product confirmed it as a member of the GT11 family. A panel of acceptors was analyzed to determine the substrate specifity of this enzyme. *Tb*FT shows the best activity with Galb1,3GlcNAc as acceptor.
Fucose has recently been observed in a high-molecular-weight glycoconjugate on the surface of procyclic form *T. brucei* [3], but it has not yet been possible to precisely identify the fucosylated glycoconjugate(s). Here we will discuss current experiments and future plans to elucidate the role of fucose in the life of this parasite.
[1] Turnock D.C., Ferguson M.A.J., (2007) *Eukaryot Cell* **6**, 1450-63. [2] Turnock D.C. *et al*., (2007) *J. Biol. Chem.* **282**, 28853-63. [3] Guther M.L.S. *et al.*, (2009) *Eukeryot Cell* **8**, 1407-17

P79 Immune detection of acetylcholinesterase in subcellular compartments of *Trypanosoma brucei evansi*

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The regulatory processes that involve the calcium ion in the trypanosomatidae family have been deeply studied perhaps the molecular protein entity by which the calcium entries to the parasites is a blank in the field. Recently we have characterized the presence of a possible nAChR in *Trypanosoma brucei evansi*. Those findings confirms the widespread presence of those receptors in the biological spectrum, from protozoans to mammals. The aim of this study was therefore to determine the presence of AChE in *T.b.evansi* through a strategy of confocal microscopy and subcellular localization by using commercial antibodies. The subcellular location studies carried out, provided data that are pioneers with respect to the biology of *T.b. evansi*, and also represent a novel contribution to the understanding of non-neuronal AChE. Analyzing the data generated by differential and isopycnic centrifugation and by confocal studies is possible to conclude that the AChE in *T.b. evansi* is predominately in the glicosoma. This is certainly a radical contribution in the area of tripanosomatids, since this organelle has been proposed as the target of choice for the rational development of drugs, because there is no counterpart in mammals and is central to the energy production of these parasite.

P80 Expression of recombinant plasmids containing LACK and TSA genes of *Leishmania major* (MHRO/IR/75/ER) in eukaryotic cells

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TSA (thiol-specific-antioxidant antigen) is the immuno-dominant antigen of *Leishmania major* considered as the most promising molecule for a recombinant or DNA vaccine candidate against leishmaniasis. LACK is the other immuno-dominant antigen of *L.major* which is considered as the most promising molecule for a recombinant or DNA vaccine against leishmaniasis. In the present work, genomic DNA of TSA and LACK proteins were amplificated by PCR method. The PCR products have cloned into pTZ57R/T vector followed by subcloning into the eukaryotic expression vector pcDNA3. The recombinant plasmids were characterized by restriction enzymes and PCR revealed a 939 bp band for pc-LACK recombinant plasmids and a 600 bp band for pcDNA3. The recombinant plasmids containing LACK and TSA genes were expressed in eukaryotic cells (CHO cells). The expression of plasmids containing TSA and LACK genes were demonstrated by SDS-PAGE and western-blotting. A band at about 36 KDa was recognized by *Leishmania* antibody-positive mice sera in protein extracts of the cells transfected with pc-LACK and a band at about 22.1 KDa was recognized by *Leishmania* antibody-positive mice sera in protein extracts of the cells transfected with pc-TSA. These results indicated successful expression of plasmids containing TSA and LACK genes in eukaryotic cells.

P81 Pterin Metabolism in *Crithidia fasciculata*

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Trypanosomatids such as *Crithidia* and *Leishmania spp.* are known pterin auxotrophs. Despite pterin metabolism being extensively studied in *Leishmania*, the function(s) of pterins within these parasites remains elusive. In the current study, pterin metabolism was studied in the model organism, *Crithidia fasciculata*. A variety of pterins were found to stimulate the growth of these parasites in a fully defined medium, with L-biopterin observed to be the most effective growth-stimulating pterin. An HPLC-based assay was developed to determine the intracellular pterin levels within these parasites and different pterins were observed to be metabolised to their respective tetrahydro-forms. These findings indicate that *C. fasciculata* is able to utilise a variety of tetrahydropterins to carry out the unknown pterin-dependent reactions. Progress towards identifying these reactions will also be presented.

P82 Adenylate kinases and related proteins in the trypanosome flagellum

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Adenylate kinase (AK) is typically a small monomeric enzyme catalysing reversible phosphotransfer from ATP (or GTP) to AMP. This classic reaction of intermediary metabolism is also ubiquitous in eukaryotic flagella. We have speculated on the function of two AKs that are targeted and anchored within the paraflagellar rod (PFR) of the *Trypanosoma brucei* flagellum, but as with the axoneme-associated AKs described in other flagellate eukaryotes the role(s) played by these trypanosome enzymes is uncertain. In contrast, three large (> 100 kDa) axonemal proteins containing degenerate-looking AK domains are required for the normal motility of procyclic trypanosomes and are each necessary for the viability of bloodstream trypanosomes. We used comparative bioinformatics and compared the ultrastructural defects seen in a range of flagellar RNAi mutants to predict how these proteins contribute to motility. Curiously, the C-termini of the three axonemal AK-related proteins resemble the N-terminal extensions that are necessary for the targeting of the paraflagellar rod AKs. Another AK-like protein (TbADKE) also contains an

extended N-terminal domain that is similar in sequence to the N-termini of the PFR enzymes, but this protein is located in the axoneme. Here, we include our attempts to perform gene-knockout of TbADKE as part of ongoing studies to understand its function. The amino acid sequence of TbADKE suggests the protein could be catalytically active, but the accuracy of this prediction is perhaps called into question by the absence in putative *Leishmania* ADKE orthologues of conserved residues thought necessary for AK activity.

P83* Characterization of a clathrin interactome in *Trypanosoma brucei*

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Trypanosoma brucei exhibits unique membrane trafficking properties. The endomembrane system is polarised, with most organelles of both exocytic and endocytic systems located at the posterior of the cell. Further, plasma membrane exchange is restricted to the flagellar pocket (FP), a small invagination surrounding the flagellum root. At the molecular level there are specific features that differentiate trypanosomes from higher eukaryotes. Importantly, in T. brucei endocytosis is strictly clathrin mediated, but the adaptor protein 2 (AP2) complex, the hallmark of clathrin recruitment and cargo selection at the plasma membrane in mammals and yeast, is absent. Further, TbEpsinR, the only endocytic adaptor-like protein characterized to-date has limited involvement in bulk internalization, but a general effect on protein endocytosis. Hence protein(s) involved in recruiting clathrin to the FP membrane remain elusive. Using immunoprecipitation, mass spectrometry, genomic tagging and immunofluorescence microscopy, we searched for novel clathrin-interacting proteins. Nine novel gene products that localize to the endocytic pathway were identified. Two of these are Hsc70 and myosin IB, factors with known roles in endocytosis, providing validation of the method. Of the remaining panel, five are upregulated in mammalian stage forms, while all demonstrate considerable colocalization with clathin and a requirement for robust growth. This analysis provides a first view of the divergent factors involved in clathrin-mediated endocytosis in trypanosomes.

P84* Characterization of a nuclear Pumilio domain protein in *Trypanosoma brucei*

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Proteins of the PUF family contain Pumilio RNA binding domains. They have been found in all eukaryotes so far examined. Some of them bind to the 3'-untranslated regions of mRNAs, repressing translation and destabilising the bound mRNA. Others are known to be involved in ribosomal RNA maturation.
The genome of *T. brucei* has eleven open reading frames containing pumilio repeats. A previous phylogenetic analysis, concentrating on the Pumilio domains alone, grouped PUF7 and PUF8 together with yeast Nop9 and Puf6, and with human KIAA0020, all of which are found in the nucleolus. Consistent with this, we have found that epitope-tagged TbPUF7 is located in the nucleolus. Tandem affinity purification of tagged TbPUF7 revealed binding to a cyclophilin-like protein, TbNCP1, which is found throughout the nucleus, but no attached RNA could be detected. The PUF7-NCP1 interaction was verified by bidirectional co- immunoprecipitation. RNAi targeting PUF7 caused a mild growth defect, and we could find a slight defect in the processing of pre-18S rRNA but not in 28S or 7SL RNA.

P85* SUMO in *Trypanosoma brucei*

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The small, ubiquitin-related protein modifier SUMO is essential in most eukaryotes and involved in many different cellular processes. It is attached to a lysine residue of its target proteins in an ubiquitin-like way and changes the properties of these targets upon binding.

In situ tagging of SUMO and subsequent Western blot analysis showed SUMO and its targets in both procyclic and bloodstream forms. The global SUMO patterns, as seen on Western blots, appeared to be similar between the two life cycle stages, however, this work is still in progress.
Generating oxidative stress in trypanosomes by adding hydrogen peroxide led to a global increase of SUMOylation, however, other environmental stresses, like heat shock, cold shock and inhibition of glucose transport had no visible effect.
Knock-down of SUMO by RNAi led to a growth defect in bloodstream trypanosomes but not in procyclics.
RNAi against the Tb927.2.2460 gene product, a close homologue to human and yeast SUMO conjugating enzyme Ubc9, resulted in a decrease of global SUMOylation. RNAi against the Tb09.160.0970 gene product, which was annotated as putative SUMO protease, led to an increase of SUMOylation. This indicates a role for these two proteins in the SUMOylation process in *Trypanosoma brucei.*

P86* Control of stumpy-form *Trypanosoma brucei* gene expression at the translational level

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Transmission of the African trypanosome between mammalian hosts depends upon the development in the bloodstream of so called stumpy-forms. These are transmission competent and arrested in cell-division, thereby limiting parasite numbers and so prolonging host survival. Although most genes are down regulated in these quiescent parasites, a subset are upregulated, presumably as an adaptation to transmission to the parasite's vector, tsetse flies. We have identified a number of stumpy enriched transcripts, most notably members of the ESAG9 family of VSG expression site associated genes. Using a conventional reporter system, regulatory sequences found within the 5'UTR or 3'UTR of one well conserved ESAG9 gene are being characterised that contribute to gene silencing in bloodstream slender forms, or gene activation in bloodstream stumpy forms. Complementing these studies, a genome-wide bioinformatic analysis of potential 5' upstream open reading frames has been carried out and genes potentially regulated by this unusual control mechanism identified. By cross reference to whole genome expression profiling data and reporter assays, characterisation of the contributions of the identified predicted upstream open reading frames to gene expression in stumpy forms is underway.

P87* Trypanosome microtubule associated proteins (MAPs) and their role in morphogenesis

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Shape and form of eukaryotic microorganisms such as yeast, algae and filamentous fungi largely depends upon functions embedded in the cell wall. By contrast in trypanosomatids, characteristic morphologies are the product of a highly structured microtubule cytoskeleton. The Trypanosoma brucei cytoskeleton, generated by an elaborate array of subpellicular microtubules, requires extensive remodeling involving microtubule nucleation/outgrowth and coordinated severing/re-establishment of inter-microtubule cross-links during cell growth and division. Combining bioinformatic, biochemical and proteomic approaches we have identified several novel trypanosomatid-specific MAPs and using RNAi demonstrated functional roles for some of these proteins in trypanosome morphogenesis. Data will be presented on the identification and characterization of MAPs currently under study in our laboratory, along with evidence for complex interdependency relationships during the cell division cycle. These data suggest trypanosome MAPs assemble as distinct complexes and/or in a defined temporal order on subpellicular microtubules. Our results provide further insight into the complexities of trypanosome morphogenesis and indicate that disruption of critical MAP interactions could conceivably provide

valid targets for the development of novel chemotherapeutic strategies against human and animal trypanosomiases.

P88* Circular RNA analysis in *Trypanosoma brucei*

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In eukaryotes, most mRNAs are degraded by initial 3´-5´exonucleolytic digestion of the poly(A) tail. Once the poly(A) has been shortened to a threshold length, there is rapid degradation of the remaining mRNA by 3´–5´ exonucleases or by decapping and 5´–3´ exonucleolytic degradation. XRNA is important for the degradation of highly unstable developmentally regulated mRNAs (Li et al., 2006). The *EP* mRNAs encode the major surface proteins of procyclic cells and are unstable in bloodstream trypanosomes. We previously showed that XRNA RNAi inhibited the degradation of a *CAT-EP* reporter mRNA (Li et al., 2006). We analyzed the effect of XRNA downregulation on degradation of *EP* mRNA and a control mRNA that encodes the ribosomal protein L37a. Like the reporter, degradation of *EP* mRNA was retarded in cells in which XRNA was depleted. By using an RNA circularization and sequencing technique, we showed that depletion of XRNA caused the accumulation of *EP* mRNA that had been degraded at both the 5' and 3' ends (NAR 2009 37(16): 5511-5528). There was clear evidence of multiple degradation pathways for the ribosomal protein L37a (*RPL37A*) and *EP* mRNAs. Current work focuses on transcriptome analysis using RNA sequencing.

P89* Characterising the role of ATM and ATR in the DNA damage response in *Trypanosoma brucei*

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DNA damage affects all cells, making detection and repair near continuous. A number of factors that catalyse DNA damage repair have been characterised in *Trypanosoma brucei*, but the mechanics of detecting and coordinating the response to damage have been less studied, including during the process of antigenic variation. Here we focus on ATM and ATR, two kinases which initiate DNA damage responses in other organisms, such as eliciting cell cycle checkpoints to allow repair to occur. ATM is primarily involved with homologous repair of DNA double stranded breaks (DSBs), while ATR is involved with repairing damage caused by UV irradiation and preventing collapse of stalled replication forks, although it can also act downstream of ATM. Initial work using RNA interference suggests that there may be differences in the importance of these proteins in different life cycle stages of *T. brucei*. We present the phenotypes observed following RNAi of these kinases in procyclic and bloodstream forms and describe the roles of the kinases in responding to different types of DNA damage to *T. brucei*.

P90* A novel *in vivo* approach to identify components of the mitochondrial tRNA import machinery in *Trypanosoma brucei*

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The mitochondrial genome of the kinetoplastid *Trypanosoma brucei* does not encode any tRNA genes. Thus in order for mitochondrial translation to occur, all mitochondrial tRNAs have to be imported from the cytosol. The observed extent of import ranges for 1 – 12% of the total cellular complement depending on the tRNA species. While all mitochondrial tRNAs derive from cytosolic ones, there are two tRNA ($tRNA^{Met}$ and the $tRNA^{Sec}$) that are cytosol-specific.
The composition of the tRNA import machinery is still unknown. Thus, we established an *in vivo* system that permits us to identify tRNA import factors. It consists of a cell line that allows separate induction of RNAi by tetracycline and expression of a tagged tRNA by IPTG at any desired time

point. This double inducible system allows us to follow import of a newly synthesized tagged tRNA during RNAi-mediated knockdown of any putative mitochondrial import factor.
In yeasts and plants, factors of the mitochondrial protein import system have been implicated in mitochondrial import of tRNAs. Thus, we tested whether this is also the case in *T. brucei*.
Our results show that ablation of the trypanosomal orthologues of Tom40 and Tim22/23 inhibits mitochondrial import of the newly synthesized tRNA, suggesting a similar a connection between mitochondrial tRNA and protein import as in yeasts and plants.

P91 The *Trypanosoma brucei* neutral sphingomyelinase has differing but equally essential functions in both bloodstream and procyclic life cycle stages

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Sphingomyelin is the main sphingolipid in membranes of *Trypanosoma brucei*, the causative agent of African sleeping sickness. *In vitro* and *in vivo* characterisation of the *T. brucei* neutral sphingomyelinase (*Tb*nSMase) demonstrates that it is essential and involved in sphingomyelin catabolism to generate ceramide. In the bloodstream form of the parasite, ceramide formation in the ER by the *Tb*nSMase influences post-Golgi sorting and exocytosis of newly synthesised GPI-anchored variant surface glycoprotein (VSG) to the cell-surface. This directly affects the corresponding rate of endocytosis of pre-existing cell-surface VSG as the parasite uses this coupled endocytic and exocytic mechanism to maintain the cell-density of its crucial VSG protective coat. *Tb*nSMase has been genetically validated as a drug target against African trypanosomes and illustrates that interfering with the trafficking of variant surface glycoprotein is a highly desirable strategy for drug development. Thus we are currently chemically validating *Tb*nSMase as a drug target. Recent findings examining *Tb*nSMase activity in procyclic insect form trypanosomes suggest that the neutral sphingomyelinase has an alternative but equally essential function in the maintenance of metabolic pathways in procyclics, particularly when extrapolated to the physiological situation in the tsetse fly vector.

P92 Necessity of specific structural features of the variant surface glycoprotein of African trypanosomes for surface coat formation

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In their bloodstream form African trypanosomes are covered by a dense coat of at least 90% of one member of a family of proteins, the variant surface glycoprotein (VSG). VSGs found in *Trypanosoma brucei* contain a larger N-terminal domain and a smaller C-terminal domain and are attached to the cell surface via a GPI-anchor. VSGs form homodimers. All known VSGs carry at least one N-linked glycan, though the amount and composition of added N-glycans differ. VSGs found in *T. congolense* are similar, but lack the structured C-terminal domain found in *T. brucei* VSGs. Although much is known about the function of VSGs, less is known about how specific structural features contribute to building a successful VSG coat. Here, we have focused on N-linked glycans and the C-terminal domain of VSGs. We have generated VSG mutants in which the protein tripeptide sequon N-X-S/T, necessary for N-glycan addition, has been changed by mutagenesis to generate VSGs lacking their native N-glycan modifications. We were able to generate and maintain trypanosomes displaying a VSG coat completely devoid of N-linked glycans. We have also generated *T. brucei* cells that solely express a *T. congolense* VSG, BeNat1.1, on their cell surface.

P93 Infection of host macrophages with Leishmania involves a new ABCG transporter implicated in phosphatidylserine exposure

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Leishmaniasis is a neglected disease produced by the intracellular protozoan parasite *Leishmania*. Transient exposure of phosphatidylserine (PS) on the outer leaflet of the plasma membrane is one of the known mechanisms used by *Leishmania* amastigotes and metaclyclic promastigotes to infect macrophages and to silencing its respond. In the present study we show that a new ABCG half-transporter (LABCG1) from *Leishmania* could be a first protein involved in this outward PS translocation. Down-regulation of LABCG1 function by overexpressing an inactive version of this transporter reduced the translocation of fluorescent short-chain analogues of PS. This dominant negative phenotype is specific for the headgroup of the phospholipid, as the movement of fluorescent analogues of phosphatidylcholine, phosphatidylethanolamine or sphingomyelin was not affected. In addition, Annexin-V–Alexa488 binding assays showed that LABCG1 "functional knock-down" promastigotes exposed less endogenous PS in the stationary phase than control parasites. The LABCG1-mediated defect in the externalization of PS correlated with significantly reduced infection of macrophages. These differences disappeared when most external PS was masked by preincubating the parasites with Annexin-V. Altogether, our results strongly suggest that LABCG1 function is required for the externalization of PS in metacyclic *Leishmania* promastigotes, a process involved in macrophage infectivity.

P94 TbVAP, a transmembrane protein involved in endoplasmic reticulum organisation in *Trypanosoma brucei*

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The single flagellum of *T. brucei* is attached to the cell body by the flagellum attachment zone (FAZ) and attachment is essential for viability. We have used cellular electron tomography to investigate the three dimensional architecture of the FAZ and the associated endoplasmic reticulum (ER) at the flagellar pocket and along the cell body. We found that the ER associated with the FAZ is regularly connected into the main rough ER of the cell. We identified a transmembrane protein with a major sperm protein (MSP) domain in our flagellar proteome. Using a combination of bioinformatics, molecular and cell biology approaches, we show that this protein is an orthologue of the mammalian VAP family and that it localises to the ER, FAZ and the flagellar pocket. We named this protein TbVAP and demonstrate that it is necessary for attachment of ER to microtubule quartet of the FAZ.

P95 Phosphodiesterases PDEB1 and PDEB2 of *T. brucei*: the role of the GAF domains

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Phosphodiesterases (PDEs) play an important role as regulators of cAMP signalling. PDEs in parasitic protozoa have not been extensively investigated yet, despite their potential as drug targets. In *Trypanosoma brucei,* RNA_i against two PDEs resulted in rapid cell death in culture and in the mouse model.
Many PDEs, including the two major PDEs of *T. brucei*, contain two N-terminal GAF domains; their role in modulating enzyme activity is still unclear. Kinetic data of the *T. brucei* enzymes are available only for the full-size enzymes. To obtain more information on the role of these GAF domains, we expressed the individual catalytic domains and investigated their biochemical

properties. We also separately expressed the GAF domains and determined their binding properties for cAMP and related substrates.
Moreover, between the two GAF domains, a putative protein kinase phosphorylation site can be found. In order to elucidate its function, the substrate serine was substituted by either alanine or aspartate. These constructs were transfected into *T. brucei* to investigate the role of phosphorylation in intracellular localization. Similar constructs were expressed in yeast to determine the effect of the mutations on the kinetic properties of the enzyme.

P96 Over-expression of the histone methyltransferase DOT1B causes continuous replication of nuclear DNA in *Trypanosoma brucei*

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Cell cycle progression has to be regulated carefully to ensure accurate propagation of genetic material to the daughter cells. Although many cell cycle regulators are conserved in trypanosomes, other regulatory mechanisms seem to have evolved. We are interested in the influence of posttranslational histone modification, in particular methylation of histone H3 on lysine 76 (H3K76), on cell cycle regulation. Di-methylation of H3K76 is only detectable during mitosis and cytokinesis and is mediated by the histone methyltransferase DOT1A. RNAi-mediated depletion of DOT1A causes severe cell cycle defects and generates a population with a potential haploid DNA content (Janzen et al., 2006). To further investigate the possible role of H3K76 methylation in cell cycle regulation, we analyzed the effect of over-expression of the methyltransferase (DOT1B), which is responsible for tri-methylation of H3K76.
DOT1B over-expression is lethal and generates a population of cells with polyploid nuclei as well as enucleated cells. Detailed analysis showed that DOT1B over-expression causes continuous re-initiation of replication which generates nuclei with increasing DNA content but does not prevent cell division. Since histone methylation has never been associated with replication control in other eukaryotes before, this could be a novel regulatory mechanism which might be unique for trypanosomes.

P97 Uracil glycosylase is important for the maintenance of genome stability in *Trypanosoma brucei*

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Cells contain low but significant amounts of dUTP as part of their normal pyrimidine metabolism. DNA polymerases do not discriminate well between dUTP and dTTP as building blocks for DNA. Thus, the net incorporation of dUMP depends on the dUTP/dTTP ratio at the time of DNA synthesis. In addition, uracil in DNA can be the result of spontaneous or enzyme mediated cytosine deamination. The molecular mechanisms that control uracil in DNA in trypanosoma are not fully understood. Most uracils in DNA are normally removed by a uracil-DNA glycosylase generating an abasic site, which is potentially mutagenic unless correctly processed prior to the next round of replication. We have generated knock-out *Trypanosoma brucei* cells for uracil glycosylase. The knockout strain is viable and exhibits a proliferation rate similar to the parental strain in both procyclic and bloodstream forms. No uracil excision activity could be detected on U:G, U:A pairs or single strand uracil-containing DNA. Sensitivity to antifolates, mutation frequency and mutation spectra have been determined and show that the enzyme has a central role in the elimination of uracil from DNA and the preservation of genome stability. Conversely, overexpression of the enzyme is cytotoxic probably due to an excessive binding to uracil-containing DNA which could hinder processes required for adequate cell proliferation.

P98 The C-terminal VSG domain is not accessible for antibodies on a functional surface coat of trypanosomes

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VSG is the major cell surface protein of bloodstream form trypanosomes and in current models the VSG is so densely packed that it shields invariant plasma membrane proteins from host antibodies. At low VSG antibody titres VSG-immunoglobulin complexes are rapidly taken up by trypanosomes and the immunoglobulin is degraded in the endosome whilst the VSG is returned to the cell surface. However, high VSG antibody titres combined with complement kill trypanosomes and antigenic variation based on the VSG has evolved to allow a subset of the population to survive.
Here, the degree to which the VSG layer acts as a barrier has been tested. VSGs have a small C-terminal domain proximal to the plasma membrane. The ability of antibodies that recognize this domain to bind to living or fixed cells was tested. Polyclonal antibodies against the C-terminal domain of VSG 118 and VSG 221 are not able to bind to live cells whereas antibodies recognising the whole VSGs do bind. All antibodies bound fixed cells expressing the cognate VSG. This data demonstrate that the VSG surface coat does block access of antibodies to the plasma membrane.

P99 *Trypanosoma cruzi* trans-sialidase activity mediates G-protein dependent cell entry

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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 intimately associated with multiple aspects of the pathogenesis of Chagas disease. Previous work has linked trans-sialidase activity to the *T. cruzi* invasion process. 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 and fluorescence imaging showed accumulation of caveolin-1 in the region of the bead. 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 internalisation, requiring actin polymerisation and raft formation but that active trans-sialidases trigger a supplemental G-protein dependent action enhancing internalisation. This evidence clarifies the role of trans-sialidases in *T. cruzi* invasion and reinforces their importance as a therapeutic target for the future.

P100 Membrane orientation and recognition nexus (MORN) domain proteins of *Leishmania*

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MORN domains were first discovered in junctophilin a protein believed to anchor the plasmalemma to the endoplasmic reticulum. It has been suggested that MORN domains interact with the plasmalemma by specific interactions with key phospholipids and proteins. MORN domains are found in multiple repeats of 23 nucleotides in several proteins of organism across the tree of life, the proteins are frequently complex and modular containing multiple functional domains. In *Leishmania major*, there are 20 proteins that contain MORN domains. Recently, a study in trypanosomes showed localization of GFP-tagged MORN domains to a bilobed structure associated with the proximal end of the flagellum. In this study, we look at two of the complete leishmanial proteins MORN-Kinesin and MORN-tkinase which have their MORN repeats at the N-terminus and C-terminus respectively. We fused both proteins in the pXG-GFP vector. Expression of the fusion proteins was confirmed by western blot. In culture of transfectants, fluorescence

microscopy reveals strong expression of the protein in a bilobed structure at the base of the flagellum and proximal to the kinetoplast consistent with the localization previously observed in *T. brucei*. Importantly though the MORN-Kinesin chimaera clearly localizes to the flagellum whilst the MORN-tkinase chimera does not. Cold triton treatment shows retentention of the fusion proteins to these structures. Both transfectants are capable of infecting macrophages, while in the MORN-Kinesin the GFP stain is restricted to the amastigotes of the parasite vacuole, in the case of the MORN-tkinase chimaera is able to reach not only the lumen of the parasitophorous vacuole but appears to give diffuse fluorescence to from the macrophage cytosol suggesting the predicted N-terminal signalling domain on the protein may direct secretion and export of the protein allowing for direct interaction with host proteins.

P101 Trafficking and secretion of the *Leishmania* HASPB protein

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The *Leishmania*-specific hydrophilic acylated surface protein (HASPB) is exclusively expressed in infective stages of *L.Leishmania* species and shows both inter- and intra-specific variation in its central repetitive domain. Recombinant HASPB is recognised by sera from leishmaniasis patients and also induces long-term protection, in the absence of adjuvant, in the mouse model of visceral disease, making it a promising vaccine candidate.
HASPB is dually acylated by the N-terminal addition of myristate and palmitate, co- and post-translational modifications essential for trafficking to the plasma membrane. We have developed live cell flow cytometry and microscopy methods to investigate presentation of full-length and GFP-tagged HASPB quantitatively at the parasite membrane and following exposure on the external surface of infective *L. major.* These approaches have confirmed that HASPB can be translocated across the plasma membrane and decorates the cell surface in a distinct pattern co-localising with lipophosphoglycan. This process correlates with entry into metacyclogenesis, while some HASPB is released from the parasite surface upon macrophage entry. Amastigotes within host macrophages initially express HASPB, although this is not surface exposed. However, following parasite replication within the parasitophorous vacuole, HASPB can no longer be detected. Further analysis of this unusual protein trafficking pathway may aid functional analysis of these unusual *Leishmania* proteins during intracellular infection.

P102* Molecular mechanisms in the controlled degradation of glycosomes in trypanosomes and a determination of the importance of this process

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Peroxisomes are highly versatile organelles; their enzymatic content is adapted to the environmental conditions of a cell, for example to the nature of the available nutrients. Increased turnover of the organelles allows such adaptation. Such turnover involves the specific, induced degradation of peroxisomes by autophagy, a process also called pexophagy. It is observed in yeasts during adaptation to changes in nutrient sources; only old peroxisomes are targeted for recycling, while new ones, still competent for import of matrix proteins, are spared and give rise to a new population of organelles with a different enzymatic content.
Glycosomes are peroxisome-like organelles present in trypanosomatids. They contain glycolytic enzymes and this compartmentalization has been shown to be essential for the parasites. An *in silico* study has found in the trypanosomatid databases around 20 genes that are homologous to yeasts genes involved in autophagy (autophagy-related genes or ATGs). We are currently studying three proteins that might be involved in pexophagy in *T. brucei* to determine the role and mechanism of glycosome turnover during differentiation between life-cycle stages and upon altered nutrient supply. RNA interference is being used together with morphological studies to characterize the encoded proteins, ATG8, ATG24 and VAC8, and their role in the cell during starvation, adaptation to changes in nutrient source and differentiation.

P103* Characterisation of unusual ATG8-like proteins in *Leishmania major*

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Macroautophagy is a degradative pathway important in development of eukaryotic organisms. It requires two ubiquitin-like conjugation systems involving ATG8 and ATG12. The protozoan parasite *Leishmania* appears to be unique in that it possesses, in addition to *ATG8,* a large number of *ATG8*-related genes, divided into the subfamilies *ATG8A, ATG8B* and *ATG8C.* Members from each family are able to complement an *ATG8* null *S. cerevisiae* strain, implying functional conservation. Previous work has shown that ATG8 associates with autophagosomes during parasite differentiation, as well as in response to starvation. ATG8A, on the other hand, is primarily involved in starvation-induced autophagy. Punctate structures containing ATG8B or ATG8C form close to the flagellar pocket in a small number of cells, suggesting roles other than autophagy. Members of the *ATG8* families are selectively cleaved by the cysteine peptidases ATG4.1 and ATG4.2, and this processing is key to their activation. In order to further characterise the roles of these proteins, we produced GFP-tagged mutants of each ATG8 family with mutations at the processing site. The rationale was that the mutant proteins might exert a dominant negative effect. *L. major* expressing the mutant proteins were analysed to determine differences in localisation and/or processing, thus providing insights into the functions of the ATG8 families.

P104 The autophagic response is recruited and required by *Trypanosoma cruzi* GP82-mediated cell invasion

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Trypanosoma cruzi expresses a number of virulence factors that allow it to invade mammalian cells. Expression of one of these *T. cruzi* invasion factors, the transialidase-like GP82, in the null invasion background of the related but non-pathogenic *Trypanosoma rangeli*, is sufficient to facilitate *T. rangeli* entry into mammalian cells. Autophagy, the innate defence mechanism in non-phagocytic cells in response to attack by pathogenic organisms and survival response in starvation conditions, has been shown to play a key role in *T. cruzi* cell invasion. Autophagosomes are recruited to envelop invading pathogens, which subsequently fuse with lysosomes for targeted degradation. We show, using GFP-tagged autophagy protein LC3 in the target cells, that the GP82-expressing *T. rangeli* trigger the formation of an autophagosome around the parasite, and that when coated onto 4 µm beads, GP82 alone is capable of stimulating this autophagic response. However, GP82 expression in *T. rangeli* is not normally sufficient to enable the parasite to exit the parasitophorous vacuole and continue its life cycle. Further, GP82-mediated cell entry is dramatically compromised either by cholesterol depletion of the target cell, or the inhibition of autophagosome formation of epithelial lines with gene knockouts in the autophagy pathway.

P105* Immunogenicity of *L. donovani* centrin-3 in *L.mexicana* mouse model

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Vaccine is one of the most effective measures to control infectious diseases with considerable number of successful stories. However, at present, there is no effective vaccine available in the world for routine use against Leishmaniasis; therefore, there is still a need to identify new highly immunogenic Leishmania antigens to be tested as a vaccine.

In this study the immunogenicity of *L. donovani* centrin-3 (Ldcen-3) was investigated in *L. mexicana* Balb/c mouse model. Ldcen-3 is a calcium binding protein that has been shown to be involved in duplication and segregation of the centrosome and basal body duplication in higher

and lower eukaryotes. Ldcen-3 cloned in two vectors, pcDNA3.1 (-) and pCR®T7/CT-TOPO (obtained from Dr H. Nakhasi, FDA), were coated on gold particles for gene gun immunisation. The pcDNA3.1 (-) vector, containing CMV promoter, is a widely used vector for DNA delivery/transfection in mammalian cells compared with the less known CR®T7/CT-TOPO vector. Gene gun immunisation of Balb/c mice with both plasmids encoding Ldcen-3 significantly protected against challenge with live *L. mexicana* parasite; the potency of protection was greater with CR®T7/CT-TOPO vector. Splenocytes from Balb/c mice immunised with pcDNA3.1(-)-Ldcen-3 or pCR®T7/CT-TOPO®-Ldcen-3 induced potent CTL response against dendritic cell targets loaded with Leishmania soluble antigen (SLA).

P106* Diagnostic biomarkers for HAT: identification and primary validation

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Human African Trypanosomiasis (HAT) is caused by two species of *Trypanosoma brucei*, *T. b. gambiense* and *T. b. rhodesiense,* which are estimated to kill 50,000 to 70,000 people every year. Many of these cases remain undetected until the disease has progressed to the second stage, where trypanosomes invade the CNS. This requires administration of more toxic drugs such as Melarsoprol, which can cross the blood-brain barrier. CATT test is the commonly used screening tool, however it lacks sensitivity and specificity and it requires trained personnel.
Our aim is to find and validate diagnostic biomarkers for *T. brucei* species for use in a lateral flow test. We have identified a number of potential diagnostic biomarkers by immunoprecipitation experiments using purified antibodies from infected and control sera. The antibody columns were mixed with *T. b. brucei* cell lysate, the eluted trypanosomal antigens were run on SDS PAGE, followed by in-gel tryptic digestion and identification of tryptic peptides by LC-MS/MS. Twenty five potential biomarkers were identified. To validate the biomarkers, a chemilluminescence ELISA assay was developed using recombinant biomarkers to test infected and control human sera.

P107 A novel mechanism to resist complement lysis and invade host: cell triggered by *Trypanosoma cruzi*

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During infection of mammals, the insect-derived trypomastigote metacyclic forms of *Trypanosoma cruzi* have to escape lysis by the complement system and invade host cells. We detected parasites inducing release of microvesicles (MVs) from monocytic (THP-1). MVs originate from the cell plasma membrane, present asymmetric distribution of phosphatidylserine, and are between 200 to 500 nm. The release of MVs is Ca^{2+}-dependent and inhibited by 5 mM EGTA, 1.5 μM thapsigargin, and 100 nM wortmannin. We investigated the role of MVs in the parasite complement lysis and invasion in eukaryotic cells. Complement lysis of *T. cruzi* epimastigote forms by human serum was inhibited by MVs in a dose-dependent manner. MVs bind to C3, but rather than inhibiting C3b deposition on the parasite surface, they inhibited further cleavage of C3b to iC3b.We detected C2a increased on the parasite surface proportional to MV concentration, and a lower dissociation of C2a in the presence of MVs, indicating that MVs could be stabilizing, C3 convertase. Monocyte-derived MVs increased invasion of *T. cruzi* in Vero and HeLa cells. The MV's effect on *T. cruzi* invasion was inhibited by pre-incubating MVs with anti-TGF-β antibodies or using SB-431542, a TGF-β receptor inhibitor. These results suggest that *T. cruzi* induce microvesiculation in blood cells allowing success at early stage of infection.
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P108* Global climate changeability: Pervasiveness of visceral leishmaniasis by sand-fly

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Lesihmania donovani, causes Visceral Leishmaniasis (VL), which is very serious disease and endemic in warmer part of the world covering almost 88 countries. It is most prevalent in India, Bangladesh, Southern Sudan, Nepal and Northeast Brazil. VL is transmitted by an insect vector i.e. Sand fly (*Phelobotomus argentipus*) It belongs to the fly family Psychodidae, characterised by their densely hairy wings which provide them a moth-like appearance .Sand flies are mostly found in the tropics but few species also occurs in the temperate regions. They have very specific habitat requirements. Leishmaniasis is developed in dry, semi-arid areas in the old world, whereas in the new world, this disease occurs mainly in tropical forests and savannas. Mostly sand fly lives in warmer places (cervices, tree hole, dung and domestic wastes) where humidity and temperature both are present at regular intervals in a day (humidity during night and temperature at day time). These conditions are essential / necessary for the survival of vector, parasite development and for their distribution. But now a day, due to global climate changes and temperature increases, which support the high degree of sand fly growth, the transmission of disease has increased manifold. The flooding also increase the transmission of larvae from one place to another place thus also increases the distribution of disease.

P109* Exploiting a luciferase reporter system for rapid *Trypanosoma cruzi* amastigote drug screening

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Nitroheterocyclic drugs such as nifurtimox are used as treatments against both African and American trypanosomiasis. However, their use is problematic due to toxic side-effects and emerging resistance. Screening for new drugs against the *T. cruzi* amastigote lifecycle stage is particularly slow and laborious. Here we report the construction of a *T. cruzi* cell line that constitutively expresses luciferase and the use of these cells in a 96-well plate format *in vitro* infection model to facilitate rapid drug screening against this clinically relevant stage. This system was initially validated with nifurtimox and benznidazole then used to determine the trypanocidal activities of other nitroheterocyclic compounds including nine aziridinyl dinitrobenzamides and 24 nitrophosphoramide mustards. In contrast to bloodstream form *T. brucei*, no mustard displayed selective killing activity against *T. cruzi* amastigotes. For the aziridinyl dinitrobenzamides, three exhibited IC_{50} values of approximately 1 μM against the amastigote stage with no cytotoxicity against mammalian cells at 100 mM. Analysis of the structure/activity relationship of the aziridinyl dinitrobenzamides identified key chemical signatures found only in the three trypanocidal compounds. Exploiting the luciferase reporter system has allowed the rapid discrimination and identification of novel anti-trypanosomal agents against the fastidious *T. cruzi* amastigote life cycle stage.

P110* Structure-activity relationship of the High Affinity Pentamidine Transporter in *Trypanosoma brucei*

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Pentamidine has a long and successful history in Human African Trypanosomosis chemotherapy. The drug is concentrated by at least three transporters (TbAT1/P2, HAPT1 and LAPT1). The recognition motif of TbAT1/P2 transporter has been well characterised [1,2] whereas HAPT1 and LAPT1are still under investigation. Characterization of natural substrates of HAPT and LAPT would be beneficial for the design of new drugs that can utilise these transporters for entry into the trypanosome. To help to identify the recognition motif of HAPT and LAPT, we studied the structure activity relationship using 55 pentamidine analogues as competitive inhibiters of pentamidine uptake in *T.b. brucei* blood stream form, determining the inhibition constant (*Ki*) of

each compound. Using molecular modelling and structure-activity relationship analysis we tried to determine the common properties and the motif needed for binding on HAPT1. We found that small changes of the main chemical structure of the ligand by the addition of minor substitutions such as Cl or OH to the benzamidine moiety causes major loss of affinity for HAPT. Other findings include the importance of the oxygen atom in the pentamidine linkage chain and of the flexibility of this chain – with rigid linkers less likely to bind strongly to HAPT.
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P111*. Complex melarsoprol: a possible oral therapy for Human African trypanosomiasis

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At present only a handful of drugs are available for the treatment of human African trypanosomiasis. Many of the drugs are difficult to administer and treatment schedules are often prolonged. Unacceptable side effects are frequently observed and resistance is an increasing problem. Novel or re-formulated trypanocides are therefore urgently needed.
Cyclodextrins are naturally occurring oligosaccharides, widely utilised by the pharmaceutical industry to improve the solubility of lipophilic drugs. Melarsoprol was incorporated into hydroxypropyl-β-cyclodextrin (HPβCD) and randomly-methylated-β-cyclodextrin (RAMβCD) to give two water soluble melarsoprol/cyclodextrin complexes. The trypanocidal activity of the two complexes was assessed.
The complexes exhibit trypanocidal activity *in-vitro* and *in-vivo*. Oral administration of mel/HPCD and mel/RAMCD at 0.05mmol/kg for seven consecutive days cured murine CNS stage *T. b. brucei* infection. QPCR studies using PFR2 as the target gene indicate that all trypanosomes are cleared from the CNS 24 hours after completion of treatment. A single Intravenous dose of 0.03mmol/kg mel/HPCD failed to cure CNS stage murine trypanosomiasis. Work is ongoing to find a minimum curative intravenous dose. The pharmacokinetic and tissue distribution profile of the melarsoprol/cyclodextrin complexes is currently being investigated using LCMS methodology.

The authors acknowledge Stéphane Gibaud for preparing the cyclodextrin-melarsoprol complexes.

P112 Bisphosphonium salt analogues against *Trypanosoma brucei brucei* bloodstream form

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Bisphosphonium salts analogues were used to determine the susceptibility in vitro of *Trypanosoma brucei brucei* wild-type and drug resistant strains (TbAT-1 knockout and B48 - resistant to pentamidine and melaminophenyl arsenical drugs). Standard methods such as alamar blue, propidium iodide and proliferation assays were used. 22 out of 37 bisphosphonium compounds tested in this study had an effective concentration to kill 50% (EC50) value below 0.5 μM, with 9 having an EC50 value under 100 nM. The most active compound was AHI17 with an EC50 value of 21 ± 1 nM. No major cross-resistance was displayed by the TbAT-1 knockout and B48 dimaidine resistant strains. In addition, some of those compounds appeared to rapidly inhibit trypanosome proliferation, with a short exposure time sufficient for eventual cell death.

P113 Synthesis and antiprotozoal activity of *N*-alkoxy derivatives as possible prodrugs of bis(2-aminolimidazolinium) lead compounds

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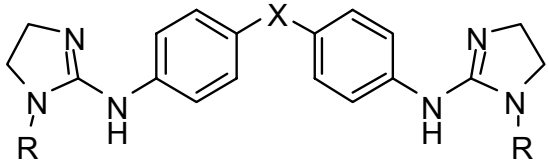
Different bis-(2-aminoimidazoline) compounds were synthesized and tested in vitro against *T. brucei*, the causative agent of human African Trypanosomiasis (HAT). Further in vivo studies showed (**1a**–**4a)** to be curative by ip administration in the acute stage of the HAT **(Fig.1)**; however, these compounds didn't show any activity in the neurological stage of the disease (i.e., GVR35 murine model)[1-3]. We have the theory that, due to the positive charge present in these compounds at physiological pH, aminoimidazolines have a very poor ability to cross the blood brain barrier. To decrease the basicity of the imidazoline heterocycle we have synthetized a series of *N*-alkoxy-derivatives (**b**-**f**) as possible prodrugs of the bis-(2-aminoimidazoline) lead compounds (**1a-4a**).
The new compounds were tested in vitro against a panel of parasites (*T. brucei rhodesiense, T. cruzi, L. donovani and P. falciparum*) and in vivo in the *T. brucei* (STIB900) mouse model. Several *N*-alkoxy derivatives showed submicromolar activity in vitro but only one (**1b**) displayed moderate activity in vivo by intraperitoneal administration.



(1) X= NH
(2) X= CONH
(3) X= NHCONH
(4) X= CH_2CH_2

(a): R= H
(b): R= OH
(c): R= OMe
(d): R= OEt
(e): R= OBn
(f): R= OTHP

Fig. 1: Structure of bis-(2-aminoimidazolines) and *N*-alkoxy-2-aminoimidazolines

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P114 African Animal Trypanosomiasis: are multidrug resistance extrusion systems involved in drug resistance?

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African Animal Trypanosomiasis affects about 10 million km2 of sub-Saharan Africa and is a primary cause of rural poverty and food insecurity as explicitly recognized by the African Union, FAO and others. The control of animal trypanosomiasis (mainly *Trypanosoma congolense*) and zoonotic Human African Trypanosomiasis (mainly *T. brucei rhodesiense*) in poor rural communities has and will continue to rely heavily on the use of trypanocidal drugs. As no new drug is expected to be available in the near future and as the resistance phenomenon is spreading very rapidly, alternatives are urgently needed to circumvent trypanocidal drug resistance. Reversal of drug resistance or chemosensitization was successfully achieved, among others, cancer cells, *Leishmania* and *Plasmodium*.
Our working hypothesis was that compounds could interfere with the extrusion of the trypanocide isometamidium chloride (ISM) from the drug resistant trypanosome allowing a prolonged

trypanocidal action. After routine screening in a mouse model (animals inoculated with *T. congolense* strains resistant to ISM, treated and untreated groups of 16 mice each), tetracycline and enrofloxacine were selected as best potentiators. Those 2 potentiators were further tested in three groups of six cattle infected with ISM-resistant *T. congolense*, group A being treated with ISM, group B with ISM associated oxyteracycline and group C with ISM associated with enrofloxacine. All animals of group A (ISM) became positive. In groups B (ISM-tetracycline) and C (ISM-enrofloxacine), 50% of the animals were considered as completely cured (PCR negative for 95 days). Animals from groups B and C that became parasitaemic presented a significantly longer prepatent period than animals in group A ($p<0.001$). The impact of the disease on the Packed Cell Volumes (PCV's) was lower in groups B (ISM-tetracycline) and C (ISM- enrofloxacine) compared to group A (ISM) ($p<0.01$). Such strategies will bring a much needed relief to African livestock breeders as they can be implemented at a reasonable price by shortcutting new compound development, toxicity studies and long clinical trials.

P115 Molecular analysis of pentamidine transporters in *Trypanosoma brucei*

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Three transporters of pentamidine are known in *Trypanosoma brucei*: the AT-1/P2 transporter, the high affinity pentamidine transporter (HAPT1) and the low affinity pentamidine transporter (LAPT1) (1). Whilst the gene for the AT-1/P2 transporter was identified in the 1990s (2), the genes for HAPT1 and LAPT1 are still to be elucidated.
We are investigating the genes encoding the remaining two transporters. Candidate genes have been identified through analysis of sequence homology to AT-1/P2 and our investigation is focusing on three genes identified as being phylogenetically similar to AT-1/P2: AT-like A, E and G (3). Sequence analysis has revealed multiple sequences for all three genes, with A and G indistinguishable from each other. Analysis of gene number is continuing, using various methods including Southern blotting and real time PCR.
In order to investigate the functions of the three genes RNA interference (RNAi) is being undertaken in both bloodstream and procyclic forms of *T. b. brucei*. Analysis of these knockdown cells is continuing, and an updated model for pentamidine uptake in *T brucei* will be presented.

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2. Maser, P, et al. (1999) *Science* **285**(5425), 242-244
3. De Koning, HP, Bridges, DJ, and Burchmore, RJ. (2005) *FEMS Microbiol Rev* **29**(5), 987-1020

P116 Assessment of choline-derived analogs as new anti-kinetoplastid lead compounds

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A series of compounds roughly based on the choline scaffold has been tested for activity against *Trypanosoma* and *Leishmania* species. Activity was particularly strong against *T. brucei* with EC_{50} values in the mid nanomolar range. Cell death would be complete some time between 2 and >24 hours depending on the particular compound and dosage. The choline-derived compounds did not affect the lipid composition of *T. brucei* plasma membranes, nor alter the cell cycle in any specific way. Instead, they induced progressive depolarisation of the mitochondrial membrane and DNA fragmentation. This type of cellular effect is reminiscent of programmed cell death and, consistent with such a response, a slow increase of intracellular calcium was observed over several hours. However, we did not find any evidence for an increase in the production of reactive oxygen species. We conclude that this class of compounds has potential as new lead compounds against African trypanosomiasis.

P117 New bisphosphonium salt derivatives are potent antiprotozoal agents: in vitro activity against *Trypanosoma brucei*, *Trypanosoma cruzi*, *Leishmania donovani*, and *Plasmodium falciparum*

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A set of 29 benzophenone-derived bisphosphonium salts was synthesized and screened in vitro against a panel of protozoan parasites. Most of them inhibited *P. falciparum* (strain K1) proliferation at nanomolar concentrations with selectivity indexes (SI)[1] from 60 to > 5000. IC_{50} in the submicromolar range of concentrations were observed against *T. brucei* (20 compounds), *L. donovani* (9 compounds) and *T. cruzi* (3 compounds). However, lower selectivities were generally found against these parasites.
A second set of compounds was synthesized to get insights into the structure-activity relationships of this class of antiprotozoal agents. Preliminary results of in vitro activity against *T. brucei* showed the diphenylmethane and diphenylether analogues to be equally or more active than the benzophenone counterparts. Most importantly, these compounds displayed similar activities against the wild type, TbAT1-KO and HAPT-deficient strain (B48) of *T. b. brucei* indicating that they are not dependant on these transporters for uptake into the parasite. Hence, these new compounds are not likely to exhibit crossresistance with existing treatments, unlike many new diamidine drugs.
[1.] SI = IC_{50} (L6-cells)/IC_{50} (*P. falciparum*)

P118 Parasitic loads in tissues of *Trypanosoma cruzi*-infected mice treated with AmBisome

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Trypanosoma cruzi is the infectious agent of Chagas disease, one of the most important public health problems in Latin America. The currently available drugs to treat *T. cruzi* infection (benznidazole and nifurtimox) are effective in recent infection, but hardly in long-term chronic infection. In addition, no rapid effective way is presently available for confirming that a parasitological cure has been achieved in infected subjects. AmBisome® (a lipid formulation of amphotericin B), has been previously shown efficient in *Leishmania* infection. We demonstrated that AmBisome treatment prevented mortality and patent parasitaemia in the acute phase and abrogated parasite DNA amounts in all tested tissues of infected mice, though complete cure could not be obtained with the used treatment protocol. Moreover, qPCR datas agree with levels of live parasites observed in acute infection of untreated mice, whereas chronically infected mouse tissues presented DNA parasitic amounts corresponding to higher parasite levels than those determined by microscopic observation. The highly sensitive parasitic DNA detection of qPCR offers a way to decipher classically undetectable chronic parasitic loads, but likely also to evaluate the DNA release resulting from the parasite destruction related to the treatment.

P119 New Anti-malarial drug therapy? Efficacy of Pyronaridine-Artesunate Combination *in vivo*

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Whilst strategies are already in place to control malaria, the growing problem of resistance has called for new drugs to be developed which can work in combination to produce new effective Artemisinin Combinational Therapies to treat multi-drug resistant *Plasmodium falciparum.* The aim of this study was to evaluate the efficacy and curative effects of pyronaridine in combination with artesunate and dihydroartemisinin against a number of rodent malaria models *in vivo,* to support its clinical registration. The combinations successfully reduced the ED90 required to clear the

parasitic infection compared to the monotherapy particularly in the drug resistant strains of *P. berghei* NPN (pyronaridine-resistant) and *P. berghei* SANA (artesunate-resistant). The combinations were also able to increase the average time of survival from 6 days (as seen with the artemisinins) to 60 days after a single curative dose. The results of this study provide strong evidence that pyronaridine-artesunate combination is a promising new artemisinin drug combination therapy (ACT) to treat malaria in multi-drug resistant areas.

P120 RNA editing as a drug target in trypanosomes: identification of inhibitors of the essential enzyme REL1 by virtual screening

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RNA editing is essential for mitochondrial gene expression in all trypanosomatids but absent from the host and therefore a potentially powerful drug target. The process is catalyzed by multiprotein complexes, the editosomes, and involves several enzymatic steps. A key component of editosomes is RNA editing ligase 1 (REL1). The crystal structure of this enzyme revealed a deep pocket that serves to bind and orient the essential ATP cofactor. Using a virtual drug screening strategy we had previously identified compounds that inhibit *Tb*REL1 with micromolar IC_{50} values (Amaro, R., Schnaufer A. et al., 2008).
To identify additional hits for drug discovery efforts we generated a library of compounds similar to the known *Tb*REL1 inhibitors by performing a substructure search against several databases of commercially available compounds. Top compounds, judged by their predicted binding energies, were docked against molecular dynamics snapshots to account for molecular flexibility. After further refinement, 34 top candidates were tested in biochemical assays. Four compounds inhibited REL1 with IC_{50} values between 1 and 10 μM. Efforts are under way to improve the *in vitro* activity and selectivity of these compounds and to establish their effect on trypanosomes.

P121 Population genetic structure of Central African *Trypanosoma brucei gambiense* isolates using microsatellite DNA markers

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Genetic variation of microsatellite loci is a widely used method for the analysis of population genetic structure of microorganisms. Seven microsatellite markers were used here to characterize *Trypanosoma brucei gambiense* isolates from Central Africa sub-region in order to improve knowledge on the population genetic structure of this subspecies. DNA was extracted from the trypanosome using the Dneasy tissue kit following the protocol elaborated in the hand book. The extracted DNA was then used as template to amplify the microsatellite makers. The allele sizes was estimated on 2% agarose gels and the alleles were then resolved on 10% polyacrylamide gel in TBE buffer and stained with ethidium bromide. Genetic diversity was measured by the number of alleles and heterozygosity at each locus. Genotype frequencies were tested against Hardy Weinberg's expectations for each locus in the pooled population and in each single population. Statistical significance was assessed by the exact probability test; exact tests for linkage disequilibrium between pairs of loci were computed in the pooled population and within each population. Differentiation between populations was assessed by F-statistics.
These markers confirmed the low genetic polymorphism within Central African *T. b. gambiense* isolates from the same focus and strong differentiation between different foci. The presence of

many multilocus genotypes of *T. b. gambiense* and the excess of heterozygotes found in this study play in favour of a clonal reproduction of this parasite. But some data may be indicative of a unique recombination event in one subsample. The high F_{ST} value indicates low migration rates between *T. b. gambiense* subpopulations (foci). Very negative F_{IS} suggests fairly small clonal population sizes of this pathogen in the different human trypanosomosis foci of Central Africa. . The microsatellite markers used in this study can be used on field samples in order to characterize trypanosomes infecting tsetse flies, domestic and wild animals. The circulation of different trypanosome stocks in the same sleeping sickness focus can partially explain the endemic or the epidemic evolution of sleeping sickness in Central African foci.

P122 Patterns of population genomic variation in *Leishmania donovani*

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The development of high-throughput sequencing platforms enables a deeper and more comprehensive exploration of genomic variability in disease-causing parasites. Using 454 and Illumina sequencing technologies, the Gemini and Kaladrug consortiums have resequenced over 20 *Leishmania donovani* field isolate genomes obtained from patients with varying disease phenotypes at reference hospitals. 14 of these strains with differing sensitivities to drug treatments and onset of visceral leishmaniasis were examined further here. These high-quality genome sequences cover over 95% of chromosomal sites based on a reference sequence constructed from the *L. infantum* genome, yielding 6,280 candidate SNP sites. 748 of these were located in coding regions and 463 of these cause changes at the protein sequence level. About one third (1, 854) of the candidate SNP sites were uniformly heterozygous, indicating that nucleotide variation may be preserved at key regions: 210 of these SNPs were in protein-coding sequences. The restricted number of amino acid-changing sites (21) with high (> 0.4) folded allele frequencies that were not uniformly heterozygous in the 14 *L. donovani* samples indicates that a limited number of key changes may be related to the variable pathogenicities of these strains in humans. Further analysis may refine and elucidate the relationship between genetic variation in *L. donovani* and clinical disease.

P123* Functional analysis of species-specific genes that may contribute to *Leishmania* tropism

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Genome sequencing of three *Leishmania* species (*L.infantum, L.major* and *L.braziliensis*) that cause different types of leishmaniasis has identified only a few species-specific genes that may contribute to *Leishmania* tropism. My project is focused on the functional analysis of one of only 5 *Leishmania infantum* species-specific genes, Linj31.3030, which codes for a putative phosphatase. Transgenic parasites deleted for this sequence have been generated by sequential gene replacement and these analyses have demonstrated the presence of 4 Linj31.3030 alleles. These data correlate with recent deep sequencing of *L. donovani* and *L. infantum* strains, suggesting that chromosome 31 might be tetrasomic.
In depth bioinformatic analysis suggests that Linj31.3030 codes for a cofilin phosphatase.
As reported for chronophin (the human cofilin phosphatase), recombinant Linj31.3030 protein has dual enzyme activity against pyridoxal 5' phosphate and a phospho-cofilin peptide. If the identification of Linj31.3030 as a cofilin phosphatase is verified, this enzyme may modulate the flagellar malfunction phenotype observed in *Leishmania* cofilin deletion mutants, impacting on parasite motility and infectivity.

P124* A genomic approach to *Trypanosoma cruzi* lineage-specific serology for Chagas disease

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Chagas disease, marked by life-long chronic infection with *Trypanosoma cruzi*, remains a major parasitic disease in the Americas. *T. cruzi* is divided into the 6 genetic lineages TcI-VI, with disparate ecologies and geographical distributions. Disease outcome may be linked to parasite lineage, complicated by mixed infections and divergent tissue distributions.
The trypomastigote small surface antigen (TSSA) has been described as the only serological marker to identify infection by TcII-VI, as distinct from TcI. Here, by analysis of the *TSSA* nucleotide and predicted amino acid sequences across a panel of reference biological clones representing all lineages, we show that the TSSA epitope previously considered to be serologically characteristic of TcII-VI is restricted to TcII, V, and VI, not TcIII or IV, and that the peptide described as TcI-specific shares key features with TcIII and IV. Notably, *TSSA* sequences inferred greater phylogenetic affinities of TcIII and IV to TcI than to TcII, V, or VI. A high ratio of non-synonymous/synonymous nucleotide substitutions (ω=1.233) indicates that the TSSA gene has been under positive selection pressure.
The demonstration of lineage-specific epitopes within TcII-VI has potential implications for sero-epidemiological studies of an individual's historical and extant *T. cruzi* infection status. Comparative genomics thus provides a means to finding new molecular epidemiology tools.
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P125 The Genome of *Leishmania mexicana*

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We report on the assembly of the genome of Leishmania mexicana, a causative agent of new world cutaneous Leishmaniasis. The genome has been assembled into 34 pseudochromosomes from 358 contigs, making up 30.8 mbps of sequence in total. The genome is overall very similar to those of L. infantum and L. major, but for the fusion of chromosomes 8 and 29, and 20 and 36. Phylogenomic analysis of a set of 1,000 concatenated protein coding genes supports a position of L. mexicana at the base of the Leishmania Leishmania clade, in agreement with previously published findings. Conversely, analysis of orthologue clusters suggests that L. mexicana shares more orthologue groups in common with L. infantum than L. major.

P126* The novel AT-hook protein ETR1 is involved in transcriptional control in *Trypanosoma brucei*

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Trypanosoma brucei evades the immune system of its mammalian host by periodically switching its Variant Surface Glycoprotein (VSG) coat. Trypanosomes show monoallelic expression of one VSG out of a repertoire of ~1200 genes, with the active VSG expressed from one of about 15 telomeric VSG expression sites (ESs). The mechanism behind the monoallelic exclusion of ESs is unclear.
ETR1 was identified as a novel and essential AT-hook protein binding transcriptionally silent simple sequence repeats in *T. brucei*. We depleted ETR1 using RNAi in various *T. brucei* reporter

lines containing an *eGFP* inserted into different transcriptionally silent areas of the genome. After blocking ETR1 synthesis we monitored the derepression of *eGFP* using flow cytometry. Blocking ETR1 synthesis resulted in 30-75 fold derepression of silent ESs, and up to 5 fold derepression of other transcriptionally silent areas. Using chromatin immunoprecipitation and quantitative PCR we found an enrichment of ETR1 in the non-transcribed rDNA spacers and other non-transcribed loci. Lastly, we found that blocking ETR1 synthesis results in a rapid decrease in levels of the active VSG transcript. We are currently attempting to determine how ETR1 operates mechanistically.

P127 GPEET procyclin regulation: a nucleolar affair?

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Trypanosoma brucei undergo major changes in the composition of their surface coat when cycling between the bloodstream of a mammalian host and the tsetse fly vector. In the midgut of a tsetse fly a dense coat formed by the procyclins GPEET and EP covers the parasite. After 7-9 days in this environment, EP remains on the cell surface but GPEET expression is repressed. This regulation is post-transcriptional and is dependent on the GPEET 3' untranslated region. In an RNAi-based genome-wide screen, we identified a negative regulator of GPEET expression. This protein is expressed in the nucleolus of procyclic cells. It co-localises with two additional proteins, both of which contain RNA binding motifs and influence GPEET expression. The exact mechanism of the strict regulation of GPEET expression is not yet understood, but our results suggest an interplay of multiple distinctly localised proteins with the specific target mRNA.

P128 HAT3 and SIR2rp1 control RAD51-dependent DNA repair in African trypanosomes

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Antigenic variation in *T. brucei* depends upon Variant Surface Glycoprotein recombination and RAD51 recombinase activity. Covalent post-translational chromatin modifications have important roles in recombination and repair in other organisms but the mechanisms remain only partially characterised. We are currently assessing the role of histone acetylation in DNA repair in *T. brucei*. Among eleven putative histone acetyltransferases (HATs) and deacetylases (DACs), several are dispensable for growth. We used an inducible meganuclease system to introduce site-speicific DNA double-strand breaks (DSB) and to assess dispensable enzymes for roles in DNA repair. This revealed defects in *hat3* (histone H4-K4 acetyltransferase) and *sir2rp1* (histone deacetylase) null cells. Further analysis revealed *hat3*-defects in DNA resection and RAD51 assembly. In contrast, the *sir2rp1* strains displayed defects in ssDNA processing and RAD51 disassembly. Neither protein was required for efficient single-strand annealing, a RAD51-independent repair mechanism. These results indicate a role for histone acetylation and deacetylation (possibly involving H4-K4) in RAD51-dependent DNA repair in trypanosomes.

P129 Visualizing mating in trypanosomes: what are they getting up to in the fly?

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Genetic exchange occurs in *Trypanosoma brucei* during the developmental cycle in the tsetse vector, but all evidence about the mechanism is indirect and no-one has yet seen trypanosome cells mating. The event definitely occurs in the salivary glands of the fly, but the salivary gland stages cannot be cultured *in vitro* and are produced in small numbers inside the fly. Investigation is further constrained by the difficulty of staining these cells *in situ* by antibodies or cell dyes. Therefore to visualize these cells, we are developing approaches based on expression of fluorescent reporter genes. Specific organelles such as the flagellum, nucleus or kinetoplast can be tagged endogenously through suitable fluorescent fusion proteins. Moreover, by using fluorescent reporters of different colours, organelles that belong to each parent can be identified in

cells undergoing mating, helping us to understand the mechanics of hybrid production. We report the results of various crosses carried out to date using such tagged cell lines and discuss implications regarding the mechanism of genetic exchange in trypanosomes.

P130* Standardisation of NASBA method by using 18S rRNA gene for identification of *Leishmania major* parasite

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Objective: *Leishmania major* is flagellate protozoan parasite, with more than 20 species diversity and global distribution. This parasite can cause skin disease cutaneous leishmaniasis (CL) in humans. In diagnosis of the parasite, molecular methods are more sensitive and convenient than microscopic method. Application of NASBA (nucleic acid sequence-based amplification) method is shown to be high efficient for diagnosis of live parasite. In the present work, molecular isothermal NASBA were evaluated to identify live *Leishmania* In vitro condition.
Materials and Methods: The parasites were cultured in RPMI then their RNA was purified from promastigote stage. For evaluation of NASBA method in diagnosis of live parasite, the 18s rRNA gene of *Leishmania* was amplified. The result band on gel agarose was investigated.
Results: The results indicated that, the 18s rRNA gene is appropriate for identification of *Leishmania* in NASBA. It was detected in the area of approximately 200bp on gel electrophoresis.
Conclusion: The application of NASBA method is suitable for diagnosis of live *Leishmania*. This method is relevant for evaluating effectiveness of course and treatment of anti *Leishmania* drugs as well as early diagnosis of *Leishmania*.

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