Welcome

Dear BSP2008 Delegates,

It gives me great pleasure to welcome you for the annual conference of the British Society for Parasitology at Newcastle University. This year we received around 350 abstracts and more than 430 delegates have already registered for the conference. Delegates from around 30 countries will be attending the conference, bringing with them their knowledge and expertise on parasites of many shapes and presenting research from the field to laboratory scale.
We hope you will have a pleasant stay and that you will actively network during our social activities allowing you to learn about new exciting developments in the field of parasitology and perhaps identify new research partners.
The organising committee will do its best to make your stay memorable and enjoyable so do not hesitate to contact us with any questions or queries during the conference (wearing purple badges).

I look forward to meeting each of you during the conference.

Best regards

Olivier Sparagano
BSP2008 Local Chair

Front picture: The celebrated 'Angel' is built from 200 tonnes of steel and was created by Antony Gormley OBE. It stands 20m high and is seen by 33 million people every year - many of whom enjoy the impressive sight while travelling on the A1 or the East Coast Mainline.

Photographer: Graeme Peacock

Source: NewcastleGateshead Media Centre

CONFERENCE INFORMATION

Sunday night:
-Registration from 3pm to 8pm in the Lindisfarne Room (King's Road Center, 1st floor)
-Welcome reception from 6pm to 9pm in the Lindisfarne Room

Monday:
-Oral presentations in four Lecture Theatres (Curtis Auditorium, LT1, LT2, LT3) all on the ground floor of the Herschel Building.
-Coffee breaks, exhibitors and Poster session 1 in the Lindisfarne room
-Lunch: 3 serving stations (Lindisfarne room (first floor), Bamburgh room (ground floor) and Bistro Café (ground floor). All three rooms are in the King's Road Center. Please go to the servicing room indicated on your ticket so we can reduce your queuing time for meals
-Evening poster session/finger buffet in the Lindisfarne Room

Tuesday:
-Morning coffee break in the Lindisfarne room
-Lunch as for Monday
-Poster session 2 in the Lindisfarne room
-Afternoon coffee break in the Herschel Building
-Evening conference dinner (sold out) at the Centre for Life near Central Station

Wednesday:
Morning coffee break in the Herschel Building
Conference ends at 1pm

The computer cluster (1st floor in the Herschel Building) will be open on Monday and Tuesday during conference hours (9am to 5pm) so that delegates can check their emails

A campus map should be in your conference bag.

The BSP2008 Organising Committee would like to thank the following sponsors

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Abstracts

BSP PLENARY LECTURE by Prof. Tim Geary

BSP340
Enabling mechanism-based screening for antiparasitic discovery in developing regions
Tim Geary

Institute of Parasitology, McGill University, 21111 Lakeshore Road, Ste-Anne-de-Bellevue QC Canada H9X 3V9

Parasite chemotherapy has been indelibly enriched by the use of drugs derived from plant and microbial secondary metabolism. Changing fashions in the pharmaceutical industry have placed less emphasis on natural products as sources of leads for new drugs, but their potential for antiparasitic drug discovery is undeniable. To identify new antiparasitic leads from the floral and microbial biodiversity in economically less developed regions of the world, it would be optimal to put the discovery process as much as possible in the same area as the natural products are obtained to facilitate the rapid feedback needed to guide purification based on bioactivity. It is also important to empower the discovery effort in the hands of the people who suffer most from parasitic infections. An impediment to the establishment of modern high-throughput, mechanism based drug discovery approaches in these regions is the difficulty of maintaining the necessary equipment. An alternative is to adapt screens based on simple measures of survival of recombinant microbes that require the function of parasite proteins (drug targets) for viability. The basis of and potential for such an approach will be the subject of this presentation.

CA WRIGHT MEDAL LECTURE by Keith Matthews

BSP341
Ready for take-off: Life cycle differentiation control in African trypanosomes.
Keith R. Matthews

Institute for Immunology and Infection Research, University of Edinburgh, UK

African trypanosomes are transmitted between mammalian hosts by tsetse flies. This involves a developmental transition between bloodstream parasites and tsetse midgut procyclic forms. The bloodstream parasite population is described as pleomorphic, being composed of morphologically slender- and stumpy-forms, as well as intermediates between these extremes. The stumpy-form population is non-dividing and accumulates in response to parasite density. By limiting the parasitaemia, this maximises survival of the host as well as maximising transmission potential of the parasite. Once in the tsetse midgut, stumpy-forms transform to procyclic-forms, a differentiation process that can be mimicked in vitro and occurs synchronously in the population. This synchrony has allowed molecular and cytological dissection of the events of differentiation, providing a framework for understanding its underlying controls. We have investigated the interaction between the cell biology and molecular biology of trypanosomes as they prepare for this transition to the tsetse fly, and as they progress through the developmental pathway once it is initiated. By focussing particularly on the fundamental biology of the parasite, specific mechanisms controlling trypanosome development are being uncovered.

MALARIA ABSTRACTS (ORAL PRESENTATIONS)

Session 1A

BSP283 (guest speaker)
The application of genetic analyses to studies of human malaria.
Lisa Ranford-Cartwright

Division of Infection and Immunity, Institute of Biomedical and Life Sciences, University of Glasgow, Glasgow Biomedical Research Centre, 120 University Place, Glasgow G12 8TA, Scotland, UK.

David Walliker took a genetic approach to understanding malaria parasites both in the laboratory and field. His research utilised classical genetic approaches to understand traits such as drug resistance in malaria parasites, as well as deepening our understanding of the population structure of malaria parasites in their natural environments. Having spent ten years working in David's laboratory, from a PhD student onwards, it is no surprise that my research group now continues these themes, specifically applying them to the human malaria parasite in the mosquito, and population genetic studies of antimalarial drug resistance. Our current work utilises genetic analysis to identify regions of the genome, and ultimately genes, which influence the ability of the human malaria parasite *Plasmodium falciparum* to infect mosquitoes. Our initial studies to establish the extent of phenotypic variation in infection levels have shed light on the biology of the human malaria parasite in mosquitoes.

BSP323 (guest speaker)
The genetics and genomics of rodent malaria parasites
Jane M. Carlton

Department of Medical Parasitology, New York University School of Medicine, New York, NY 10010, USA

Rodent malaria species have provided the malaria research community with a robust model system for the study of human malaria. In particular, David Walliker and colleagues at the University of Edinburgh, developed *Plasmodium chabaudi* as a model system for genetic analysis of malaria parasites. David constructed genetic crosses between parental clones with different phenotypes, for example, crossing drug sensitive clones with genetically distinct parasites that had been selected for resistance to various antimalarial drugs. Subsequent analysis of the progeny clones revealed the mode of inheritance of the traits and led to hypotheses concerning the evolution of drug resistance. Since leaving David's lab in 1996, my research has continued to focus on the genetics of malaria parasites, along the way morphing into the logical extension of that work, genomics. Plasmodium genomics took off in 2002 with the publication of the *P. falciparum* genome and the sequence of the first rodent malaria species, *P. yoelii yoelii*. Sequences of two more rodent malaria species followed in 2005, and now on the

eve of the publication of the *P. vivax* and *P. knowlesi* genomes, comparative genetics and genomics of multiple malaria genomes has finally come of age.

BSP339 (guest speaker)
Remarkable diversity of *Plasmodium falciparum* merozoites
David Conway

MRC Gambia, and LSHTM

Genotypic, phenotypic, and transcriptional data strongly endorse the characterisation by David Walliker and his colleagues.
Firstly, balancing selection maintains allelic diversity of particular genes encoding merozoite antigens. A scan of available genome-wide polymorphism data indicates that a number of non-telomeric 'oases' of diversity map to such genes. Allele frequency-based tests of neutrality effectively detect those genes under strongest balancing selection in endemic populations, and the approach is being scaled up to prospectively identify targets of acquired immunity.
Secondly, merozoites vary in erythrocyte invasion phenotypes, relying on glycophorins A, B or C, or other receptors that differ in sensitivity to experimental enzyme treatments. Phenotypic diversity in a single population in Africa approaches that seen among a diverse worldwide collection of isolates, suggesting that it may be adaptively maintained in populations.
Thirdly, African clinical isolates vary in the relative abundance of transcripts encoding different merozoite proteins, including known ligands for erythrocyte invasion such as EBA and Rh molecules, and other unrelated surface-accessible proteins. Whether parasites carry different 'hard-wired' merozoite gene expression profiles, or there is frequent switching and thus true antigenic variation of the merozoite is under investigation.

Session 2A

BSP330 (guest speaker)
Group A var genes are transcribed by *Plasmodium falciparum* rosetting strains
J. Alexandra Rowe, Ahmed Raza and Jean-Philippe Semblat

Institute of Immunology and Infection Research, University of Edinburgh, EH9 3JT, UK.

Rosetting (binding of *P. falciparum*-infected erythrocytes to uninfected erythrocytes) is a parasite virulence phenotype associated with severe malaria. The molecular mechanisms of rosetting have not been fully described, although the variant surface antigen PfEMP1, encoded by specific var genes, is known to be the parasite rosetting ligand in two laboratory strains. We have investigated the var genes transcribed by 6 rosetting laboratory strains and found that they all transcribe "Group A-like" var genes (characterised by their large size, complex domain structure and upstream sequence). The rosetting-associated var genes from different strains show high levels of sequence diversity, however certain common features in gene structure and domain type are

apparent. Antibodies raised to a rosette-mediating PfEMP1 domain of one strain show cross-reactive rosette-inhibiting activity against some (but not all) of the other strains. These results show that group A var genes/PfEMP1 variants play a major role in rosetting, and are consistent with previous work linking group A var genes to the most severe clinical forms of malaria.

BSP110

The export of *Plasmodium falciparum* adhesion antigens.

David E. Arnot, Kordai M. Sowa & Dominique Bengtsson.

Centre for Medical Parasitology, University of Copenhagen Medical School, 1014 Copenhagen K, Denmark.

The transportation and anchoring of *Plasmodium falciparum* adhesins in the erythrocyte plasma membrane is not understood. Laser scanning confocal microscopy has been used to obtain high resolution immuno-fluorescent images of parasitized cells illustrating the movements of parasite antigens from the parasite cytoplasm, across the parasitophorous vacuole membrane, through the cytosol to the plasma membrane. A novel staining technique has been developed which permits clear distinction between erythrocyte surface PfEMP1 and the intracellular pool of this antigen. KAHRP antigen moves to its sites of deposition underneath the erythrocyte membrane independently of PfEMP1, which arrives at the erythrocyte membrane in a later wave of antigen export. PfEMP1 is not a continuously present component of knobs, but progressively inserted at a limited number of sites in the membrane. KAHRP is not transported in Maurer's clefts, nor does it appear to be strongly bound to these vesicular structures. PfEMP1 is not stored or transported in Maurer's clefts but associates with peripheral Maurer's clefts underneath the erythrocyte membrane. Soluble, intracellular PfEMP1 is concentrated within the intra-erythrocytic parasite.

BSP142

Cytoadherence of drug treated *Plasmodium falciparum* infected red blood cells.

Katie Hughes, Giancarlo Biagini, Alister Craig

Liverpool School of Tropical Medicine, Liverpool, L3 5QA, UK

The development of severe disease in *Plasmodium falciparum* malaria is thought to be, at least in part, due to the sequestration of trophozoite-stage infected red blood cells in microvasculature. We show that infected red blood cells are capable of cytoadherence to endothelial cells for many hours after addition of antimalarials to trophozoite-stage parasites. The parasite protein responsible for cytoadherence, PfEMP-1, is detectable on the surface of infected red blood cells long after antimalarial treatment has been administered. Similar results are seen using a range of antimalarials, although an advantage for artesunate is demonstrated due to its activity against early pre-cytoadherent ring stages. Signaling responses by endothelial cells to infected red blood cells are also maintained after antimalarial treatment of infected red blood cells. These findings show that cytoadherence and post-adhesion responses are not dependent on the presence of a viable parasite. We show an advantage for drugs that are active against

ring stage parasites and illustrate the need for adjunct therapies to support patients during the first 24 hours after administration of anti-parasitic drugs.

BSP148

Variation in the human host response may influence malaria pathogenesis.

M. Griffiths[1,2], C. Dolecek[3], S. Popper[2], H.D.T. Nghia[3], D.X. Sinh[3], N.H. Phu[3], T.T.T. Hien[3], J. Farrar[3], D. Relman[2]

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[2] Department Infectious Disease and Geographic Medicine, Stanford University, California, USA.
[3] Hospital for Tropical Diseases, Ho Chi Minh, Vietnam.

Uncomplicated malaria is observed following either *Plasmodium falciparum* or vivax infection. Severe malaria is almost exclusively observed following *P. falciparum* infection. Consequently, uncomplicated malaria (*P. falciparum* or *P. vivax*) may be associated with a distinct host response compared to severe malaria (*P. falciparum*).
A genome-wide study of transcript abundance was undertaken. Whole-blood samples obtained from Vietnamese adult patients on hospitalisation, and during disease course (Day 0 (admission), Day 5, Day 30), with either acute uncomplicated (*P. falciparum* (n=9) or *P. vivax* (n=8),) or severe malaria (*P. falciparum* (n=18)) were examined.
Patients with severe malaria (*P. falciparum*) demonstrated divergent patterns of transcript abundance compared to uncomplicated P.falciparum or P.vivax infection. These changes in transcript patterns related to B-cell, cell-cycle, erythropoiesis, innate and cellular immune responses. The differences remained after controlling for peripheral parasite density. In contrast, patients with uncomplicated malaria (*P. falciparum* or vivax) demonstrated overlapping transcript patterns.
Variations in human host response, and not just variation in parasite characteristics, may influence malaria pathogenesis.

BSP052

Alleles -308A and -1031C in the TNF-α gene promoter do not increase the risk but associated with circulating levels of TNF-α and clinical features of vivax Malaria in Indian patients

Mohd Sohail [1,2*], Asha Kaul[1], Prerna Bali[1] M.Raziuddin[2], M.P. Singh[2], O.P. Singh[1], A.P. Dash[1] and T. Adak[1]

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The biological significance of TNF promoter polymorphism and infectious disease association prompted us to investigate whether TNF-α -308 G/A and -1031 T/C promoter polymorphisms are associated with *P. vivax* infection, cellular TNF-α level and possibly with clinical symptoms by employing PCR-RFLP methods. An overall significant elevation of serum TNF-α, IL-6 content (p=0.0002, p=0.002 respectively), whereas highly significant depletion of IL-10 content (p=0.0001) was observed in vivax patients. In addition, TNF-α concentration in patients with and without fever were

found to be significant (p=0.0001, p=0.0004, respectively).The genotypic distribution for -308 G/A and -1031 T/C positions were found non significant, but it was clinically potent to observe statistically significant distribution of genotypes (p=0.032) in patients with and without fever. Furthermore, the TNF-α level in TNF1 and TNF2 genotype for -308 position was significantly higher (p=0.010, p=0.006 respectively). In case of -1031 position TNF-α level was significant in ancestral (TT) genotype (p=0.0007) in patients compared to healthy subjects and significantly higher in rare (CC) genotype (p=0.021) as compared to ancestral genotype. In addition, the two polymorphisms 308G/A and -1031T/C were in highly significant LD (D' =0.7992, r2 = 0.6005, p = 0.0001) in the patients as well as it is interesting to report that the distribution of novel 308A: 1031C alleles associated haplotypes are nearly the same in patients (0.2610) and in healthy subjects (0.2636).

In view of present observation of promoter polymorphism with TNF-α level and other clinical parameters of vivax infection, we suggest that evaluation of TNF level and its polymorphisms in the promoter region may be considered to be reliable molecular and immunological markers, possess promising rational for diagnostic potential and immunotherapeutic interventions in clinical vivax malaria. Genetic variation in the promoter region is of biological significance and may play important roles in host defense mechanisms against vivax infection by enhancing cell-mediated immunity and stimulating the protective immunological cascade.

Session 3A

BSP280 (guest speaker)

Is receptor mediated endocytosis involved in host cell invasion by *Toxoplasma gondii* ?

Carolina Agop-Nersesian, Manuela Breinich, Angelika Herm-Goetz, Henning Kessler, Manuel Rauch and Markus Meissner

Hygieneinstitute, University Hospital Heidelberg, Germany

Apicomplexan parasites rely on sequential secretion of specialised secretory organelles (micronemes and rhoptries) for the invasion of the host cell. We recently demonstrated that micronemal protein MIC8 of *T. gondii* plays an essential role for the formation of a moving junction, possibly by triggering rhoptry secretion via a signalling cascade. Receptor mediated endocytosis is a critical step during the activation of surface receptors and its ablation can result in inactivation of the signalling cascade. In order to test if a similar mechanism exists in *T. gondii*, we established a novel ectopic regulation system that allows rapid regulation of protein levels. Using this system we expressed dominant negative versions of proteins required for receptor mediated endocytosis in other organisms. Interestingly specific upregulation in extracellular parasites interferes with the capability of the parasite to invade the host cell, indicating that these factors play a dual role during replication and invasion. We are currently analysing if the observed block in invasion is caused by the ablation of endocytosis in extracellular parasites.

BSP138

Characterisation of Eukaryotic initiation Factor 2Alpha stress-reponsive kinases in *Plasmodium falciparum*

Clare Fennell, Luc Reininger, Christian Doerig.

INSERM U609, Welcome Centre for Molecular Parasitology, Glasgow Biomedical Research Centre, 120 University Place, University of Glasgow, G12 8TA, UK.

Eukaryotic cells typically respond to stress by coordinated changes in gene expression, achieved by modulating transcription. An alternative is to modulate translation by the action of four independently regulated eukaryotic initiation factor 2alpha (eIF2alpha) kinases.

An analysis of the *P. falciparum* kinome published by our laboratory (Ward et al., 2004) identified three eIF2alpha kinase-related enzymes (PFA0380w, PFF1370w, PF14_0423). We also identified the orthologue of the substrate eIF2alpha (PF17_0117), suggesting that stress-dependant translation regulation may occur, as in mammalian cells. It has previously been shown that a recombinant protein encompassing the catalytic domain of PFF1370w is active in vitro, indicating it is a bona fide protein kinase (Mohrle et al., 1997). Here, we report activity of the PF14_0423 kinase domain and confirm the identity of the target regulatory serine of PfIF2alpha. Further, we show by gene disruption that neither PF14_0423 nor PFA0380w are required for asexual growth. In contrast PFF1370w, which is expressed throughout the life cycle, appears to be essential for asexual growth.

BSP133

Significant differences in genetic diversity and recombination frequency in ama1genes of sympatric *Plasmodium vivax* and *P. falciparum* from the Venezuelan Amazon

Colin Sutherland[1], Rosalynn Ord[1], Adriana Tami[2]

[1] London School of Hygiene & Tropical Medicine, UK
[2] Royal Tropical Institute, Amsterdam, The Netherlands

Genetic analyses of full-length gene sequences encoding the AMA1 surface antigen are presented for sympatric populations of the human malaria parasites *Plasmodium vivax* and *P. falciparum* from the Venezuelan Amazon.

Pvama1 sequences (N = 73) exhibited significantly greater genetic diversity than did pfama1 sequences (N = 30). Levels of recombination were also significantly higher for pvama1. In contrast, patterns of nucleotide substitutions provided evidence that polymorphisms in the ama1 gene of both species are maintained by balancing selection, particularly in domain I.

The two distinct population structures observed are unlikely to result from different selective forces acting upon the two species, but suggest a much larger effective population size for *P. vivax*. These results highlight the need for future control interventions to employ strategies targeting each of the parasite species present in endemic areas.

BSP049

Prevalence of Plasmodium species in malaria asymptomatic African immigrants assessed with real-time quantitative nucleic acid sequence-based amplification

Gaetano Scotto[1], Marianna Marangi[2], Rocco Di Tullio[1], Vincenza Fazio[1], Gioacchino Angarano[1], Annunziata Giangaspero[2] & Henk D. Schallig[3]

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[2] Dipartimento PrIME, Università di Foggia, Italy;
[3] Royal Tropical Institute, Amsterdam, The Netherlands.

A sensitive and specific real-time Quantitative Nucleic Acid Sequence Based Amplification (real-time QT-NASBA) assays, based on the small-subunit 18S rRNA gene, was used to assess the prevalence of human Plasmodium species in 195 volunteers with no clinical signs related to malaria and coming from sub-Saharan African regions to Southern Italy. Sixty-two subjects (31.8%) were found positive to Plasmodium infections. Twenty-four samples (38%) of the 62 NASBA positive subjects were found positive with a Pfs25 mRNA Real-time QT-NASBA, which is specific for the detection of gametocytes of *P. falciparum*. This study shows a relative high prevalence of Plasmodium infection in the studied population. These data suggest that careful monitoring of the investigated population is needed to avoid future health problems. Furthermore, the presence of gametocytes poses the need for better monitoring of the presence of the vector in Southern Italy in order to circumvent possible future transmission.

BSP009

Molecular diagnosis of malaria: development of a novel, one-step Nucleic Acid Lateral Flow Immuno-Assay detecting human Plasmodium species and field evaluation in Kenya.

Pètra F. Mens[1,2], Aart van Amerongen[3], Patrick Sawa [4], Piet Kager[2] & Henk Schallig[1]

[1] Royal Tropical Institute, Amsterdam, The Netherlands;
[2] Centre for Infection and Immunity Amsterdam, The Netherlands;
[3] Wageningen University Research Centre, The Netherlands;
[4] International Centre for Insect Physiology Ecology, Mbita, Kenya

Microscopical malaria diagnosis becomes insensitive at low parasitaemia and time consuming. Molecular tools allow for specific/sensitive diagnosis but current formats, like PCR combined with gel-electrophoresis, are difficult to implement in resource poor settings. Development of a simple, fast, sensitive and specific detection system, Nucleic Acid Lateral Flow Immunoassay (NALFIA) for amplified Pan-Plasmodium PCR products is reported. NALFIA lower detection limit is 0.3-3 parasites/µl, ten-fold more sensitive than gel-electrophoresis analysis. Evaluating 650 clinically suspected malaria cases with the assay under field conditions (rural Kenya), revealed that NALFIA detected more positives than microscopy (agreement: 95%; k-value 0.85) and there was an excellent agreement between gel-electrophoresis and NALFIA (98.5%; k-value: 0.96). NALFIA is more sensitive than microscopy and a good alternative to detect PCR products whilst circumventing using electricity or expensive equipment, making NALFIA the first step towards molecular field diagnosis.

Session 4A

BSP115 (Guest speaker)
Ecological and Evolutionary Determinants of Host Choice by African Malaria Vectors
Heather Ferguson[1], Valeliana Mayagaya[2] and Issa Lyimo[1,2]

[1]Division of Infection and Immunity, University of Glasgow, G12 8TA, UK;
[2]Public Health Entomology Unit, Ifakara Health Research and Development Centre, Tanzania

The propensity of mosquito vectors to feed on humans instead of other available animal hosts is one of the most important determinants of malaria transmission intensity and stability. Despite the clear epidemiological importance of mosquito host choice, there remains a poor understanding of the evolutionary and ecological factors that have caused malaria vectors to specialize on humans, and whether these selective pressures could be weakened by environmental manipulation. We are currently conducting a programme of laboratory experiments and field work in a malaria endemic region of southern Tanzania to evaluate competing hypotheses for why humans are the preferred vertebrate hosts of African malaria vectors: (1) human blood gives rise to higher mosquito fitness than animal blood, (2) humans exhibit less defensive behaviour than other available host species, and (3) humans are more available than alternative animal sources of blood. We summarize preliminary findings from this research and its implications for malaria-control strategies based on mosquito host-choice manipulation.

BSP258
The development and field trial of a molecular age-grading assay for Anopheline mosquitoes.
Mikhail, A.F.W.[1], Martin, S.A.M.[1], Illian, J.[2], Mayer, C.[3], Drakeley, C.J.[4], Mordue, A.J.[1], Billingsley, P.F.[5]

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[3] The Rowett Research Institute, Greenburn Road, Bucksburn, AB21 9SB, Aberdeen, Scotland.
[4] London School of Hygiene & Tropical Medicine, London WC1E 7HT, UK
[5] Sanaria Inc., 9800 Medical Center Drive, Suite A209, Rockville, MD 20850, USA.

The lifespan of a mosquito is a critical determinant of its vector capacity. By examining the transcriptome of ageing mosquitoes with Affymetrix™ microarrays, we have identified 30 candidate genetic markers for chronological age and lifespan prediction in a key malaria vector, *Anopheles gambiae*. A multiple gene expression assay based on real time reverse transcriptase PCR for a sub-set of five markers was constructed, with which the age class of a wild mosquito can be determined. Field validation the assay was undertaken with the (known age) F1 offspring of wild *An. gambiae sensu stricto*

from Muheza, Tanzania. The performance of this assay and its potential application as a vector monitoring tool will be discussed.

BSP281
Quantifying Sex Ratios in *P. falciparum*
Samana Schwank, Chris Drakeley & Colin Sutherland

LSHTM, Immunology Unit, London WC1E 7HT, UK.

Malaria parasites, like many other eukaryotes, are able to alter the sex ratio of their gametes to maximize the reproductive output and thereby inevitably influence the epidemiology of this disease. The standard method for quantifying sex ratios in *P. falciparum* is based on the visual identification of male and female gametocytes by light microscopy. Limitation of gametocyte availability from field samples and difficulty of sexing gametocytes have been restricting sex ratio studies, despite their important role of transmitting this disease. An assay is being established that quantifies sex specific proteins, such as Mal7P1.162, α-tubulin II, and Pfg377, via rtq-PCR, which is designed to measure more precise sex ratios data from gametocyte-positive field samples, whether for examining the effect of treatment on sex ratio, or for longitudinal monitoring of changes in sex ratio over extended periods of gametocyte carriage. The IFAT technique (Immuno-flourescent antibody test) has also been used to observe expression of α-tubulin II and Pfg377 to provide validated sex ratios in vitro and to elucidate the timing of the expression of sex specific proteins during gametocyte development.

BSP107
Site-specific integrations in *Anopheles gambiae*
Sanjay Basu, Janet Meredith and Paul Eggleston

Institute of Science and Technology in Medicine, Keele University, ST5 5BG, UK.

Anopheles gambiae is a major vector of malaria throughout Africa. One strategy of control concentrates upon population replacement of native susceptible mosquitoes with transgenic refractory populations. Techniques to generate transgenic strains have progressed with success over the last 20 years but many fundamental issues remain. By employing a site-specific mechanism adapted from φC31 Streptomyces bacteriophage it has been possible to generate transgenic *Anopheles gambiae* with increased efficiency. To this end we have successfully identified, sequenced and cloned an anopheline anti-platelet protein salivary gland promoter to drive expression of an artificially designed anti-malarial peptide. Current work involves the integration of this new construct and its effect upon infection studies of the salivary glands. We have previously generated a transgenic mosquito with gut-specific AMP expression and hope to combine the two constructs to give the first transgenic mosquito employing two different promoters driving an AMP.

BSP004
Apoptosis-like death in *Plasmodium berghei* ookinetes in vitro and in vivo
Medhat Ali, Anne Loweth and Hilary Hurd

Centre for Applied Entomology and Parasitology, ISTM, Keele University, Staffordshire, ST5 5BG, UK.

Several studies have now demonstrated the occurrence of apoptosis-like cell death in protozoan parasites, including Plasmodium. This study confirmed our previous findings that ookinetes of *P. berghei* exhibit chromatin condensation, DNA fragmentation and have molecules that bind caspase substrates. Our objective is to identify triggers of ookinete-apoptosis present in the mosquito midgut. Nitric oxide (NO) and reactive oxygen species (ROS) were potential candidates. NO is toxic to ookinetes but their mode of death had not been investigated. Two NO donors, S-Nitrosoglutathione (SNOG) and Sodium Nitroprusside (SNP) were used. Significant increases in ookinetes expressing markers of apoptosis occurred with 2mM SNOG (24h incubation) and 100µl SNP (1h incubation, ~30 % increase). *P. berghei*-infected mice had significantly elevated levels of NO in the plasma. Other potential apoptosis inducers such as tumour necrosis factor and interferon gamma will be investigated. Treating ookinetes with 3, 4-dihydroxy-L-phenylalanin (L-DOPA), a ROS donor, did not induce apoptosis-like death but cause death attributed to necrosis. The effects of NO donors on ookinete apoptosis in vivo will be reported.

Session 5A

BSP345
Regulation of *Plasmodium berghei* development in the mosquito blood meal
Oliver Billker

The Wellcome Trust Sanger Institute, Hinxton Cambridge, CB10 1SA, UK.

The transmission of malaria parasites to the mosquito is initiated when sexual precursor stages, the gametocytes, become ingested with the blood meal. From their location within the erythrocyte, they respond to cues from the mosquito environment, which trigger their rapid differentiation into free gametes. Following fertilisation, the zygote transforms into a motile ookinete. One of the developmental triggers of gamete formation is a small mosquito molecule, xanthurenic acid. While the parasite's receptor for xanthurenic acid has remained elusive, a screen of small molecule libraries has provided valuable insights into structure activity relationships of gametocyte activators and has generated probes for the analysis of signal transduction during sexual development. The same secondary messengers, including calcium and cyclic guanosine monophosphate (cGMP), are involved in regulating both, gametocyte activation, and gliding motility of the ookinete. In each case they act through different, stage-specific signal transduction and effector pathways. Recent evidence suggests, for instance, that in *P. berghei* a cGMP phosphodiesterase and a guanylyl cyclase act specifically in ookinetes to control gliding motility through cellular cGMP levels.

BSP250 (Guest Speaker)

How to investigate the function of merozoite surface proteins in *Plasmodium falciparum*

Ellen Knuepfer [1], Rita Tewari [2], Madhusudan Kadekoppala [1] & Anthony A. Holder [1]

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Merozoite surface proteins (MSPs) of *Plasmodium falciparum* have been regarded as promising vaccine candidates for decades. The role of these proteins in the parasite's life cycle however is still unexplained.
Plasmodium is haploid during the asexual blood stage when it is most easily manipulated and as a consequence essential genes at this stage cannot be deleted because the resulting mutants are not viable. In addition even when successful in deleting parasite genes after extended drug cycling periods the phenotype is often elusive. Functional redundancy at the protein level appears to be common. We are investigating the function of peripheral merozoite surface proteins in the asexual blood stage of *P. falciparum* by means of double recombination. We are concentrating on investigating the role of MSP3, MSP6, MSP7 and MSP9 in erythrocyte invasion and parasite growth.

BSP013

Revealing the complexity of the erythrocyte invasion machinery of *Plasmodium falciparum*: Global screening of putative invasion-related proteins

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One of the key processes in the pathobiology of the malaria parasite is the invasion and subsequent modification of the human erythrocyte. In this complex process a large and unknown number of parasite proteins are involved; some are promising blood stage vaccine candidates. The majority of the proteins that play a pivotal role in invasion are either stored in the apical secretory organelles, or located on the surface of the merozoite, the invasive stage of the parasite. Using transcriptional and structural features of these known proteins we performed a genome wide search that identified 67 hypothetical proteins with a high probability to be located in the secretory organelles or on the surface. These predictions were validated by cloning, tagging and subsequently tracking these proteins with green fluorescence protein in transgenic parasites. We show that indeed most of these proteins are trafficked to their predicted subcellular localisation. Our data indicates that a significant part of the parasites invasion machinery remains to be characterized and our screen provides an important step

towards this goal. The putative function of some of these new proteins within the invasion process will be discussed.

BSP064

A central role for *Plasmodium falciparum* cGMP-dependent protein kinase in schizogony.

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We have demonstrated a central role for *Plasmodium falciparum* cGMP-dependent protein kinase (PfPKG) in schizogony of the malaria parasite. Treating erythrocytic stages of *P. falciparum* with a specific anti-coccidial PKG inhibitor (a trisubstituted pyrolle, compound 1) had the greatest effect during schizogony, leading to persistence of large dysmorphic schizonts that did not progress to ring stages. IFAs of treated schizonts revealed that several apical organelle and merozoite surface proteins were correctly localised, but EM demonstrated marked structural abnormalities. Using genetically manipulated *P. falciparum* parasites expressing a compound 1-insensitive PfPKG introduced by allelic replacement, we showed these effects could be attributed to the selective action of compound 1 on PfPKG alone. Parasites with the mutated enzyme could complete schizogony in the presence of compound 1 or the related inhibitor, compound 2, but not in the presence of non-specific kinase inhibitors. The aim now is to identify targets of PfPKG whose phosphorylation is essential to schizogony.

BSP067

Sequence requirements for the export of the PEXEL-negative protein REX2 in *P. falciparum*

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The identification of the PEXEL motif allowed a preliminary prediction of the Plasmodium "exportome". However, an increasing number of proteins without a PEXEL motif are exported, indicating the presence of a PEXEL-independent export pathway.
In order to shed light on PEXEL-independent export we analysed the integral Maurer's cleft protein REX2 in detail. REX2 does not contain any PEXEL-like sequences. Taking advantage of the short sequence of REX2, which must contain the whole export information, we generated serial deletions and domain replacements and assessed the sequence requirements for the export of this protein. We show that a sequence resembling the second half of a PEXEL-motif is necessary for REX2 export

and can be complemented by a similar region found in SBP1, demonstrating that this region is found in other PEXEL negative exported proteins. Furthermore we show that the type of TM also has a pronounced effect on REX2 export.

BSP040
Biochemical characterisation of lipoic acid protein ligase A1, an essential protein from *Plasmodium falciparum*
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Lipoic acid is an essential cofactor of α-keto acid dehydrogenase complexes (KADH) and the glycine cleavage system (GCS), which are involved in oxidative metabolism. Plasmodium possesses two distinct pathways to lipoylate these complexes; an apicoplast located biosynthesis and a mitochondrial salvage pathway. This study focuses on the biochemical characterisation of the mitochondrial lipoic acid protein ligase A1 (LplA1), a protein essential for the erythrocytic development of *P. falciparum* and *P. berghei*. Recombinant LplA1 was expressed in *E. coli* and purified protein was analysed for its substrate specificity, utilising the detection of bound lipoic acid to the lipoyl-domains of all three Plasmodium KADH and the H-protein of the GCS. In addition, a spectrophotometric assay was developed which detects ATP hydrolysis during the LplA1 reaction. Released pyrophosphate was detected colorimetrically and corresponded linearly to the catalytic activity of LplA1. Kinetic parameters for *P. falciparum* and *E. coli* LplA were determined and validate this assay for future drug-screening use.
This research was funded by the Wellcome Trust and Boehringer Ingelheim Fonds.

Session 6A

BSP111 (Guest Speaker)
Between protection and pathology: Regulation of T cells during malaria
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T-cells have to be tightly controlled during malaria. Immunity against the liver-stage critically depends on CD8+ T-cells. Infection with sporozoites induces a transient increase of these cells, but they rapidly decline after onset of the blood-stage. Here, large numbers of T-cells were activated and especially CD8+ T-cells were sequestered in the brain and contribute to pathology. Thus the immune response against malaria is a double-edged sword, since it controls parasites but is also involved in pathology. Our work is focussed on the analysis of immune regulatory mechanisms that modulate either protective T-cell responses against the liver-stage and on mechanisms that control inflammation during the blood-stage. To this end we studied the function of the negative costimulators CTLA-4, BTLA and PD-1 but also of Foxp3+ regulatory T-cells on the immune response against *P. berghei*-infection. Our data suggest that immune

modulation can limit the protective capacity of T-cells during the liver-stage but are beneficial by dampening inflammation in peripheral organs during the blood-stage. Thus these mechanisms might represent host-derived strategies to prevent overwhelming inflammation rather than parasite derived immune-escape strategies.

BSP259

Demyelination in experimental cerebral malaria.

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Cerebral malaria (CM) is a serious and often fatal complication of *Plasmodium falciparum* malaria. It is an acute encephalopathy with loss of consciousness, convulsions and fever. The underlying pathology includes dysregulated cytokine expression, increased permeability of the blood-brain barrier and sequestration of host cells.
We used the murine CM model (*P. berghei* infection in C57BL/6 mice) to study the possibility that CM results in demyelination. Experimental murine and human CM share several clinical and pathological characteristics. Brains were after transcardial perfusion, immersion fixed in Zamboni's fixative and embedded in paraffin. Myelin was visualised immunohistochemically with antibodies specific for myelin basic protein.
Extensive demyelination was detected in terminally ill mice. Demyelination was particularly evident in the cerebellum and in the corpus callosum. The underlying mechanisms remain to be elucidated as well as the extent of remyelination and neuronal damage.

BSP216

Malaria specific memory B cells and plasma cells in the *Plasmodium chabaudi* mouse model of malaria: kinetics, longevity and effect of chronic infection

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There is longstanding evidence for the presence of strong anti-parasitic as well as anti-disease effects of anti-malaria antibodies in immune serum adoptively transferred into sick children suggesting that antibodies play an important role in naturally acquired immunity to falciparum malaria. However, the antibody response to some malaria antigens is short-lived and clinical immunity is only acquired after several years. Here we have investigated the kinetics, longevity of malaria specific memory B cells (MBCs) and plasma cells (PCs) in the presence and absence of persisting "live-antigen". Malaria-specific MBC and PC were sustained for more than eight months during the

course of a primary infection. Using cyclophosphamide treatment, it was demonstrated that some of these PS are long-lived malaria. Chronic parasitisation modulated secondary memory B-cell responses to *P. chabaudi* antigens, in that faster antibody responses were generated in response to a second challenge of mice that had been drug-cured during the chronic phase of the primary infection.

BSP118

Is MSP-1 the only target of Strain Specific Protective Immunity in *Plasmodium chabaudi chabaudi*?

Elaine O'Mahony, Sandie Cheesman, Richard Carter.

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The genetic target(s) of Strain Specific Protective Immunity (SSPI) are being investigated in mouse malaria using Linkage Group Selection, a technique based upon classical genetic analysis. Independent analyses of two genetic crosses between the *P.c. chabaudi* lines AS and CB and between CB and AJ have identified a single locus on chromosome 8 as containing the major target antigen of SSPI. Within this locus of approximately 200kb lies the major malaria merozoite surface antigen, MSP-1. We wish to determine whether MSP-1 is the only important target of SSPI by a combination of backcrossing and semi-cloning of the immune-selected progeny of such a backcross. By backcrossing the original SSPI- selected cross progeny to the SSPI-sensitive parent and then repeating the immune selection, the size of the locus under selection should be reduced. This will correspondingly reduce the number of genes under scrutiny and that will need to be investigated by subsequent semi-cloning analysis. We report here the results of the first stage of this process, the backcross and immune selection analysis.

BSP274

Production and immunological validation of MSP-1 Block1/Block2 hybrid as vaccine candidate

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The polymorphic N-terminal Block 2 of Merozoite Surface Protein 1 (MSP-1) contains human antibody epitopes associated with protective immunological responses in African children. Possession of antibodies to Block 2 is associated with reduced risk of malaria symptoms and can protect immunized Aotus monkeys from experimental challenge infection with virulent *P. falciparum*. Block 2 is a vaccine candidate, but the high degree of polymorphism of the Block 2 region within the parasite population is still a big challenge. In order to cover the sequence diversity of all three Block 2 serotypes, a new antigen derived from MSP-1 Block 1/Block 2, called MSP-1 hybrid, was expressed as soluble protein and purified. The protein is thermostable and has unique biochemical properties. Immunisation of mice and rabbits show that the MSP-1 hybrid is immunogenic. The protein elicits multiple-strain specific antibody responses, is recognised by sera from African individuals who have experienced natural infection,

and promisingly, human antibody responses to this new antigen have a statistically negative correlation with the time to the next clinical malaria episode.
Further immunisations are underway to test the potential of this vaccine candidate in combination with various human-safe adjuvants. Investigation of the immunogenicity of the MSP-1 hybrid is currently being assessed. In parallel, optimisation of antigen production is underway to produce a standard protocol for industrial scale-up. Functional assays such as parasite growth inhibition assays will be performed in order to validate this vaccine for clinical trials.

Session 7A

BSP293 (guest speaker)
Exploiting the malaria parasite mitochondrion for drug development
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Plasmodium falciparum malaria is a major cause of global mortality. In the absence of vaccines, therapy is totally reliant on drugs, however tragically, many of the current therapies are failing due to drug resistance. To counter this, the international scientific community is urgently seeking new drug target leads.
A compounding problem to the development of new therapeutic interventions is the lack of basic knowledge of parasite biology. This alarming situation is illustrated in the case of the malaria parasite mitochondrion. This organelle has previously been shown to: (i) possess different functional states which are believed to be correlated with parasite virulence and clinical outcome of disease, and (ii) be essential for parasite survival as demonstrated by the antimalarial activity of MalaroneTM, a mitochondrial bc1 complex inhibitor. Yet despite these significant observations implicating a major role for the mitochondrion in malaria pathogenicity and chemotherapy, there are virtually no biochemical data regarding individual mitochondrial components. Data will be presented on the characterisation of a novel mitochondrial component, the type II NADH:quinone oxidoreductase and the development of small molecule inhibitors with selective toxicity to the malaria parasite electron transport chain.

BSP086 (guest speaker)
Vitamin B6 biosynthesis by *Plasmodium falciparum*
Martin Gengenbacher[1], Bianca Derrer[1], Niclas Riechert[1], Teresa Fitzpatrick[2], Thomas Raschle[2], Karlheinz Flicker[3], Ivo Tews[4], Sylke Müller[5], Peter Macheroux[3], Kai Matuschewski[1], Barbara Kappes[1]

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Most organisms synthesize vitamin B6 via a recently discovered pathway employing the proteins Pdx1 and Pdx2. We will present an in depth characterization of the respective orthologs from the malaria parasite, *Plasmodium falciparum*. Pdx1 and Pdx2 form a glutamine amidotransferase, with Pdx2 as the glutaminase and Pdx1 as pyridoxal-5`-phosphate synthase domain. Pdx1 and Pdx2 are expressed in blood stages and in gametocytes.
To assess the potency of parasite B6 de novo biosynthesis as novel drug target, knockouts of the pdx1 and pyridoxal kinase (pdxK; B6 salvage) genes were generated in *Plasmodium berghei*. The phenotypes of the knockout parasites will be presented and a conclusion for parasite vitamin B6 homeostasis will be discussed.

BSP165
The characterisation of CK2 (Casein Kinase II) in *Plasmodium falciparum*, and validation as a potential drug target
Zoe Holland, Luc Reininger, Christian Doerig

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P. falciparum is the causative agent for the most severe forms of malaria. The parasite has a complex lifecycle, encompassing sexual reproduction, differentiation between multiple lifecycle forms, and three stages of abundant proliferation. The signaling mechanisms underpinning proliferation and differentiation in the parasite are not well understood. CK2 is a eukaryotic serine/threonine protein kinase with multiple substrates and roles in diverse cellular processes including differentiation, proliferation, and translation. The mammalian holoenzyme consists of two catalytic alpha subunits, and two regulatory beta subunits. One gene encoding the alpha subunit, and two genes encoding different beta subunits have been identified within the *P. falciparum* genome (Ward et al., BMC Genomics, 2004).
We present the biochemical characterisation PfCK2α, demonstrate the essentiality of the three PfCK2 subunits to asexual parasite survival, and show that differential inhibition between the human and *P. falciparum* CK2α enzymes is possible. We therefore present PfCK2 as a validated drug target.

BSP236
Chloroquine resistance in the rodent malaria parasite, *Plasmodium chabaudi*
Kasia Modrzynska, Alison Creasey, Paul Hunt

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Following the withdrawal of chloroquine from Malawi, malaria parasites with pfcrt76K genotypes have re-emerged. This raises questions regarding the fitness costs of mutations and whether chloroquine could be re-introduced, at least for a limited period.
We are investigating similar questions using a lineage of drug resistant mutants of the rodent malaria parasite *Plasmodium chabaudi*. We use a linkage-disequilibrium-based method - Linkage Group Selection - to identify new loci underlying the resistance, and competition experiments to explore their fitness cost.
We present here the results of crosses performed between a chloroquine-sensitive and –resistant parasite strain. Uncloned recombinant progeny were treated at different chloroquine concentrations. The analysis of allele frequencies revealed loci under selection. The comparison of treated and untreated parasite populations indicates loci involved in chloroquine resistance.
We have also grown a resistant clone with its sensitive progenitor, and evaluated the effect of a specific mutation upon the mutant's growth in mice in the presence and absence of drugs.
This system represents a valuable experimental tool for understanding the genetic basis of drug resistance in malaria.

BSP037
Glutathione biosynthesis of *Plasmodium falciparum*
Eva-Maria Patzewitz and Sylke Müller

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Glutathione (γ-glutamyl- cysteinyl- glycine, GSH) is the most abundant low molecular weight thiol in most eukaryotic cells and serves a number of important functions. Plasmodium falciparum and its host cell utilize GSH as a cofactor for enzymes, for detoxification of xenobiotics and as a sulfhydryl buffer.
The parasite and its host cell both possess the biosynthesis pathway for glutathione, consisting of the two enzymes γ-glutamylcysteine synthetase (γGCS) and glutathione synthetase (GS). Inhibition of γGCS of both parasite and host cell with L- buthionine-(S,R)- sulphoximine has an antimalarial effect. However, results of this study show that the parasite is independent of a functional biosynthesis pathway, as shown by knockout of the γGCS gene, as long as sufficiently high GSH concentrations are available in its environment. These results show that *P. falciparum* can compensate for the loss of the first step of glutathione biosynthesis through an active uptake system of the tripeptide itself or its precursor γ- glutamylcysteine and suggests that this uptake system might be a potential way to interfere with parasite survival.

BSP005
The structure of a chondroitin sulphate-binding domain important in placental malaria.
Matthew K. Higgins

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The PfEMP1 proteins of *Plasmodium falciparum*, are expressed on the surface of infected erythrocytes, where they adhere to human receptors. This protects the parasite from spleen-mediated destruction and causes some of the most severe symptoms of the disease. PfEMP1 proteins also undergo antigenic variation, allowing them to evade immune detection.
Placental malaria kills an estimated 75,000-200,000 foetuses a year. It is caused by the var2csa encoded PfEMP1 protein binding to chondroitin sulphate A (CSA) on the placenta surface, leading to placental inflammation and a reduction in blood flow to the developing child.
Here we present the structure of a CSA-binding DBL domain from a var2csa encoded PfEMP1 protein, both alone and in the presence of sulphate or CSA disaccharide. We consider both the location and nature of the carbohydrate-binding site and the distribution of polymorphic residues on the domain surface. This suggests a model for how the domain maintains CSA binding while evading the immune response and will guide future drug and vaccine development.

Session 8A

BSP300 (guest speaker)
Genome evolution in Plasmodium: ancient, recent and present day
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Since the genome of *Plasmodium falciparum* was sequenced 5 years ago there has been a host of other sequenced genomes published from related species and genera. This has allowed us to classify which genes are shared among all eukaryotes, Which are specific to Apicomplexa and which are specific to Plasmodia. While very few gene are species specific, there are some gene families, located in the subtelomeres that are rapidly evolving. New high-throughput sequencing technologies will allow us to sample within species much more deeply than before and enabling us to study the evolutionary forces acting on Plasmodium genomes in natural populations and inform us of what genes are important in defining how the parasite interacts with the host. An example of a preliminary study of antigen evolution in a Ghanan population will be presented.

BSP269
A determinant of monkey infectivity in the human malaria parasite *Plasmodium falciparum*
Karen Hayton, Deepak Gaur, Anna Liu, Jonathan Takahashi, Bruce Henschen, Rachel Bouttenot, Michelle Doll, Fatima Nawaz, Tetsuya Furuya, Subhash Singh and Thomas E. Wellems

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Some human Plasmodium falciparum parasites but not others cause malaria in Aotus monkeys. To identify the basis for this variation, we crossed two clones that differ in A. nancymaae infectivity and mapped A. nancymaae erythrocyte invasion in the progeny. The major invasion pathway is linked to polymorphisms in a newly-identified erythrocyte binding protein, PfRH5, found in the apical region of merozoites. Polymorphisms of pfrh5 from the A. nancymaae-virulent parent (GB4) transformed the non-virulent parent (7G8) to a virulent parasite. Conversely, replacements to remove these polymorphisms from pfrh5 converted a virulent progeny clone (LC12) to a non-virulent parasite. Our results show that PfRH5 is a parasite ligand for erythrocyte invasion.

BSP119

How many parasite proteins are targeted by strain-specific protective immunity against malaria?

Sandra Cheesman, Elaine O'Mahony, Kathryn Degnan and Richard Carter.

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We have previously shown that the principle target of strain-specific protective immunity (SSPI) against malaria lies within a 200kb region of *Plasmodium chabaudi chabaudi* chromosome 8. Within this locus is a gene encoding the Merozoite Surface Protein 1. Identification of the causative locus was achieved using a genetic approach called 'Linkage Group Selection' (LGS). LGS involves crossing two genetically distinct parasite strains, then subjecting the uncloned progeny to strain-specific immune selection in mice previously made immune to either of the parental strains used for the cross. AFLP analysis of the DNA harvested from parasites surviving immune selection revealed the msp1 selection valley. Due to limitations of the AFLP technique, we were unable screen all of the parasite chromosomes at sufficient density to confirm that only one locus was involved. To enable us to screen all of the chromosomes at much higher density, we used a SNP quantification method that allowed us to obtain a much more detailed picture of which genes are or are not targeted by strain-specific immunity.

BSP160

Design of Selective Inhibitors of Dihydroorotate Dehydrogenase

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We are exploiting the strict requirement of malaria parasites for de novo pyrimidine biosynthesis. All Plasmodium contain the five enzyme pathway but do not contain pyrimidine salvage enzymes. In contrast, human cells contain both synthetic and salvage enzymes. The central enzyme in pyrimidine biosynthesis dihydroorotate dehydrogenase (DHODH) is essential for growth based on targeting the gene with dsRNA (McRobert and McConkey, 2002*). P. falciparum* DHODH has also been validated chemically by inhibition of parasite growth with well-described DHODH inhibitors (Boa et al. 2005) and genetically (Painter et al. 2007). We have shown that selective inhibitors of the parasite enzyme can be developed targeting a species-specific

hydrophobic channel in the enzyme. (Boa, et al. 2005). We have identified high potency inhibitors that inhibit parasite growth and are selective for *P. falciparum* DHODH (Heikkila, et al. 2007). Applying in silico design approaches we are assessing the molecular interactions (Heikkila, et al. 2006). The most recent generation of inhibitors and development of co-crystals to examine the inhibitor-enzyme interactions will be presented.

BSP205

Towards Identifying Genes Controlling Growth Rate in the Rodent Malaria Parasite *Plasmodium yoelii yoelii*

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The growth rate of a malaria parasite in the blood can often be positively correlated with the pathological severity. The underlying genetic determinants are unknown. We here describe a genetic approach, Linkage Group Selection (LGS), to locate regions in the *Plasmodium yoelii yoelii* genome containing genes for blood stage growth rate. A slow-growing line, 33XC, and a faster-growing line, 17XYM, of *P. y. yoelii* were crossed and the uncloned progeny were selected by growing as blood infections in mice. The selected progeny was analysed with genome-wide quantifiable genetic markers to identify a linkage group, or linkage groups, under growth selection. A single genomic region under selection containing a gene, or genes, controlling growth rate in this parasite was identified on chromosome 13. This finding is in agreement with the results of a classical linkage analysis by Walliker *et al*., (1976) that a major genetic determinant of growth rate in *P. y. yoelii* is controlled by a single locus.

MALARIA ABSTRACTS (POSTERS)

BSP022

Sphingolipid biosynthesis in *Toxoplasma gondii*: novel drug targets and roles in pathogenesis

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Sphingolipids are essential constituents of the plasma membrane, the establishment and maintenance of which is key to the survival and proliferation of the intracellular parasite *Toxoplasma gondii*. This study seeks to gain understanding of parasite sphingolipid biosynthesis, the scavenging of these lipid species from the host and their role(s) in pathogenesis. To these ends we have identified several key enzymes in the biosynthetic pathway, including a sphingolipid synthase (TgSLS). TgSLS complements an auxotrophic Saccharomyces cerevisiae inositol phosphorylceramide (IPC) synthase mutant and in vitro analyses demonstrate that it possesses IPC synthase activity. Mammals do not harbour this activity and IPC synthase is an established target for anti-fungals. Localisation and functional analyses are being undertaken in order to fully understand the sphingolipid biosynthetic pathway in Toxoplasma and to elucidate the complex balance between de novo sphingolipid biosynthesis and the scavenging of host lipids.

BSP044

Antimalarial effects of nitric oxide synthetic metabolite (S-Nitrate) as a novel therapy for in vivo treatment of *Plasmodium berghei* NY

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Potential effector mechanisms of immunity in malaria include antibodies, macrophages, T-cells, cytokines and a variety of soluble mediators. In addition to cytokines, nitric oxide (NO) is thought to be a critical molecule in infections and a target for immunomodulation. This study has investigated the involvement of stable NO metabolites, which is increased by synthetic S-nitrate in murine malaria (*Plasmodium berghei*), in order to evaluate whether NO metabolite are beneficial or detrimental to the host. The effects of *P.berghei* was studied on NO production and essential trace Zn/Cu elements and alterations during successful S-nitrate therapy in vivo. NO was measured by Griess Microassay and Zn/Cu levels were detected by Flame Atomic

Absorption Spectrophotometer. Synthetic S-nitrate therapy was used for its ability to modify malaria infection, in order to evaluate the effects of NO metabolite on immunobiochemistry of infected host. Results of this study indicated a partial involvement of NO in the cytotoxic activity of host against malaria. Data of NO and Zn/Cu values indicated the efficacy of S-nitrate therapy on malaria infected animals. Synthetic nitrate is introduced as a novel malaria therapy; it was able to decrease parasitaemia and increase survival rate and had no pathophysiological effects on liver and spleen.

BSP051

Effects of internal deletions of hydroxymethylpteridine pyrophosphokinase-dihydropteroate synthase from *Plasmodium falciparum*.

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Folates are synthesised de novo in many eukaryotic and in plants. The bifunctional enzyme hydroxymethylpteridine pyrophosphokinase-dihydropteroate synthase (HPPK-DHPS) is a target for sulfadoxine. While both full length HPPK-DHPS and its separate HPPK part could complement an HPPK knock-out of *E. coli*, the full-length gene was required for complementation of a DHPS knock-out. The *P. falciparum* HPPK is much larger than corresponding bacterial HPPK enzymes. The extra amino acids in the plasmodial enzymes are contained within two extensive insertions. Even very small deletions within insertion 1 led to loss of HPPK activity, while some DHPS activity remained. A *P. falciparum*-specific part of insertion 2 could be deleted without affecting the complementation of either HPPK or DHPS knockouts. Further deletions into the part of insertion 2 common to all plasmodia led to diminished activity of both enzymes. The conclusion is that apart from a *P. falciparum*-specific amino acid sequence, the extended HPPK is necessary for both activities of the bifunctional enzyme.

BSP082

An analysis of the temporal expression of *Plasmodium falciparum* PCNA using the Bxb1 integrase system

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Little is known about the fundamental mechanisms controlling temporal patterns of gene expression in *P. falciparum*. To investigate the role of DNA sequences flanking coding regions in the temporal expression of a gene, we adopted Pfpcna (PF13_0328) as a model gene. Pfpcna is an essential gene that is under temporal control. Using the bxb1 integrase system, homogeneous populations of parasites carrying various deletions of the 5' flanking sequences of Pfpcna driving a luciferase reporter were produced. Integration of reporter constructs was confirmed by PCR and Southern

blotting, before the temporal expression of the reporter gene was investigated using northern blotting and luciferase assays.
The temporal expression of the reporter gene was found to be the same as the endogenous Pfpcna gene. Serial deletion of the 5' flanking sequences resulted in a significant decrease of 74% of the absolute level of luciferase activity (P=0.003). These data suggest that whilst the control of absolute promoter activity may lie in the 5' region of a gene, temporal control of transcript accumulation lies elsewhere.

BSP150

In-vitro study of apoptosis-like cell death in *Plasmodium berghei* ookinetes

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This study confirms that *Plasmodium berghei* ookinetes show multiple features of apoptosis-like cell death including nuclear chromatin condensation, DNA fragmentation, activation of caspase-like molecules and phosphatidylserine translocation. The proportion of ookinetes expressing each of these markers is similar over time post-incubation, with the exception of phosphatidylserine translocation. Inhibitor studies suggest the caspase-like molecule is a clan CD cysteine protease. Metacaspases (MCP) are clan CD proteases and are implicated in apoptosis-like cell death in other protozoans. However, PbMCP1 and PbMCP2 knockout parasites did not differ from wild type ookinetes in the proportion exhibiting apoptotic markers. Proteomic approaches are now being used to identify the caspase-like molecule. A proportion of ookinetes exhibit loss of mitochondrial membrane potential and current studies correlating changes in mitochondrial membrane potential with release of cytochrome c and subsequent caspase-like activity will be reported. These results contribute to our understanding of the morphological and cellular pathways involved in the death of ookinetes in the midgut lumen.

BSP169

Transcriptome analysis of *Plasmodium falciparum* parasites selected for cytoadherence to human brain endothelial cells.

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Cerebral malaria is characterised by a blockade of brain microvessels due to an accumulation of infected erythrocytes. Human Brain Endothelial Cells (HBEC-5i) are used as a blood-brain barrier model. The parasite adhesion ligands have not been identified.
We would like to identify variant surface antigen (VSA) that are differentially regulated after selection for cytoadherence to HBEC-5i.
P.f. laboratory strain HB3 has been selected using a panning assay for binding to HBEC-5i. The transcriptome of this selected strain is being characterised by microarray.

As the microarray chip is based on the 3D7 genome, variant gene family sequences (var, rif, stevor) were extracted from the sequenced HB3 genome and added to the microarray chip.
Interestingly, the extraction of genomic sequences reveals that a few rif and stevor genes as well as pseudogenes are conserved among strains. Some of these conserved sequences are transcribed, including many pseudogenes.
Microarray will allow us to identify VSA associated with cytoadherence to HBEC-5i.

BSP177

Origin of pyrimethamine resistant *P. falciparum* in eastern Sudan.

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In a cross sectional study carried out during the transmission season 2003, we examined dihydrofolate reductase (dhfr) haplotypes of *Plasmodium falciparum* in Asar village in eastern Sudan, an area of distinct seasonal transmission. dhfr gene of 94 *P. falciparum* isolates was sequenced and examined for mutations in codon 51, 59, 108 and 164. In addition 3 microsatellites located, 0.3, 4.4 and 5.3 upstream codon 108 of dhfr were examined. Two dhfr genotypes were detected in Asar, the wild type dhfr allele (51N 59C 108S) and a double mutant allele (51I, 59C, 108N), existed at a frequency of 11% and 89%, respectively.
Twenty distinct dhfr haplotypes (each composed of the dhfr and alleles of the above microsattelite loci) were identified, 8 haplotypes (1 to 8) existed among 10 sensitive *P. falciparum* isolates carried the wild type dhfr allele (51N 59C 108S), while 12 haplotypes (9 to 20) were seen among 84 resistant isolates harobured the mutant dhfr allele (51I, 59C, 108N). All the 8 sensitive and 11 of the 12 resistant haplotpes existed at limited frequency (1- 4%). However, one resistant haplotype (haplotype 12) was found in 69 (82%) out of the 84 resistant isolates carrying the mutant allele (51I,59C,108N). This haplotypes is different from high-level resistant dhfr haplotypes detected, using similar methods, in Kenya, Senegal, Tanzania and South Africa.
The present findings do not support the hypothesis of a common ancestor of pyrimethamine resistance in Africa. The results were discussed in the context of factors influencing emergence and spread of pyrimethamine resistance in the region.

BSP182

Simultaneous visualisation of protein distribution on the surface of and within *Plasmodium falciparum*-infected erythrocytes.

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We have developed a protocol that enables the simultaneous visualisation of the distribution of proteins on the surface of and within *Plasmodium falciparum* infected erythrocytes. Using this method we can estimate the quantity of surface protein within the cell (i.e prior to transportation and deposition on the infected erythrocyte external surface) and on the surface of the cell. This assay is particularly useful for monitoring the movement and distribution of various life-cycle stage specific proteins of *P. falciparum* infected erythrocytes and provides valuable information about the rate at which antigens are transported from the parasite membrane to the infected erythrocyte surface. The protocol can be completed in approximately 7 hours and can be used to visualize the distribution of proteins in a wide range of cells.

BSP185

Genetic factors affecting sporozoite production in *Plasmodium falciparum* malaria

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The malaria parasite is haploid for most of its life cycle, with a brief diploid stage when the zygote is formed after fertilisation between haploid gametes in the mosquito midgut. Fertilisation can take place between genetically identical gametes arising from human infections of a single parasite genotype (selfing) or between genetically dissimilar gametes arising from human infections of mixed parasite genotypes (crossing). The zygote develops into an oocyst on the mosquito midgut wall within which sporozoites subsequently form. Sporozoites then make their way to the salivary glands ready for inoculation into the next host. Relatively little is known about the factors which influence sporozoite production within a single oocyst and the variation in sporozoite numbers from different clones and fertilisation events.
The numbers of sporozoites in individual oocysts have been assessed using a quantitative polymerase chain reaction technique. Data will be presented on variation in sporozoite numbers related to clone and whether the oocysts were formed from cross-fertilisation or self fertilisation events.

BSP206

Congenicity and genetic polymorphism in cloned lines of the rodent malaria parasite *Plasmodium yoelii yoelii* isolate 17X

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Many of the most commonly studied lines of the rodent malaria parasite *Plasmodium yoelii yoelii* originated from a single parasite isolate designated 17X from the Central African Republic. Amongst these lines are parasites that exhibit variation in genotype and phenotype (e.g. growth rate). We describe here the results of a comparative genetic analysis between cloned lines of 17X that differ in growth rate, using nucleotide sequence data on specific genes and patterns of genome-wide amplified fragment length polymorphism (AFLP). Our findings indicate that the original stock of 17X comprises two completely unrelated genotypes. Within genotype-1 are parasite lines

with a slow growth phenotype (e.g. 17X (NIMR)) and others with a fast growth phenotype (e.g. 17XYM). Within this genotype, there are also genomic differences manifest as a small number of AFLP bands that differentiate the fast- and slow-growing lines from each other. The other genotype, genotype-2, is represented only by parasites with a slow growth phenotype (e.g. 17XA).

BSP273

IL-17 in immunity and immunopathology in a mouse model of malaria, *Plasmodium chabaudi*

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Immunity and immunopathology in malaria infections are not completely understood, but involve the cytokines TNFα and lymphotoxin, and CD4 T cells. Since IFNγ is also a dominant feature of acute malaria in most mouse models, it is assumed that the important pro-inflammatory CD4 T cells are TH1 cells. However, it has recently been shown that pro-inflammatory responses in many infections belong to the TH17 subset of CD4 T cells. These cells trigger potent pro-inflammatory responses by up-regulating chemokine production, and are characterized by their production of IL-17A leading to increased recruitment of neutrophils. We are investigating the role of IL-17A, TH17 cells and the cytokines involved in their induction or maintenance in mice infected with *Plasmodium chabaudi*. Neutrophilia increase in numbers of TH17 cells and low level of expression of IL-17A mRNA, consistent with a Th17 response, were observed in a primary infection. However, reduction or removal of IL-6, and expression of a dominant negative TGFbRII which disables TGF-β signalling in CD4 T cells, both major requirements for TH17 differentiaton had little effect on either parasitemia or pathology. Also neutralisation of IL-17A in vivo did not influence these parameters, suggesting that TH17 cells and IL-17A do not play major roles in acute stage *P. chabaudi* infections.

BSP279

Human monoclonal antibodies recognizing pregnancy-specific variant surface antigens can inhibit adhesion of *Plasmodium falciparum*-infected erythrocytes to chondroitin sulphate A

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Pregnancy-associated malaria (PAM) is an important health problem in *Plasmodium falciparum*-endemic areas. The accumulation of parasite-infected erythrocytes (IE) in the placenta is due to parasite-encoded variant surface antigens (VSA) binding to chondroitin sulfate A (CSA). Acquired immunity to PAM is mediated by IgG with

specificity for VAR2CSA and possibly other VSAPAM. We have previously reported the production of VSAPAM-specific human monoclonal IgG1 antibodies (HumAbs). We now report results on the ability of eight HumAb supernatants to inhibit adhesion of IE to purified CSA in static adhesion inhibition assays.
Three HumAb supernatants inhibited IE adhesion in a concentration dependent manner, whereas five HumAb supernatants showed no blocking ability on the whole. Pooled HumAb supernatants were comparable to plasma from multigravidae and more effective than single HumAb supernatants.
Our data indicate that we have antibodies of functional importance in protection against placental IE sequestration. These HumAbs could have satisfactory impact for vaccine development.

BSP289

Inhibitor Design for the Pyrimidine Biosynthesis Enzyme Dihydroorotate Dehydrogenase Based on Antiparasitic Compounds

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Malaria parasites depend exclusively upon de novo biosynthesis to supply pyrimidines, whereas human cells have pathways for both pyrimidine synthesis and salvage. Indeed, the human biosynthetic pathway is not essential as potent inhibitors (e.g. Arava®) are used as drugs. Hence, the malaria pyrimidine biosynthetic pathway is a logical target for drug design. Dihydroorotate dehydrogenase (DHODH) is essential for parasite growth based on dsRNA treatment (McRobert and McConkey, 2002) and genetic evidence. Inhibitors of DHODH have been described (Boa et al. 2005, Heikkila, et al. 2007, Baldwin, et al. 2005) validating it as a drug target. We noted that a described antimalarial compound (Marrero-Ponce et al, 2005) has features of known DHODH inhibitors. The compound was found to dock into the structure of the parasite enzyme in silico and inhibited *P. falciparum* DHODH in vitro. Applying SPROUT software for design of inhibitors, compound structures were modified to increase affinity for the enzyme binding pocket. These compounds are undergoing testing and results will be presented.

BSP291

***Plasmodium falciparum* RON3 is present in ring-stage parasites.**

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Rhoptry organelles have a role in erythrocyte invasion by the malaria parasite, which may include cell recognition, invasion and the formation of the parasitophorous vacuolar membrane. Rhoptries contain a number of proteins, such as RhopH2 which has been shown by immuno-electron microscopy to be in the bulb of the rhoptry.

Another class of rhoptry proteins has been identified in *Toxoplasma gondii* and localised to the rhoptry neck. Five such proteins have been described and have been called TgRONs 1 to 5. A search of the *P. falciparum* genome revealed 5 genes for putative RON proteins, which have been designated PfRON 1 to 5 according to their similarity to the respective Tg RON protein.
We describe here the identification of PfRON3 using a monoclonal antibody. We localise the protein in rhoptry organelles by comparison with antibodies of known specificity, and show that it is transferred to the ring stage following invasion.

BSP324

The Role of IgE antibodies in protection against *P. falciparum*

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More than 40% of the people in the world are at risk of getting malaria. Immunity to malaria is complex partly due to the complicated life cycle of the parasite. In endemic malaria areas, infection with malaria is associated with elevation of strong specific and non- specific antibody responses with humoral immune responses involving production of predominately IgM and IgG comparing both total IgE and anti malarial antibodies has been reported in a several studies carried out in a number of different malarial endemic areas. It is also well known that IgE mediates activation of various effectors cells such as monocytes/ macrophages). This data may suggest that IgE may play a role in protection against malaria. The main objective of my research project is to determine the role of IgE in protection against acute *P. falciparum* malaria in an area characterized by highly seasonal but stable malaria transmission in Sudan. To archive this goal Total IgE, antimalarial IgE antibodies and number of cytokines will be determined in my study subjects.

BSP326

Chemically modified heparins disrupt *Plasmodium falciparum* mediated rosetting, observed in severe malaria, with highly attenuated side-effects

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Severe malaria is a serious manifestation of the disease caused by the parasite *Plasmodium falciparum*, and is often fatal. Parasite infected erythrocytes aggregate together to form rosettes via host heparan sulfate (HS) and the parasite protein PfEMP-1, which is expressed on the surface of erythrocytes. Rosettes are thought to block the vasculature of the brain leading to apoxia. Inhibitors of rosetting caused by the rosetting strain R29 (IC50=$1.9x10^{-2}$ and $3.05x10^{-3}$ mg.ml-1), based on chemically modified heparin polysaccharides and their depolymerised, low molecular weight (LMW) derivatives were identified with reduced anticoagulant and protease (renin, pepsin and

cathepsin-D) activities. Low molecular weight derivatives of the two most effective inhibitors were shown to require distinct minimum size and strain-specific structural requirements. These formed distinct complexes in solution when bound to platelet-factor IV. They also showed different minimum size requirements for rosette disruption.

SPRING MEETING ABSTRACTS

Session 1B

BSP028

The characterisation of amidating enzymes in the blood fluke *Schistosoma mansoni*

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Many bioactive neuropeptides require post-translational C-terminal amidation by the sequential actions of PHM (peptidylglycine α-hydroxylating monooxygenase) and PAL (peptidyl-α-hydroxyglycine α-amidating lyase). In most eukaryotes PHM and PAL are expressed as separate domains on the bifunctional protein PAM (peptidylglycine α-amidating monooxygenase). Previous work has characterised schistosome PHM, suggesting that PHM and PAL are expressed as separate monofunctional proteins in *Schistosoma mansoni*. Here we identify a cDNA encoding a novel schistosome PAL, reveal functional properties by transient expression of the protein, and examine expression patterns using immunocytochemistry. Expression analysis of schistosome PHM reveals widespread expression throughout the central and peripheral nervous system, and co-expresssion with selected amidated neuropeptides. This study further investigates the role of schistosome PHM and PAL using RNA interference (RNAi) in cultured schistosomula employing electroporation as a mode of dsRNA delivery.

BSP035

***Fasciola hepatica* cathepsin L cysteine proteases: a close and sharing family**

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Fasciola hepatica parasites secrete a family of cathepsin L cysteine proteases to invade and feed on host tissues. Although phylogenetic studies show the family arose by a series of gene duplications followed by divergence they still form a close monophyletic group. Proteomic studies support our phylogenetic data indicating that some family members are specific to the infective larval stages while others are expressed in the adult stages. Functional divergence within the group results from changes in active site residues that are critical in determining the substrate specificity of the proteases and has given rise to a repertoire of enzymes with overlapping specificities. Cathepsin L1 proteases are the most abundant sub-group and display a broader substrate specificity

and greater turnover than the cathepsin L2 proteases which exhibit an unusual ability to degrade native collagen. By sharing the workload this family creates a comfortable living for the parasite inside the host!

BSP070

Function of the translationally controlled tumor protein in *Ostertagia ostertagi* & *Caenorhabditis elegans*

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The search for a vaccine against the parasitic cattle nematode *Ostertagia ostertagi* has led to the discovery of a protective excretion-secretion (ES) antigen mixture, named ES-thiol. Mass spectrometry analysis revealed the presence of an Ostertagia translationally controlled tumor protein (TCTP). The immunoregulatory importance of parasite TCTP has previously been described in *Schistosoma mansoni*, *Brugia malayi* and *Wuchereria bancrofti* infections. Therefore, the objective of this study was to evaluate the function of Ostertagia TCTP. The transcription pattern of TCTP indicated an upregulation in adult female worms and eggs. Western blot analysis showed the presence of TCTP in extract of adult female worms, eggs and adult ES material. Immunolocalisation on sections of *O. ostertagi* and *Caenorhabditis elegans* using crossreactive antibodies demonstrated high concentrations of TCTP in the eggs. In addition, a RNA-interference experiment targeting the Ce-tct-1 gene rendered a 90% reduction in egg production. In conclusion, these findings suggest that TCTP fulfils a crucial role in the production and development of nematode eggs.

BSP088

Functional genomics of parasitic nematodes: learning lessons from *C. elegans*

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Genome sequencing projects are providing significant quantities of data for parasitic nematodes, with the potential to identify novel targets for drug or vaccine development. However defining gene function in parasitic species remains a challenge, with the applicability of high-throughput RNAi screens yet to be established. A variety of alternative approaches based on *C. elegans* technology have been used to define gene function in nematodes such as *Haemonchus contortus*, but their application to more distantly related species remains to be determined. We have used a comparative genomics approach to study a highly conserved essential protein (Hsp90) in nematodes. Ce-Hsp90 is unique amongst eukaryotes as it fails to bind Geldanamycin (GA) a specific Hsp90 inhibitor. We have examined the ability of a range of additional parasitic and free-living nematode Hsp90s to bind GA. In an attempt to further analyse the GA-resistance of Ce-Hsp90, we have attempted to express Bp-Hsp90 in *C. elegans* to determine whether this confers GA susceptibility on the free-living species.

BSP131

Characterisation of the cytochrome P450 family in the parasitic nematode *Haemonchus contortus*

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Infection by parasitic nematodes is one of the most serious health problems of grazing livestock. Dosing with anthelminitics is currently the mainstay of treatment, but resistance to these drugs is becoming widespread. The mechanism of resistance to the commonly used anthelmintic, ivermectin, is unknown.
Cytochrome P450s (CYPs) are a family of drug-metabolising enzymes, present in many organisms. A CYP is responsible for multi-drug resistance in *Drosophila melanogaster* and metabolises ivermectin in humans.
The role of CYPs in drug metabolism by nematodes is unclear. Bioinformatic analysis, facilitated by comparative analysis with the model organism *Caenorhabditis elegans*, indicates *Haemonchus contortus* has at least 45 CYPs. Phylogenetic analysis indicates 4 of these enzymes share most homology with the drug-metabolising CYPs in Drosophila and humans, and full length cDNAs are being isolated for these candidate genes.
RTQ-PCR is also being used to compare expression levels of all identified CYPs in non-drug-exposed and drug-exposed *Haemonchus*, using both susceptible and resistant isolates.

BSP221

Inheritance studies of *Haemonchus contortus* using microsatellites

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There is a need to develop approaches to genetic analysis of parasitic nematodes in order to study problems such as anthelmintic resistance. *Haemonchus contortus* is potentially one of the most amenable parasitic nematodes for genetic analysis and genomic resources are currently being developed. We are undertaking inheritance studies using microsatellite markers to investigate the genetics of this organism. Genotyping of individual female worms and progeny from their respective broods with a panel of autosomal microsatellite markers demonstrated Mendelian inheritance, a general high frequency of null alleles and that female worms mate with more than a single male. We have also developed a panel of microsatellites on the X-chromosome and determined the sex karyotype to be XX/XO for male and female worms respectively. Haplotype analysis of the X-chromosome markers has revealed up to five

different male worms contribute to a single brood. These studies have important implications for inbreeding strategies and genetic analysis in this organism.

BSP298

Stop the signal! - evidence for *Fasciola hepatica* cathepsin L as a signal termination protease

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Neuromuscular activity in the liver fluke, *Fasciola hepatica*, is modulated by myoexcitatory neuropeptides with RFamide C-termini [neuropeptide F (NPF), FMRFamide-like peptides (FLPs)]. Although nothing is known about signal termination processes in flatworms, several lines of evidence implicate F. hepatica cathepsin L (FheCL) in the termination of FLP-signalling. Firstly, FheCL has been observed in nerves and muscle, secondly recombinant FheCL can efficiently cleave FLPs in vitro, and finally FheCL inhibitors can enhance FLP-induced myoexcitation. Recently, RNA interference has been successfully used to silence cysteine proteases, including cathepsin L, in the infective stage of F. hepatica. This study uses the RNAi platform and immunocytochemistry to examine the effects of silencing cathepsin L on NPF- and FLP-expression. Results reveal an increase in RFamide/NPF expression following silencing of cathepsin L, further supporting a role for FheCL in neuropeptide signal termination.

Session 1C

BSP069

Impact of weaning age on helminth worm burdens in the wild rabbit (*Oryctolagus cuniculus*)

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The age of rabbits is difficult to ascertain since young rabbits are born and suckled underground. However there is a close relationship between age and weight in young rabbits and therefore weight has been used to estimate rabbit age in this study. The absence of milk in the stomach was used to ascertain when weaning had occurred.
Four thousand one hundred and seventy rabbits, which had been collected monthly between January 1977 and December 2006 from farms in eastern Scotland, were weighed, sexed and examined for internal parasites especially those in the stomach and small and large intestine. The five commonest parasites were the three nematodes *Graphidium strigosum*, *Trichostrongylus retortaeformis* and *Passalurus ambiguus* and two cestodes *Cittotaenia denticulata* and *Mosgovoyia pectinata*.

The reason for the reductions in some of worm burdens (possible enhanced immunity and/or a reduction in food intake) and their impact on the future survival of the rabbits will be discussed.

BSP271

Innate immune responsiveness covaries with macroparasite infection in a wild vertebrate

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Innate signalling responses to pathogen-associated molecular patterns (PAMPs) can generate rapid non-specific defence and influence the development of adaptive immunity in vertebrates. An effect of one infection on innate responsiveness to heterologous PAMPs therefore provides evidence that assemblages of disparate pathogens can form interactive communities. We surveyed >100 wood mice for macroparasite infection and innate immune function. Heterologous innate responsiveness was measured by splenocyte cytokine responses to TLR-2, 4, 5, 7 and 9 agonists of bacterial, fungal or synthetic origin. In preliminary analyses, the respective sets of immune and parasite variables were reduced to overall "grouped" measures using PCA and then analysed with REML models accounting for host biometric variables, spatial position, and correlation arising from temporally aggregated sampling and assaying. Individual TLR response variables showed strong positive intercorrelation and overall innate responsiveness was significantly negatively associated with overall infection levels. A causal link underlying this covariation should now be confirmed by experiments in a laboratory model and/or by manipulative field experiments in the wood mouse system.

BSP084

Gyrodactylids on the freshwater fishes of Senegal

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During 2004 – 2007 the parasitological survey of African freshwater fishes in National park Niokolo Koba, Senegal, West Africa were realised. Altogether 471specimens of fishes from 20 families and 55 species were investigated for the presence of parasites. Three genera of the viviparous gyrodactylids were collected from 12 fish species. Five species of the genus Macrogyrodactylus Malmberg, 1957 were found on two host fishes, *Polypterus senegalus* and *Clarias anguillaris*. This finding represents the first record of monogenean parasite of the genus Macrogyrodactylus in Senegal. Members of the genus *Gyrodaclytus* Nordmann, 1832 were recorded from 11 host species and for seven of them represent new parasite records. From the gills of *P. senegalus* a new

genus of viviparous monogenean was discovered. General anatomy of the parasite body is similar to the description of the genus *Gyrodactylus* but the morphology of the sclerites of the attachment apparatus is different.

BSP172

Guppies-Gyrodactylus as model organisms for evaluating the effects of prior exposure to parasites in reintroductions programs of wildlife

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Reintroduction of captive-bred individuals for supplementation of wild populations has become increasingly important to prevent the extinction of many endangered species. Nevertheless, despite its relevance for conservation biology, few studies have empirically evaluated the potential impacts of reintroductions on disease outbreaks in native wild populations. In this study, we use a model organism, the guppy (*Poecilia reticulata*) and its commonly found parasite in the wild (*Gyrodactylus turnbulli*), to evaluate: (i) whether pre-exposure to the parasite decreases susceptibility during the reintroductions; (ii) the effect of the reintroduction of naïve individuals on parasite dynamics and host mortality, and (iii) the most effective release procedure to prevent outbreaks (en mass vs. gradual release). Interestingly, we show that gradual release reduces host mortality whereas pre-exposure does not seem to guarantee host resistance.

BSP338

Oomycete parasites of animals

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It is now known that the oomycete fungi are part of the Chrome-Alvelolate lineage and probably evolved from marine parasitoid nanoflagellates. Although mostly thought of as saprophytes and as pathogens of plants, oomycetes are significant parasites of both invertebrate and vertebrate animals. Furthermore it appears that all the members of the early branching clades of the lineage are parasites are either marine algae, nematodes or marine crustacea. The ways these organisms infect animals will be illustrated with reference to parasites of nematodes and fish. The former have evolved a number of specialised infection spores, including the elaborate gun cells of Haptoglossa, to a variety of sticky budded infection spores produced by members of the genus Myzocytiopsis. The fish pathogenic water mould, *Saprolegnia parasitica* is characterised by producing infective secondary cysts with bundles of ‘boathook’ spines

BSP068

An outlook of Lernaea species infection in culturable carps in Punjab, Pakistan

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The purpose of this study was to survey *Lernaea sp*. infection in culturable carps and to identify infection level of this parasite in the fish. The study revealed that *Lernaea cyprinacea* is the most common parasite of the carps along other *Lernaea sp*. A total of 290 fish were examined from private fish farms and carp hatcheries throughout the province of Punjab. All the culturable carps were found infected; however *Catla catla*; *Ctenopharyngodon idella* and *Labeo rohita* were more susceptible species. Prevalence and abundance of *L. cyprinacea* infecting these fish was high. A seasonal pattern of infection showed that *L. cyprinacea* was abundant from winter to spring and less common in summer and autumn. All size of fish was infected, but 11-20 and 21-30 cm length class of all species of fish showed highest infection. The control and prevention method of *L. cyprinacea* is also discussed.

BSP018

First Record of *Tritrichomonas agusta* in the intestine of green toad, *Bufo viridis,* in Neinava Governorate

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During parasitological investigation of vertebrates living in Neinava governorate small microscopic flagellates were encountered in the intestine of the green toad, *Bufo viridis* Laurenti, 1768. After careful microscopical examination of freshly prepared faeces and after staining with Giemsa stain, specimens are identified as *Tritrichomonas agusta* (Alexeieff). Despite of extensive protozoan investigation of Iraqi vertebrates this represent the first record of this flagellate in Iraq. The important characters of *T. agusta* recovered in this study are presence of numerous shallow folds in the undulating membrane , axostyle and axostylar granules surrounded by periaxostylar rings, and the axostyle become pointed as it leave the body proper, parabasal body is rod like or finger like. Further investigation especially ultrastructural may reveal more details about organelles may present in this protozoan.

BSP336

Molecular analyses of Gyrodactylus infecting benthic and limnetic populations of *Gasterosteus aculeatus* L. from Texada island, Canada.

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We use parasites as a method to study the evolution of their host, the three spined sticklebacks (*Gasterosteus aculeatus*) from British Columbia. Gyrodactylus parasites were collected from stickleback populations in lakes situated on the coast of British Columbia, Vancouver Island and Texada Island. This latter island harbours populations of the famous benthic-limnetic stickleback species pair. We sequenced the complete ITS rDNA region and a partial cox2 mitochondrial DNA fragment and compared our results with the Gyrodactylus species found on European sticklebacks. The results are discussed in the light of two hypotheses postulated for the origin of the stickleback species pair: the 'double invasion' theory (two separate invasions of marine stickleback, thus an initial phase of allopatry) and the hypothesis of sympatric speciation (one invasion followed by ecological speciation).

Session 2B

BSP344

Investigation of vertical transmission of *Toxoplasma gondii* parasite in human in Libya

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Toxoplasma gondii parasite is single celled parasite that infects all warm blooded animals including human, it's an important disease causing agent that is responsible for abortion in human and farm animals. It can be transmitted in three ways: via cat faeces, eating raw meat and vertical transmission (mother to baby). As it is known which of these is most important, the objectives of this study are to measure vertical transmission. To achieve this 150 umbilical cord samples were collected from volunteer subjects in Misurata central hospital and Alsaied hospital in Misurata /Libya for subsequent DNA extraction and testing. The clinical records from these subjects were analysed for factors such as mothers' age number of previous children, pregnancy success and number of previous abortions. These data will be presented. A molecular detection system is being developed to test for the parasite and determine the levels of vertical transmission. The progress of this will be discussed.

BSP021

A genetic analysis of two strains of *Plasmodium chabaudi adami* that differ in growth rate and virulence

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Rodent malaria parasite strains vary in growth rate and virulence in their host; we aim to establish what genetically determined parasite-specific factors cause these differences. Two cloned lines of *Plasmodium chabaudi adami* produce different types of infection in inbred mice: the DS line is fast-growing and highly virulent, whereas the DK line is slow-growing and avirulent. In this study we have used Linkage Group Selection (LGS) to locate genes underlying the difference in growth rate between DS and DK. Following selection for fast growth in mice, the progeny of genetic crosses between DS and DK were screened with genome-wide quantitative AFLP markers. Markers with greatly reduced intensity were found on *P. c. adami* chromosomes 6, 7 and 9. We then looked in more detail at these regions using SNP-based markers quantified by Pyrosequencing™. We find that a 600kb region of DS chromosome 9 is consistently inherited as a single non-recombining unit in cross progeny selected for high growth rate. The genetic basis of this finding is under further investigation.

BSP331

Functional characterization of the minimal sequence requirements for trafficking, surface localization and function of the blood stage vaccine candidate AMA1

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The malaria parasite invades erythrocytes by using a set of proteins stored in specialised secretory organelles termed micronemes and rhoptries. One of these proteins is the leading blood stage vaccine candidate apical membrane antigen-1 (AMA-1). Functional analysis of this essential protein was hampered due to the fact that genetic manipulation of the endogenous gene was not feasible. To overcome this hurdle, we used a transgenic approach that enables us to study the precise role of domains or sequence motifs of AMA-1 in trafficking and invasion. AMA-1 function in the invasion process can be blocked by a strain specific inhibitory peptide. By episomal expression of a peptide insensitive GFP-tagged version of an unrelated AMA-1 that can complement endogenous AMA-1 function, we directly assessed the results of introduced mutations. Using this technique in combination with life- and video microscopy we investigated the role of different domains for trafficking, surface translocation and the invasion process.

BSP346

Macro-array probing to discriminate equine cyathostomes

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Cyathostomes or cyathostomins (Nematoda, Strongylida) are the most important helminth parasites of horses. They cause intestinal syndromes with symptoms of different degrees of severity. Moreover, the larval cyathostomes encyst in the large intestinal mucosa and their synchronous emergence cause a life-threatening syndrome known as "larval cyathostomosis" or "larval cyathostominosis". Drug resistance is a serious threat for the control of cyathostomes and for the health management of horses in several countries. Nonetheless, information at species level on the diffusion, biology, epidemiology and pathogenic role of resistant cyathostomes are few, mainly due to inherent difficulties in their identification at any biological stage. In this work the Intergenic Spacers (IGS) of the ribosomal DNA (rDNA) of thirteen species of cyathostomes (*Coronocyclus coronatus*, *Coronocyclus labiatus*, *Coronocyclus labratus*, *Cyathostomum catinatum*, *Cyathostomum pateratum*, *Cylicocyclus ashworthi*, *Cylicocyclus goldi*, *Cylicocyclus insigne*, *Cylicocyclus nassatus*, *Cylicostephanus calicatus*, *Cylicostephanus longibursatus*, *Cylicocyclus leptostomum*, *Cylicostephanus minutes*) and of the *Strongylus* genus were characterized, and the ability of a Reverse Line Blot (RLB) assay for their unequivocal identification and discrimination demonstrated. A further application of this highly sensitive and specific RLB assay would be useful in the detection and monitoring for the presence of anthelmintic resistant cyathostomes and their distribution at species level, thus opening new options for the control of equine cyathostomosis.

Session 2C

BSP094
Unlocking the Secrets of the Schistosome Sex Chromosomes:
Mining the genome for markers to be used in evolutionary and ecological studies
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Unlike most other trematodes the schistosomes have evolved true separate sexes that are predetermined by the presence of differentiated heterogametic sex chromosomes (Males ZZ, females ZW). By referring to the available genome assembly for *Schistosoma mansoni* it was possible to use the concordant BAC library of the sex chromosomes to identify the scaffold sequences that should reside upon them. Using gene predictor models and Artemis software several potential sex linked markers have been identified and the divergence between the Z and W homologs in *S. mansoni* and other African species is being investigated. Such molecular genomic tools may give us insights into the evolutionary significance of the development of separate sexes in these parasites and the population processes that are influencing evolution of the schistosome genome.

BSP145

The highly polymorphic Arctic charr: what role for immune genes?

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The highly variable Arctic charr (*Salvelinus alpinus*), present in freshwater lochs across Scotland, provides an opportunity to study host-parasite interactions and the role of major histocompatibility (MH) genes as well as the dynamics of MH genes in allopatric and sympatric speciation. Within and between lochs, these salmonid fish show great variability in many aspects of their biology, including diet and parasite fauna. It is this variation in parasite fauna, and the selection pressures associated with dissimilar parasite assemblages, that may result in differentiation at loci involved in the immune responses, particularly MH class I and class II genes. Here we characterize MH class I and class II genes obtained from our cDNA library and genotype *S. alpinus* populations from different localities to examine genetic differentiation at these immune response genes. The data on these MH genes are discussed in the context of parasitism, adaptive radiation and ecological speciation.

BSP168

Trading health for reproduction: Haematozoan infections of wild birds

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In wild animal populations, it is commonly observed that reproduction carries a fitness cost. One suggestion is that parasites could mediate this cost, if making a large reproductive effort reduces immune control of pathogenic parasitic infections. We tested this hypothesis in a wild blue tit population infected by avian malaria (Plasmodium) parasites. By manipulating brood size, we experimentally altered reproductive effort and monitored the effect this had on Plasmodium parasitaemia of parents using real-time PCR techniques. We also conducted a meta-analysis of similar published studies, to assess the overall level of support for a negative relationship between reproductive effort and haematozoan parasitism.

BSP209

Evolutionary Basis of Host-Parasite Immunity

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Calliphoridae (blowflies) is one of the oldest recorded insect families, being found in almost all localities on all continents (Otranto, Stevens, 2002). The defining characteristic of Calliphoridae is their necessity to lay eggs on a proteinaceous substrate, often including the tissue of living animals; larvae then develop by feeding on the protein rich tissue of the host, a process known as myiasis. Within Calliphoridae a

range of ectoparasitic larval feeding habits exist, namely: saprophagous, obligate parasitism, and facultative ectoparasitism.
Accordingly, our research aims to investigate this range of ectoparasitic life strategies through immunological proteins present in the larvae of several blowfly genera, including taxa of major veterinary and economic significance.
In addition to permitting the evolutionary history of blowfly host-parasite immunology to be studied for the first time, by attempting to resolve how key antigens and immunogenic proteins evolved in the blowfly, this research will also allow the potential of immunological characters to act as phylogenetic markers to be explored.
Otranto, D.; Stevens, J. R. (2002). Molecular approaches to the study of myiasis-causing larvae. Int. J. Parasit. 32 (11), 1345-1360.

Session 3B

BSP024 (guest speaker)
Microarray analysis of nematode-vertebrate interactions
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Interactions between vertebrates and nematode parasites are widespread and likely to shape the ecology and evolution of both antagonists. Gene expression analysis using microarrays provides the opportunity to rapidly identify host and parasite genes involved in nematode-vertebrate interactions and the response of such genes to their environment. We have used a cDNA microarray to identify genes involved in the fecundity of *Strongyloides ratti* (a nematode parasite of rats), the plastic responses of these genes to different host immune environments, and the heritable changes in these genes following a selection experiment. We identify a group of genes involved in egg production that may be critical the commonly observed phenomenon of density-dependent fecundity in *S. ratti*. We also analysed host responses to infection. In experimental infections of red grouse and sheep, we show that tissues at the site of infection exhibit a large number of responding genes, and that these genes can be modulated by the physiological environment of the host. The potential impact of new technological and bioinformatic methods are briefly discussed.

BSP065
Comparative genomics of nematodes: *Caenorhabditis elegans* as a tool to study the *Haemonchus contortus* genome.
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The *H. contortus* genome is being sequenced at the Sanger Institute and currently 7x coverage is complete. The overall aim of the project is use comparative genomics along with functional analysis in *C. elegans* to help annotate the *H. contortus* genome and study gene function in this parasite. We have focussed initially on members of the β-

tubulin gene family, some of which are important targets of benzimidazole anthelmintics and are involved in the development of anthelmintic resistance. From our studies so far we have been able to find an additional 2 β-tubulin loci within the *H. contortus* genome which now gives a total of 4 genes for this family. Our findings on the organisation of the β-tubulin gene family in *H. contortus* will be presented. We are also interested in identifying genes involved in the RNAi silencing pathway in *H. contortus* and comparing these with *C. elegans.*

BSP102

Secretory peptides in phylum Platyhelminthes

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Given the proven biological importance of helminth neuropeptides, neuropeptide signalling in parasitic flatworms is an appealing focus for drug target discovery. However, essential receptor deorphanisation (ligand-receptor matching) studies are hindered by an ignorance of endogenous flatworm secretory peptides (putative neuropeptides). This study aimed to address this matter by identifying flatworm secretory neuropeptides through exploitation of genomic resources. Degenerate search strings were used to interrogate flatworm expressed sequence tags, yielding 28 secretory peptide-like sequelogs from 11 flatworm species, encoding peptides belonging to 9 structural families. Except for neuropeptide F and several FMRFamide-like peptides (FLPs), none of the putative neuropeptides display similarity to those known from other species. Several of the peptides have been localised within the nervous system of *Schistosoma mansoni*, revealing restricted expression patterns. Functional investigations employing physiology and gene silencing methods in *S. mansoni* are ongoing. This work was funded by The National Institutes of Health grant ROI-AI49162.

BSP167

Evidence for a long terminal repeat (LTR) retrotransposon in *Fasciola hepatica.*

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Retrotransposons are mobile genetic elements that integrate into new sites within a genome via reverse transcription of an RNA intermediate. Amongst the parasitic helminths, long terminal repeat (LTR) retrotransposons have been characterised in *Ascaris lumbricoides*, *Clonorchis sinensis*, *Schistosoma mansoni* and *Schistosoma japonicum*. We identified a 1.2 kb sequence from *F. hepatica* that encodes a homolog of the retrotransposon pol polyprotein. The sequence encompasses the reverse

transcriptase (RT) and RNAse H domains of pol and phylogenetic analysis of the RT domain showed the putative retrotransposon belongs to the Gypsy/Ty3 family. To reconstruct the full-length retrotransposon, a genomic DNA library was constructed in lambdaZapII and analysed by a PCR-based batch screening procedure. Preliminary results show that the gag domain encodes the conserved CHCC motif specific to Boudicca (*S. mansoni*), CsRn1 (*C. sinensis*) and Kabuki (*B. mori*), confirming the placement of the *F. hepatica* retrotransposon in the Gypsy/Ty3 family.

BSP272

Off target RNAi in plant parasitic nematodes (PPNs)

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PPNs represent a threat to sustainable crops, accounting for >$US150 billion in annual agricultural loss worldwide. We have discovered that neuronally-expressed genes in both Meloidogyne incognita and *Globodera pallida* J2s are susceptible to RNAi through simple soaking procedures, resulting in aberrant behavioural phenotypes. As specificity concerns become realized in a range of model organisms, we have found evidence of 'off-target' effects in *M. incognita*. Negative control dsRNAs that shared no significant regions of identity with known nematode targets, were created from a range of plant, and non-plant-derived constructs, including sucrose synthase, a cysteine protease, a G-protein beta subunit, and a chloroplast-specific ribosomal protein, all from the tomato *Lycopersicon esculentum*; an ADP-ribosylation factor and an ethylene receptor from the potato, *Solanum tuberosum*; green fluorescent protein (GFP); and neuropeptide F (NPF) from *Moniezia expansa*. Surprisingly, the control dsRNAs negatively impacted *M. incognita* motility, although *G. pallida* remained unaffected.

BSP312

Effects of PF1 on voltage-activated currents of *Ascaris suum* muscle.

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The quest for knowledge about actions of novel anthelmintics continues as resistance increases to existing anthelmintics. Emodepside is a recently introduced anthelmintic that has been suggested to mimic inhibitory neuropeptides like PF1. We have investigated further effects of PF1 in muscle cells of *Ascaris suum* and examined effects of PF1 on voltage-activated currents. Previous investigators have found that PF1 produces hyperpolarization of muscle membrane, an effect explained by an increase in potassium conductance. Under voltage-clamp however, we found that PF1 also reduced both peak and steady state inward calcium currents as well as increasing voltage-activated potassium currents. The effect on potassium currents did not occur when calcium currents were inhibited with cobalt, suggesting that the potassium

current effect required entry of calcium. Interestingly, very high concentrations of PF1 did not increase the voltage-activated potassium currents but still inhibited the calcium currents. These observations show that the inhibitory effects of PF1 also involve an inhibitory effect on calcium currents and suggest further experiments on the mode of action of emodepside.

BSP322

Single-channel properties of levamisole activated ion-channels in *Caenorhabditis elegans* muscle.

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The nematode nicotinic acetylcholine receptor (nAChR) is the site of action of levamisole. We have used single-channel recording to examine this receptor in the model nematode *C. elegans*. In wild type worms nAChRs aggregate at the neuromuscular junction and are not accessible for single-channel recording. LEV-10 is a protein essential for receptor aggregation. In lev-10 mutants the nAChRs are dispersed over the muscle membrane and are accessible for patch-clamp recording. The *C. elegans* nAChR has a conductance of ~30 pS and brief mean open times of 0.2-0.4 ms. Previous studies have demonstrated that loss of either the LEV-1 or LEV-8 nAChR subunits confers resistance to levamisole. We constructed lev-10:lev-1 and lev-10:lev-8 double mutants to examine the properties of these levamisole resistant nAChRs. In both mutants the single-channel conductance was unaffected. In lev-10:lev-8 mutants the mean closed time of the channels increased (control 133ms, lev10:lev-8 410ms). In lev-10:lev-1 mutants the channel properties were unaltered but the number of functionally expressed channels was reduced.

Session 3C

BSP071

Predicting the impact of long-term temperature changes on the epidemiology and control of schistosomiasis: a mechanistic model

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Many parasites of medical importance are transmitted by cold-blooded intermediate hosts or vectors, the abundance of which varies with ambient temperatures, potentially altering disease prevalence. In particular, if global climate change increases mean ambient temperature in a region endemic with a human pathogen then disease incidence may similarly increase. Here we use a mathematical model to explore the effects of increasing ambient temperature on the prevalence and abundance of *Schistosoma mansoni*. We show that the impact of increasing temperature on disease occurrence is not straightforward, resulting in non-linear effects on mean infection burdens and

potentially altering disease dynamics from stable, endemic infection to unstable, epidemic cycles. Sensitivity analyses reveal that temperature increases also affect the relative importance of the model's parameters, indicating that the optimal control strategy will change as temperatures change. It is only through a mechanistic approach, incorporating the combined effects of temperature on all stages of the life-cycle, that we can begin to predict the consequences of climate change on the incidence and severity of schistosomiasis.

BSP092

Why does protective immunity against schistosomes develop slowly in humans?

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Protective immunity against schistosomes develops slowly in humans despite repeated re-infection. Mathematical models describing levels of infection intensity and immunity in endemic populations were used to explore two different hypotheses for the slow development of protection: (1) parasite-induced immunosuppression delays the development of acquired immunity; (2) only dying worms release the right type and amount of antigen sufficient to stimulate a protective response. Patterns of infection intensity and immunity predicted by the models were compared with field data from Zimbabwe.

Parasite-induced suppression delayed the development of protective immunity in the model, but entirely overwhelmed protective responses in more heavily infected populations. This model predicted patterns in infection intensity not seen in field data.

With protective immunity resulting from exposure to dying worms, model outputs were critically dependent upon the assumed worm survival curve. Reduced variation in worm lifespan delayed the development of protective immunity. Predicted patterns of infection intensity were consistent with field data. This hypothesis is able to explain observed patterns of infection intensity and immunity, which parasite-induced immunosuppression cannot.

BSP109

Spatial aggregation of *Ascaris lumbricoides* infective stages; identification of clusters within human hosts using finite mixture models

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In both human and wildlife helminth-host systems, the distribution of worms in the host population is aggregated; a large proportion of worms occur in a small proportion of hosts. One possible cause of aggregation is heterogeneity in exposure to infective stages, for instance due to their spatial aggregation within the environment. A question of importance is whether or not worms are acquired in a fashion resembling a trickle infection in a continuous manner, or they are acquired in clumps during a few infection events.

This study analyses data collected by Hall and co-workers from a three-round chemo-expulsion study of humans infected with *Ascaris lumbricoides*. It is assumed that infection events may be indentified by clusters of worms of similar mass in individual hosts. Finite mixture models are used to identify clusters. The results from the analysis of the data are presented. The reliability and robustness of this approach is also analysed and discussed using stochastically simulated data under variable epidemiological parameters.

BSP123

Investigation into vertical transmission of *Toxoplasma gondii* in humans.

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The protozoan *Toxoplasma gondii* is one of the most common parasitic infections of man and other warm blooded animals, it has been found worldwide in nearly one-third of the human population. *T. gondii* may be transmitted horizontally by ingestion of infectious oocysts from the environment or ingesting tissue cysts present in many different animal intermediate hosts. *T. gondii* can also be transmitted vertically from mother to baby, however this route is not thought to be common in humans. Samples of human umbilical cord from healthy pregnancies have been collected from a hospital in the UK. Presence of *T. gondii* was tested for using SAG1 PCR and the results showed a high percentage of samples were positive which suggests vertical transmission is more common than previously thought. All three strain types have also been identified using SAG3 PCR.

BSP235

Zoonotic transmission of *Schistosoma japonicum*: which hosts count the most?

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Schistosoma japonicum can infect over 40 species of mammalian hosts and is the only species of schistosome for which zoonotic transmission is considered important. In order to characterise levels of parasite gene-flow across host species, and identify the most important zoonotic reservoirs, we genotyped *S. japonicum* isolates from a range of definitive host species in both hilly and marshland regions of Anhui Province, China, using microsatellite markers. F-statistics and clustering analyses showed strong genetic structuring according to geography and habitat type. However, there was generally very little parasite genetic differentiation among host species within sympatric villages, suggesting frequent *S. japonicum* transmission across species, with rodents and dogs

potentially very important infection reservoirs in hilly regions, in contrast to bovines in marshland regions. These results have important implications for *S. japonicum* control, particularly in hilly regions where control of infection among wild rodent populations could be challenging.

BSP255
Sex-specific heritability of human hookworm infection
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Papua New Guinea Institute of Medical Research

The role of host genetics in determining human hookworm infection is not well understood. Here, we use variance components analysis to investigate additive genetic effects on the intensity of hookworm infection, and whether these effects vary by sex, in a Papua New Guinean population. The heritability of hookworm infection was estimated as 15 ± 4% ($P<0.001$). Allowing the variance components to vary between the sexes revealed consistently larger additive genetic effects in females than in males, reflected by heritabilities of 18% in females and 8% in males. Household effects were also higher in females than males, though the overall household effect was not significant. The results show that additive genetic effects are an important determinant of the intensity of human hookworm infection. However, despite similar mean worm burdens, the factors responsible for generating variation in intensity differ markedly between males and females.

Session 4B

BSP146
"Killing" of nonparasitized(np) RBC by 4-hydroxynonenal (4-HNE) produced by parasitized(p) RBC. Role in malarial anemia
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We show that pRBC produce 4-HNE, a lipoperoxidation product that forms covalent adducts with proteins and crosslinks adjacent proteins. 4-HNE is generated from membrane arachidonic acid by hemozoin-elicited non-enzymatic heme-catalysis. 4-HNE accumulates in membranes and diffuses to neighboring cells. We show that co-incubation without physical contact of pRBC with npRBC induced changes in npRBC, fully superimposable to changes induced by direct supplementation of HNE (5-15microM) to npRBC. Those changes lead to enhanced phagocytosis ("killing") of npRBC. Modifications observed in co-incubated npRBC and 4-HNE-treated npRBC were: 1) formation of 4-HNE-protein adducts on cell surface, localized on spectrin, band-3, band-4.1/4.2 and actin; 2) increased IgGs binding and phagocytosis by human

monocytes (+48.8%); 3) decreased deformability and elongation index at low shear-stress proportionally to 4-HNE adducts. In conclusion, changes (4-HNE-protein adducts and protein-protein crosslinks) induced by pRBC in npRBCs may increase their phagocytic removal in vivo and thus contribute to malarial anemia.
Supported by Compagnia di San Paolo.

BSP225
Characterization of seven clonal and one recombinant *Toxoplasma gondii* strain from Uganda
Irene Lindström, Johan Lindh and Judith Smith

Leeds University, Leeds, UK and Swedish Institute of Infectious Diseases Control, Stockholm, Sweden

In spite of its global distribution, wide host range and multiple transmission routes, *Toxoplasma gondii* exhibit little genetic variation. Sexual recombination occurs only in the intestine of felids, where a single cross between two different strains can potentially yield millions of genetically different progeny. Curiously, very few of these recombinants appear to be successful in nature and three clonal lineages are predominant in most parts of the world. We have isolated and characterized eight Toxoplasma strains from Uganda and found that seven of these closely resemble European and US strains of lineages II and III, while the last one appears to be a natural type II/III recombinant. All these strains were avirulent in their chicken hosts, but showed very different tissue densities in mice. Growth in cell culture also showed great divergence and remained remarkably slow for most strains even after many months of continuous passage in fibroblasts. Extensive genetic characterization of the recombinant strain is currently in progress and preliminary results will be discussed.

BSP328
Rapid Response of Dendritic Cells to *Trichuris muris* Infection
Sheena Cruickshank, Matthew Little, Richard Grencis & Kathryn Else

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Dendritic cells (DC) direct immune responses by responding to a pathogen and directing the appropriate T cell action. A key question is how DC initiate immunity in tissue sites. The colon is a major site of infection and diseases that affect millions worldwide yet little is known about how colon DC function. Using *Trichuris muris* as a model large intestinal infection I studied DC recruitment into the colonic epithelium in mice that are resistant (BALB/c) or susceptible (AKR) to infection. Within 24 hours of infection, immature DC were recruited into the epithelial layer of resistant but not susceptible mice. DC localization in the epithelium was confirmed by confocal and electron microscopy. The number of DC associated with the epithelium remained higher in BALB/c mice after 2 and 7 days infection compared with AKR. The DC recruited in BALB/c early post-infection were immature (MHCII, CD86, TLR2 and TLR4 low). Thus the DC/epithelial/*T. muris* interaction differs dramatically between resistant and susceptible mice and this difference may underlie the subsequent effectiveness of the immune response.

Session 4C

BSP294 (guest speaker)
Strategies of *Trichomonas vaginalis* to establish a successful infection
Paola Rappelli, Daniele Dessì, Nicia Diaz, Antonella Mura, Federica Riu, & Pier Luigi Fiori

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Trichomonas vaginalis can be considered a particularly intriguing mucosal pathogen not only because it is the causative agent of the most common nonviral sexually transmitted disease, but also because the infection is strictly related with higher susceptibility to HIV, invasive cervical cancer, and increased risk for preterm delivery during pregnancy. In addition, *T. vaginalis* is the only known human parasitic protist establishing a stable symbiosis with a pathogenic prokaryote (*Mycoplasma hominis*).
In order to adapt to host environment, counteracting the host immune system, *T. vaginalis* have evolved different pathogenicity strategies, based on colonization of vaginal mucosa, production of toxic molecules, and modulation of innate and adaptive immunity. The most recent data on pathogenicity mechanism of *T. vaginalis* are discussed, particularly with respect to the recent publication of the draft genome sequence of the protist, also focusing on relationships with host innate and specific immune response.

BSP029
Anti-inflammatory properties of *Nippostrongylus brasiliensis* L3 larvae excretory-secretory products
Min Zhao, Janis MacCallum and Lorna Proudfoot

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Previously, in the pulmonary phase of *N. brasiliensis* infection, we observed a lack of TNF-α production and minimal recruitment of neutrophils. Photomicrographs of lungs showed the damage was rapidly repaired after the larvae left the lungs. The immune regulatory properties of helminths have been largely linked to their excretory-secretory (ES) products. In rats, we observed that instillation of *N. brasiliensis* L3 larvae ES (L3 NES) can inhibit the recruitment of neutrophils by approximately 45%. Real-time PCR using bronchoalveolar lavage cells showed suppressed mRNA expressions of TNF-α, IL-1and ICAM-1 on a background of LPS induced inflammation. Using the rat alveolar macrophage cell line NR8383, such inhibition of TNF-α production was strongly associated with the protein fraction of L3 NES. Inhibition of TNF-α production was linked with the reduction of NFκB p65 and TNF-α mRNA in vitro. Western-blot results showed degradation of rat TNF-α by

L3NES protein, which suggests the inhibition of pro-inflammatory cytokines could also occur post-translationally.

BSP072

Gene family phylogeny in *Candida spp*.: Initial results from genome comparisons.

Andrew P. Jackson

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Genome sequences for four species of the fungal pathogen Candida (*C. albicans, C. dublinensis, C. tropicalis, C. parapsilosis*) have recently become available. Primary sequence and positional information was extracted from these resources (as well as from two non-pathogenic relatives *Lodderomyces elongisporus* and *Debaryomyces hansenii*), and used to estimate phylogenies for various gene families operating at the host-pathogen interface, and potentially active in pathogenesis. Various evolutionary patterns were observed, ranging from specific acquisition of secretory lipases in *C. albicans* and *C. dublinensis* to the relative conservatism of 'Candida Surface Antigen' (CSA) loci across all Candida species. Among the Agglutinin-Like Sequences (ALS) and PIR-like genes, the analyses identified both ubiquitous loci, suggestive of more reliable drug targets or vital functions, and species-specific loci, which could contribute to differences in pathology. Comparisons of chromosomal positions in relation to phylogeny revealed the importance of chromosomal rearrangements in creating new genes; for example, segmental inversions were associated with novel secretory aspartyl proteases in *C. albicans*, while *C. parapsilosis* has evolved tandem gene arrays with considerable sequence diversity. These phylogenies of key *Candida* gene families have identified priorities for further functional classification.

BSP166

Bioinformatics analyses of GP63-like proteins from the mucosal pathogen *Trichomonas vaginalis*

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GP63 proteases, major surface glycoproteins in several infectious kinetoplastids, play crucial roles in host-parasite interaction and pathogenicity. 77 GP63-like proteins encoding genes were identified in the genome of *Trichomonas vaginalis*. Bioinformatics analyses on *T. vaginalis* GP63 reveals extracellular zincin catalytic motif (HEXXH). Unlike the proteins from Leishmania that adhere to cell membrane using a GPI anchor, homologs in *T. vaginalis* are inferred to have transmembrane domains at their C-terminal end. Only 12 conserved cysteine residues are found in the proteins from *T. vaginalis*, compared to the 18 cysteines conserved among kinetoplastid GP63. Together with structural modelling, this suggests important structural differences. Phylogenetic analysis of the eukaryotic GP63 protein family indicates local gene duplication occurred after speciation during eukaryote diversification. Sequence divergence between *T. vaginalis* proteins are more important than those among other

eukaryotes, likely reflecting the functional importance of these proteins for *T. vaginalis* mucosal life style.

BSP333

Complete absence of the glycosylphosphatidylinositol (GPI) biosynthetic pathway in the human parasite, *Trichomonas vaginalis*

Yuk-Chien Liu, Angela Mehlert, Terry Smith, Tomas Hermoso, Mark Field, Jeremy Mottram & Alvaro Acosta-Serrano.

Wellcome Centre for Molecular Parasitology, University of Glasgow; School of Life Science, University of Dundee; Department of Pathology, University of Cambridge, UK.

GPIs are considered ubiquitous in eukaryotic organisms. GPI-anchored molecules are particularly abundant in parasitic protozoa, where they play essential roles in parasite survival, infectivity and pathogenesis.
Trichomonas vaginalis expresses on its surface abundant lipophosphoglycans (TvLPG), which are important virulence factors. It has been assumed that this glycoconjugate is anchored to the parasite membrane via a GPI. However, none of the conserved genes involved in GPI synthesis are present in the genome of this organism. Using a combination of cell-free systems and metabolic incorporation of radioactive sugars, we corroborated that *T. vaginalis* is unable to make GPIs. Interestingly, TvLPG contains inositol and fatty acids, suggesting that this molecule is anchored via a novel type of PI-glycolipid.
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.

Session 5B

BSP060

Possible parasite and bacteria combination cause bacterial meningitis

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This is a case report that points out the importance of looking at the whole clinical picture. The patient had had epigastric pains but was admitted with a probable bacterial meningitis due to *E. coli*. He had classical symptoms of bacterial meningitis, stiff neck, raised white cell count in cerebral spinal fluid (CSF) and an *E. coli* isolated from the CSF. He did however have a pneumonic process initially thought to be bacterial but on examination of a bronchiolar lavage specimen the pneumonic condition was due to *Strongyliodes stercoalis*. The patient's stools were checked and *Strongyloides stercoalis* was present. Due to the initial epigastric pain caused by the parasitic infection it is probable that the *E. coli* was carried on the back of a larva as it migrated through the

central nervous tissue. The patient was treated with ceftriaxone for the bacterial meningitis and albendazole for the parasitic infection. After treatment patient recovered and was discharged with no sequels.

BSP081

Novel control strategies for the root-knot nematode *Meloidogyne minor*

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The root knot nematode *Meloidogyne minor* is causing increasing economic difficulties throughout Western Europe due to its wide host range including sports turf, pasture grass and potatoes. Infestation by the nematode results in stunted development of turf-grass and consequently the appearance of yellow-patch disease. This study aims to examine two novel approaches to the control of *M. minor*; firstly biostimulants are believed to disrupt the interactions between the nematode and host plant; thereby restricting nematode development (Whapham et al 1993), here we have examined various biostimulants for their ability to reduce parasitism due to *M. minor*; secondly, the use of naturally derived nematicidal compounds, in particular furfural, could prove to be a more environmentally friendly alternative to chemical nematicides. This study has also examined the effects of furfural on *M. minor* infestation. Most recently we have investigated the sensitivity of *M. minor* to RNA interference, with a view to additional molecular based control strategies.

BSP093

Induction of metabolic enzymes following exposure of *Caenorhabditis elegans* to ivermectin.

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Resistance to anthelmintics is a major problem in parasites of small ruminants, affecting both the economic viability of farming and animal welfare. As a result there has been a great deal of research into the mechanisms by which parasites develop this resistance. However, there has been little research into drug metabolism by parasites, despite this being a major mechanism of insecticide resistance We are using the model organism *Caenorhabditis elegans* to examine how nematodes metabolise ivermectin and identify new candidate genes which can then be tested for potential roles in ivermectin resistance in parasitic nematodes. In general, drug metabolising enzymes are specifically induced by their substrates; therefore we have used a whole genome microarray to assess the transcriptome of *C. elegans* after exposure to ivermectin. A number of genes show dramatic increases in expression levels following ivermectin exposure including several metabolic enzymes. Experiments are

currently underway to assess the potential role of these genes in metabolising/detoxifying ivermectin and identify functional homologues in parasitic nematodes.

BSP284

Effect of multiple stool sampling and different diagnostic techniques for detection of soil-transmitted helminth infections

Stefanie Knopp, Ali F. Mgeni, I. Simba Khamis, J. Russel Stothard, David Rollinson, Hanspeter Marti, Jürg Utzinger

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National History Museum, London, United Kingdom

In view of enhanced global efforts to control soil-transmitted helminthiasis, there is a need to better understand the reliability of standard diagnostic methods. Our focus is on Zanzibar, in 2 settings where anthelminthic drugs have been administered over the past decade. We examined 3 stool samples from a group 342 schoolchildren, aged 7-20 years, using the Kato-Katz, Koga agar plate and Baermann techniques for the diagnosis of *Ascaris lumbricoides*, hookworm, *Strongyloides stercoralis* and *Trichuris trichiura*. By examination of multiple stool samples, a considerable increase in the prevalence of each helminth species was observed, and hence diagnostic sensitivity of single stool sampling was poor to only moderate. The highest sensitivity was observed when the different methods were combined and using these data in a mathematical model, 'true' prevalences for *T. trichiura*, hookworm, *A. lumbricoides* and *S. stercoralis* were 50.1%, 24.1%, 16.5% and 15.8%, respectively. For more precise epidemiological surveillance, multiple stool samples bolstered by different diagnostic techniques are warranted for accurate diagnosis of helminth infections.

BSP316

A metabolic effect of Schistosoma mansoni infection on mouse using 1H NMR spectroscopy

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Schistosomiasis is a chronic debilitating parasitic disease caused by blood flukes of the genus Schistosoma. Here we report the metabolic characteristics of biofluids (urine, stool and plasma) and organs (jejunum, ileum, colon, liver, spleen and kidney) of mice infected with *S. mansoni*, using 1H nuclear magnetic resonance spectroscopy and multivariate data analysis. We infected 10 female NMRI mice with 80 *S. mansoni* cercariae each. Another 10 mice served as a control. Biofluid samples were collected at 1 day preinfection and fortnightly postinfection until day 74. At day 74, all mice were sacrificed and tissues were removed. Metabolic profiles of biofluids showed increased creatine, pyruvate and phenylacetylglycine, and decreased hippurate in the

infected mice. Tissues obtained from *S. mansoni*-infected mice were characterized by an elevation of amino acids, creatine, glycerophosphoryl choline and cytosine. The metabolic signature of *S. mansoni* indicated an enhanced glycolysis process, a disturbance of amino acid metabolism, microbial changes and organ dysfunction.

BSP318

Integrative metabolic phenotyping of *Echinostoma caproni* in the mouse

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Food-borne trematode infections are a growing public health problem, yet they are neglected. We assess and characterise the effect of Echinostoma caproni infection in the mouse, using 1H nuclear magnetic resonance (NMR) spectroscopy and multivariate data analysis. Biofluids and tissue samples of 12 infected mice and 12 non-infected controls were obtained at 7 time points post-infection up to day 33. Major biological changes were observed related to metabolites linked to osmolytic changes coupled with perturbed Na+-coupled transmembrane transport, which are reflected by inhibited amino acids re-absorption in ileum and changes in osmolytically active substances such as glycerolphosphocholine and scyllo-inositol in the kidney, and myo-inositol in the ileum. Additionally, changes in various gut microbiota-related metabolites were observed particularly in urine and stool. Combinatorial diagnosis based on biofluids delivers the most comprehensive fingerprint of infection. However, for practical purposes, a single biofluid may be preferred. Here urine and plasma would be the first choice based on the ease of collection and number of perturbed metabolites.

Session 5C

BSP313 (guest speaker)

Protection of *Schistosoma mansoni* infection against allergic asthma depends on intensity and chronicity of infection.

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An inverse correlation between chronic schistosome infections and allergic diseases has been described. In this study, we addressed the issue of infection intensity or chronicity in a well-defined experimental model. *S. mansoni* infection in C57/Bl6 mice was set up, followed by sensitization and challenge with ovalbumin, such that different stages and intensities of infection could be studied. Airway resistance, lung

eosinophilia and OVA-specific Th2 cytokines were decreased in chronically, but not in acutely infected mice. In chronically infected mice both splenic B and T cells produced elevated IL-10 in comparison to acutely infected mice. Adoptive transfer of splenic B and T cells from chronically infected mice both reduced airway inflammation in recipient OVA-sensitized mice. This suppression proved to be IL-10-dependent. Activation profiles of splenic dendritic cells were hardly affected, while MHCII-expression was decreased on splenic B cells during the course of schistosome infections. In vitro cocultures of OVA-specific T cells and splenic DC induced IL-10-producing T cells, while B cells from chronically infected mice strongly upregulated FoxP3 expression, in contrast to DC or B cells from allergic control mice. During chronic schistosome infections suppressive mechanisms are induced that protect against allergic airway inflammation.

BSP031

Inflammatory Bowel Disease (IBD) and *T. muris* infection

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Prevalence of inflammatory bowel disease (IBD) is far more common in developed countries as compared to the developing world, where infection with intestinal parasites is common. The hygiene hypothesis suggests lack of infection predisposes to the development of IBD in genetically susceptible people. However, little is known immunologically about how gut parasites alter IBD progression. P-glycoprotein (PGP) present in the apical membrane of gut epithelial cells is encoded by the mdr1a gene. This protein inhibits absorption of many drugs and acts like a transport protein. Mice which lack the mdr1a gene (mdr1a-/-) develop spontaneous colitis in the presence of enteric bacteria. We have used mdr1a-/- mice on an FVB background to investigate any change in progression of colitis in mice infected with *Trichuris muris*. Our results reveal that mdr1a-/- mice have more Th1 type of gut environment and delayed worm expulsion compared to wild type. Some infected mdr1a-/- mice show increased gut inflammation. Thus, mice prone to colitis are also prone to *Trichuris* infection.

BSP104

Do regulatory T cells contribute to the survival of the S isolate of *Trichuris muris*?

Riccardo D'Elia[1], Jerzy Behnke[2], Janette Bradley[2] and Kathryn. J. Else[1]

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The mouse whipworm *Trichuris muris* is a useful model of the human parasite *T. trichiura*. Studies using *T. muris* typically focus on the E (Edinburgh) isolate. However, two other isolates exist: J (Japan - sub-cultured from E) and S (Sobreda - isolated from wild mice in Portugal). Earlier experiments have shown that following infection with

the S isolate of *T. muris* the C57BL/6 mouse becomes susceptible and the worm survives, whereas the E and J isolates are still expelled. The reason for the survival of the S isolate to chronicity has yet to be elucidated.
Data suggests that mice infected with the S isolate of *T. muris* increases the number and percentage of natural regulatory T cells (CD4+CD25+Foxp3+) in the MLN and gut compared to mice infected with the E isolate.
Antibodies to CD25 and GITR have been used in vivo to determine whether S isolate survival is linked to these increases in Treg numbers.

BSP213

Effect of *Haemonchus contortus* derived products on eosinophil function *in vitro*.

Darryl G. Turner, Louise A. Wildblood & Doug G. Jones

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Soluble and excretory-secretory products of *H. contortus* have previously been shown to have a chemokinetic effect on ovine eosinophils in vitro. Characterisation studies have attributed this effect potentially to parasite derived galectins. This study attempted to characterise further effects on eosinophil function in vitro, namely survival. An initial study showed that soluble *H. contortus* derived extracts could enhance eosinophil survival in the presence of the apoptotic inducer dexamethasone. Further studies showed that this activity was inhibited in the presence of lactose to a greater degree than sucrose. These results suggest that the *H. contortus* produces a factor which can affect eosinophil survival. The results also suggest that the factor may be the same as the chemoattractant as they are both inhibited by lactose, and therefore is likely to be a galectin. This would suggest that *H. contortus* is producing a factor which may be mimicking the action of the mammalian galectin-9, which has been shown to effect both eosinophil migration and survival.

BSP260

***Schistosoma mansoni* eggs regulate hepatic stellate cell-mediated fibrogenesis**

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Parasite interactions with host cells at sites of infection, such as that caused by Schistosoma mansoni eggs in human liver, can induce fibrosis with significant host pathology. Since liver fibrosis requires transdifferentiation of hepatic stellate cells (HSC) from a quiescent to an active myofibroblastic phenotype, we have developed a model in which a human HSC cell-line (LX-2) is manipulated to yield either phenotype, prior to co-culture with *S. mansoni* eggs or purified soluble egg antigen (SEA). Treatment of quiescent cells cultured in wells for up to 7 days with eggs (0-1,650 per well) or SEA (0-15μg/ml per well), was compared with transforming growth factor (TGF)-β, as a positive control. Gene expression of pro-fibrogenic collagen type I (col1A1), connective tissue growth factor (CTGF), α-smooth muscle actin (αSMA) and anti-fibrogenic peroxisome proliferator-activated receptor (PPAR)γ

were evaluated by realtime PCR. SEA or eggs, either directly or indirectly in contact with host cells, suppressed αSMA, CTGF and col1A1 gene expression and upregulated PPARγ expression, indicating that liver HSC are directly regulated by *S. mansoni* eggs.

Session 6B

BSP059

The role of the excretory system during skin penetration and surface membrane damage of schistosomula of *Schistosoma mansoni*

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Cercariae (*Schistosoma mansoni*) have a nephridiopore which is exposed to the environment after the rupture of the tail from the body during skin penetration. We have observed that during this process there is a massive uptake of Lucifer Yellow (LY) a highly fluorescent membrane impermeant molecule into the excretory tubules through the nephridiopore and surrounding orifices. Ultraviolet (UV) irradiation of the cercariae (400microwatt mins/per square cm) decreases the uptake during skin penetration. The UV dose is critical since larger doses of UV can massively increase the uptake, an effect likely to be due to membrane damage. This critical UV dose restricts the distribution of propidium iodide to regions close to the nephridiopore which suggests that adjacent cells have become less permeable than in non-irradiated forms. We are currently investigating the role of the sense organs and nervous system in this effect. The implications for the immunogenicity of the irradiated parasite will be discussed.

BSP090

Transmission dynamics and genetic epidemiology of *Schistosoma mansoni* and *Schistosoma. haematobium* in Nder, Senegal.

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The epidemiology of human schistosomiasis along the Senegal River Basin has been extremely dynamic with a recent increase in the prevalence of *S. haematobium* and a decline of *S. mansoni.* In order to investigate the re-infection dynamics of these two species, a group of children from the village Nder, North Senegal, carrying infections

of both *S. haematobium* and *S. mansoni* were treated twice, 3 weeks apart with praziquantel. Stool and urine samples were taken from each child before treatment, 6 weeks after the 1st treatment and 6 months later. Egg counts were taken and eggs excreted by individual children were hatched and single miracidia stored on Whatman FTA cards. The partial COX1 DNA barcode of each miracidia was sequenced to genotype the schistosomes within each child to monitor changes over six-months. The data provide surprising insights into treatment strategies, transmission dynamics and the genetic diversity of these schistosomes.

BSP130

***Biomphalaria glabrata* haemolymph modulates tyrosine phosphorylation in *Schistosoma mansoni* miracidia.**

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Molecular interplay during snail-schistosome interactions is poorly understood. Using the *Biomphalaria glabrata – Schistosoma mansoni* system, the effects of exposure to haemolymph, derived from schistosome resistant and susceptible snail strains, on protein tyrosine phosphorylation in miracidia were investigated. Western blotting revealed several tyrosine phosphorylated proteins in this larval stage. Exposure of miracidia to haemolymph from susceptible snails for 60 min resulted in a striking, 5-fold, increase in the tyrosine phosphorylation of a 56 kDa (p56) S. mansoni protein. In contrast, haemolymph from resistant snails had little effect on protein tyrosine phosphorylation levels in miracidia. Confocal microscopy revealed that tyrosine phosphorylation was predominantly associated with proteins in the tegument. Finally, treatment of miracidia with the tyrosine kinase inhibitor genistein impaired their development into primary sporocysts. Our results open avenues for research that focus on the potential importance of phospho-p56 to the outcome of schistosome infection in snails, and the significance for protein tyrosine kinase-mediated signalling events to the transformation of *S. mansoni* larvae.

BSP178

***DNA barcoding of Ugandan Schistosoma mansoni* reveals a genetic division between Lake Victoria and Albert populations**

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Vector Control Division, Ministry of Health, P.O. Box 1661 Kampala, Uganda

Intestinal schistosomiasis is widespread throughout Uganda but the disease is particularly rife along the shorelines of Lakes Victoria and Albert. To shed light on the population genetic structure of Ugandan Schistosoma mansoni, DNA barcodes (COI haplotype sequences) have been determined from both adult worms and eggs from a total of 9 villages, representative of Lake Victoria and Albert populations. Eighteen

COI haplotypes have been encountered with slightly greater haplotype diversity within samples from Lake Victoria. Strong genetic partitioning was observed between samples such that the collection of COI haplotypes was unique to each lake environment with none co-occurring between. Comparing these data with that from GenBank has revealed a very distinct phylogeographical split between parasite populations which may also permit a new interpretation of disease heterogeneity between locations.

BSP223

The outer-surface of adult *Schistosoma mansoni* and transfer of antigens to host lipoproteins

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Schistosomes are long-term inhabitants of the mesenteric veins and employ multiple mechanisms for their survival in the host. The outer-surface of the parasite (tegument) forms the contact-site with the host and plays an important role in immune evasion. Characterization of the tegumental lipidome resulted in identification of many tegument-specific lipids, of which lysophosphatidyl-serine was demonstrated to activate toll like receptor 2 on dendritic cells, a process that results in the induction of a regulatory T-cell response. Furthermore, GPI-anchored schistosomal antigens (such as Sm200) were shown to circulate on host lipoproteins, which are subsequently bound by host antibodies that then allow endocytosis of lipoproteins by immune cells via the Fc-receptor pathway, resulting in induction of apoptosis in neutrophils. We showed that transfer of parasite antigens to host cells via host-lipoproteins disrupts lipid homeostasis in immune cells, promotes neutrophil apoptosis and probably induces aberrant antigen presentation, resulting in inefficient immune response of host.

BSP232

Circadian rhythm of *S. japonicum* from hilly areas and marshland: indication of contrasting definitive host reservoirs by habitat?

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Schistosomiasis japonicum involves the transfer of cercariae from snails to vertebrate hosts. This temporal distribution of cercarial may follow a rhythmic pattern shaped by their definitive hosts. Our previous investigation in two different geographical regions in China revealed a significant difference in main definitive hosts, bovine vs rodents, for the parasite. Therefore, it may be predicted that there should be a difference in circadian rhythm of cercarial emergence between the two regions. Three chronobiological tests of cercarial emergence were performed. Two shedding modes, at the level of snails, were identified, with a late afternoon shedding pattern found in the hilly areas, a morning shedding pattern combined with an initial shedding pattern

and a late afternoon shedding pattern in the marshland. These results provide strong evidence for the previous investigation of prevalence, and also provide a convenient method in determination of main definitive hosts when wildlife suspected as reservoirs.

Session 6C

BSP159
Characterisation of a new aromatic amino acid hydroxylase from the protozoan parasite *Toxoplasma gondii*.
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T. gondii is an obligate intracellular protozoan parasite that can inhabit many different host cell types including muscle and brain tissue. Here we present data on a new aromatic amino acid hydroxylase (AaaH) from *T. gondii*.
The AaaH's are a highly conserved family of enzymes. They catalyse the successive conversion of phenylalanine to tyrosine to l-dopa, and from tryptophan to 5-hydroxytryptophan (neurotransmitter precursors). *T. gondii* possess two parologous AaaH genes that are not present in the rest of the apicomplexa with the exception of Neospora caninum. We have cloned and sequenced these two genes and shown that they are differentially expressed in different developmental stages. Recombinant proteins of TgAaaH1 and TgAaaH2 have been expressed, purified and biochemically characterised. Both proteins are predicted to contain an N-terminal signal peptide, suggesting that these proteins may be exported to a separate cellular compartment or the parasitophorous vacuole. Here we present data on the developmental expression, biochemical characterisation and possible localisation of these proteins.

BSP183
Analysis of the functions and interactions of the RAD51 paralogues in *T. brucei.*
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Trypanosoma brucei evades host acquired immunity by antigenic variation, involving periodic switches in the variant surface glycoprotein (VSG) coat. DNA recombination is critical in this process. A key enzyme of homologous recombination, RAD51, plays a role in VSG switching. Trypanosomatids encode four proteins distantly related to RAD51; termed RAD51 paralogues, though their functions remain poorly understood. Two *T. brucei* RAD51 paralogues, RAD51-3 and RAD51-5, play roles in homologous recombination, DNA repair and RAD51 re-localization into foci following DNA damage. Surprisingly, however, only RAD51-3 appears to act in VSG switching.
To examine the functions of all the RAD51 paralogues in *T. brucei*, we have used reverse genetics to generate mutants in Rad51-4 and Rad51-6. This indicates that these factors also play critical roles in RAD51-directed recombination and repair in the parasite. In addition, we have examined the physical interactions of all the paralogues and find that they form at least two complexes. These analyses shed light on the

evolution and role of eukaryotic RAD51 paralogues in DNA recombination and repair in general, as well as the contribution that recombination makes to antigenic variation in *T. brucei.*

BSP266

The expressed protein repertoire of *Toxoplasma gondii* tachyzoites

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We report on the use of three complementary strategies to characterise the proteome of *Toxoplasma gondii* tachyzoites: Using one-dimensional SDS-PAGE coupled with tandem mass spectrometry (Gel-LC MS/MS), two-dimensional electrophoresis and Multidimensional Protein Identification Technology (MudPIT), approximately 2,500 non-redundant proteins were identified. These proteins represent approximately 1/3 of the entire predicted proteome of *T. gondii*. Bioinformatic approaches such as BlastP, GO, SignalP, TMHMM, PATS, PlasMit and WoLFPSORT were applied to interpret the functional significance of these expressed proteins. We have also undertaken an analysis of our protein expression data in the light of other expression data, such as evidence of mRNA expression and the significance of these results is discussed.

BSP267

Proteome analysis of the Apicomplexa: implications for genome annotation and expression analysis

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Although extensive genome sequence is available for many important protozoa, inadequacies still exist in its annotation. Proteomics can inform both genome annotation and gene expression analysis, but the integration of proteomics, genomics and expression data on a single platform represents a significant challenge. We have completed whole proteome studies on the invasive stages of a range of Apicomplexa including *Toxoplasma gondii*, *Cryptosporidium parvum* and *Neospora caninum* and obtained peptide evidence for the expression of approximately one third of the entire predicted proteome of these organisms. We have developed a platform to integrate these data with other genomic resources and made this available as a community tool (www.toxodb.org; www.cryptodb.org). We describe how our data corroborates existing gene annotations, but also suggests that some alternative gene models may be more appropriate. We discuss the value of proteomics to understanding the

relationship between mRNA expression data (ESTs and microarrays) and protein expression in the Apicomplexa.

BSP055
Prevalence of *Cryptosporidium* parasite in children in south of IRAN in 2005
Mohammadreza Foroutani

Iran

Cryptosporidium" parasite is from Coccidia group, which causes digestive diseases in people who have a weak security system or suffer from AIDS. Although, the infection is usually stopped spontaneously in normal individuals, the quality of self-pollution and extension of this parasite is possible to continue the infection. The parasite can produce acute and chronic digestive infections in children. The continuation and intensity of illness can cause much harm in children. It is certainly influential for the health of the society to know about the ill children.
In this research, we collected 541 samples of faeces from eight area of south of IRAN. 64 samples were watery as having diarrhoea. We used the colour method (Ziehl-Neelsen's modified by Henriksen) for diagnosis.
To colour these samples didn't show any sign of pollution with "*Cryptosporidium*" in these children. It was probably because either the facet samples were not sufficient or at the time of survey (Autumn and Winter), the rate of pollution had been less.
It is nevertheless a requirement to continue carefully this research and to find its prevalence which is a real danger for the infants' health and security.

Session 7B

BSP098
The Effects of Adjuvants on the Course of *Fasciola hepatica* Infection in Sheep.
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F. hepatica causes a serious clinical disease in ruminants. We are interested in the possibility of immunoprophylaxis and one strand of research involves characterizing the effect of adjuvants on the immune response of ruminants to infection. We intend in particular to investigate the "adjuvant effect" that we have seen in several vaccination trials. We carried out a study in which cross-breed sheep were injected with one of three adjuvants – Quil A, TiterMax Gold or FICA (Freund's incomplete adjuvant), or a PBS control. Two weeks post final immunization; all animals were infected with 100 metacercariae of *F. hepatica*. CD4+/CD8+ cell ratio, plasma biochemistry-hematology parameters, antibody and cytokine responses were analyzed. The antibody and cytokine levels were higher in the Quil A group, relative to the other groups. The Quil A group offered the best protection, as measured by lower fluke burden and faecal egg count. This work will be used in the design of future

vaccine trials and will also advance our understanding of the immunology of liver fluke infection.

BSP161

***Angiostrongylus vasorum* and Toxocara in Surrey.**

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Surrey is a recently recognized hot-spot for French Heartworm *Angiostrongylus vasorum*. An epidemiological study commenced December 2007 set out to discover levels of infection in dogs visiting parks in three local authority areas within the county. Fresh samples were collected from dog-bins at the end of days when they had been emptied and temperatures were above 0C. They were processed within 24 hours by the 'Gold Standard' Baermann test to detect larvae of *A. vasorum*. Standard McMaster egg counts for 'other ' nematode infections were also conducted.
From 333 initial samples, of 1200 to be reported, initial prevalence of A. vasorum was at 1.5% and 3 of 26 parks contained positive samples. *Toxocara canis* and leonina were at a prevalence of 1.2%. A significant association of Toxocara infection with areas of multiple deprivation (MD) found in Hampshire will be re-run with our Surrey data.

BSP197

Genetic approaches to define protective antigens of the protozoan *Eimeria maxima*

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Control of the Eimeria species, causative organisms of the disease coccidiosis, has largely been based upon chemotherapy or vaccination with live parasites. Sustainable alternatives are being sought but, in common with other protozoan parasites, the identification of genuinely immunoprotective antigens has proven difficult. The majority of screens for vaccine candidates protective against protozoan infection have been largely empirical, based upon criteria other than protection. We have developed an approach based upon genetic mapping of immune-relevant loci with *Eimeria maxima* which identified five distinct sub-chromosomal regions, representing ~1% of the genome in total, whose retention is correlated with susceptibility to immune killing. The sequencing of relevant BAC clones is facilitating the discovery of genes encoded by *E. maxima* that confer susceptibility to strain-specific immune selection. The significance of such a small number of putative protective antigens (in the face of a large cohort of antigens which may be responded to by the host) and the potential for application of similar approaches to development of vaccines against other parasites will be discussed.

BSP211

Prevalence, transmission and performance impacts of *Toxoplasma gondii* in lambs

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Toxoplasma gondii is economically important in sheep. The sheep is a secondary host which can become infected by ingesting *T. gondii* oocysts from the faeces of the parasite's definitive felid host (horizontal transmission). In addition, *T. gondii* can cross the ovine placenta (vertical transmission). Foetal or neonatal death can result but vertically infected, asymptomatic lambs have also been reported. Flock to flock variation might exist in the relative frequencies of these outcomes and in the frequency of horizontal transmission to lambs. This study aimed to estimate vertical and horizontal transmission rates, and to measure impacts on growth, in one cohort. Prevalence in neonatal viable and non-viable lambs was estimated using a more standardised PCR protocol than previously reported, and prevalence in surviving lambs at age 16 weeks was estimated by serology. Lambs were weighed at birth and at age eight weeks. PCR positivity and seropositivity occurred independently of one another, suggesting that vertically transmitted *T. gondii* was non-immunogenic. Associations with lamb weight and with litter membership suggested that both transmission routes contributed to the maintenance of endemic infection.

BSP224

Suppressive subtractive hybridisation analysis of genes upregulated during feeding in the sheep scab mite, *Psoroptes ovis*

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Sheep scab is a major welfare concern in the UK, due to its persistent detrimental effects on the host. The disease is controlled by dipping in acaricides, but there are problems with these, and alternative methods are being sought. One alternative is the development of a vaccine, which is plausible as sheep do develop immunity to the disease. Although the causative agent of the disease, *Psoroptes ovis*, is not a blood feeding parasite, it has been observed to ingest host immunoglobulins, so in theory hidden antigens in the gut could be targeted.

In an attempt to identify potential hidden antigens, a suppressive subtractive hybridisation (SSH) experiment was carried out to compare patterns of gene expression between actively feeding and starved mites. This analysis revealed that a variety of interesting genes were upregulated in feeding mites, including homologs of house dust mite antigens, salivary gland proteins, and several proteins potentially involved in immunomodualtion; whilst mostly housekeeping genes were upregulated in the starved mites.

Session 8C

BSP002 (poster requested in Schistosoma session)
Schistosoma mansoni disrupts defence-cell signalling in schistosome-susceptible _Biomphalaria glabrata_
Zahida Zahoor[1,2], Angela J. Davies[1], Ruth S. Kirk1, David Rollinson[2] and Anthony J. Walker[1]

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The snail *Biomphalaria glabrata* is an important intermediate host for *Schistosoma mansoni*, the cause of human intestinal schistosomiasis. *Biomphalaria glabrata* can be used to study snail-schistosome interactions as susceptible and resistant snail strains exist. During the transformation of *S. mansoni* miracidia to mother sporocysts, Excretory-Secretory Products (ESPs) are produced; which interfere with snail defence responses. Our research has shown a cell signalling pathway in haemocytes from schistosome-susceptible *B. glabrata* that is modulated by *S. mansoni* ESPs. Western blotting and fluorescence confocal microscopy with anti-phosphospecific antibodies have revealed that the phosphorylation (activation) status of mitogen-activated protein kinase (MAPK) was significantly down-regulated in haemocytes of susceptible, but not in resistant, *B. glabrata* snails. This study is the first to demonstrate an effect of *S. mansoni* ESPs on signalling process in *B. glabrata* haemocytes, furthering our understanding of molecular interplay between schistosomes and their hosts.

BSP039
Schistosoma haematobium antigen recognition patterns of human antibody subclasses: IgE vs. IgG4 and IgA vs. IgG1.
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Schistosoma haematobium is a parasitic trematode, the causative agent of urinary schistosomiasis and the most prevalent species of human schistosome in Africa and the Arabian Peninsula. In endemic regions parasite-specific acquired immunity develops with age and current research supports a role for humoral immunity in resistance to infection. Theories on the development of schistosome-specific antibody responses propose that IgG4 can block IgE responses by binding onto the same antigens. Therefore this study of Zimbabwean participants resident in a schistosome endemic area compared specific antigens recognised by the two subclasses. Furthermore we have previously reported a reciprocal relationship between parasite specific IgA and IgG1, therefore the antigen recognition profile of these subclasses was also compared.

The gel based proteomic approach used allowed unequivocal identification of specific parasite antigens recognised by the sera from the study population. The study shows that while all 4 isotypes recognise some common antigens, other antigens are subclass-restricted suggesting that these antibodies play differential roles in the immune response to *S. haematobium.*

BSP095

Triclabendazole Response in the Liver Fluke *Fasciola hepatica*.

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* Equal contribution.

The trematode, *Fasciola hepatica*, is the causative agent of fasciolosis, a foodborne disease affecting livestock and humans worldwide at significant economic cost. With continued absence of commercial vaccines, fasciolosis control relies largely on chemical flukicides.
Triclabendazole (TCBZ), a novel benzimidazole-derivative, as the major flukicide, offers exclusively high activity against both pathogenic juveniles and adults of chronic infection. Worryingly, global reports of TCBZ resistance are increasing, thus compromising control efforts. Despite observed changes in different biological systems of TCBZ exposed fluke, the mechanism of action and route of resistance to TCBZ remains unknown.
Elucidation of complex interactions between responding biological systems requires global approaches to identify linking factors. Two-dimensional gel electrophoresis (2-DE) and gel-image analysis allows resolution and quantification of different proteins within complex mixtures. We investigate TCBZ-susceptible and TCBZ-tolerant *F. hepatica* proteomes responding to the major metabolite TCBZ-SO under laboratory conditions.

BSP222

Host choice and penetration of *Schistosoma haematobium* miracidia.

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Schistosome miracidia show specificity to their intermediate snail hosts and this specificity can vary between parasite strains and geographical location. Here the role of miracidia behaviour in host specificity of *Schistosoma haematobium* on the islands of Zanzibar is investigated. In choice chamber experiments, *S. haematobium* miracidia moved towards *Bulinus globosus* snail hosts in preference to empty chambers and miracidia preferred uninfected over patent *B. globosus*. Miracidia also

moved towards and discriminated between the host B. globosus and the sympatric, non-host species *Cleopatra* spp. In contrast, *S. haematobium* miracidia did not discriminate against the allopatric *Bulinus nasutus* in either choice chamber or penetration experiments. We suggest that these differences in preference reflect selection pressure to avoid sympatric non-hosts which represent a transmission dead end; whereas the distribution of *B. nasutus* on Zanzibar will result in little contact with and hence little selection pressure to avoid this non-host snail.

BSP270

Schistosoma mansoni and _Schistosoma haematobium_ co-infections; genotypic and phenotypic implications

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Schistosoma mansoni and *Schistosoma haematobium*, digean trematodes infecting humans, are the most widely distributed and prevalent schistosome species in Africa. Forty countries are endemic to both species raising the likelihood of mixed infections. In the developing world polyparasitism is common. Control strategies and public health measures should consider how synergistic or antagonistic interactions of co-infections may clinically affect individuals and how co-endemicity may affect the epidemiology of the parasites.

The research presented combines field-based surveys with tightly controlled laboratory-based experiments, aimed to: 1) elucidate the effects of *S. mansoni* and *S. haematobium* co-infection and inter-specific interactions on human morbidity, 2) establish the impact of *S. mansoni* and *S. haematobium* co-infections and inters-specific interactions on parasite genetic and phenotypic diversity, 3) determine the impact of sympatry on *S. mansoni* and *S. haematobium* behavioural ecology. Preliminary results from research in progress will be presented and discussed in terms of their implications for targeted control programmes and policy.

SPRING POSTER ABSTRACTS

BSP001

Toxoplasmosis in pregnant women in the Nenavah Governorate

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The present study included the serological screening of as one of the abortion causes in (463) pregnant women whose aging between (18-40) year during the period from December 2002 to May 2003. Tests included latex agglutination test LAT and Modified MLAT by using 2-Mercaptoethanol (2-ME) compound to distinguish between two types of infection acute or chronic. The ELISA tests for Toxoplasmosis were also used as a high specific and sensitive test. The level of Lactate Dehydrogenase (LDH) and Anti Cardiolipin antibodies type IgM (ACL-IgM), Anti-Sterptolysin-O (ASO), Rheumatoid factor (RF), Anti-Nuclear Factor (ANF) were also estimated. The results showed that frequency of infected cases was (79%) using LAT and the frequency of active positive cases using MLAT was (46%) while ELISA detected (37%) .The percent of Latent cases as detected by MLAT was(33%). The rate of abortion was (76%). The percentage of reactivation cases was (35%) while the rate of persist IgM was at(28%). Eighty nine of pregnant women who have positive (ACL–IgM) also gave positive result for LAT. (71%) some of them were active cases by using MLAT and (63%) by using ELISA. The result also revealed that (78%) of infected women were positive to ASO test and only (9%) to RF test but no interaction between infection and ANF test was noticed.

BSP025

An anti inflammatory factor in the saliva of the sheep tick *Ixodes ricinus*

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The tick saliva contains many molecules that prevent their rejection from the host, ranging from lipids to peptides and proteins. These factors have therefore become attractive pharmaceutical targets.

The present study focuses on a protein in the salivary glands of Ixodes ricinus. We have shown this protein has a hydrolase activity, using a fluorescent substrate and confirmed using the inflammation mediator platelet activating factor (PAF) as a competitor.

The enzyme was partially purified using a two step anion exchange chromatography. The activity fraction was analysed by LC-MS/MS. By screening the peptides against an EST database from *Ixodes scapularis*, three proteins of interest were identified, all having a hydrolase folding and/or catalytic site. Primers were designed to screen an *I. ricinus* cDNA library to get the full length sequence of the genes. mRNAs extracted from various tick organs were analysed by RT-PCR to assess whether those genes were expressed in other tissues. One of the candidates was present only in the female salivary glands, suggesting its implication in feeding.

BSP036

Function and Regulation of *Haemonchus contortus* genes

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RNA interference is an extremely effective technique to knock out specific genes to examine their function. RNAi has been used extensively in *C. elegans* but has proven to be less effective in parasitic nematodes. However we have obtained successful knockdown for some genes of the sheep gastrointestinal nematode *Haemonchus contortus*. Initial analysis of *H. contortus* genome data suggests that some parts of the RNAi pathway are conserved whilst other parts may be missing/not conserved. We are currently testing components of this pathway to confirm knockdown is an RNAi effect and to try to improve on current methods.
At present, little is known of the spatial and temporal regulation of parasitic nematode genes. The regulation of *H. contortus* genes will be examined by two main methods; analysing sequence data to identify promoter regions and regulatory motifs, and by promoter reporter constructs to examine expression patterns of specific genes in transgenic *C. elegans*.

BSP062

Hsp90 and the biology of parasitism

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Hsp90 is essential in all eukaryotes and plays a central role in multiple cellular processes. Recently much effort has been targeted at the identification and development of drugs that can alter Hsp90 function. The best-characterised inhibitor of Hsp90 is Geldanamycin (GA). Previous studies have shown that Hsp90 from the filarial nematode Brugia pahangi is susceptible to GA while Caenorhabditis elegans Hsp90 fails to bind GA. We set out to determine whether C. elegans Hsp90 is unique or whether the GA-resistant phenotype is shared with other nematodes. Our studies demonstrate that the ability of Hsp90 to bind GA is correlated with the phylogeny of the species. Most Clade III nematodes bound GA, while all Clade V species (the same clade as *C. elegans*) failed to bind GA, as did *Strongyloides ratti* (Clade IV). However Trichinella spiralis (Clade I) Hsp90 did bind to GA. These results suggest that species with free-living larval stages in the environment fail to bind GA, while obligate parasites or some species enclosed within an egg while in the environment have retained GA binding.

BSP063

Characterisation of *Biomphalaria spp*. from Lake Victoria, Uganda with field observations on infections with *Schistosoma mansoni*

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Biomphalaria snails are the obligate intermediate host of the parasite *Schistosoma mansoni,* which causes intestinal schistosomiasis in humans. The disease is a scourge to millions of people worldwide, and is rife in East Africa, although transmission is not homogenous. One suggestion is that differences in compatibility of the snail host may account for this focality of distribution. With the intention to investigate snail-related aspects of disease transmission and population genetic characterisation of encountered forms, we present preliminary findings of a field trip to the Lake Victoria shoreline of Uganda. Samples were collected using timed-search techniques from over forty populations and examined for patent *S. mansoni* infection; initial morphological and molecular analyses are also reported here. Three further trips are envisaged to collect more Biomphalaria specimens to enable a better appraisal of this genus across the Lake and in so doing hope to identify areas where there is heightened risk of schistosomiasis allowing better local control.

BSP080

Drug target discovery in the tick synganglion

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Current control strategies for ticks and tick-borne diseases rely heavily on the use of ectoparasiticides, most of which target the central nervous system. However, with increasing resistance, new drugs are urgently needed. Therefore, we are attempting to identify potential new drug targets in ticks, an area with a relatively limited knowledge base, especially in the area of neurobiology.
Here, we describe the analysis of a normalised full-length cDNA library prepared from the synganglion of the brown dog tick *Rhipicephalus sanguineus*. To date, 960 ESTs have been identified from this library. BLASTx analysis has identified four potentially interesting transmembrane receptors including leukokinin-like receptor, two glutamate gated chloride channel subunits and a nicotinic acetylcholine receptor (nAChR) subunit. The *R. sanguineus* nAChR is the first identified full length arachnid α subunit and we report the characterisation of this receptor using two-electrode voltage clamp in *Xenopus* oocytes.

BSP096

Proteomic Profiling of *Haemonchus contortus*: searching for drug resistance biomarkers in a non-genome verified parasitic nematode.

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Nematode infection remains the greatest health and economic burden to the sheep industry worldwide. In the absence of viable vaccines, chemotherapy remains the major method of nematode control in intensively farmed livestock. However, inappropriate anthelmintic drug usage has brought development of widespread nematode resistance. In some areas, sheep farming is already economically unfeasible. Resistance may arise through gene mutation and expression change. Protein interaction equilibrium may thus be affected, altering overall dynamic status of the phenotype-inducing proteome. Comparative proteomic analysis may reveal profiles of resistant and susceptible nematodes, with subsequent application in biomarker and novel drug-target discovery, elucidation of drug action and resistance monitoring. However, proteomic profiling of parasitic nematodes is limited by genomic sequence availability. This study combines expressed sequence tag (EST) data, bioinformatic and proteomic technologies to initialize investigations of resistant and susceptible *Haemonchus contortus* parasitic nematodes of significant veterinary importance.

BSP126

Pterin Metabolism in *Crithidia fasciculata*

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Tetrahydrobiopterin is a biologically significant pterin which acts a cofactor in several enzymatic reactions in mammalian cells. These reactions include the hydroxylation of aromatic amino acids and the biosynthesis of nitric oxide, an essential signalling molecule in the cardiovascular and nervous systems, which is also known to have antimicrobial properties. Crithidia and *Leishmania spp*. have previously been shown to be pterin auxotrophs, unlike mammalian cells which synthesize biopterin from GTP. It is doubtful that either pterin-dependent aromatic amino acid hydroxylases or nitric oxide synthases exist in trypanosomatids; hence the role of pterins in trypanosomatids is unknown. In our ongoing studies, we have cultured *Crithidia fasciculata* in a fully defined media in order to identify specific pterins that can support growth and we have developed an HPLC based assay to determine the intracellular pterin levels within these parasites. Using these techniques, we hope to elucidate the possible roles of pterins within trypanosomatids.

BSP140

Uneven distribution of sandflies in areas with contrasting incidence of classic Kaposi's sarcoma

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Classic Kaposi's sarcoma (KS) and KS associated herpesvirus (KSHV/HHV-8) have unexplained geographical distributions. A possible reason is the presence of certain species of blood-sucking insects which induce intense skin response to bites and might promote the reactivation of KSHV/HHV-8 and facilitate human-to-human KSHV/HHV-8 transmission and KS development ("promoter-arthropod hypothesis"; Coluzzi et al., Parassitologia 2002). To test this hypothesis, we carried out an entomological survey in two areas of Sardinia with contrasting incidence rates (IRs) of classic-KS. We collected 10,821 promoter-arthropods (99.9% Phlebotominae; 0.1% Culicidae), and found a highly significant difference in their geographical distribution that is strongly correlated to classic-KS IRs by area ($r=0.53$, $p<0.01$). Promoter-arthropods were more likely to be captured in areas with limestone, volcanic-soil and cereal cultivation, higher past prevalence of malaria and cutaneous-leishmaniasis. The study supports the association between promoter-arthropods and classic-KS, and highlights the link with a number of variables previously associated with KS incidence. The overlap in Phlebotominae distribution, cutaneous leishmaniasis and classic-KS has not previously been noticed and requires further research.

BSP141

Presence of disease vectors in inland Sardinia

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An entomological survey was carried out in inland Sardinia (Sassari province; July 2006) using CDC UV-light traps to evaluate the presence/density of disease vectors. 16,103 specimens of three groups of blood-sucking Diptera were captured: 11,069 Plebotominae, 4815 *Culicoides spp*. (32 species), and 219 Culicidae (8 species). Phlebotominae specimens included *Phlebotomus perfiliewi* (58.3%), *P. perniciosus* (38.9%) and damaged *Phlebotomus spp.* (2.8 %). The high number of Phlebotominae is not surprising since human/canine leishmaniasis is hypoendemic in Sassari province since the beginning of last century. The principal bluetongue vector (*Culicoides imicola*) and the secondary potential vectors (*C. obsoletus* and *C. pulicaris*) represented 9,9% of all midges. In the past years (2000–2006) several bluetongue outbreaks have been recorded in Sardinia. The yield of human malaria vector *Anopheles labranchiae* was represented by 4.3% of all mosquitoes (malaria has been eradicated from Sardinia about 50 years ago).

BSP158

Does parasite prevalance and impact vary between native and invasive alien crayfish?

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Parasites that affect native and alien species differently can influence the outcome of invasions. In the UK, the invasive alien signal crayfish, *Pacifastacus leniusculus*, and the native white-clawed crayfish, *Austropotamobius pallipes*, can both be infected by microsporidian species. One such species, *Thelohania contejeani*, causes porcelain disease which destroys muscle tissue and causes mortality. It is not known if this parasite is affecting the crayfish species differently. We are investigating the role of parasitism in the ongoing invasion in the River Wharfe in Yorkshire using a long-term survey of parasite prevalence in native and alien crayfish populations. Behaviour experiments using crayfish of both species show the impact of parasitism on each species' activity, responsiveness, and habitat choice. Here we present data on *T. contejeani* prevalence in native and alien crayfish populations, and the impact of parasitism on the behaviour of native crayfish.

BSP191

Activation and initiation of feeding in vitro in infective third-stage larvae of *Nippostrongylus brasiliensis*

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Developmentally arrested and non-feeding infective larvae (L3) of strongylid nematodes resume their development in response to specific host-derived cues during invasion. This has been studied in most detail in the hookworm *Ancylostoma caninum*, which responds to host serum and glutathione in vitro, activating signalling pathways which result in novel gene expression, protein secretion and resumption of feeding. We are examining larval activation in *Nippostrongylus brasiliensis*. Our data show that these processes are activated by temperature (37°C) independently of serum or glutathione. Infective larvae began feeding after a lag period of 3 to 6 hours at 37°C, reaching a maximum of 90% of the population feeding by 48 hours. Use of neurotransmitters suggests that both cholinergic and serotonergic pathways act synergistically with the elevated temperature cue to stimulate feeding, but cannot initiate the process independently. We are examining this process with a view to understanding the transition to parasitism and in order to enhance uptake of macromolecules for RNA interference.

BSP196

The development of RNA interference protocols in the cestode, *Moniezia expansa*

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Cestodes of livestock pose a significant burden on the agricultural sector with resistance to anthelmintic treatments now emerging, particularly to benzimidazoles in *Moniezia expansa* (Coles, 2005). This demonstrates there is an impending need for characterization of new targets for drug screening in cestodes, with the exploitation of neuropeptide signalling being one potential option. Neuropeptide F (NPF) is abundantly expressed in the nervous system of *M. expansa* and it has been identified in related flatworms where it modulates physiological processes including muscle motility. This study aims to develop RNA interference (RNAi) protocols for the NPF encoding gene in *M. expansa*. RNAi is an accepted method for probing gene function via post-transcriptional gene knockdown and helping to identify and validate novel drug targets in parasites. Similar studies have provided novel data on gene silencing in trematodes and a monogenean; thus far there are no published data on RNAi in cestodes. Various methods of double stranded RNA (dsRNA) delivery have been investigated in adult stages of *M. expansa* to facilitate optimization of RNAi protocols in tapeworms.

BSP203

The swimbladder nematode *Anguillicola crassus* in eel populations from rivers in southern England.

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A decline of up to 90% in eel recruitment has occurred across most of Europe since the 1980's. The endangered status of eels has been largely attributed to a combination of factors including overfishing, pollution and infection with *Anguillicola crassus*. The migratory and blood feeding activities of *A. crassus* cause severe structural changes to the swimbladder of the eel. The parasite was accidentally introduced into the UK via the eel trade but little is known about the impact of *A. crassus* on eel stocks in major river systems in the UK. In the present study the epidemiology and pathology of both larval and adult parasites in up to 120 eel samples has been undertaken in river catchments in Sussex during 2005-2006 in conjunction with the Environment Agency's National Fisheries Monitoring Programme. Patterns and levels of infection of *A. crassus* are discussed in relation to concentrations of contaminant loads in eels including heavy metals, polychlorinated biphenyls and chlorinated organic pesticides.

BSP204

flp-11 knockdown in *Globodera pallida, Panagrellus redivivus* and *Haemonchus contortus* using RNAi

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FMRFamide-like peptides (FLPs) represent the largest known family of nematode neuropeptide signalling molecules. Their roles in diverse aspects of motor and sensory functions in nematodes have highlighted the potential of their associated signalling systems as targets for novel chemotherapeutics. Although there is some evidence for differences in the expression and function of individual flp genes in different nematodes, no concerted efforts have been made to compare flp function across nematode life styles and clades. Here, RNA interference (RNAi) is used to probe the function of the highly conserved flp-11 in a free-living cade III nematode (*Panagrellus redivivus*), a plant parasitic clade IV nematode (*Globodera pallida*) and a clade V animal parasitic nematode (*Haemonchus contortus*). Detailed investigations of motility phenotpyes were carried out using a state-of-the-art nematode movement analysis system.

BSP240

Evidence for sex in monomorphic diplokaryotic microsporidia

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Microsporidia are single-celled, obligate, intercellular parasites of animals. Many microsporidian species are binucleate for part or all of their life cycles, with the two nuclei closely appressed in a diplokaryotic state. Microsporidia that are diplokaryotic for their entire life cycles are usually considered to be asexual. However, recent evidence of synaptonemal complexes suggests that recombination may occur between the nuclei of the diplokaryon. The possible existence of sex in monomorphic, diplokaryotic microsporidia is of practical relevance since several commercially and medically important pests, pathogens and biological control agents possess life cycles of this type. We present preliminary molecular data supporting the presence of sex in the monomorphic, diplokaryotic microsporidian *Paranosema grylli*. In contrast, there is evidence for long-term asexuality of another monomorphic, diplokaryotic microsporidian *Nosema bombycis*.

BSP252

Assessment of intraspecific variation among *Bulinus truncatus* and *Biomphalaria pfeifferi* using random amplified polymorphic DNA technique (RAPD)

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The present study was carried out to investigate the genetic variation within Bulinus truncatus and Biomphalaria pfeifferi in Sudan, intermediate host snails of urinary and intestinal schistosomiasis, respectively. Snails were collected from field sites and were identified morphologically. Random amplified polymorphic DNA assay was used to determine the intraspecific variation of collected snails using the following four primers: OPA02 (5' TGCCGAGCTG 3'), OPA10 (5' GTGATCGCAG 3'), OPA11 (5' CAATCGCCGT 3') and APY12 (5' AAGCCTGCGA 3'). From analysis of RAPD profiles there was high similarity (i.e. low genetic diversity) for both *Bulinus truncatus* and *Biomphalaria pfeifferi* from the sampled areas.

BSP292
H2 Production in the Fish Pathogen *Spironucleus vortens*.
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The fish parasite *Spironucleus vortens* causes major problems in aquaculture of ornamental fish. The organism studied here was isolated from an angelfish (Sarah Poynton, 1995). A membrane-inlet mass spectrometer was employed to monitor H2, O2 and CO2. When introduced in air saturated buffer, Spironucleus vortens consumed O2 and produced CO2. H2 production started under microaerophile conditions ([O2] = 60 µM) with a rate of 20 ± 11 nmoles/min/107 cells. KCN (15 mM) inhibited H2 production by 85 % and 20 mM by 96 %, suggesting that an Fe-only hydrogenase is responsible for H2 production. Metronidazole (1mM) inhibited H2 production by 50%, while CO2 production was not affected. A higher concentration (1.5 mM) inhibited H2 production by 87 % and CO2 production by 36%, suggesting that metronidazole is reduced by an enzyme of the H2 pathway, thus competing for electrons with H+. The question of the source of H2 requires discrimination between the various inclusions evident in confocal and Normarski direct images. Antibodies raised to *Trichomonas vaginalis* hydrogenosomes and their enzymes have failed to reveal hydrogenosomes in this organism, but transmission electron microscopy suggests the presence of small (100nm) double-membraned organelles, which resemble mitosomes.

BSP301
***Babesia canis* in dogs and ticks (Acari: Ixodidae) in western Slovakia**
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Canine babesiosis, serious veterinary problem occurs throughout Europe. Increasing clinical cases were diagnosed in the Slovak Republic formerly in a period of 2000 - 2007. The presence of *Babesia* sp. was confirmed principally by microscopic examination of blood smears. The apparent vector of the infection was *Dermacentor reticulatus* ticks. We report molecular evidence of *B. canis* infections in *D. reticulatus* and *D. marginatus* ticks and blood samples of dogs with acute clinical form of babesiosis. By amplification of a part the 18S rRNA gene was confirmed the presence of babesia in 10.2 % of tested adult *Dermacentor* ticks. The 12-year-old female cocker spaniel with clinical symptoms (pyrexia, anemia, dark-brown urine) was successfully treated with enoxacin, broad-spectrum fluoroquinolone antibacterial agent, infusion and other supportive therapy. The dog was infected with *B. canis*-positive *D. reticulatus* tick in a focus in western Slovakia, where babesiae and other tick-borne microorganisms (*Rickettsia* sp., *Anaplasma/Ehrlichia* sp.) circulate.
The study was financially supported by VEGA No. 6151 and APVV 51-009205.

BSP335

A population genetic analysis of *Schistosoma mansoni* in the Senegal River Basin, 20 years after the epidemic outbreak.

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About twenty years ago, two dams were constructed in the Senegal River Basin (SRB) in order to improve the agricultural conditions in Northern Senegal. The subsequent ecological changes stimulated the growth and spreading of Bulinus and Biomphalaria snail species, intermediate hosts of *Schistosoma haematobium* and *S. mansoni*, respectively. This resulted in a major outbreak of intestinal schistosomiasis. Here we report on the population genetic structure of *S. mansoni*. In March 2006 and 2007, urine and stool samples were collected along the SRB. Individual miracidia were collected on Whatman FTA® cards and genotyped for 9 microsatellite loci (multiplex). Additional samples have been sequenced for ITS1 rDNA and partial mitochondrial cox1. A thorough population genetic analysis has been carried out, and combined with the sequence data for interpretation.

BSP347

Clinical isolates of anthroponotic species of Cryptosporidium show lower than expected sequence divergence.

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Cryptosporidium is an apicomplexan parasite causing diarrhea worldwide. Two species are of particular interest to public health: *C. hominis*, infecting humans primarily and *C. parvum* infecting humans and a wide range of mammals. The genomes of these two pathogens are sequenced and show only 3-5% sequence divergence between the two species. Using comparative genomic tools, we investigated this sequence divergence, identifying 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 collection of six clinical isolates (three *C. hominis* and three *C. parvum*). These samples were isolated from symptomatic human cases of Cryptosporidium and were previously genotyped at the Cryptosporidium Reference Unit using the standard methods (PCR-RFLP, microsatellite and Real time PCR). Our results showed that the divergence between the clinical isolates of *C. hominis* and *C. parvum* is less than predicted from reference strain sequence. In fact, among the twelve genes, only one was species specific. All the others amplifying from all of the clinical isolates and showing limited but distinct divergence of a few nucleotides per gene. We discuss the potential reasons for the low degree of diversity observed.

LEISHMANIA/TRYPANOSOMA ABSTRACTS

Session 1D

BSP189 (guest speaker)
Treatment failure and drug resistance in visceral and tegumentary leishmaniases: diversity of scenarios
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Worldwide prevalence of leishmaniasis is estimated at 12 million, with 59.000 deaths each year. Treatment is a major pillar of control programs, but the arsenal of available drugs is limited and their use is jeopardized by the emergence of drug resistance. Combination regimens are under clinical development, but it will take several more years to change the drug policy. Meanwhile, the effectiveness of current drugs needs to be safeguarded to ensure unremitting sustainment of leishmaniasis control. Therefore, factors underlying treatment uneffectiveness need to be better understood. These may be related to (i) the parasite (intrinsic insensitivity -i.e. species- or drug resistance), (ii) the treatment (drug quality, compliance, dosage), and (iii) the host (clinical presentation, immunological response, host genetics).
During a multi-disciplinary project on antimony therapy, we explored some of these factors in two different epidemiological settings: anthroponotic visceral leishmaniasis (VL, Nepal) and zoonotic cutaneous leishmaniasis (CL, Peru). Average treatment failure rate was 11.4% in Nepal and 23.9% in Peru, and it was associated with a series of risk factors, differing according to the type of disease. By in vitro susceptibility testing, we found SbV-resistant strains among the 6 species endemic in the respective study areas, but surprisingly, we did not find a correlation between the clinical outcome and the in vitro resistance of the parasite. Two different resistance phenotypes were encountered in vitro, some strains being resistant to SbV and SbIII (the reduced and active drug), and others only resistant to SbV; this suggests that antimonial resistance might be a stepwise process, involving different mechanisms. Molecular exploration showed an altered expression of specific genes among resistant strains, but expression patterns differed according to Leishmania species and also populations, suggesting a pleomorphic adaptation of the parasites. This hypothesis was reinforced by a parasite population genetic study in Nepal.
Lessons from this study are discussed and situated in the context of the current elimination programme in the Indian sub-continent.
The Leishnatdrug-R consortium was funded by EC (ICA4-CT-2001-10076).

BSP184
A new mechanism for multi-drug resistance in trypanosomes
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Nifurtimox and benznidazole are the front line drugs used to treat Chagas disease, the most important parasitic infection in the Americas. These agents function as pro-drugs and must be activated within the parasite to have trypanocidal effects. Despite more than 40 years research, the mechanism(s) of action and resistance have remained elusive. Here, we report that in trypanosomes, both drugs are activated by a NADH-dependent, mitochondrially-localised, bacterial-like, type-I nitroreductase (NTR), and that down-regulation of this enzyme readily explains how resistance may emerge. Loss of a single copy of this gene in *Trypanosoma cruzi*, either through in vitro drug selection or by targeted gene deletion, is sufficient to cause significant cross-resistance to a wide range of nitroheterocyclic drugs. In *Trypanosoma brucei*, loss of a single NTR allele confers similar multi-drug resistance without affecting parasite growth rate or the ability to establish an infection. This potential for drug-resistance by a simple mechanism has important implications, since nifurtimox is currently undergoing phase III clinical trials against African trypanosomiasis.

BSP103
Roles of trypanothione S-transferase and tryparedoxin peroxidase in resistance to antimonials
Susan Wyllie and Alan H. Fairlamb

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Using the model organism *Leishmania tarentolae*, we have examined the role of trypanothione S-transferase (TST) in trivalent antimony (SbIII)-resistance. TST has S-transferase activity with substrates such as chloro-dinitrobenzene as well as peroxidase activity with alkyl and aryl hydroperoxides, but not with hydrogen peroxide. Although S-transferase activity and TST protein levels were unchanged in SbIII -sensitive and resistant lines, rates of metabolism of hydrogen peroxide, t-butyl hydroperoxide and cumene hydroperoxide were significantly increased. Elevated peroxidase activities were shown to be both trypanothione and tryparedoxin-dependent; and associated with the over-expression of classical tryparedoxin peroxidase (TryP) in the cytosol of *L. tarentolae*. Over-expression of the recombinant *L. major* TryP in SbIII-sensitive promastigotes resulted in a two-fold increase in the level of TryP activity which was accompanied by a significant decrease in sensitivity to SbIII. Overexpression of an enzymatically inactive TryP failed to result in SbIII resistance. These findings indicate that enhanced anti-oxidant defences may well be a key feature of clinical resistance mechanisms to antimonial drugs.

BSP180
Biosynthesis of phosphatidylcholine in *Trypanosoma brucei*
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Phosphatidylcholine (GPCho) is the major phospholipid species (50-60 % of total phospholipids) and is presumably an essential structural component and metabolite in the protozoan parasite *Trypanosoma brucei*. *T. brucei* lack the ability to de novo synthesise GPCho via methylation of phosphatidylethanolamine, and thus seemingly generate GPCho solely via the Kennedy pathway utilising CDP-choline and diacylglycerol. This limited diversity of biosynthetic capability to synthesise GPCho suggests *T. brucei* parasites, unlike man, will be vulnerable to the inhibition of key enzymes in GPCho biosynthesis.
Thus the parasite is totally dependent upon the acquisition of choline from their host, however choline is not actively taken up by trypanosomes, leaving the question "how does *T. brucei* obtain this necessary substrate?" Candidate sphingomyelinases are being investigated for their potential role in maintaining lipid homeostasis by releasing choline phosphate (Cho-P), which can re-enter the Kennedy pathway for the formation of GPCho.
Recent findings will be discussed in relation to the validation of novel drug targets in *T. brucei*, the causative agent of African sleeping sickness.

BSP047 (Guest speaker)
Inhibition of ABC Transporters Abolishes Antimony Resistance in Leishmania Infection
Jayati Mookherjee Basu and Syamal Roy

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In the present study we show that infection with Sb-resistant *Leishmania donovani* (SbR-LD) unlike that with Sb-sensitive (SbS-LD) not only induces elevation of GSH levels but also up regulation of multi-drug resistance-associated protein-1 (MRP1) and permeability glycoprotein (P-gp) in both in vitro and in vivo experimental infections. This results in clearance of Sb from the infected cells following sodium antimony gluconate (SAG) treatment and favors parasite replication. Inhibition of MRP1 and P-gp with resistance modifying agents (RMA) such as lovastatin allows Sb accumulation and parasite killing within macrophages and offers protection in an animal model in which infection with SbR-LD strains is otherwise constantly lethal. The occurrence of a similar scenario in clinical cases is supported by the findings that monocytes from SAG unresponsive kala-azar (KA) patients not only have elevated GSH levels but also overexpress P-gp and MRP1 as compared to monocytes from SAG sensitive KA patients. These observations usher in a new strategy for treatment of Sb resistant KA patients.

BSP264

Novel substrates and inhibitors of the third alpha-mannosyltransferase of GPI biosynthesis in Trypanosoma brucei.

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The glycosylphosphatidylinositol (GPI) anchor biosynthetic pathway is essential in bloodstream form *Trypanosoma brucei*. The third alpha-mannosyltransferase (MT-III), which catalyses addition of a third mannose to ManManGlcNAc-PI, has previously been shown to be essential for GPI biosynthesis in yeast. The MT-III is a transmembrane protein localised in the ER and has nine predicted transmembrane domain, hence there is little chance of obtaining high resolution structural data.
In order to examine the substrate specificity of the *T. brucei* MT-III towards ManManGlcNAc-PI substrates and discover potential inhibitors we synthesised a range of ManManGlcNAc-PI analogues containing systematic modifications at the first and second mannose. The analogues were tested for their ability to act as substrates or inhibitors of the *T. brucei* MT-III using a novel radiographic cell-free system assay.

BSP234

From hit to target: Toward a new therapeutic strategy against Human African Trypanosomiasis

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Compound 4-[5-(4-phenoxyphenyl)-2H-pyrazol-3-yl]morpholine was found to exhibit antitrypanosomal activity with an IC50 of 1µM on *Trypanosoma brucei rhodesiense*, the causative agent of the acute form of Human African Trypanosomiasis. Three derivatives of this compound, each containing an additional amine group, were synthesized and immobilized via the amine group on epoxy-activated agarose to perform affinity chromatography to elucidate the corresponding target within the unicellular parasite. Ligand-bound matrices were incubated with the total cell lysate, washed and separated by SDS-PAGE. Bound proteins were detected by silver staining and identified by trypsin digestion followed by LC/ESI/MS/MS-QTOF mass spectrometry.
Using this chemical proteomics approach *T. b. rhodesiense* adenosine kinase (TbrAK), a key enzyme of the parasite purine salvage pathway, was identified as putative intracellular target. For subsequent chemical validation, the ak gene was cloned, recombinantly expressed in *E. coli* and purified to homogeneity. Specific compound binding to TbrAK was confirmed by ITC, CD and fluorescence spectroscopy, and kinetic measurements showed lack of substrate inhibition conferred by the compound, thus leading to hyperactivation of TbrAK.

Session 2D

BSP006 (guest speaker)
Sophisticated, but inflexible, differentiation programs modulate intracellular transport during life cycle progression in *Trypanosoma brucei*.
V. Lila Koumandou, Senthil K. A. Natesan, Tatiana Seergenko and Mark C. Field

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Trypanosoma brucei has a complex life cycle, involving two distinct hosts and several life stages, the latter of which includes both proliferative and non-proliferative forms. Intracellular transport is essential for maintenance and turnover of the cell surface, and in the mammalian stage likely also contributes directly to virulence by removing surface antibody. Here we show, by a combination of cytological and transcriptome analysis, that the trypanosome trafficking system is highly attuned to the host. In particular we find that high levels of endocytosis always accompany expression of surface VSG and also mammalian infectivity. Further, there is substantial remodelling of expression of the genes that comprise the trypanosome trafficking system. In contrast, all evidence suggests a near complete absence of any transcriptional responsiveness to environmental change. These data indicate a very sophisticated, but rigid, adaptation to the specific host conditions encountered during the life cycle.

BSP245
Global translation arrest triggered by blocking VSG synthesis in *T. brucei*
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[4] Peter Medawar Building for Pathogen Research, University of Oxford, UK.

The African trypanosome *Trypanosoma brucei* is covered with a dense protective layer of GPI-anchored Variant Surface Glycoprotein (VSG). We have discovered a novel translation arrest, whereby blocking VSG synthesis results in a reduction in total protein synthesis to less than 1% normal. This translation arrest is correlated with disassociation of ribosomes from the endoplasmic reticulum. Surprisingly, despite the fact that blocking VSG synthesis leads to arrested cells that are not changing in volume, rates of lipid and GPI-anchor synthesis appear unaffected. Ultrastructural analysis of the stalled cells shows distortion of the Golgi cisternae, which coincides with an increase in sphingomyelin. We propose that a feedback mechanism senses significant reductions in the formation of mature GPI-anchored VSG. The response to this is a precise cell cycle arrest coupled to a global protein synthesis shutdown.

BSP032

Regulation of Autophagy during Leishmania Differentiation

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Autophagy is a highly conserved degradative pathway in eukaryotes in which cytosol and organelles are sequestered within double membrane-bound vacuoles called autophagosomes, which deliver their contents to the lysosome / vacuole for degradation. It has recently been shown that *Leishmania major* deficient in autophagy are unable to undergo metacyclogenesis (Bestiero et al., 2006), leading to an interest in studying further the roles of potential components of the autophagy machinery in Leishmania. *L. major* uniquely possesses twenty five putative apparent homologues of the autophagy marker ATG8, which have been classified into three families designated ATG8A, ATG8B and ATG8C. The subcellular localisations and potential roles of these proteins in the lifecycle of L. major will be described. In addition, phenotypic analyses of a mutant deficient in a putative aspartyl peptidase with sequence identity to the mammalian Presenilin-1, a potential regulator of autophagy, will be presented.

BSP099

LmxMPK3, a MAP kinase homologue of *Leishmania mexicana*, is essential for flagellar length regulation

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LmxMPK3 is one of 15 MAP kinase homologues of *Leishmania mexicana*. In mouse infection studies the LmxMPK3 null mutant showed the same infectivity as the wild type. However, the null mutant displays flagella reduced to an average length of only 2 µm. Moreover, the paraflagellar rod (PFR) is either completely absent or its assembly is severely affected.

GFP-LmxMPK3 mutants were generated and subjected to fluorescence microscopy showing that GFP-LmxMPK3 is mainly located around the nucleus, in the flagellum and at its basis.

In order to study the activation mechanism of LmxMPK3, mutations were introduced into the TXY-activation motif of LmxMPK3. The phosphorylation status and the activity of the mutants were investigated both in vitro and in vivo.

Using in silico analysis we found a potential substrate of LmxMPK3. First evidence for the candidate protein being a real substrate could be gained showing that a peptide containing the potential phosphorylation site is phosphorylated by the activated LmxMPK3.

BSP310 (guest speaker)
The increasingly complex coat of African trypanosomes
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African trypanosomes actively remodel their surface during their life cycle. In addition to regular switching of the variant surface glycoprotein (VSG) coat by bloodstream forms, there are several changes of coat in the tsetse fly. Midgut forms of *Trypanosoma brucei* are covered by several million copies of procyclins; it has been proposed that these protect parasites against proteases and/or are required for tissue tropism. In the course of migrating to the salivary glands, epimastigote forms lose procyclins and replace them by a set of proteins known as brucei alanine-rich proteins (BARP). These, in turn, are exchanged for VSG when the parasite develops into the metacyclic form. Other, less abundant surface molecules are also developmentally regulated and at least one is required for efficient transmission. Unexpectedly, in contrast to VSG, procyclins are not essential in vivo. The 14 BARP genes have so far defied deletion. We are currently testing RNA interference as a means to investigate the function of BARP in the tsetse fly.

Session 3D

BSP186 (guest speaker)
Sand fly – Leishmania interaction: specific versus permissive vectors
Petr Volf

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Phlebotomus papatasi and *P. sergenti* display remarkable specificity for the Leishmania species they transmit. Most other sand fly species examined to date support the development of a broad range of Leishmania species and are called permissive vectors. These include species transmitting parasites of the *L. donovani* complex. Attachment of Leishmania promastigotes in permissive vectors does not arise from interactions of lipophosphoglycan (LPG) with sand fly lectins. Instead, O-glycoproteins localized on the microvillar border of the midgut are involved in a novel mechanism of attachment as they bind to Leishmania. The concept of LPG-dependent attachment in specific vectors and LPG-independent attachment in permissive vectors was confirmed by experiments with natural genetic hybrids between *L. infantum* and *L. major*. The presence of a conserved O-glycoprotein ligand in permissive species has important epidemiological consequences; examples are the introduction of *L. infantum* from the Mediterranean to Latin America or transmission of atypical *L. tropica* strains with modified LPG.

BSP256

Exposure to sandfly saliva and disease in canine visceral leishmaniasis

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Domestic dogs are the most important reservoir hosts of zoonotic visceral leishmaniasis. There is great variation in disease presentation between infected dogs, but the factors responsible are largely unknown. Laboratory models suggest that prior exposure of hosts to sandfly saliva affects the outcome of Leishmania infection. Here, we examine the role of sandfly saliva in determining disease status and immune responses in a cohort of naturally infected Brazilian dogs. Exposure to sandfly saliva prior to infection was measured as anti-saliva IgG by ELISA, using dissected salivary glands from the vector *Lutzomyia longipalpis*. Severity of disease was assessed by clinical score and parasite isolation, and immune responses by specific IgG and cytokine production (IL-10 and IFN-γ) in stimulated peripheral blood mononuclear cells. Analysis will test the hypotheses that high exposure to saliva prior to infection protects against severe disease, and stimulates protective immune responses.

BSP262

Leishmania promastigote secretory gel facilitates infection by promoting the alternative activation of macrophages.

Matthew E. Rogers [1,2], Rod J. Dillon [2], Beak-San Choi [1], Pascale Kropf [1], Ulrich Schaible [3], Petr Volf [4], Paul A. Bates [2] & Ingrid Muller [1]

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Leishmania transmission from sand fly to mammals occurs by the regurgitation of parasites from a blocked sand fly midgut. Accompanying the parasites is a gel of parasite origin which contributes to the blockage of the sand fly vector. Specifically, promastigote secretory gel (PSG) is a protoephosphoglycan-rich gel that has been previously shown to significantly exacerbate cutaneous leishmaniasis in a low dose model of infection designed to replicate transmission by bite. Now working with an in vitro model of macrophages infection we show that the gel synergises with IL-4, an anti-inflammatory cytokine produced in response to exposure to sand fly saliva, to promote the alternative activation of these host cells and parasite survival. Macrophage arginase levels are enhanced in the presence of PSG and deglycosylation of the gel

ablates this effect. This effect of PSG results in a facilitated macrophage infection with higher parasite burdens and will be discussed in the context of natural infection.

BSP125
Urate production and xanthine dehydrogenase knockdown by RNAi in *Lutzomyia longipalpis*
Mauricio R. V. Sant'Anna, Bruce Alexander, Paul A. Bates & Rod J. Dillon

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Females of the phlebotomine sand fly *Lutzomyia longipalpis* ingest large amounts of protein when they take a blood meal. Xanthine dehydrogenase is thought to be a key enzyme involved in protein catabolism through the production of urate. Large amounts of heme are also released during digestion, with potentially damaging consequences, as heme can generate oxygen radicals that damage to lipids, proteins and nucleic acids. However, urate is an antioxidant that may prevent such oxidative damage by heme. We investigated urate production in *Lu. longipalpis* fed on rabbit and chicken blood. We also developed the RNAi technique for sand flies using this technique to knock down the Lu. longipalpis xanthine dehydrogenase gene, to evaluate its role in urate production and bloodmeal digestion.
We described the gene sequence of *Lu. longipalpis* xanthine dehydrogenase together with its expression in different life cycle stages and RNAi knock down. Semi quantitative RT-PCR of xanthine dehydrogenase expression showed a significant increase in expression after bloodmeal ingestion. Micro-injection of dsRNA via the thorax of 1 day old adult female sand flies resulted in approximately 40% reduction of xanthine dehydrogenase gene expression in comparison to flies injected with a control dsRNA. A significant reduction of urate in the whole body and excretions of *Lu. longipalpis* was observed after dsRNA xanthine dehydrogenase microinjection and feeding 96h later on rabbit blood. The demonstration of xanthine dehydrogenase knock down by dsRNA microinjection, low mortality of micro-injected insects and the successful bloodfeeding of injected insects demonstrated the utility of RNAi as a tool for functional analysis of genes in phlebotomine sand flies.

BSP139 (Guest Speaker)
What *Trypanosoma brucei* does in the tsetse fly
Wendy Gibson

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Trypanosoma brucei undergoes a complex cycle of development in its tsetse vector. Contrary to previous belief, *T. brucei* is not imperfectly adapted to the fly – the insect simply has well-developed and efficient antimicrobial immune responses, which serve to lower the chance of successful establishment of infection at each point along the trypanosome's developmental route. While following its tortuous journey through the fly, the trypanosome undergoes a succession of developmental steps, notably an asymmetric division, for which the underlying necessity is obscure. In addition, given suitable circumstances, trypanosomes can mate and undergo genetic exchange either inter- or intraclonally. Major obstacles to studying the tsetse-trypanosome interaction

have been the difficulty of culturing most tsetse-developmental stages in vitro and of working with trypanosome-infected tsetse in the lab. However, improvements in digital image analysis, coupled with the use of fluorescent reporter genes and immunocytochemistry, now offer the means to explore this relatively unknown part of the trypanosome's life.

Session 4D

BSP297 (guest speaker)
Role of host galectin-3 and annexin 2 in the cell invasion by *Trypanosoma cruzi*
Luciane Ganiko, Ernesto S. Nakayasu and Igor C. Almeida

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T. cruzi trypomastigotes secrete vesicles that are heavily α-galactosylated (TcαGalVes). We recently showed that parasite host-cell invasion is considerably facilitated by TcαGalVes via a mechanism dependent on the recognition of α-galactosyl (α-Gal) epitopes by host-cell surface α-Gal-binding proteins and associated protein complexes (i.e., annexins A1, A2, A4, and A5, annexin-binding proteins S100A10 and S100A11, and galectin-3). Here we show that anti-A2 (anti-p36), anti-S100A10 (anti-p11), and anti-galectin-3 antibodies, alone or combined, inhibited up to 54% the RAW macrophage infection by *T. cruzi*. Down-regulation of p36, p11, and galectin-3 in RAW macrophages by siRNA reduced the number of infected cells in 52, 42, and 51%, respectively. By immunolocalization analysis, we detected an overexpression of both galectin-3 and p36 at the parasitophorous vacuole of infected macrophages, suggesting that both molecules could also be involved in the parasite internalization and phagolysosome formation. Taken together, our data strongly indicate that galectin-3 and A2 play a role in *T. cruzi* host-cell invasion. Supported by NIH/NIAID Grant # 1RO1AI070655-01A1.

BSP129 (guest speaker)
Cellular basis of IL-10-dependent immune regulation in experimental visceral leishmaniasis
Paul Kaye, Lynette Beattie and Asher Maroof

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Although IL-10 is believed to play a critical role in determining the outcome of both human and experimental visceral leishmaniasis (VL), the cellular events that regulate IL-10 production remain to be fully characterized. Here, we will also discuss new data which suggests that the microenvironment created as a result of ongoing VL supports the generation of a population of CD49b+NKp46+ NK cells, with potent capacity to secrete IL-10 and inhibit host resistance in vivo. We will also discuss the results of an extensive analysis of the expression of IL-12p70 and IL-12-related cytokines by CD4+, CD8+, and DN subsets of conventional myeloid dendritic cells (cDC). Together, these data lead us to propose a model whereby infection-associated changes in the cytokine-

producing capacity of cDC promotes the differentiation of both NK cells as well as CD4+ T cells towards a regulatory, IL-10-producing phenotype.

BSP143

Leishmania infection modulates macrophage protein-tyrosine-phosphatases in a GP63-dependent manner.

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The balance of protein phosphorylation, maintained by the concerted action of protein-kinases and protein-phosphatases, is essential in the outcome of cellular functions. Leishmania induces the activation of the macrophage protein tyrosine phosphatase (PTP) SHP-1, as a mechanism to downregulate host cell functions, promoting the successful establishment of infection. Herein we sought to determine whether other host PTPs were involved in this event. PTP profiling of Leishmania infected macrophages by in-gel PTP activity assay, showed the appearance of low molecular weight (LMW) PTP bands, further characterized as active PTP cleavage products of SHP-1, PTP1B and TCPTP. PTP cleavage is independent of parasite internalization, but dependent on the expression of Leishmania GP63. Interestingly GP63 can directly cleave target PTPs. Preliminary confocal microscopy analysis suggest changes in the subcellular localization of some target phosphatases. Results here presented show that multiple macrophage PTPs, in addition to SHP-1, are modulated upon Leishmania infection, contributing to understand the mechanisms that this clever parasite utilizes for its successful intracellular survival.

BSP058

Evaluation of Safety and Protective Immunity of Live Attenuated, Centrin-knockout, *Leishmania donovani* Parasites.

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Leishmania donovani causes visceral leishmaniasis. Low level of parasite persistence may be essential for maintenance of an effective protective response against leishmaniasis. We have addressed whether centrin deleted *L. donovani* parasites that have growth defects in human macrophages, can persist in mice and are both safe and protective. Safety of such parasites was judged by the maintenance of expression of biomarkers of attenuation after 5 weeks of recovery from spleen or liver of BALB/c mice. Further, such parasites do not persist in mice beyond 6-7 weeks. Protective immunity was demonstrated by a significant increase in CD4+ T cells producing IFNg, IL-2 and TNFa cytokines either individually or simultaneously, indicating a Th1-type immune response after challenge with the virulent *L. donovani* parasites. This immune

response correlated with reduced parasite burden (~40 to 100 fold) in spleen and liver respectively compared to non-immunized mice 8 weeks post challenge. These results indicate that live attenuated centrin-knockout *L. donovani* can be a safe and effective vaccine candidate against VL.

BSP074

Parasite-induced B-cell apoptosis results in loss of specific protective anti-trypanosome antibody responses, and abolishment of vaccine induced protective memory responses.

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African trypanosomes evade the host immune response by continuous antigenic variation of their surface coat. Here we demonstrate that in addition trypanosomes cause the permanent loss of various B cell populations, and disable the hosts' capacity to raise a protective anti-parasite antibody response. The analysis of different B cell populations showed that during early onset of a *T. brucei* infection, spleen remodeling results in the rapid and permanent loss of the marginal zone (MZ) B cell population characterized as B220+IgMHighIgDIntCD21HighCD23LowCD1d+CD138-. These cells, when isolated during the first peak of infection, stained positive for Annexin V and had increased caspase-3 enzyme activity, indicating the onset of apoptosis. Elevated caspase-3 mRNA levels coincided with a decrease in mRNA levels for the anti-apoptotic Bcl-2 protein and BAFF receptor (BAFF-R), both factors involved in B cell survival and homeostasis. In vitro, parasites abolished the proliferative capacity of an infection-derived IgM+ B cell hybridoma, and induced B-cell apoptosis through cell-cell contact. In vivo, infection-induced loss of IgM+MZB cells coincided with the disappearance of protective variant-specific T-independent IgM responses, rendering mice rapidly susceptible to re-challenge with a previously encountered *T. brucei* variant antigenic type. Finally, we show that infection with *T. brucei* can also abrogate vaccine-induced protective response to a non-related pathogen such as *Bordetella pertussis* using the human diphtheria, tetanus and *B. pertussis* (DTPa) vaccination model in mice.

Session 5D

BSP014 (Guest speaker)
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. By means of RNAi, we have down-regulated several evolutionary highly conserved components of the Fe-S cluster assembly pathway, such as cysteine desulfurase IscS, metallochaperone IscU, frataxin, ferredoxin, IscA1, and IscA2. With the exception of IscA1, all are essential for the parasite and their down-regulation results in reduced activities of the marker Fe-S enzyme aconitase, succinate dehydrogenase and fumarase, affected mitochondrial membrane potential and induced generation of reactive oxygen species. Isd11, another highly conserved component of the pathway, appears to exist in the *T. brucei* genome in several isoforms, at least one of which seems to be non-essential for the procyclics. Another unique feature of trypanosome Fe-S assembly is that combined RNAi against IscA1 and IscA2 is lethal. Our data supports the hypothesis that the mitochondrion plays a fundamental and evolutionary conserved role in cellular Fe-S cluster assembly throughout the eukaryotes.

BSP122
Responses to chromosomal double-strand breaks in African trypanosomes
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The African trypanosome relies upon rearrangement of subtelomeric variant surface glycoprotein (VSG) genes to achieve antigenic variation, a process of mitotic double-strand break-repair (DSBR) typically involving homologous recombination (HR). Previous recombination studies relied upon analysis of rare recombinants following transfection. We have employed a I-SceI, meganuclease-based system and monitored temporally constrained DSBR at specific chromosomal sites. In response to a chromosome-internal lesion, repair kinetics were revealed by the generation of adjacent single-stranded DNA; focal accumulation of the homologous strand-exchange factor, Rad51, and G2M checkpoint activation. More than 50% of cells achieved repair and quantitative analysis of the pathways employed indicated that interchromosomal HR dominated. HR displayed a strong preference for the allelic template but also the capacity for interaction among heterologous chromosomes. Rarer intrachromosomal joining was dominated by microhomology-mediated repair, a situation unique among organisms examined to date and a finding with major implications for VSG rearrangement and expression control. We are currently using the system to begin genetic dissection of repair pathways, to monitor repair within VSG expression sites

and within a tandem gene-array and to facilitate the high-efficiency targeting of exogenous DNA.

BSP195

BRCA2 in DNA replication, recombination and antigenic variation in *Trypanosoma brucei*

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Trypanosoma brucei escapes killing by acquired immunity through periodic switches in the Variant Surface Glycoprotein (VSG) coat. RAD51-directed recombination is important in this, though the detailed pathway(s) and its potential regulation is unknown. BRCA2 binds RAD51 via BRC repeats, exerting control on recombination. *T. brucei* BRCA2 is remarkable amongst eukaryotes in possessing up to 12 tandemly arrayed BRC repeats. *T. brucei* BRCA2 mutants provide evidence for a role in general recombination: impaired growth, sensitivity to DNA damage and impaired recombination. Several potentially novel phenotypes are also seen. First, the mutants display chromosome rearrangements involving loss of genes from the silent VSG archive. Second, the mutants show a delay in cell division and accumulation of cells with aberrant DNA content that is not due to BRC-RAD51 interaction via BRC repeats, but is provided by a function of the protein's C-terminus. Finally, BRCA2 acts in antigenic variation, but remarkably only a single BRC repeat is needed in this reaction, though that the expanded *T. brucei* BRC repeat number is critically important in general repair.

BSP132

Changes in trypanosomal mRNA metabolism during heat shock: Stress granules, P-bodies and fireflies.

Susanne Kramer, Rafael Queiroz, Louise Ellis, Jörg Hoheisel, Christine Clayton and Mark Carrington

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Regulated mRNA decay is important in trypanosomes in the absence of transcriptional control. Two types of cytoplasmic ribonucleoprotein particles are involved in eukaryotic mRNA decay. P-bodies are thought to be the site of 5' to 3' mRNA decay. Stress granules are stress-inducible complexes involved in mRNA storage, sorting and decay.

We used a reversible heat-shock treatment as a model to study mRNA decay pathways in trypanosomes, as the cellular response included a decrease in the steady state levels of most mRNAs, resulting, in part, from an increased decay rate. We found that heat-shock induced stress granule formation via a novel pathway that was independent of eFI2A phosphorylation on Ser51, but required translational exit. P-bodies increased in number, but only partially co-localized with stress granules and the P-body component XRNA re-localized to the posterior pole of the cell. This is the first report showing that trypanosomes have distinct P-bodies and stress granules. Our data suggest that either type of granule might be involved in the increased decay following heat shock.

BSP152

Transcription and precursor RNA processing are only minimally involved in regulation of PGK isoenzyme gene expression.

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In many organisms it is becoming increasingly apparent that post-transcriptional processes are as important, in some cases more important, in the regulation of mRNA and protein levels than transcription and precursor RNA processing. Trypanosomes contain an ideal environment in which to study these regulatory processes due to unique molecular processes, including polycistronic transcription and trans-splicing.
Using in vivo quantitation and computer-model based Metabolic Control Analysis for the PGK isoenzymes, we have calculated the relative contributions of transcription, RNA processing, translation and protein degradation to control of gene expression. Regulation of gene expression in different life cycle stages was also calculated by Regulation Analysis. Results have shown that although the control exerted by transcription on mRNA levels is high, this process does not regulate mRNA levels. Furthermore, the differential regulation of mRNA levels is brought about predominantly by degradation, the contribution of precursor processing being slight.

BSP199

Monitoring amastigote-specific signaling in *Leishmania donovani* by quantitative phosphoproteomics and 2D in-gel kinase assay

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We investigated the signaling networks underlying *Leishmania* differentiation analyzing purified phosphoproteins by gel-based proteomic approaches. Differential 2D gel electrophoresis (DIGE) allowed us to establish a repertoire of 174 distinct Leishmania phosphoproteins implicated in stress- and heat shock response, RNA/protein turnover, metabolism and signaling. Based on protein quantification and statistical analysis, 44% of the *Leishmania* phosphoproteome showed significant stage-specific differences with a strong bias towards increased protein phosphorylation in amastigotes. Utilizing 1D and 2D activity assays, we could correlate this increase in phosphotransferase activity to the presence of amastigote-specific protein kinases. Amastigote-specific phosphoproteins were mostly implicated in chaperone functions, including the co-chaperones HOP/Sti1, cyclophilin 40, and various isoforms of HSP83. These chaperones are known to form a multi-protein complex termed ‘foldosome’, which has been implicated in other organisms in cell cycle control and signaling and

thus may play a crucial role in the maintenance of the amastigote differentiation state and virulence.

BSP121 (Guest Speaker)
Role of extinct retroposons located in the 3-UTRs of *Leishmania major* mRNAs
Frédéric Bringaud[1], Michaela Müller[2], Gustavo Coutinho[3], Cerqueira, Najib[3], M. A. El-Sayed[3], Barbara Papadopoulou[2] and Elodie Ghedin[3]

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Retroposons are mobile DNA sequences, which are quite abundant in the genome of *T. cruzi* and *T. brucei* (~3% of nuclear genome), while no potentially active retroposons have been characterized in L. major. We identified in the L. major genome a new large family of small mobile elements (LmSIDER) that fulfills all the characteristics of extinct trypanosomatid retroposons. SIDERs are ~70-times more abundant in L. major compared to *T. brucei* and have been found almost exclusively within 3'-untranslated regions of L. major mRNAs. We showed that LmSIDERs fulfill important biological functions such as the regulation of gene expression, whereas *T. brucei* developed other mechanisms to maintain such a cellular function. This is the first example in eukaryotes of the domestication and expansion of a whole family of mobile elements involved in the maintenance of a critical cellular function.

Session 6D

BSP287 (Guest speaker)
Structure and biosynthesis of glycoproteins in *Trypanosoma brucei*: basic and translational research
Michael Ferguson

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Trypanosoma brucei synthesises different glycoproteins in its disease-causing bloodstream stage and disease-transmitting insect stage. The organism is notable for its high copy number of glycosylphosphatidylinositol (GPI) anchored glycoproteins (1E7 variant surface glycoproteins per cell in the bloodstream stage and 3E6 procyclin glycoproteins per cell in the insect stage). GPI biosynthesis is essential for the bloodstream form of the parasite and a validated drug target. Surprisingly, GPI biosynthesis is not essential for the growth of the insect stage of the parasite and recent analysis of GPI null mutants has revealed other coat glycoproteins.
The glycoproteins of both life cycle stages are N-glycosylated with conventional oligomannose and/or complex glycans but the bloodstream stage parasites also express unique giant poly-N-acetyllactosamine (poly-LacNAc) containing structures throughout

their endosomal/lysosomal system. Poly-LacNAc side-chains are also found in insect stage GPI anchors.
I will provide an overview of glycoprotein structure, biosynthesis and sugar nucleotide metabolism in *T. brucei* and describe some of our recent attempts to translate this basic research into a drug discovery programme.

BSP193
Glycosomal ABC transporters of *Trypanosoma brucei*
Mariana Igoillo-Esteve & Paul Michels

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Three ABC transporters have been identified in the glycosomal membrane of *Trypanosoma brucei*. They have been designated GAT1 - GAT3 for Glycosomal ABC Transporters. They are so-called half ABC transporters, comprising only one transmembrane domain and a single nucleotide-binding domain. For GAT1 and GAT2 it was shown by immunofluorecence studies of trypanosomes transfected with deletion constructs fused to GFP that a short sequence motif adjacent to a transmembrane segment comprises the glycosome-targeting determinant. Protease protection assays indicated that the nucleotide-binding domain is at the cytosolic face of the membrane, suggesting that the transporters function as importers of substrates into the glycosome. GAT1 and GAT3 are expressed in both bloodstream-form and procyclic *T. brucei* while GAT2 is only found in bloodstream forms. Complementation studies with yeast mutants and phenotypic analysis of RNAi mutants suggest that the GATs transport fatty acids. Knocking down GAT1 resulted in overexpression of GAT3. Nevertheless this could not counteract the deleterious effect of the RNAi in procyclics grown in the absence of glucose, suggesting that both transporters have different substrate specificities.

BSP116
Vitamin C is essential in *Trypanosoma cruzi*
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Vitamin C (ascorbate) functions widely in eukaryotes as an antioxidant and enzyme co-factor. It is an essential dietary component of humans, who lack the last enzyme in the biosynthetic pathway. In *Trypanosoma cruzi*, the corresponding enzyme is a glycosomal FMN-dependent galactonolactone oxidase (GAL). *T. cruzi* lacks an ascorbate uptake capacity and GAL is essential. In contrast, GAL is dispensable in bloodstream-form *Trypanosoma brucei*, parasites which are also deficient in ascorbate uptake. *T. cruzi* possess an ER-localised ascorbate-dependent peroxidase (APX), an enzyme absent from *T. brucei*. APX is expressed constitutively and plays an important role in protecting *T. cruzi* against hydrogen peroxide. Double-knockout of APX is achievable only in the presence of an ectopic copy. The essential nature of this enzyme may therefore explain the requirement for vitamin C biosynthesis in *T. cruzi*.

BSP226

Acetate production in trypanosomatids and other parasitic protozoa

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Acetate:succinate CoA-transferases (ASCT) are acetate-producing enzymes found in hydrogenosomes, anaerobically functioning mitochondria and in the aerobically functioning mitochondria of trypanosomatids. We identified the enzyme for acetate production in the hydrogenosome-containing protozoan parasite *Trichomonas vaginalis*, which is the first hydrogenosomal acetate-producing enzyme to be identified. Interestingly, TvASCT does not share any similarity with the mitochondrial ASCT from *Trypanosoma brucei*, the only other eukaryotic succinate-dependent acetyl-CoA-transferase identified so far. The trypanosomal enzyme clearly belongs to a distinct class of acetate:succinate CoA-transferases. Apparently, two completely different enzymes for succinate-dependent acetate production have evolved independently in ATP-generating organelles. The kinetic differences and similarities in ASCT and the effects on acetate production in trypanosomatids will be discussed and compared with other parasites.

BSP295 (guest speaker)

Uracil incorporation and antifolate action in trypanosomes.

Dolores González-Pacanowska, Luis M. Ruiz-Pérez, Victor Castillo-Acosta & Antonio E. Vidal

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Antifolate mediated inhibition of thymidylate biosynthesis leads directly to depletion of dTMP and indirectly to an accumulation of dUMP which is converted to dUTP. Perturbations in the intracellular levels of dUTP and dTTP are detrimental and interrupt DNA replication. The enzyme deoxyuridine 5'-triphosphate nucleotidohydrolase (dUTPase) is responsible for the control of intracellular levels of dUTP and prevents the incorporation of uracil into DNA. In trypanosomes dUTPase is a member of the family of dimeric dUTPases which belongs to the newly described superfamily of all-alpha NTP pyrophosphatases. Here we report that treatment with antifolates induces DNA fragmentation and increased dUTPase activity while over-expression of the enzyme confers resistance. In addition, using RNAi we show that trypanosomal dUTPase is essential for DNA replication and cell viability. The knockdown of activity renders defects in G2/M progression, a pronounced increase in uracil-DNA glycosylase activity and hypersensitivity to the antifolate methotrexate. We conclude that in trypanosomes, dimeric dUTPase is an essential enzyme involved in the control of dUTP misincorporation.

Session 7D

BSP286 (guest speaker)
Trypanosome motility and the cell cycle
Markus Engstler

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In the world of low Reynolds numbers inertia does not exist. Here, hydrodynamic forces have properties that are very different from our common perception. We have shown that trypanosomes utilize hydrodynamic flow in a particularly clever way. By continuous and directional swimming, flow forces are generated on the cell surface that drag host-derived antibodies to the posterior cell pole, where they are rapidly internalized and destroyed. However, directional swimming is essential for bloodstream stage trypanosomes also in the absence of antibodies. Thus, a strong selective pressure other than immune attack must act on the parasites. We propose that trypanosome motility and the concomitant continuous intracellular deformation are critically required for cell cycle progression. The forces acting on the swimming trypanosome cell body have been quantified and modelled. We found that extrinsic mechanical forces are crucially involved in at least two distinct steps of the cell cycle, namely organelle segregation and final stage cytokinesis.

BSP207
The flagellum attachment zone of Trypanosoma: localisation and function during the parasite cycle.
Brice Rotureau, Thierry Blisnick, Sabrina Absalon & Philippe Bastin

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The *Trypanosoma brucei* flagellum is attached along the length of the cell body and defines cellular morphogenesis. The flagellum attachment zone (FAZ) is a complex structure found in the cell body that follows the flagellum. It comprises a FAZ filament and a specialised microtubule quartet. FLA1 is a transmembrane protein required for flagellum attachment although its mode of action is unknown. We localized FLA1 to the FAZ filament by immunofluorescence and scanning electron microscopy. Analyses of mutants lacking the flagellum revealed that FLA1 accumulated in cytoplasmic vesicles similar to the flagellar pocket protein CRAM. In contrast, FAZ filament constituents were still assembled in the cytoplasm. Thus, Fla1 appeared to directly contribute to flagellum adhesion along the cell body by docking on the FAZ filament via its cytoplasmic portion and to unknown flagellar membrane components via its external region. FLA1 and FAZ1 expression and localisation evolved during the parasite lifecycle. In experimentally infected tsetse flies, we found that they were co-expressed except in asymmetrically dividing long epimastigotes.

BSP108

A Kinesin involved in the assembly of an extra-axonemal structure in *Trypanosoma brucei*.

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Flagella and cilia are widespread organelles in the living world, where they are implicated in many cellular processes. Frequently they are composed only of a microtubular structure, the axoneme, surrounded by the membrane. This is constructed by an active machinery of intra-flagellar transport that brings precursors to the assembly site. In some instances extra-axonemal structures are present, such as the paraflagellar rod (PFR) in trypanosomes or the fibrous sheath in sperm cells. Nothing is known about the assembly of such structures. Here we report the involvement of a novel flagellar kinesin in the construction of the PFR. Functional analysis by RNAi revealed defects in PFR construction and cell survival. The axoneme is properly assembled, but the PFR is dramatically perturbed. Some regions of the flagellum exhibit large accumulation of PFR material, whereas others are only made of a naked axoneme. This is the first report of a protein essential for the assembly of an extra-axonemal structure.

BSP135

Characterisation of the putative glycogen synthase kinase 3beta LmxGSK3β of *Leishmania mexicana*

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The glycogen synthase kinase from *L. mexicana* LmxGSK3β is highly conserved in the different Leishmania species and has close homologues in other kinetoplastids. The serine/threonine kinase GSK3β is a key regulator of numerous cellular processes in eukaryotes. It has been identified to be involved in flagellar assembly and maintenance in *Chlamydomonas rheinhardtii**. These results sparked our interest in GSK3β as a potential interaction partner of other kinases from *L. mexicana* with a proven role in the regulation of flagellar length, particularly LmxMKK and LmxMPK3**. In vitro kinase assays demonstrated the kinase activity of recombinant LmxGSK3β. Using recombinant protein, we were able to show that LmxMPK3 is not an interaction partner of LmxGSK3β in vitro. Kinase assays using recombinant LmxGSK3β and *L. mexicana* cell lysates suggest the existence of several potential substrates. We are currently investigating the most promising of these proteins.

* Wilson N. F. and Lefebvre P. A., Eukaryot Cell, 3;5: 1307-1319, 2004

** Erdmann M., Scholz A. et al., Mol Biol cell, 17: 2035–2045, April 2006

BSP218

The prokaryotic proteasome is essential for the biology of Trypanosomatids

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The proteasome is a highly conserved proteolytic machinery that participates in the regulated turnover of proteins involved in essential biological processes such as the cell cycle. In Trypanosomatids, like in several other parasitic protozoa, two proteasomes coexist: the classical eukaryotic one, and a prokaryotic proteasome. We identified the genes encoding both components of this proteasome, namely HslV and HslU, with, surprisingly, two genes encoding the latter (HslU1 and HslU2). Using GFP-fused proteins expressed in *Leishmania major*, we could localise this complex at the mitochondrion. Further, RNAi inhibition of the expression of each of the three components lead to a cell growth arrest and to an accumulation of aggregated material in the mitochondrion of *T. brucei*. This shows that this 'ancestral' proteasome plays a specific and essential role in Trypanosomatids. Also, the coexistence of two necessary HslU proteins suggest a peculiar function of this complex in these organisms. Finally, our results allow a new approach for the development of specific drugs targeting this essential protease complex.

BSP181

Processing and localization of *Leishmania major* metacaspase

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Leishmania major expresses metacaspase (LmjMCA), a caspase-related cysteine protease. This essential protein is important in the life cycle of the parasite especially under stressful conditions and could play a role in programmed cell death. Structurally, LmjMCA is characterized by the presence of an N-terminal mitochondrial localization signal and a proline-rich C-terminus flanking the catalytic domain. We have previously shown that the catalytic domain of LmjMCA is generated by auto-processing. In this study, we characterized LmjMCA processing sites and subcellular localization. By biochemical fractionation and confocal microscopy, we showed that LmjMCA is present in the cytoplasm and in the mitochondrion and could be differentially processed and localized in the life-cycle or upon H2O2 treatment. Furthermore, over-expression of LmjMCA enhances susceptibility of parasites to oxidative stress. This differential expression is likely to be important in the interaction of the metacaspase with its hypothetical substrates and in its physiological role in the parasite.

BSP200
Inhibition of active nuclear transport is an intrinsic trigger of apoptosis in Trypanosomatids
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The link between nucleocytoplasmic transport and apoptosis remains controversial: nucleocytoplasmic exchange of molecules seems essential for the initiation and execution of apoptotis but inhibition of nuclear transport may also represent a powerful apoptotic trigger. The GTPase Ran plays multiple key roles: nucleocytoplasmic transport, spindle assembly, kinetochore function and nuclear envelope assembly, all controlled by the constitution of a RanGTP/RanGDP gradient. Among Ran partners, NTF2 appears to be solely involved in nucleocytoplasmic transport.
Here, we localised Ran and its partners RanBP2, CAS and NTF2 at the nuclear membrane in the Trypanosomatid *Leishmania major*. All decorated a perinuclear collar of a dozen beads colocalising with the nuclear pores. In *Trypanosoma brucei*, RNAi knockdown of the expression of the corresponding genes resulted in cell apoptosis. Our data support the hypothesis that active nucleocytoplasmic transport is not essential for the initiation and execution of apoptosis and that, in the absence of proapototic external stimuli, the impairment of the nucleocytoplasmic RanGTP/RanGDP gradient constitutes the intrinsic signal for triggering apoptosis.

Session 8B

BSP144 (Guest Speaker)
Stamp out sleeping sickness-An intersectoral approach to disease control
Sue Welburn

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Two areas in Uganda are affected by sleeping sickness; *Trypanosoma brucei rhodesiense* occurs in the east of the country and *T b gambiense* in the northwest. The geographic distribution of these parasites is separate, although there are concerns regarding overlap. The epidemic of *Trypanosoma brucei rhodesiense* sleeping sickness in eastern Uganda, which began in 1998 in Soroti District as a result of movements of the livestock reservoir of the parasite, has continued to spread. Limited resources committed to control interventions in Soroti District in 1999 failed to contain the outbreak and the disease spread to five further Districts: Kaberamaido, Dokolo, Amolitar, Lira and Apac.
The continued high prevalence of the parasite in cattle presents a significant risk for transmission to humans and for further spread of this neglected zoonotic disease.

The magnitude the animal reservoir for human sleeping sickness needed to be defined and quantified, together with delineated policy implications for targeted control and facilitation of training of medics, scientists and veterinarians. Interventions were urgently required to control outbreaks and reduce the high mortality resulting from sleeping sickness. This led to the formation of a Public Private Partnership aimed to 'Stamp Out Sleeping Sickness' (SOS). Phase 1 of the SOS campaign is being financed and supported by the veterinary pharmaceutical company CEVA Santé Animale and Industri Kapital (IK), a pan-European private-equity fund and is being implemented by Makerere University with inputs from the University of Edinburgh, the Co-ordinating Office for Control of Trypanosomiasis (COCTU) in Uganda and with support from WHO.
New PCR based diagnostics have helped identify accurately the reservoir of disease in cattle and demonstrate that restocking activities were responsible for the disease spreading around Lake Kyoga, Uganda. This led to development of a cattle-based approach to halt the spread of the acute form of sleeping sickness towards the Gambiense disease focus, complimenting efforts to trap tsetse flies or treat humans with the disease. In phase I, 220,000 head were targeted for trypanocide treatment in 5 districts in the overlap zone with follow on application of insecticide applied using restricted application technology (RAP) to prevent re-infection. The cost-effectiveness of this new approach attracted private funding from IK, to help underwrite Makerere University's veterinary program to prevent the disease in cattle using an inexpensive spray-on insecticide developed by CEVA.

BSP247

The population structure of *Trypanosoma cruzi* TCI displays extensive genetic diversity, multiclonality, and bottleneck or founder effect-associated human infections.

Martin Llewellyn[1], Michael Miles[1], Matthew Yeo[1], Michael Lewis[1], Hernan Carrasco[2], Jorge Vargas[3], Patricio Diosque[4], Sebastiao Valente[5], and Michael Gaunt[1]

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Trypanosoma cruzi infection is the most important parasitic disease in South America. We present the sylvatic population structure of *T. cruzi* (TCI; TCIIc) from over seven different countries using a panel of genome-wide microsatellite loci across ~200 isolates, in context with domestic *T. cruzi* populations from Venezuela and Bolivia (TCI). Sylvatic TCI parasites from arboreal ecotopes show extensive genetic diversity and multi-clonality, independent of geographic focus size. This observation is mirrored in TCIIc. However, terrestrial TCI ecotopes, associated with rodents 'sympatric' with arboreal TCI ecotopes, demonstrate genetic bottlenecks or founder effects. Moreover, a significant proportion of human TCI infections in Bolivia and Venezuela are associated with bottlenecks or founder effects with respect to sylvatic strains despite, in Venezuela, repeated potential exposure to TCI from incursion of arboreal vectors. The implications of these findings, in terms of sylvatic disease transmission, human disease transmission, and parasite pathogenicity, are discussed.

BSP220

Antibodies against sand fly saliva: efficient marker of exposure

Iva Rohousova, Jitka Hostomska, Michaela Vlkova & Petr Volf

Department of Parasitology, Charles University in Prague, Czech Republic

We found host antibodies as an efficient marker of exposure to sand flies. Antibody response to sand fly saliva was studied in experimental dogs and mice and in humans from an endemic focus of *Leishmania tropica* in Turkey. In repeatedly bitten hosts, anti-saliva antibodies are produced in large quantities and are highly specific. In experimental conditions, the levels of IgG and its subclasses reflect the intensity of exposure; the differences between high- and low-exposed hosts are detectable more than 6 months after the last exposure. In humans living in the endemic focus we demonstrated a positive correlation between the levels of IgG against saliva of the vector *Phlebotomus sergenti* and the presence of active *L. tropica* lesions. In conclusion, these results proved that monitoring host antibodies to vector saliva could be a useful tool in epidemiological studies, e.g. for evaluation of anti-vector campaign effectiveness or as a risk marker for transmission of vector-borne diseases.

BSP154

Evidence for acquired immunity against *Trypanosoma brucei*

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Trypanosomes cause disease in humans and livestock throughout sub-Saharan Africa. Although wildlife and cattle show evidence of clinical tolerance to trypanosomes, until now there has been no evidence of acquired immunity to natural infections. We discovered a distinct peak and decrease in age prevalence of *T. brucei s.l.* infection in wild African lions that appears to be driven by an exposure-dependent increase in cross-immunity following infections with the more genetically diverse species, *T. congolense s.l.* The causative agent of human sleeping sickness, *T. brucei rhodesiense*, disappears by 6 yrs of age apparently in response to cross-immunity from other trypanosomes, including the non-pathogenic subspecies, *T. brucei brucei*. These findings suggest novel pathways for vaccinations against trypanosomiasis despite the notoriously complex antigenic surface proteins in these parasites.

BSP239

Development of fluorescent fragment length barcoding for identification of African trypanosomes

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Accurate identification of trypanosome species in tsetse flies is necessary to understand the epidemiology of African tsetse fly-transmitted trypanosomes. To address this problem, we have developed a novel method for trypanosome identification, fluorescent fragment length barcoding (FFLB). FFLB uses an approach similar to that used for high-throughput microsatellite analysis. Four primer sets, with one of each pair fluorescently tagged, were designed to amplify small regions of ribosomal DNA with interspecies size variation, and an automated sequencer was used to determine the sizes of the PCR-amplified DNA. FFLB is a sensitive, high-throughput method and is able to identify all species of tsetse-transmitted trypanosome in single and mixed infections. The use of FFLB has led to the discovery of trypanosomes related to *Trypanosoma brucei* and *T. vivax* and has revealed the presence of trypanosomes related to *T. cruzi* in African terrestrial vertebrates.

BSP308

A spectrum of population structures in *T.b. rhodesiense.*

Annette MacLeod, Liam Morrison, Lindsay Sweeney, Anneli Cooper, Craig Duffy, Lorna MacLean, Martin Odiit, John Chisi, Peter Kennedy, Jeremy Sternberg, Mike Turner and Andy Tait

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The African trypanosome, *Trypanosoma brucei*, has been shown to undergo genetic exchange in the laboratory, but controversy exists as to the role of genetic exchange in natural populations. Here we have used multilocus microsatellite genotyping to determine if there is evidence of genetic exchange in 3 foci of disease of *T.b. rhodesiense* sleeping sickness. The results reveal dramatic differences in parasite population structure in different locations within a single sub-species. These include (1) a clonal population structure, where there is significant linkage disequilibrium and no evidence for mating; (2) an epidemic population structure, in which linkage disequilibrium results from temporal expansion of a few clones in an otherwise sexual population and (3) a panmictic or randomly mating population structure. Clearly *T.b. rhodesiense* utilizes a variable mating strategy in which the proportion of clonal propagation can vary probably depending on environmental conditions.

Session 8D

BSP041 (Guest speaker)
Biogenesis of the Trypanosome Endo-exocytotic Organelle is Cytoskeleton Mediated
Mélanie Bonhivers, Sophie Nowacki, Nicolas Landrein and Derrick R. Robinson

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Trypanosoma brucei is a protozoan parasite that causes African sleeping sickness. In *T. brucei*, end-exocytosis occurs exclusively through a sequestered organelle called the flagellar pocket (FP), an invagination of the pellicular membrane. The pocket provides the sole site for specific receptors and maintains them inaccessible from components of the innate immune system of the mammalian host. The pocket is also responsible for sorting of protective parasite glycoproteins targeted to, or recycling from, the pellicular membrane and the removal of host antibodies from the cell surface.
Here we describe the first cytoskeleton flagellar pocket protein BILBO1. BILBO1 functions to structure a cytoskeleton framework upon which the flagellar pocket is formed and is essential for FP biogenesis and cell survival. Remarkably, RNAi mediated ablation of BILBO1 in insect form procyclic parasites prevents FP biogenesis and induces vesicle accumulation, Golgi swelling, new flagellum repositioning and cell death. Cultured bloodstream forms are also not viable when subjected to BILBO1 RNAi. These results provide the first molecular evidence for cytoskeleton mediated flagellar pocket biogenesis.

BSP242
Katanins are essential regulators of the *Trypanosoma brucei* cell division cycle.
Caroline Clucas, Russell Eason, Jeremy C. Mottram and Tansy C. Hammarton

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Katanins are microtubule severing agents belonging to the AAA ATPase family. They are heterodimers, made up of a catalytic subunit and a regulatory subunit, which is important for substrate recognition by, and activity of, the catalytic subunit. A single putative regulatory subunit (KATR) and several putative catalytic subunits (KAT1-5) have been identified in *T. brucei*, which contrasts with other organisms, which have only a single catalytic subunit. Given the abundance of microtubule-based structures in the *T. brucei* cell, and the number of katanins present, it is likely these molecules are important regulators of microtubule dynamics throughout the trypanosome cell cycle. RNAi of KATR, KAT1 or KAT2 rapidly arrested growth of bloodstream form trypanosomes, resulting in the accumulation of post-mitotic cells (2N2K cells) lacking cleavage furrows, 2N1K and 0N1K cells, and at later time points, multi-nucleate cells, indicating that katanins are essential cell division regulators. Progress on this project will be reported.

BSP306

Identification of a palmitoyl acyltransferase regulating flagellar membrane trafficking by RNAi-based screening

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Flagellar membrane trafficking of the *T. brucei* calflagins depends critically on protein lipid modifications. Cotranslational myristoylation of calflagin is sufficient for simple membrane association, while postranslational palmitoylation seems to provide a secondary cue that sorts the protein from the pellicular into the flagellar membrane. To identify the palmitoyl acyltransferase (PAT) that catalyzes calflagin palmitoylation, we undertook a candidate-based RNAi screening approach. The *T. brucei* genome contains 12 genes predicted to encode proteins with the PAT canonical DHHC motif. We engineered *T. brucei* mutants targeting these genes individually and screened them for calflagin mislocalization. One of these mutants, depleted of TbPAT7, displayed calflagin mislocalization to the pellicular membrane, identical to what is observed in a calflagin C3A mutant that lacks palmitate. Identification of TbPAT7 will enable further investigation of calflagin structure and function as well as trafficking of acyl proteins to different membrane domains. Further, the PAT mutant library created in this investigation should allow PAT-substrate mapping and functional analysis of this important modification.

BSP175

Dissecting the centromeric-specific activity of topoisomerase-II in trypanosomes.

Samson Obado, Christopher Bot and John Kelly.

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Centromeres are the chromosomal loci where kinetochores are assembled. In Trypanosoma cruzi, transcriptional “strand-switch” domains, composed predominantly of degenerate retrotransposons, are central features of chromosomal regions required for mitotic stability. Etoposide-mediated topoisomerase-II cleavage, a biochemical marker for active centromeres, is concentrated at these domains. In Trypanosoma brucei, topoisomerase-IIα activity is also focussed at single chromosomal loci which encompass regions between directional gene clusters containing transposable elements and additionally, domains composed of AT-rich repeats. RNAi-mediated knockdown of topoisomerase-IIα in bloodstream-form *T. brucei* results in the abolition of centromere-specific activity and is lethal within 48 hours. Both phenotypes can be rescued by co-expression of the *T. cruzi* enzyme. This suggests that the elements governing centromere-specific topoisomerase-II activity have been conserved within the trypanosomes. The variable C-terminal domain of topoisomerase-II is thought to modulate function. We generated *T. brucei* lines expressing *T. cruzi* topoisomerase-II enzymes containing C-terminal truncations, and examined centromere-specific enzyme activity after the RNAi-mediated knockdown of endogenous topoisomerase-II. This has allowed us to delineate the region necessary for centromere-specific activity to six amino acids, which interestingly includes a putative SUMOylation motif.

BSP253

Genetic exchange in *Trypanosoma cruzi*: insights from analysis of DNA content variation and microsatellites

Michael Lewis[1], Martin Llewellyn[1], Michael Gaunt[1], Nidia Acosta[2], Hernan Carrasco[3], Patricio Diosque[4] & Michael Miles[1]

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Hybridisation events have shaped the evolution of *Trypanosoma cruzi* and may continue to do so given the experimental demonstration of an extant capacity for genetic exchange, which, in vitro, involves hybridisation by fusion followed by gene loss. Flow cytometric analysis of fluorescently labelled, cloned *T. cruzi* stocks (n=48) allowed quantification of relative nuclear DNA content and revealed wide variation both within and between genetic groups. Naturally occurring hybrid strains (TcIId/IIe) are approximately diploid, whilst experimental hybrids are aneuploid with a mean DNA content 1.73-fold higher than their parents. Long term culture of experimental hybrids resulted in significant reduction of DNA content in 4/6 hybrid clones. Multilocus microsatellite (MLMT) analysis confirmed that TcIId and TcIIe lineages are the products of distinct hybridisation events. It remains unclear whether natural hybrids are the products of meiosis or the apparent parasexual cycle identified in vitro; application of MLMT potentially provides a means to resolve this question.

TRYPANOSOMIASIS/LEISHMANIASIS ABSTRACTS (POSTERS)

BSP007

The Epidemiology of Human and Animal Trypanosomiasis on the Jos Plateau, Nigeria

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The Jos plateau in North-Central Nigeria is historically free of tsetse flies and trypanosomiasis.The lack of trypanosomiasis and abundant pasture has attracted large numbers of cattle herding pastoralists. The plateau is home to millions of cattle and plays a significant part in the economy of the cattle industry in Nigeria.

Beginning in 1982, increasing reports of animal African trypanosomiasis and tsetse fly infestation on the Jos plateau indicate that it has lost its tsetse and trypanosomiasis (T & T) free status over the past two decades.

This study aims to characterise the current epidemiology of the disease and its vector on the plateau and develop theories as to how it originally became infested with tsetse. A presurvey visit was conducted in September 2007 to gather information on human and livestock populations, animal husbandry practices and T & T occurrence using a Rapid Rural Assessment (RRA) questionnaire. The results of the RRA questionnaire point to 3 significant factors affecting T & T distribution on the plateau: cattle migration, topography and the Jos Crisis – an outbreak of religious conflict in 2001.

BSP008

Effect of treatment on different trypanosome species ratios especially mixed infections in cattle during mass treatment programme, Uganda.

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Human and animal African trypanosomiases are caused by a range of trypanosome species in sub-Saharan Africa. Field studies have revealed that many wildlife and domestic animals serving as natural host of Glossina often carry mixed infections of *T. brucei* s.l., *T. congolense* species and/or *T. vivax* species. This study has been conducted in centre Uganda, as a part of a large project, Stamp Out Sleeping Sickness (SOS) control programme. The aim of this work is to determine the extent of mixed infections in cattle and the effect of treatment programme on such mixed infections in terms of the establishment of relationships between these parasites. The results have shown that before treating the animals there is a predominance of *T. brucei*, but a shift to increased *T. vivax* prevalence is noticed 9 months after the implement of a block treatment programme. The shift in the balance between these parasites and the relation between the different trypanosomes will be discussed in this presentation.

BSP010

Detection of *Trypanosoma brucei* parasites using quantitative nucleic acid sequence based amplification assay (NASBA)

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Currently, conventional diagnosis of Human African trypanosomiasis (HAT) is by microscopic demonstration of trypomastigotes in blood, lymph and/or cerebrospinal fluid of clinically or serologically suspects. However, microscopic diagnosis of HAT is not sensitive enough and may give false negative results, thus denying patients necessary treatment of the otherwise fatal disease. For this reason, a highly sensitive technique needs to be developed to enhance case finding. Real-time NASBA assay based on amplification and concurrent detection of small subunit rRNA (18S rRNA) of *Trypanosoma brucei* has been developed. The analytical sensitivity of the assay was evaluated on nucleic acid from in-vitro cultured parasites, and blood spiked with parasites. The assay detected 10 parasites/ml using cultured parasites or spiked blood. Using clinical samples of appropriate controls and confirmed HAT cases NASBA specificity (100%) was determined. Statistical analysis showed that the developed real-time NASBA detected *T. brucei* in significantly more blood samples than microscopy did ($P<0.0001$); such a sensitive assay may become an alternative tool to confirm diagnosis of HAT.

BSP011

Cutaneous leishmaniasis in Suriname

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Cutaneous leishmaniasis (CL) is an increasing problem in Suriname, but a control programme is lacking in the country. CL puts a significant economic burden on affected people, often the poorest of endemic areas, and has a great social impact on quality of life as patients are often mutilated by CL, leading to severe stigmatization. The clinical picture is very diverse from single lesions to extensive ulceration as will be illustrated with some clinical cases. Diagnosis is difficult and treatment options are few, as Pentamidine the only available drug in the country has serious site effects, and failing (due to emergence of drug resistance?). Alternative drugs. like miltefosine, are needed and show promising efficacy as will be presented. Furthermore, insight in epidemiology and biology of the disease is lacking in the country. *L. guyansis* is thought to be the causative agent of CL in Suriname, but other species will now be reported. An inventory of CL distribution over the country is now being prepared and will be discussed. The reservoir remains unknown.

BSP012

Innate immunity in *Lutzomyia longipalpis*: putative genes and identification of nonspecific antiviral response

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Lutzomyia longipalpis is the major vector for visceral leishmaniasis in Brazil. Here we present the expression profile of immune/development related genes in different stages and Leishmania-infected insects. One defensin gene was expressed after de 4th larval stage, with increased expression in adults. MAP-K and V-ATPase genes had low expression levels in final stages of development and high levels in adults. Cactus and TGF-beta, with dual roles in development and immunity, had constant expression levels. RNAi has recently arisen as a convenient way of performing functional studies in insects. To establish RNAi assays in *L. longipalpis*, we have transfected cultured cells with double stranded RNAs (dsRNA), using West Nile virus-virus like particles (VLPs) expressing luciferase as model. Luciferase dsRNA caused a lowered production of VLPs as expected. Surprisingly, we found that unrelated dsRNAs, that included the *E. coli* β-galactosidase sequence, diminished the production of VLPs. This is the first report of a non-specific anti-viral response in an insect cell line.

BSP019

Possible role of Mismatch Repair components on differential genetic variability in *Trypanosoma cruzi*

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Three distinct isoforms of the TcMSH2 protein, which plays a major role in DNA mismatch repair pathway, can be identified amongst *T. cruzi* strains: TcMSH2a, TcMSH2b and TcMSH2c. Various studies indicated lower levels of genomic sequence variability in *T. cruzi* I strains compared to *T. cruzi* II and hybrid strains. A possible role of Tcmsh2 gene polymorphisms in generating such genetic variability in *T. cruzi* was evaluated. We measured the levels of oxidative stress-induced DNA damage and cell viability in the presence of different genotoxic agents. Our analyses support the hypothesis that strains belonging to *T. cruzi* I strains (which express the TcMSH2a isoform) present a more efficient MMR. We also performed similar analyses using Tcmsh2 single knockouts (CL-Brener strain). Attempts to knocking-out the second allele were unsuccessful, suggesting that the Tcmsh2 gene is essential, and may be multifunctional. Support: CNPq, FAPEMIG and HHMI.

BSP020

Molecular characterization of ribonucleoproteic antigens containing repeated amino acid sequences from *Trypanosoma cruzi*

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Protein components of ribonucleoproteic complexes and proteins containing repetitive amino acid sequences are among the most prominent antigens expressed in *Trypanosoma cruzi* amastigotes. Here we characterized two antigens, named TcRpL7a and TcRBP28, which carry similar repetitive amino acid motifs and share homology to L7a ribosomal protein and two *T. brucei* RNA binding proteins, respectively. Western blots and ELISA showed a strong humoral response in patients with Chagas disease, directed to the repetitive regions. Western blot using subcellular fractions and parasite transfections with GFP fusion proteins indicated that TcRBP28 is found dispersed in the cytoplasm whereas TcRpL7a is found in the nuclei and as well as in the polysomes. Current experiments are being carried out to evaluate the influence of the repetitive amino acids in the capacity of generating a protective immune response during *T. cruzi* infection. Support: CAPES, CNPq AND FAPEMIG (BRAZIL) and HHMI (USA).

BSP023

Kinetic analyses of the Leishmania inositol phosphorylceramide (IPC) synthase: a novel drug target.

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Sphingolipids are essential components of eukaryotic membranes. Notably organisms such as the pathogenic fungi and protozoa synthesize inositol phosphorylceramide (IPC), from the substrates ceramide and phosphatidylinositol, as the primary phosphosphingolipid rather than sphingomyelin, ceramide and phosphatidycholine, as do mammals. This reaction is catalysed by IPC synthase, an enzyme with no mammalian equivalent encoded by the AUR1 gene in yeast and recently identified functional orthologues in the pathogenic kinetoplastid protozoa. As such this enzyme represents a promising target for novel anti-fungal and anti-protozoal drugs with minimal toxic side effects. Given the paucity of effective drugs treatment for kinetoplastid diseases such as Leishmaniasis, there is a need to characterize the protozoan enzyme. To this end we have established a fluorescent-based activity assay protocol in a 96-well plate format, using CHAPS-washed microsomal material isolated from auxotrophic AUR1 mutant yeast complemented with the *Leishmania major*

enzyme, LmIPCS. Using this system the full kinetic parameters of the enzyme have been established.

BSP026

Molecular characterization for detection and typing of the recovered leishmania species from *Nesokia indica* (Rodentia; Muridae) in Ghasre-Shirin in the boundary of Iran and Iraq.

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Zoonotic Cutaneous Leishmaniasis (ZCL) is an important health problem at the half of 30 provinces of I.R.Iran. A study was made both rural and urban localities in the west part of Iran during June and September 2007. The study area was previously encountered as a *Tatera indica*. In this investigation, 19 rodents was caught at outdoor and identified as *Nesokia indica* (52.6%), *Tatera indica* (26.3%) and *Mus musculus* (21.1). The finding was revealed that one out of ten *Nesokia indica* was naturally infected to amastigote form of *Leishmania spp*. This isolate identified by RAPD-PCR method using 5 primers (AB1-O7, 327, 329, A4 and M13) as *L. major*. This is the first record for incrimination of *N. indica* as reservoir host for *L. major* which occurred at hypo-endemic foci of ZCL at Ghasreshirin district, Kermanshah province, Located in the boundary of Iran and Iraq.

BSP027

Tamoxifen in the treatment of leishmaniasis: in vivo and in vitro analysis of drug activity

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Tamoxifen (TAM) is a triphenylethylene widely used in the treatment of breast cancer. We have previously demonstrated that TAM killed Leishmania promastigotes and amastigotes with EC50% in the micromolar range. We have also provided evidence that TAM induces a rapid and long-lasting alkalinization of the vacuolar environment in *Leishmania amazonensis*-infected macrophages. Here we show, by oligonucleosomal fragmentation analysis, anexin V/propidium iodide labeling and ultrastructural studies, that TAM-treated promastigotes and amastigotes do not die by programmed cell death. On the other hand, using HPTLC and RP-HPLC analysis, we verified that 10 µM of TAM reduced the incorporation of [14C]-leucine into dolichol, ergosterol and ubiquinone in promastigotes. We have also tested TAM's activity in vivo. *Leishmania amazonensis* and *Leishmania braziliensis*-infected BALB/c mice treated with TAM for 2 weeks presented a significant reduction in lesion size and parasite burden. Given that clinical safety is well established for this drug, we suggest

TAM should be considered as a new alternative in leishmaniasis chemotherapy. Support: FAPESP, CNPq.

BSP030

Lipoic acid metabolism in *Leishmania major*

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Lipoic acid plays an essential role as a cofactor of a-ketoacid dehydrogenase complexes (a-KADHs), which are central to energy production. Lipoylation of a-KADHs occurs in Leishmania major, as shown by Western blotting using an antibody directed against protein-bound LA. The level of lipoylation of the enzyme complexes varies during the development of the parasite. Analyses of the variations provide insight into the utilisation of carbon sources by the parasite under different environmental conditions and stresses.
Genes for both biosynthesis and salvage of LA are present in the *L. major* genome. Attempts to ablate either pathway in promastigotes by gene knockout have proved unsuccessful. This implies that both biosynthesis and salvage of LA are important to parasite fitness. Studies are underway to investigate their importance to the amastigote form of the parasite.

BSP033

Genomic context affects the expression of genes in *Leishmania major*

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We have previously generated a *L. major* heterozygous mutant for the RPC2 gene, in which a selectable marker (SAT) was integrated at one of the telomeres of chromosome 20. Unsuccessful efforts to generate a RPC2 null mutant did not lead to amplification of the locus suggesting that maintenance of copy number and expression of this telomere-located gene is under strict control. Our results show that the expression of SAT integrated into the genome may vary depending on its location. We have compared the heterozygous RPC2 cell line with a mutant carrying SAT integrated within chromosome 23.The expression of the marker located at the chromosome end was notably dependent on the presence of the selective drug in the culture. The transcriptional activity in higher eukaryotes genes can be highly influenced by variations in chromosome packaging. Based on these results we hypothesized the presence of silencing mechanism associated with the telomeres of *Leishmania major*. Supported by FAPESP, CAPES and CNPq.

BSP034

Hus1-like gene of *Leishmania major* protects DNA from genotoxic stresses.

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The amplification of *Leishmania major* H locus is involved in drug resistance. The parasite LmHUS1 gene is encoded within H-region and is widely conserved. In yeast and mammals, the Hus1 protein is a checkpoint protein that forms a complex with Rad1 and Rad9 (911 complex) to encircle damaged DNA participating in maintenance of genome integrity. LmHUS1 transfectants presented resistance to genotoxic drugs Phleomycin and Hydroxyurea. The phleomycin resistance correlates to the maintenance of chromosome integrity, as seen in molecular karyotype analysis by pulse field gel electrophoresis and TUNEL assays. The involvement in the cell cycle progression was initially investigated in synchronized cultures treated with the phleomycin and analyzed by flow cytometry. These experiments showed that the increase in the expression of LmHUS1 alters the pattern of progression through the cell cycle. Considering that LmHUS1 functions in DNA damage repair and in the control of replication, we hypothesize that this gene participates in the phenomenon of DNA rearrangement and in the formation of amplicons in this parasite. Supported by: FAPESP, CAPES and CNPq.

BSP038

***Trypanosoma cruzi* modulates the immune system via binding to Siglec-E**

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Trypanosoma cruzi, the aetiological agent of Chagas disease, causes a strong immunosuppression leading to dissemination and persistence of parasites in host cells. Thereby the sialylation of the parasite surface is crucial for its survival in the mammalian host. Here we provide evidence that murine Siglec-E directly interacts with sialylated ligands on *T. cruzi*. Siglecs are sialic acid-binding Ig-like lectins that are expressed on immune cells and act as inhibitory receptors, ascribed to the presence of tyrosine-based inhibition motifs in their cytoplasmic regions. Using a Siglec-E-Fc fusion molecule we demonstrate that Siglec-E binds with high affinity to pathogenic parasites of the Tulahuen strain, whereas only a weak binding of Siglec-E to apathogenic parasites of the Tehuantepec strain was detectable. Cross-linking Siglec-E on dendritic cells results in a significantly decreased production of IL-12, a cytokine that is essential for the control of *T. cruzi* infection. These results raise the possibility that pathogenic parasites dampen immune responses via binding to the inhibitory receptor Siglec-E, allowing the parasites to persist and to establish chronic infections.

BSP042

Molecular and physical regulators of cytokinesis in *Trypanosoma brucei*

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Cytokinesis in *Trypanosoma brucei* occurs by the ingression of a cleavage furrow along the length of the parasite, following the helical path of the microtubule cytoskeleton. Polo-like kinase (PLK) is known to regulate furrow ingression in bloodstream form trypanosomes (Hammarton et al., 2007 Mol. Microbiol. 65:1229-1248), and we believe it likely that cytoskeleton alterations will also play a regulatory role.

The biochemistry and function of PLK are being probed further using recombinant 6xHis-tagged PLK purified from *Escherichia coli*. His-PLK, but not a kinase dead variant, exhibits autophosphorylation and transphosphorylates α and β-casein in vitro. His-PLK will be used to search for binding partners, substrates and inhibitors.

Investigations into how cytoskeletal modifications affect cytokinesis are also in progress. Treatment of *T. brucei* with microtubule stabilising or destabilising agents has been found to affect furrow ingression. Additionally, functional analysis of an AIR9 homologue, (a plant microtubule-associated protein and cytokinesis regulator) suggests that this protein is essential for trypanosome cell division.

Progress on these projects will be reported.

BSP043

Immunobiochemical evaluation of antileishmanial effects of trinitroglycerin as nitric oxide donor In BALB/C mice infected with *leishmania major* MRHO/IR/75/ER

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The recommended drugs used for the treatment of both visceral (VL) and cutaneous leishmaniasis (CL), the pentavalent antimonials, were first introduced 60 years ago. The effects of CL were investigated on Nitric Oxide (NO), essential trace elements (Zn/Cu) and liver enzymes [Alkaline Phosphatase (ALP), both Serum Glutamic Oxaloacetic Transaminase (SGOT) and Pyruvic Transaminase (SGPT)] and alterations during successful trinitroglycerine (TNG) therapy in vivo in susceptible Balb/c mice. TNG as NO donor was used for its ability to increase NO and to modify leishmania pathophysiology. Liver, spleen and lymph nodes were also studied as target organs to detect amastigotes. NO was detected by Griess Microassay (GMA), Zn and Cu levels were measured by Flame Atomic Absorption Spectrophotometer (FAAS) and liver SGOT, SGPT and ALP were determined by Auto Analyzer RA1000. Results of this study cleared that differences between control and test groups were correlated with immuno-biochemical factors and point to a partial involvement

of NO in the cytotoxic activity of macrophages against leishmania. Statistical analysis of data revealed the survival of leishmania inside the macrophages and its proliferation was affected by TNG as a novel chemotherapy, which indicated an association between NO increases with the pathology decrease.

BSP045

The Serine Peptidase Inhibitor (ISP2) of *Trypanosoma cruzi*: influence on host cell invasion

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Ecotins are bacterial high affinity inhibitors of family S1 serine peptidases, such as trypsin and neutrophil elastase (NE). In *E. coli*, ecotin was found to exert a protective role against NE. Genes similar to ecotins were identified in the genomes of *T. cruzi*, *T. brucei* and Leishmania, and were designated Inhibitors of Serine Peptidases (ISPs). No S1 peptidases are encoded in the *T. cruzi* genome raising the possibility that ISP could modulate host enzymes. We cloned and expressed *T. cruzi* ISP2, showing that it is a potent inhibitor of trypsin and NE. Western blot of parasite lysates revealed that ISP2 expression is higher in epimastigotes than in trypomastigotes and amastigotes. The addition of rec-ISP2 during the interaction of trypomastigotes with epithelial or muscle cell lines or its overexpression in the parasite significantly reduced parasite entry, suggesting that host serine peptidases might play a role in parasite invasion.

BSP053

Functional Characterization of Recombinant *Trypanosoma cruzi* DNA Polymerase eta

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Analysis of the complete genome of *Trypanosoma cruzi* revealed the existence of four polymerases families: A, B, X and Y. The Y family is composed by translesion synthesis DNA polymerases, inside this family we find DNA polymerase eta. Through localization assays, we confirmed that Tcpoleta is a nuclear polymerase. Tcpoleta showed to be an active enzyme, being capable to synthesize from different DNA templates in vitro, including those with an 8oxoG lesion. In vivo experiments showed that parasites overexpressing poleta are more resistant to H2O2 and MMS agents than the wild-type. Wild-type and transfected strains showed similar behavior against cisplatin and UV light. Interestingly, overexpressing poleta cells were unable to recover the cell growth after gamma radiation. These data indicate that this

polymerase present different functions in *T. cruzi* when compared with other eukaryotic cells.

BSP054

Analysis of cysteine biosynthesis in Leishmania

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Leishmania acquire cysteine through two distinct pathways – the so-called trans-sulfuration pathway and the de novo biosynthesis pathway. The latter does not exist in mammals and therefore is potentially attractive as a drug target. In order to test this hypothesis, *Leishmania donovani* promastigotes were genetically manipulated to generate cysteine synthase null mutants, and lines re-expressing the gene. The phenotype of these lines with respect to thiol content and susceptibility to sodium stibogluconate will be presented. A parallel study revealed that thiol-dependent reductase 1 (TDR1), an enzyme that preferably uses glutathione as a reductant, might have a role in the resistance of the parasite to antimonials, perhaps through detoxification of Sb(III).

BSP056

The phenotype of J-binding protein 2 (JBP2) knock-out in Leishmania

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The genomic DNA of kinetoplastid parasites contains a hypermodified base, □-D-glucosylhydroxymethyluracil, or J. Base J localizes to repetitive DNA elements; in Leishmania, it is found almost exclusively in the telomeres. To unravel the function of base J, we seek to target proteins involved in its synthesis. We have identified two proteins, JBP1 and JBP2, which are likely to be the enzymes catalyzing the first step in J biosynthesis. JBP1 is essential in Leishmania, but JBP2 turned out to be dispensable. The JBP2-/- cells gradually lose J, eventually reaching a ~8-fold reduction in comparison to WT. Under standard conditions, these cells grow normally, but they are highly sensitive to bromodeoxyuridine (BrdU), a thymidine analogue that lowers J-levels in kinetoplastids. BrdU-treated JBP2-/- cells grow ~100-fold slower than controls and their J-levels are almost undetectable. The treatment does not affect the cell cycle profile, but cell death increases considerably at later time points. This phenotype is completely rescued by ectopic expression of JBP2, and we are currently further analyzing it.

BSP061

A survey of Aluminium phosphate effects on efficacy of plasmid DNA encoding thiol- specific antioxidant (TSA) against *Leishmania major*

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TSA is the immuno-dominant antigen of *Leishmania major* which is considered as the most promising molecule for a recombinant or DNA vaccine against leishmaniasis.
In this study ,we evaluated antibody responses after immunization with pcTSA and pcTSA+AlPO4 and after the challenge with *L. major*, which were significantly higher than in control groups($p<0.05$).Although pcTSA +AlPO4(vaccinated group) elicited IgG antibody value that were greater than in the sera of the immunized mice with pcTSA alone, respectively and there was no statistical significant difference between each related two groups ($p>0.05$) following immunization and after the challenge infection.
IFN-γ values were markedly increased in the pcTSA and pcTSA+AlPO4 groups, which were significantly higher than in the control groups ($p<0.05$) following immunization and after the challenge with *Leishmania major*. Although pcTSA+AlPO4 elicited IFN- γ values were greater than the immunized mice with pcTSA alone, there were no statistically different between these two groups ($p>0.05$). IL-4 values were increased in all groups, but there was no statistical difference between the groups ($p>0.05$) following immunization and after the challenge with *Leishmania major*.

BSP066

The role of TbZC3H4 in *Trypanosoma brucei* differentiation

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Trypanosomes rely mainly on post-transcriptional mechanisms to control gene expression, which is exemplified by the paucity of transcription factor homologs and an overrepresentation of certain classes of RNA-binding proteins, e.g. CCCH zinc finger proteins in their genomes. TbZC3H4, a *T. brucei* tandem CCCH zinc finger protein was identified in a screen for Tb14-3-3 binding partners using the TAP-tagging approach. Co-purification of phosphorylated TbZC3H4 with Tb14-3-3 was observed in procyclic form cells only. TbZC3H4 is not essential in monomorphic bloodstream or procyclic form cells, whereas depletion of the protein in the pleomorphic AnTat 1.1 cell line affects differentiation to the procyclic form as assessed by the gain of EP1 procyclin. Microarray analyses were used to identify differentially expressed genes in cells depleted of TbZC3H4 compared to cells expressing wildtype levels of the protein during differentiation. Moreover, transcripts specifically binding to TbZC3H4 are currently being investigated and results will be presented.

BSP073

New curcumin derivatives against Trypanosomes and Leishmania.

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Leishmaniasis and Human African Trypanosomiasis are diseases caused by Kinetoplastid parasites: *Leishmania sp*. and *Trypanosoma brucei* sp. respectively. Over 98 new analogues of curcumin have been screened on three protozoan parasites: *Trypanosoma brucei* (bloodstream forms), *Leishmania major* (promastigotes) and *Leishmania mexicana* (axenic amastigotes). The compounds have been also tested on the Human Embryonic Kidney (HEK) cell line. The results showed that some of the curcumin analogues have a clear activity, with EC50 values of 21 compounds between 50 nM and 3 µM. Using resistant trypanosome lines, no cross-resistance was observed with existing trypanocides. Little effect was observed on HEK cells. The most promising compounds have been assessed for their effects on the viability, GSH content and protein content of rat hepatocytes. None of the test compounds displayed a significant effect on hepatocyte viability or protein content, but one of the lead compounds rapidly depleted GSH. Some of the compounds act very rapidly and non-reversibly on trypanosomes in vitro. The effect of some these drugs on DNA content and cell death were also analyzed using FACS. No toxicity has been noticeable up to 50 mg/kg BW intraperitoneal administration of these analogues.

BSP076

The UDPGlcNAc biosynthesis pathway in *Trypanosoma brucei*

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N-Acetylglucosamine and glucosamine are the only aminosugars identified so far in *Trypanosoma brucei.* It was recently demonstrated (Stokes and Ferguson, unpublished) that UDPGlcNAc biosynthesis is essential for the bloodstream form of the parasite but the complete UDP-GlcNAc biosynthesis pathway has not yet been described. *Trypanosoma brucei* expresses many glycoproteins rich in these hexosamines. In the bloodstream form the major surface protein, VSG, contains glucosamine in GPI anchors and GlcNAc in N-linked glycans, and there are other glycoproteins with giant poly-N-acetylactosamine N-linked glycans. In the procyclic form, some procyclins are N-glycosylated and all have GPI structures containing poly-N-acetylactosamine chains. Bioinformatic analysis of the trypanosome genome indicated a conventional eukaryotic pathway for UDPGlcNAc biosynthesis involving four enzymes. These are Glutamine:fructose-6-phosphate amidotransferase (GFA1), Glucosamine-6-phosphate N-acetyltransferase (GNA1), Phosphoacetylglucosamine mutase (PAGM) and Glucosamine 1-phosphate uridylyltransferase (UAP), but only the last enzyme has been biochemically characterized. Here we present results obtained from cloning and expressing the first three enzymes (GFA1, GNA1 and PAGM) involved in

Trypanosoma brucei UDPGlcNAc biosynthesis. These results support the bioinformatics-predicted eukaryotic pathway.

BSP078

Cloning and Sequencing of *Leishmania major* Thiol-specific-antioxidant Antigen (TSA) gene

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Leishmaniasis is caused by an intracellular protozoan parasite. *Leishmania major*, is widespread throughout the world. Infection with HIV/AIDS can increase the risk of developing leishmaniasis by 100- to 1000-fold.
Treatment of this disease is difficult due to toxic and side effects and resistance of available drugs, resistant parasite variants in cases of cutaneous and visceral leishmaniasis have become more common and reinfection occurs rapidly. development of either new anti-leishmania drugs or a vaccine is an attractive alternative. Immunity against reinfection is acquired following cutaneous infection with *Leishmania spp*., suggesting that prophylactic immunization is feasible.
In the present work, first *Leishmania major* was producted. The following strain of *L. major* was used (MHRO/IR/75/ER) (Friedlin strain). Then genomic of DNA was extracted and used for amplifying of TSA gene as a template. Then PCR product extracted from agarose gel was cloned into PTZ57R/T vector and plasmid containing TSA gene (PTZ57R/T-TSA) was extracted from transformed bacteria (TG1 strain) and sequenced. Compare of extracted plasmids on 0.8% agarose gel.
PCR amplification of TSA gene with plasmid extracted from white colonies bacteria (pT-TSA)
Enzyme digestion of plasmid extracted from white colonies bacteria (pT-TSA) by restriction enzymes. The plasmids extracted from white colonies bacteria (PT-TSA) were sequenced.
Nucleotide sequence analysis of the TSAcloned in pTZ57R/T vector revealed 90% sequence identity and high homology with strain (GenBank Accession No.AFO44679or Lmjf15.1080).
TSA is the immunodominant antigen of *Leishmania major* promastigote and amastigote being considered as the most promising molecule for a recombinant vaccine or such as DNA vaccine against Lieshmaniasis.
Among the vaccine candidates, TSA (an enzyme homologous to eukaryotic thiol-specific antioxidant proteins) is one of the predominant vaccine candidates. TSA is L.major recombinant protein homologue to eukaryotic thiol –specific-antioxidant protein. TSA elicites a Th1 response, stimulated high titers of IgG2a in *L. major* infected BALB/C mice .Compared to selected other antigens. TSA DNA – vaccinated mice showed excellent and stronger protection than the mice vaccinated with the other antigens DNA-vaccinated. TSA production could be a preliminary step for further research in designing diagnostic kit or effective vaccine against Leishmaniasis.

BSP079

Comparison chemical drugs with plant drugs (Alkanna tinctoria-Peganum harmala-Ferula assa feotida) on sore and progressive recovery of leishmaniasis in Balbc mice infected with *L. L. major.*

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Leishmaniasis contains two kinds: cutaneous and visceral that are reported in Iran. L.L.major is appeared in Balb/c mice inform of visceral leishmaniasis with sores in place injection. Leishmania are intracellular protozoa and multiplied in macrophages.
The aim of this research was to study comparison chemical drugs with plant drugs on sore and progressive recovery of Leishmaniasis in Balb/c mice infected with *L.L.major*.
We choice 5 groups of Balb/c mice (n=6) that theses groups contain: the healthy group as the control and infected group as the other control. Two groups treated with chemical drugs (Glucantime & Amphotricine B).group that treated with plant drugs (Alkanna tinctoria-Peganum harmala-Ferula assa feotida) .with injection 2*106 parasites in tail of mice the sore created after 30 days. treatment with chemicals drugs was 28 days & with plant drugs was 35 days .mice were treated once daily .diameters of sores were measured weekly by caliper. Mice were maintained 4 months after treatment.
Measure death & recure in group treated with plant drugs was lesser than groups treated with chemical drugs. The difference between chemical groups and plant group were not significant P>0.05.
In group treated with Glucanime 70% cured & 30% recurred in group treated with Amphotricine B 65%cured &35%recurer.in group treated with plant
group (Alkanna tinctoria-Peganum harmala-Ferula assa feotida) 75%cured & 25% recured.use of plant drugs is effective in treatment of leishmaniasis.

BSP083

Identification of a novel putative glycosyltransferase in *Trypanosoma brucei*

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The protozoan parasite *Trypanosoma brucei* causes human African sleeping sickness. Parasites survive in their host through immune evasion aided by their unique Variant Surface Glycoprotein (VSG) coat. Additionally, Trypanosomes contain other less abundant glycoproteins. Major glycan structures in Trypanosomes are poly-N-acetyllactosamines. Their components galactose and N-acetylglucosamine are essential. Therefore UDP-Gal and UDP-GlcNAc-dependent glycosyltransferases (GTs) are potential drug targets. We identified novel putative Gal/GlcNAc GTs by bioinformatic analysis of the genome. One of these GTs, TbGT15, is a single copy gene, expressed in both parasite life cycle stages. To determine gene function we created null mutants in bloodstream-form parasites. Since knock-out cells were able to infect mice and were viable in culture TbGT15 is not essential. However, total glycoprotein extracts from TbGT15 mutant cells had different lectin binding

properties. The glycan composition of ricin-binding GPs was determined by methylation linkage analysis. According to our data TbGT15 is involved in the synthesis of poly- N-acetyllactosamine containing GPs in bloodstream-form cells.

BSP087

Evaluation of the host response to infection with *Leishmania guyanensis* parasites

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Infections with *L. guyanensis* are distinguished by their ability to disseminate from the initial site of infection to the nasopharyngeal tissues forming destructive secondary lesions. It is known that the outcome of infection is influenced by both the host immune response and the infecting parasite. The factors involved in parasitic dissemination are, however, poorly understood.

To further understand the mechanism by which *L. guyanensis* parasites form Mucocutaneous Leishmaniasis in the host, metastatic and non-metastatic disease causing parasites were used to infect macrophages and the differential gene expression was analysed using cDNA microarrays. Real-time PCR and protein analyses in macrophages from C57BL/6 and BALB/c mice confirmed that two chemokines important for a Th1 immune response were differentially expressed together with cytokines including TNFα, and IL6. Further investigation is underway to investigate the role of TLRs in the macrophage response to infection with *L. guyanensis* parasites.

Modulation of the macrophage response with the metastatic parasites is likely to facilitate parasitic survival, and promote the development of Mucocutaneous Leishmaniasis.

BSP089

The casein kinase 1 (CK1) family of *Leishmania mexicana*

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The casein kinase 1 (CK1) family of serine/threonine protein kinases play a variety of crucial roles in eukaryotic cells including DNA repair, cell cycle control and membrane trafficking. Three homologues of CK1 have been cloned from *L. mexicana* (CK1.1, 1.2 and 1.3). Recombinant CK1.2 has been expressed and purified from bacteria. Full-length recombinant CK1.2 phosphorylates α-casein but is insensitive to CK1-specific inhibitors. This is in contrast to mammalian CK1 which is inactive when expressed as the full-length form and whose C-terminally truncated active form is sensitive to these inhibitors. We have successfully prepared an active C-terminal truncation mutant of CK1.2 and compared its inhibitor sensitivity to full-length CK1.2 and the C-terminally truncated active form of rat CK1δ. The role that the C-terminus plays in inhibitor sensitivity and substrate binding will be discussed. In addition to this, targeted gene knockout has generated CK1.1 null mutants and CK1.2 heterozygote KO mutant parasites. Phenotype analysis of these mutants will be discussed and related to the role of CK1 in Leishmania.

BSP091

Intracellular trafficking of potential virulence factors in *Leishmania major*

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Leishmania resides inside mammalian macrophages from where it is thought to manipulate the host immune system. The cysteine peptidase CPB has been shown to be released by the parasite and act as a virulence factor. CPB is released through the flagellar pocket, while trafficking through there to the lysosome, or through direct secretion from the lysosome. We are interested to discover if other peptidases are secreted and whether they also act as virulence factors. To study lysosome function, we are analysing the location and role of a potential lysosomal membrane (LAMP like) protein. Lysosome-related organelles like acidocalcisomes might also be involved in virulence, as defects in trafficking of acidocalcisome membrane proteins such as the proton pyrophosphatase (V-H+-PPase) cause loss of *L. major* virulence (Besteiro et al. 2008, J. Cell. Sci., in press), so we are now investigating trafficking of acidocalcisomal proteins in more detail. Localisation analyses using GFP-tagged protein and fluorescence microscopy, together with generation of mutants and analysis of phenotypes will be discussed.

BSP097

A mouse model to evaluate the role of CTLs in leishmania DNA vaccines

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Leishmaniasis is a worldwide disease prevalent in tropical and subtropical countries. The exact role of cytotoxic T lymphocytes (CTL) has not yet been fully established. In the present study, a model was developed to assess the CTL and antibody response induced by immunisation with *L. mexicana* gp63 cDNA administered either by i.m injection or using gene gun. The CTL activity was evaluated against CT26 tumour cells transfected with *L. mexicana* gp63 cDNA as target cells in a short term 51Cr-relased cytotoxicity assay. Dendritic cells (DCs) loaded with leishmania soluble antigen (1) were also shown to be a suitable target to measure CTL activity. The results clearly demonstrated that higher protection to *L. mexicana* infection was induced by gene gun DNA-immunisation versus i.m injection. CTL activity of splenocytes was observed in mice immunised with *L. mexicana* gp63 cDNA irrespect to the method of immunisation. Long-term CTL activity was also observed in immunised and re-challenged mice but not control mice infected with the parasite.

BSP100

Glycoconjugate analysis of the *Trypanosoma brucei* transferrin receptor.

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The transferrin receptor of *Trypanosoma brucei* is found only in the flagellar pocket and is a heterodimer of gene products ESAG6 and ESAG7. Host transferrin is the only source of iron for trypanosomes so the transferrin receptor is essential in blood stream form. The trypanosome transferrin receptor binds host transferrin and the complex is endocytosed. The transferrin-transferrin receptor complex becomes disassociated in the endosomal compartment, the receptor being recycled back to the flagellar pocket. The transferrin receptor proteins, ESAG6 and ESAG7, contain several N-linked oligosaccharides and the ESAG6 protein is attached to the flagellar pocket membrane via a glycosylphosphatidylinositol (GPI) anchor.
Proteomic scale studies revealed that the oligosaccharides of the ESAG6 GPI are similar to the complex structure found in MITat 1.2, variant surface glycoprotein (VSG) 221. Using proteomic scale analysis and precursor ion scanning mass spectrometry we also found that the GPI anchor of the ESAG6 protein carries the same di-myristoyl lipids found in all VSG anchors. Analysis of the N-linked oligosaccharides is underway and initial indications suggest they are mostly high-mannose structures.

BSP101

A Serine-Threonine Phosphatase involved in Nuclear Positioning in Trypanosomes

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Phosphorylation and dephosphorylation of cellular proteins are important steps in the control of major biological events in many eukaryotes. It was shown previously that treatment of *Trypanosoma cruzi* with CalyculinA, an inhibitor of PP1 and PP2a, caused a change in morphology from trypomastigotes to amastigotes, accompanied by the nucleus and the kinetoplast coming closer together. At the onset of this project only one PP1 gene was known. In view of the difficulties to genetically manipulate *T. cruzi*, we decided to silence the expression of this gene by RNAi in the related organism *T. brucei*. The knockdown cells grew at a normal rate, but displayed a clear phenotype with the nucleus and the kinetoplast in unusual close proximity. We determined that the nuclear position was faulty: it was found too posterior when compared to wild type (or control 29-13) cells. PP1 could therefore be an important regulator of nuclear positioning in trypanosomes.

BSP105

Identification of proteins interacting with the metacaspase of *Leishmania major*

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The *Leishmania major* metacaspase (LmjMCA) has recently been characterized as a caspase-related cysteine protease able to cleave substrates after and arginine in P1. This enzyme was found to be involved in programmed cell death (PCD) when expressed in yeast cells knocked-out for the endogenous metacaspase YCA1. However, the physiological role of LmjMCA in Leishmania parasites is not known. In order to identify the L. major proteins that may function as substrates, inhibitors, or may bind and recruit LmjMCA, a yeast two-hybrid screening with cDNA libraries from different life cycle stages of the parasite was conducted. Inactive complete sequence and catalytic domain of LmjMCA were used as bait proteins. Five proteins annotated in the *L. major* genome as "hypothetical" were reiteratively found as interacting with LmjMCA. Interestingly, two proteins putatively involved in PCD were also identified as interacting with LmjMCA. Additionally, the interaction of LmjMCA with proteins involved in other physiological processes such as vesicle transport, suggests that LmjMCA could have additional roles during the life cycle of Leishmania parasites.

BSP106

The discovery of a new species of trypanosome in Tanzania closely related to *Trypanosoma brucei*.

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A new trypanosome genotype was discovered during the analysis of trypanosome infections in wild-caught tsetse flies in Tanzania using the novel technique of fluorescent fragment length barcoding (FFLB). The trypanosome was found in infected *Glossina pallidipes* caught in 2006 and 2007 from the coastal region of Tanzania at Msubugwe near Tanga, and appears to be relatively common in tsetse, although at present its mammalian host range is unknown. The phylogenetic position of the new trypanosome was determined by sequencing both the gGAPDH gene and 18S rDNA from trypanosomes in single midgut infections. In phylogenetic trees the new trypanosome fell into a strongly supported subclade within the clade of Salivarian trypanosomes, as a sister group of the subgenus Trypanozoon. This makes the new trypanosome of considerable interest both as a potential new pathogen and for comparative biological and genomic studies of the Salivarian trypanosomes.

BSP112

Protein turnover and differentiation in *Trypanosoma brucei*

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Autophagy is the primary intracellular mechanism for the degradation of long lived proteins and organelles in eukaryotes. During autophagy portions of the cytoplasm are engulfed in double membrane vesicles, called autophagosomes, which target the vacuole/lysosome causing degradation of the cargo. The process of autophagy has been implicated in a wide variety of physiological roles, including starvation adaptation, type II programmed cell death and cellular development and differentiation. In *Leishmania major* it has recently been demonstrated that autophagy is crucial for parasite differentiation during its lifecycle (Besteiro et al., 2006 J Biol Chem 281: 11384–11396). *In silico* analysis has revealed candidate genes for the key components of the autophagy machinery in *T. brucei*, including several ubiquitin-like ATG8 genes. Autophagy in *T. brucei* is being characterised by expressing ATG8-GFP as a marker to monitor formation of autophagosomes. The importance of autophagy in protein turnover during cellular differentiation and it's influence on parasite survival and adaptation to new host environments will be discussed.

BSP113

LmxMPK4 and LmxMPK6, two potentially essential mitogen-activated protein kinases of *Leishmania mexicana*

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LmxMPK4 has been known to be essential in *Leishmania mexicana* promastigotes and amastigotes and therefore poses an interesting target in the search for anti-leishmanial drugs. To investigate the biological function of this signalling protein, our lab uses the technique of an inhibitor-sensitised mutant, thus bypassing the fact that there is no knock out mutant. We could show that the inhibitor induced deactivation of LmxMPK4 leads to an unspecific growth arrest in promastigotes and are currently investigating the function of LmxMPK4 in amastigotes.
Another seemingly essential MAP-kinase of *L. mexicana* promastigotes is LmxMPK6. This protein contains an unusually long C-terminus and is homologous to the *Trypanosoma brucei* ERK-like, CRK-like protein kinase TbEck1, the C-terminus of which has previously been shown to have a negative autoregulatory function. We were able to show that the LmxMPK6 C-terminus does not have a negative autoregulatory function on the activity of the kinase in vitro, but instead supports the enzymatic activity. The in vivo function of the C-terminus is currently under investigation.

BSP114
Functional analysis of the putative mitochondrial RNA binding complex 1
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U-insertion/deletion RNA editing is required for the maturation of mRNAs in *Trypanosoma brucei*, a process involving a multi-protein complex, called the editosome, and small guide RNAs. The participation of RNA binding proteins has been predicted to have a role in this process as well. Tandem affinity purification of one such protein, TbRGG1, reveals that it is associated with a novel macromolecular complex in an RNA mediated manner. This complex has been provisionally named the mitochondrial RNA binding complex 1 (MRB1) based on this observation and because some putative subunits have motifs typically found in proteins involved in RNA metabolism. Here, we describe the impact of RNAi-silencing of selected subunits on mitochondrial RNA

BSP117
Potassium channels of *T.brucei*
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Cellular uptake of K+ can be mediated by a variety of transporters including ATPases, antiporters, carriers and channels. As extracellular parasites, *Trypanosoma brucei* need to actively concentrate K+ from their environment. Despite their pharmacological potential, very little is known about the molecular nature of K+ transporters from *Trypanosoma brucei*.
We combined pharmacological characterization of K+ homeostasis with genome-wide surveys for K+ transporters in *T. brucei*. Trypanosomes are more resistant than mammalian cells to known K+-channel blockers like charybdotoxin and also to toxic substrates such as caesium (Cs+). However, they are much more sensitive to the specific ionophores valinomycin and gramicidin. We have cloned and functionally characterized the first K+ transporters from *T. brucei*, TbHKT1, a member of the HKT/Trk family and TbKC1, a shaker-type like potassium channel. We present their validation as drug targets by RNAi silencing and gene knock-out.
This work was supported by the Swiss National Science Foundation.

BSP120
Target Validation of *Trypanosoma brucei* DHFR-TS.
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Using homologous gene replacement we have generated gene knockouts of dihydrofolate reductase–thymidylate synthase (DHFR-TS) in bloodstream forms of *T.*

brucei. A single copy of DHFR-TS is sufficient for growth and survival compared to wild-type (WT), but with the removal of both copies the parasites are unable to survive in vitro without thymidine supplementation. Removal of thymidine results in "thymidineless death", giving rise to gross cellular morphological changes in the double knockouts (DKO). The DKO cells were also unable to establish a murine infection due to the low thymidine plasma levels. The inhibitory effect of known anti-cancer agents in the presence of high and low folate medium on WT and DKO cells will be presented. *T. brucei* pteridine reductase (PTR1) levels do not change with loss of DHFR. These results together demonstrate that DHFR-TS is essential for parasite survival and may represent a promising drug target in the African trypanosome.

BSP124

The occurrence and need for autophagy in Leishmania

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Autophagy occurs naturally during differentiation of the life cycle stages of the protozoan parasite Leishmania. The ATG8 lipidation pathway exists and has been validated as a molecular marker for monitoring autophagy in Leishmania (Williams et al., 2006; Mol Microbiol 61:655-74). However, *in silico* analysis of the parasite's genome suggested that the ATG12-ATG5 pathway may not be present (Herman et al., 2006; Autophagy 2: 107-118). We have identified genes possibly encoding LmjATG5, LmjATG10 and LmjATG12, each with low sequence identity with yeast orthologues, but with the key residues thought to be vital for activity present. These genes complement their respective yeast mutants suggesting that the ATG5-ATG12 conjugation pathway exists in Leishmania. The parasite contains two ATG4 genes. The individual roles of each gene in ATG4-deficient mutant lines for their ability to form autophagosomes, undergo metacyclogenesis, infect macrophages, induce lesion pathology in BALB/c mice and withstand stress due to starvation will be discussed.

BSP127

Characterization of Glyoxalase II from *Trypanosoma brucei*

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The glyoxalase system, composed of glyoxalase (GLX) I and II, catalyzes the glutathione-dependent detoxification of 2-oxoaldehydes like methylglyoxal, an inevitable by-product of glycolysis.
Recombinant *T. brucei* GLX II shows a high preference for thioester substrates based on trypanothione rather than glutathione. Different trypanothione-thioesters were synthesized using yeast GLX I, which were hydrolysed by *T. brucei* GLX II with comparable efficiency.

Depletion of GLX II by RNA interference resulted in 10 to 25 % of wildtype activity, but did not influence the growth rate of bloodstream and procyclic *T. brucei*. Even the deletion of both alleles in procyclic *T. brucei* had no effect on cell viability. GLX II is located in the cytosol and probably the mitochondrion, while the production of methylglyoxal should take place in the glycosomes. Therefore it is unlikely that *T. brucei* GLX II has a function in methylglyoxal detoxification. The enzyme hydrolyzes propionyl- and acetyl-trypanothione, which can be formed in a GLX I-independent manner. These results open up new perspectives for yet unknown functions of GLX II in an organism lacking GLX I.

BSP128

Creation of a Vegetation Map of Luambe National Park, Zambia, for use in a GIS and Applications for Research into Trypanosomiasis

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Satellite imagery and geographical information systems are powerful tools with many applications for the study of parasitic diseases. Accurate ecological data about vegetation and land cover is essential for improving understanding of tsetse fly (Glossina species) habitats and trypanosomiasis transmission. This study utilised supervised classification techniques to develop a vegetation map of Luambe National Park, Eastern Province, Zambia. Erdas Imagine 8.4 software was used to process a Landsat ETM+ satellite image of the area. The overall accuracy of the image produced was 67.2%, with an overall kappa statistic of 0.62. The resultant data layer was suitable for the development of robust study designs for surveys of both trypanosomiasis prevalence in tsetse flies and abundance of the large mammal population.

BSP137

Transgenic, fluorescent *Leishmania mexicana* for analysis of intracellular amastigotes proteome

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Leishmania are obligate intracellular parasites causing a variety of diseases that affect 12 Million patients worldwide.
Until now it wasn't possible to isolate the intracellular life cycle stage for direct proteomic studies. Here we combined standard procedures with fluorescent particle sorting to gain very pure *L. mexicana* amastigotes from macrophages. We compared the

proteome of the intracellular parasites with that of the extracellular promastigotes and identified 509 different proteins by mass spectrometry. This reflects ~6% of the proteins predicted from the reference genome of *L. major*. Samples from intracellular amastigotes contained significantly more proteins with basic pI and showed a greater abundance of enzymes of fatty acid catabolism which may reflect living in acidic habitats and metabolic adaptation to nutrient availability, respectively. Bioinformatic analyses of the genes corresponding to the protein data sets produced clear evidence for skewed codon usage and translational bias. Furthermore, we identified characteristic motifs within 3' untranslated regions of mRNAs encoding proteins more abundant in amastigotes. Thus, the dataset could be useful to identify regulatory elements in mRNAs and provides a valuable resource for selection of candidate vaccine antigens as it contained almost all presently evaluated vaccine antigens.

BSP147

Dissecting the role of TNF in the induction of trypanosomiasis-associated anaemia

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Anaemia is a hallmark of both livestock and experimental trypanosome infections in mice. Data obtained using gene-deficient mice show that both soluble and membrane bound TNF, as well as TNF-receptor I and TNF-receptor II, all contribute to the induction of anaemia in *T. brucei* and *T. evansi* infections. Results suggest that sTNF, contributes to the TNF-R1 mediated inhibition of erythropoiesis at the level of the bone marrow. In addition, mTNF is involved in the induction of excessive erythrophagocytosis in the liver, through a TNF-R2 signalling. In contrast to these results, TNF was found to benefit the host in *T. congolense* and *T. vivax* infections, as mice lacking TNF or TNF-R1 suffered from excessive parasite load and extreme anaemia, leading to the accelerated death of the animals. These results suggest that Trypanozoon, Nannomonas and Dutonella trypanosome species interact with the host immune system in a very different manner, and that 'African trypanosomias' as such is a meaningless term from an immunological point of view.

BSP149

Correlation between cAMP and Genes of the VSG Expression Site

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Cyclic AMP (cAMP) is a ubiquitous signalling molecule described in a large variety of organisms. Regulation of cAMP level within the cell is controlled by two enzymes: adenylate cyclases (AC) and phosphodiesterases (PDE). The cyclases convert ATP to cAMP which in turn activates signalling pathways controlling various cellular effects. The PDEs hydrolyse cAMP to AMP and thereby down-regulate the cAMP signal.
In trypanosomes, action and effects of cAMP signalling are only little investigated. Nevertheless, there is evidence that cAMP plays a role in differentiation of bloodstream

form trypanosomes. When bloodstream-form trypanosomes prepare to be taken up by an insect vector, parasites stop dividing and change their shape from long slender to short stumpy. One assumes that differentiation is triggered by a trypanosome-released molecule which operates through the cAMP pathway.
Here we describe a putative key player of cAMP signalling, the adenylate cyclase ESAG4. ESAG4 is expressed in bloodstream-form trypanosomes exclusively and therefore might play an important role in triggering long-slender to short-stumpy differentiation. Currently we investigated localization, expression, importance and structure of ESAG4.

BSP151
Salivary hyaluronidase of bloodsucking insects and its effect on pathogen transmission
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Salivary hyaluronidase of bloodsucking insects plays an important role in blood meal acquisition as it degrades hyaluronan and other components of extracellular matrix. Hyaluronan breakdown products were found to have immunomodulatory properties. Previously, we demonstrated hyaluronidase activity in sand fly saliva. Thus, in recent work we assessed the effect of hyaluronidase coinoculation on the outcome of *Leishmania major* infection using a mouse ear infection model. Mice coinoculated with *L. major* and hyaluronidase developed bigger lesions than controls inoculated with parasites only. Parasite numbers in draining lymph nodes collected 1 day p.i. were similar in both groups. Furthermore, we screened salivary gland material of different bloodsucking insects for hyaluronidase activity. Pronounced activity was found in black flies, biting midges, sand flies and deer flies, lower activity in cat fleas and in Culex mosquitoes, whereas no activity was detected in human lice, Anopheles and Aedes mosquitoes, tse-tse flies and stable flies. In conclusion, we showed that hyaluronidase is a common constituent of saliva of bloodsucking insect and may serve as an enhancing factor in pathogen transmission.

BSP155
Central point versus household sampling for trypanosomiasis in Western Kenya
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The socio-economic impact of animal African Trypanosomiasis transmitted by the tsetse fly (*Glossina spp*) across sub-Saharan Africa is severe. Additionally, domestic cattle are of epidemiological importance as a reservoir for *Trypanosoma brucei rhodesiense*, which causes sleeping sickness in East Africa. The prevalence of trypanosomiasis is normally investigated in cattle gathered at central points, such as community crush pens, however any complexity of disease distribution within the surrounding area is obscured at this resolution
This study was conducted in Busia, Western Kenya, an area endemic for Rhodesian sleeping sickness. The prevalence of trypanosomiasis (by PCR) in cattle presented at

central points was compared to that of all cattle in all households within the catchment area.
Central point samples were representative of the mean prevalence of animal trypanosomiasis across the catchment area. However, a higher proportion of *T.b. rhodesiense* infections were detected through household sampling. Investigation of the geographical distribution of trypanosomiasis revealed significant heterogeneity of trypanosomiasis infection rates over the households of the sampling area.

BSP156
Leishmanicidal activity of endemic Mexican plants.
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The leishmanicidal activity of 15 crude extracts and fractions, together with four pure metabolites, obtained from *Urechites andrieuxii*, *Colubrina greggii*, *Dorstenia contrajerva* and *Tridax procumbens* was evaluated. The newly developed MTS assay was optimized and used for promastigotes of *L. major*, *L. tropica* and *L. aethiopica* as well as for L. aethiopica axenic amastigotes. The three most active samples, two crude fractions (TPZ-2A and DCG-3A) from *C. greggii* and *T. procumbens* and a pure metabolite (NCG-5C) isolated from *C. greggii* were further evaluated and found to possess an LD50 of 18.5, 62.4 and 7.2 µg/ml, respectively, on stationary promastigotes, and an LD50 of 95.2, 94.2, and 27.1 µg/ml on amastigotes of *L. aethiopica*. Moreover TPZ-2A and DCG-3A significantly reduced the percentage of infected monocytes-derived macrophages, without significantly decreasing the number of human cells. These findings indicate the presence of potentially active secondary metabolites in extracts of *C. greggii* and *T. procumbens*.

BSP163
Intraclonal mating in *Trypanosoma brucei*
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Genetic exchange occurs when two different strains of *Trypanosoma brucei* are co-transmitted through tsetse. In our highly efficient system to visualise hybrid production by the use of green or red fluorescent markers, successful mating is indicated by the production of yellow hybrids in fly salivary glands. To examine whether intraclonal mating is possible, as previous research suggests it only occurs in the presence of outcrossing, green and red fluorescent clones of each of two parental lines used in a successful outcross were co-transmitted via tsetse. After dissection, yellow trypanosomes were observed in some salivary glands containing both red and green trypanosomes. These trypanosome populations were recovered and examined in detail. Since the red and green fluorescent reporter genes were linked to different genes for

antibiotic resistance, yellow trypanosomes were selected by double drug resistance; however, none were recovered. Notwithstanding, hybrids were demonstrated by genotypic analysis of individual clones from single strain transmissions, confirming intraclonal mating but without the requirement for outcrossing trypanosomes reported previously.

BSP164

Fly transmission and mating of *Trypanosoma brucei brucei* strain Lister 427

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Lister 427 has been widely used for studies of trypanosome molecular biology, but was not selected for the genome sequencing project as it is monomorphic and unable to complete development in the insect. Instead, fly transmissible, mating-competent strain TREU 927 was used, although not as easily grown or genetically manipulable as 427, and of possible human infectivity. Variant 3, a 40-year-old cryopreserved line of 427, is fly transmissible and also able to undergo genetic exchange with another strain of *T. b. brucei* after co-transmission through tsetse. Comparison of Variant 3 with lab isolates of 427 shows that all have VSG genes 221, 121 and 117 and identical alleles for 3 microsatellite loci. Therefore, despite small differences in molecular karyotype, Variant 3 is an ancestral line of present day 427 lab isolates. Since Variant 3 grows fast both as bloodstream forms and procyclics and is readily genetically manipulable, it may prove useful where a fly transmissible 427 is required.

BSP170

Biochemical Studies of Rad51 paralogues from *Trypanosoma brucei*

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In model organisms Rad51 paralogues associate with each other to aid the function of the Rad51 DNA recombinase. In *T. brucei* Rad51 has an important role in DNA repair and switching of the VSG coat, allowing evasion of the host immune response. We are therefore interested to study the activities of the 4 Rad51 paralogues from *T. brucei*. We have expressed and purified these factors using *E. coli* and investigated their interactions and properties.

BSP171

***Leishmania mexicana* pyruvate kinase: Preliminary crystallographic and structural analysis.**

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The tetrameric enzyme pyruvate kinase (PYK) is final enzyme in glycolysis, yielding ATP and pyruvate from ADP and phosphoenolpyruvate. The exact mechanistic action required for PYK to perform this reaction remains unclear, although snapshots of PYK in two conformations (T-state/inactive and R-state/active) have been captured by X-ray crystallography.
Here we report the preliminary analysis of two new X-ray structures of PYK from *Leishmania mexicana* (*Lm*PYK), which have been trapped in different conformations. These, together with the previously reported structure of *Lm*PYK in its inactive (T-state) conformation, allow comparisons of various different conformers of the same species of PYK. We have obtained crystals of *Lm*PYK with ammonium sulphate as precipitant, and shown that the structure of the homo-tetrameric enzyme corresponds to a pseudo-active conformer with sulphate ions at the active and effector sites. *Lm*PYK has also been cocrystallized as a complex in its active (R-state) conformation with Mg^{II}ATP, oxalate, Mg^{2+} and K^{+}. The conformational changes observed show rotations of the $(\alpha/\beta\square)_8$-barrel A domains which act like cogwheels and provoke significant changes in the substrate binding pocket. These new structures provide insight into the various structural transitions experienced during catalysis and demonstrate potentially new drugable enzyme states for the development of novel anti-trypanosome drugs.

BSP173

Drosophila S2 cells as a model system for studying *Leishmania donovani* trafficking within macrophages.

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Upon inoculation into the mammalian host, Leishmania parasites are phagocytosed by monocytes into a membrane bound compartment, the phagosome. This structure interacts with endocytic organelles and acquires late endosomal and lysosomal markers (e.g. LAMP1), forming a mature phagolysosome or parasitophorous vacuole. Relatively little is known about host factors that regulate phagosome maturation.
Drosophila melanogaster macrophage-like S2 cells have been used to facilitate the genetic analysis of host-pathogen interactions within intracellular compartments (Derré et al., 2007). We are applying a similar approach to the study of Leishmania intracellular survival within S2 cells, to support whole genome screening for host genes that influence this process. We have shown that *Leishmania donovani* amastigotes are phagocytosed, maintained and can replicate within S2 phagosomes. Using a dsRNA library, methods for RNAi analysis of 7000 host genes using high-content screening microscopy are now in development, for identification of sequences that impact on phagosome maturation. Positive hits from this screen will be confirmed using siRNA methods in mammalian macrophages.

BSP174

CDP-Ethanolamine Synthesis in *Trypanosoma brucei*

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Trypanosoma brucei is the causative agent of African sleeping sickness, a parasitic disease that threatens millions of people in sub-Saharan Africa.
Ethanolamine (EtN) is a major component of the trypanosome membrane phospholipids, in the form of phosphatidylethanolamine (PtdEtN). PtdEtN constitutes 3-10% of the total membrane phospholipid of *T. brucei* and has an important role in maintaining membrane homeostasis; because membrane phospholipids determine membrane fluidity and charge of cell surfaces, EtN utilization and PtdEtN biosynthesis are likely to control a variety of cellular processes. PtdEtN is also the donor for an integral component of the glycosylphosphatidylinositol (GPI) anchor that is required for membrane attachment of cell surface proteins, most notably the Variant Surface Glycoprotein, in the bloodstream form of the parasite, where it protects the parasite from the host's immune system.
The analysis of how EtN is metabolized in *T. brucei* could unravel novel targets for the development of chemotherapeutic drugs and give novel insights on how eukaryotes assemble glycolipid components of membranes and membrane-bound proteins at the cell surface.

BSP176

The brain entry of anti-trypanosomal drug across the murine blood-brain barrier in health and disease.

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The lack of efficacy of blood (first) -stage drugs in the treatment of CNS (second)-stage Human African Trypanosomiasis (HAT), is assumed to be due to their inability to cross the blood-brain barrier (BBB), but this has never been directly investigated. We used an in situ perfusion technique to measure brain uptake of stage 1 (suramin, pentamidine) and stage 2 (eflornithine, nifurtimox) drugs in healthy mice and in mice infected by *Trypanosoma brucei brucei* (Antimicrob. Agents and Chemother. (2007) 51 3136-3146; Parasitol. Int. (2002) 51 381-388). Results revealed that suramin did not cross the BBB. However, although transport mechanisms are involved in the uptake of efornithine, pentamidine and nifurtimox, eflornithine does not cross the BBB well. Also efflux transporters can remove pentamidine and nifurtimox from brain and trypanosome infection alters the distribution of certain drugs. This overview of our ongoing research illustrates the complexity of designing drugs to treat HAT. Wellcome Trust funded (grants: 073542; 080268).

BSP179
***T. brucei* CTP synthetase – all eggs in one small pool**
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The intracellular concentration of cytidine triphosphate (CTP) is extremely low in *T. brucei*, with respect to the other intracellular NTP pools, and CTP pools in other organisms studied.
Scavenging of extracellular cytidine or cytosine in *T. brucei* is not observed, suggesting the parasite is totally dependent upon de novo synthesis of CTP from UTP, in an ATP and glutamine-dependent manner via CTP synthetase. CTP is central in the production of nucleic acid and all phospholipid biosynthetic precursors.
The gene encoding the *T. brucei* CTP synthetase has been identified, with the aim of creating RNAi cell lines in bloodstream and procyclic parasites, in order to observe a phenotype upon disruption of the gene product. Additionally, *T. brucei* CTP synthetase has been recombinantly expressed in *E. coli*, and purified for functional assays.
An extraction protocol, in conjunction with multiple reaction monitoring mass spectrometry has been developed, which allows rapid quantification of intracellular nucleotide triphosphates in wild type and genetically modified *T. brucei*, as well as in the presence of CTP synthetase inhibitors.

BSP187
Characterization of *Trypanosoma brucei* PEX5 and PEX7, receptors for import of proteins into glycosomes
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Peroxisomes of the protozoan parasite *Trypanosoma brucei* have as a special feature that they harbour the major part of the glycolytic pathway. These organelles are hence called glycosomes. Proteins are incorporated in the peroxisomal matrix after their synthesis in the cytosol and transport across the peroxisomal membrane by a process involving various proteins designated peroxins (acronym PEX). The proteins are recognized through their peroxisome-targeting signal (PTS) by either PEX5 or PEX7 which are cytosolic receptors. PEX5 recognizes a motif present at the C-terminus called PTS1 and PEX7 a sequence near the N-terminus called PTS2. After binding the PTS-protein, the cargo-loaded receptor docks at a membrane-bound complex comprising PEX13 and PEX14, before being transported through the membrane. Previously we published the identification and characterization of several *T. brucei* peroxins including PEX5. Here we report the identification of T. brucei PEX7, provide indications for its interaction with PEX5 and show by RNAi interference that both receptors are essential for the parasite.

BSP188
Novel techniques for stage diagnosis of Trypanosomiasis
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Human African Trypanosomiasis (HAT) treatment requires accurate stage diagnosis; early stage drugs do not penetrate the blood-brain-barrier and late meningoencephalitic stage therapy requires highly toxic arsenicals. Hence correct staging is essential for effective treatment and minimal exposure to toxic therapy. Currently stage diagnosis relies on analysis of cerebrospinal fluid (CSF) for white cells and trypanosomes. Due to the invasive nature of lumbar puncture as well as ambiguity regarding the most appropriate cut-off values, an alternative diagnostic would be desirable. Several central nervous system (CNS) derived biomarkers may be detected in serum after neurological trauma and we are investigating their potential as HAT stage diagnostics. These include glial fibrillary acidic protein (GFAP), neuronal-specific enolase (NSE), and neurofilament heavy and light (NF-H & NF-L). Initial results indicate that these proteins are detectable in blood samples and in some cases show a clear diagnostic potential, with significantly higher concentrations detected in late stage disease.

BSP190
The terbinafine resistance protein HTBF of *Leishmania major* belongs to the YIP1 family
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The HTBF gene of *L. major* confers resistance to terbinafine, an inhibitor of Esqualene epoxidase. HTBF predicted aminoacid sequence revealed significant homology to the Yip1 protein of Saccharomyces cerevisiae. In the yeast, Yip1p participates in vesicle trafficking by interacting with Ypt, a rab/GTPase, allowing it to insert into membranes. Our hypothesis is that HTBF is involved in the formation and/or redirection of vesicles, improving mechanisms of drug extrusion or membrane repair. The studied members of the YIP1 family localize in the Golgi complex and are widely conserved. A c-Myc::HTBF fusion was expressed in mammalian cells and localized in the Golgi complex, confirming its conservation. We further expressed a GFP::HTBF fusion in the parasite. Subcellular localization revealed that the fusion product was present in a region corresponding to the Golgi complex. Our results sugst not only that HTBF is the YIP1 of *L. major*, but also that the terbinafine resistance observed in HTBF overexpressors involves the vesicle trafficking machinery of the parasite. Supported by FAPESP, CAPES and CNPq.

BSP192

Using Fourier Transform Mass Spectrometry to elucidate metabolic changes in trypanosomatids.

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The trypanosomatids – *Trypanosoma brucei* and *Leishmania mexicana* – are responsible for the neglected diseases human African trypanosomiasis and cutaneous leishmaniasis. Previous research has shown a metabolic shift that accompanies differentiation between the insect and human infectious forms of *T. brucei*. To mimic this shift, procyclic trypanosomes were grown in the presence of either proline or glucose. Proteomic analysis of these cells revealed no significant modulation in proteins associated with energy metabolism despite clear changes in metabolism.
Using an Orbitrap FT Mass Spectrometer we have analysed global metabolomes of trypanosomes. Using customised software that is able to de-convolute the raw data gained from the Orbitrap MS we identified thousands of metabolites within the trypanosomatids. Advanced visualisation tools were employed to explore ab initio networks based on the de-convoluted mass lists and known biochemical transformations. This enabled us to outline a large number of condition-specific differences in levels of metabolites between cells grown in different carbon sources.

BSP194

Control of expression phosphoglycerate kinase genes in Leishmania

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Leishmania presents two phosphoglycerate kinases, the cytosolic (PGKB) and glycosomal (PGKC) isoforms. PGKB or PGKC genes were independently cloned into pX63Neo and transfected into Leishmania to investigate the participation of untranslated regions on the control of PGK expression. Under increasing concentration of drug of selection, PGKB transfectants and control cells gained copies of the episome. In contrast, under the same condition, PGKC episome copy number was kept at ~1/10th of that observed for PGKB. Northern analyses revealed that PGKC transcript was kept at lower levels when compared to PGKB RNA. Chimeric constructs were engineered to investigate the effect of 5'- or 3'-UTRs of PGKC on RNA stability. Southern and northern analyses indicate that 5'- and 3'-UTRs of the PGKC gene are not involved in PGK transcript stability but seem to be needed to maintain the low number of the episome copies. Evaluation of glycosomal and cytosolic protein isoforms relative levels indicates the lack of a positive correlation between protein abundance and transcript levels. Differences on translation initiation or protein turnover rates are currently under investigation.

BSP198

Over-expression of the *Leishmania major* MAP kinase LmaMPK7 leads to increased phosphorylation of endogenous phosphoprotein substrates and interferes with parasite growth and virulence

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Based on phosphotransferase activity studies, we recently implicated the Leishmania MAP kinase LmaMPK7 in environmentally induced parasite differentiation. LmaMPK7 escaped classcial biochemical and genetic analysis due to low expression and a lethal null-mutant phenotype. To circumvent these limitations we generated the transgenic lines GFPK7 and GFPK7D, over-expressing GFP-fusion proteins of functional and inactive LmaMPK7, respectively. Pull-down experiments revealed increased activity of GFP-LmaMPK7 in growth arrested promastigotes at stationary phase. LmaMPK7 over-expression was associated with a substantial delay in cell-cycle re-entry following inocculation of stationary GFPK7 into new medium, and strongly reduced parasite virulence due to an amastigote-speific growth defect. Quantitative phospho-proteomic analysis of GFPK7 by DIGE revealed over-phosphorylation of a Leishmania ortholog of methionine aminopeptidase (MAP), establishing a potential kinase-substrate relationship in situ. In conclusion, in absence of access to endogenous protein and loss-of-function analysis, our well-controlled Leishmania recombinant expression system proved to be useful to gain insight into potential LmaMPK7 functions and interactions.

BSP201

Pyrimidine transport in *Trypanosoma brucei* and *Leishmania major*

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While much is now known about the purine transporters of the main protozoan parasites, pyrimidine transport has been much less studied.

We have now characterised a number of pyrimidine transporters from Leishmania major and *Trypanosoma brucei*. The most efficiently salvaged pyrimidine by *T. brucei* procyclics is uracil, which is mostly taken up by the TbU1 transporter, using the protonmotive force for energy. This transporter also has a moderate capacity for uridine uptake. A second uridine transporter, TbU2, has higher affinity for both substrates but, under standard culture conditions, a very low capacity. *T. b. brucei* procyclics further express a very high affinity transporter for cytosine (TbC1), but we found no evidence for uptake of [3H]-cytidine, [3H]-thymine or [3H]-thymidine. In *L. major*, we found a uracil transporter (LmU1) very similar to TbU1. Using a series of uracil analogues, a model for substrate binding by LmU1 was constructed.

BSP202

Flagellum elongation contributes to maturation and function of the flagellar pocket of *Trypanosoma brucei*.

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Trypanosoma brucei presents an invagination at the base of flagellum, called the flagellar pocket (FP). This structure plays an important role in parasite development since it is the exclusive site for endocytosis and exocytosis.
We have investigated the structure and function of the FP in IFTRNAi mutants that present a short flagellum or no flagellum. Conventional transmission electron microscopy (TEM) revealed FP shape modifications and presence of vesicules inside FP. A novel scanning electron microscopy approach on cytoskeleton of control and mutant cells allowed to identify the flagellar pocket collar (FPC) which is less elaborated in mutants. Moreover the FPC protein BILBO is less abundant. Location of the FP transmenbrane protein CRAM is observed at the surface of short dilated flagella in IFTRNAi mutants. Localisation of reticulum endoplasmic and lysosomes is affected and TEM revealed presence of aberrant vesicules in Golgi and FP region in mutants. Finally, IFTRNAi mutants are less efficient to capture the lipophilic dye FM4-64.

BSP208

Identification of a putative PEX13 involved in glycosome biogenesis in *Trypanosoma brucei*

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The glycosome biogenesis pathway in trypanosomatids is homologous to that of peroxisomes in other eukaryotes. Peroxisomal and glycosomal proteins are synthesized in the cytosol and imported through a cascade of transient interactions between protein complexes (‘peroxins’; acronym ‘PEX’). A cytosolic receptor, PEX5 or PEX7, associated with a newly synthesized matrix protein docks on a membrane-bound complex comprising PEX14 and, in mammals and yeasts, also PEX13. Previously, we characterized PEX5, PEX7 and PEX14 of *Trypanosoma brucei*. Trypanosomatid peroxins show generally a very low sequence similarity with their homologues in other organisms precluding until now the identification of PEX13 in the Tri-Tryps databases However, we show here the identification of the trypanosomal homologue of PEX13. This protein possesses a C-terminal SH3 domain, a transmembrane segment, a Pro-rich region, a PTS-1 and is localized in the glycosomal membrane. It interacts with PEX14 and PEX7 in a yeast two-hybrid system but an interaction with PEX5 needs still to be confirmed. It has an essential role for the parasite as shown by RNAi.

BSP210

Involvement of an ABC transporter in heme trafficking in Leishmania.

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An "Achilles heel" of the Trypanosomatids is their auxotrophy for heme. Proteins involved in porphyrin trafficking are therefore attractive drug targets. A number of ABC proteins from the ABCB and ABCG subfamily have been very recently implicated in heme transport in mammal cells. We have evidences suggesting that one of the five ABCG transporters of Leishmania (LABCG5) could be involved in intracellular heme trafficking in this parasite: i) dominant negative parasites overexpressing an inactive version of LABCG5 showed a high growth inhibition unless an hemin supplement was added; ii) hemin-agarose pull-down assays precipitated LABCG5, the interaction being dose-dependently inhibited by free hemin. Inmmunocytochemistry analysis using transmission electron microscopy after sample high pressure freezing showed that LABCG5 localized in a multivesicular body close to the flagellar pocket. Leishmania can obtain heme from hemoglobin, which is taken through receptor mediated endocytosis, and routed via early rab5- and late rab7-endosomes. The multivesicular body where LABCG5 localise probably corresponds to this late rab7-endosomes, an issue currently under study.

BSP212

Deficiency in the heme biosynthesis pathway enzymes is the base of Photodynamic Therapy (PDT) for the treatment of cutaneous leishmaniasis

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Cutaneous leishmaniasis occurs 1-12 weeks after exposure with a small papule on the exposed site and finally ulcerates. The drugs of choice, pentavalent antimony or meglomine antimoniate are characteristically moderately toxic and in some patients there are risks of recurrence and unsatisfactory side effects. In this study, we used photodynamic therapy for the treatment of cutaneous leishmaniasis caused by Leishmania major. Leishmania was found deficient in at least five enzymes in the heme biosynthesis pathway. The first enzyme was delta-aminolevulinic acid (ALA). 5 pateints (7 lesions) with confirmed leishmaniasis, (Leishmaniasis sore in arm, forearm or leg), were injected 10% of ALA locally and after 4 hours per treatment session at a light intensity of 150 mW/cm2 (approximately 21 min) was delivered, using red light (570-620 nm), 100 J/cm2. Treatments were repeated weekly for 4 times. After 1or 2 sessions, the direct smears showed no amastigotes. Healthing and cosmetic outcome after photodynamic therapy excellents.only mild local inflammatory reaction was noted with no scarring and 4 months after the last treatment session, there were no clinical signs of recurrence. Therefore photodynamic therapy can be recommended for the treatment of cutaneous leishmaniasis.

BSP214

Enzymes of mannose activation in *Trypanosoma brucei*

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The synthesis of Dolichol-phosphate-mannose (Dol-P-Man) from dolichol-phosphate and GDP-mannose (GDP-Man) is catalysed by the ER resident Dol-P-Man synthase (DPMS).

Both GDP-Man and Dol-P-Man are key activated mannose donors in eukaryotic glycosylation pathways. These mannose donors are used directly and indirectly in both N-glycosylation and GPI biosynthesis, which are of particular importance in the formation of mature variant surface glycoprotein (VSG), the protective coat of the bloodstream parasite *Trypanosoma brucei.*

By forming conditional knockout and RNAi cell-lines, we have investigated the biochemical phenotype caused by the reduction in these mannose donors in bloodstream *T. brucei*. In particular, the associated consequences to N-glycosylation and GPI-anchoring of VSG, as the trypanosomes tries to maintain the integrity of the VSG coat.

Recombinant expression of the mannose activating enzymes has allowed enzymatic characterization including; kinetic analysis, substrate/inhibitor specificity studies and future development of high-through-put assays.

These studies have already revealed differences between the mammalian and *T. brucei* homologues, highlighting *T.brucei* DPMS as a promising drug target in the fight against African sleeping sickness.

BSP215

Transcriptional analysis of genes involved in the actin cytoskeleton of *T. brucei*

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Actin is a ubiquitous, highly conserved cytoskeletal protein which is involved in cell movement, intracellular traffic and cell division. Actin is expressed at similar levels but in different locations in the two main proliferative forms of *T. brucei* the bloodstream and procyclic forms. RNAi has shown that actin is an essential protein in bloodstream but not in procyclic forms.

The primary organizing processes of the actin cytoskeleton, polymerization and depolymerization are mediated by specific sets of proteins. Since the levels of actin are the same in both lifeforms the differential regulation of actin function may occur at the level of nucleation/depolymerization via the regulation of the expression of the proteins involved in these processes. Screening of the genome of *T. brucei* identified 22 proteins likely to be involved in the actin cytoskeleton, based on sequence comparisons with other organisms. We have carried out transcriptional analysis of these genes in both lifecycle forms by qRT-PCR. Of the 22 genes examined, 4 are highly upregulated in the bloodstream form.

BSP217

Modified histones are associated with initiation of divergent transcription in the human parasite *Trypanosoma cruzi*

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Most genes in trypanosomes are organized in clusters polycistronically transcribed in a divergent manner from strand-switch regions possibly harbouring bidirectional transcription initiation sites. The rarity of canonical transcription factors and regulatory sequences identified in trypanosomes could suggest a possible regulation of gene expression through modulation of chromatin structures. Posttranslational modifications of histones are associated with regulation of several key processes in various organisms. Histone H3 and H4 acetylation as well as histone H3 K4 trimethylation (H3K4me3) are typically associated with active genes in eukaryotes. By using chromatin immunoprecipitation (ChIP) we have determined a strong correlation of acetylated histones and H3K4me3 to initiation sites of divergent transcription in *T. cruzi*. A more detailed analysis revealed that modified histones present a bimodular distribution with peaks localizing to the ORFs flanking the strand switch region separated by an unmodified intergenic region, similar to patterns found for bidirectional promoters in higher eukaryotes. These results suggest the presence of a functional 'histone code' in trypanosomes with potential implication in transcriptional regulation.

BSP219

Role of myosin in *T. brucei*

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A functional cytoskeleton requires actin in combination with the molecular motor protein myosin. Genome analysis reveals the presence of only two myosin proteins for *T. brucei*. This number is considerably less than found in mammalian cells, where ~20 classes are present and myosins are considered as a large superfamily. The aim of this study is to elucidate the role of myosin in *T. brucei*. The two myosins in *T. brucei* can be differentiated by their tail region and are described as Myo A (Tb11.01.7990) on chromosome 11 and Myo B (Tb927.4.3380) on chromosome 4. Expression analysis using antibodies and qRT-PCR indicated upregulation of Myo B in the bloodstream form whereas Myo A was expressed at the same level for both life forms. The results of functional and localization studies will be presented and discussed.

BSP227

Immunological Role of Leishmania Secreted and Nonsecreted Antigens

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Leishmania infection consists in two sequential events, the host cell colonization followed by the proliferation/dissemination of the parasite. The mechanism of infection involves two distinct sets of molecules, the secreted and/or surface and the nonsecreted antigens. The importance of the immune response against secreted and surface antigens is noted in the establishment of the infection. Moreover, the contribution of the nonsecreted antigens in the immunopathology associated with leishmaniasis, shows the importance of these panantigens during the course of the infection. In this work, we stablish the relation between several laboratorial observations on Leishmania Sir2 and LicTXNPx as excreted/secreted proteins and LmS3arp and LimTXNPx as nonsecreted/panantigens. These observations lead to a possible role of these two groups of antigens in the immune response during the infection.

BSP230

Investigating the life cycle of avian trypanosomes by molecular phylogeny.

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Avian trypanosomes are heteroxenous parasites transmitted by bloodsucking Diptera. To elucidate host-parasite relationships of avian trypanosomes and their insect vectors, we performed RAPD analysis and SSU rDNA sequencing with 50 trypanosome isolates. According to the results of sequence analyses, it was possible to ascribe all isolates to three clades representing already known trypanosome species *Trypanosoma corvi*, *T. bennetti* and *T. avium*. Only *T. corvi* clade was monophyletic, with two groups supported by both methods. The other two major clades were polyphyletic due to RAPD analysis. The *T. avium* clade was splitted into several groups that contained mainly isolates from black flies and raptors which corresponded to our previous study. However, the sequence analysis revealed also distinct group of isolates from mosquitoes and insectivorous songbirds inside the *T. avium* clade. Interestingly, this group was also found in RAPD analysis as a separate clade. Our results show that avian trypanosomes are complex and most probably several cryptic species exist within them.

BSP231

Sitamaquine, a new oral drug to treat leishmaniasis, overcomes miltefosine resistance mediated by LMDR1 overexpression in Leishmania.

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Miltefosine is an important weapon against leishmaniasis, but the appearance of resistance is a serious threat. LMDR1, a protein included in the ABC (ATP-binding cassette) family of transporters, was the first molecule shown to be involved in experimental miltefosine resistance. LMDR1 pumps the drug out of the parasite decreasing its intracellular accumulation, a process that could be reverted by using

LMDR1 inhibitors. Sitamaquine is another promising oral drug for leishmaniasis currently in clinical trial phase III. The physico-chemical features of this drug suggested us that it could be considered as a LMDR1 inhibitor, an issue that we have confirmed as sitamaquine: i) increases the uptake of miltefosine in drug resistance MDR Leishmania lines, ii) completely overcomes miltefosine resistance in this line at concentrations lower than the needed to kill the parasites. In addition, we have shown that sitamaquine is not a LMDR1 substrate and that it does not efficiently inhibit human MDR1, two interesting features for any inhibitor of LMDR1.

BSP233

Leishmania-macrophage interaction, a proteomic study.

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Macrophages are the only host cells where intracellular Leishmania can multiply and are therefore crucial in the development of the disease.
Changes in host cell protein expression at relatively late stages (48 hours) of infection were investigated. A proteomic approach was chosen in order to take into consideration post-transcriptional and post-translational modification. DIGE analysis limited technical variations between sample analysis and insured optimal identification of differentially expressed proteins. Differentially expressed proteins were identified by MALDI-TOF analysis.
Of particular interest was the differential expression of various proteins directly and indirectly involved in the host apoptotic pathways (Annexin A1, Annexin V etc.), and of proteins involved in Leishmania survival (Ferritin light subunit, FBA etc.). For example, down-regulation of ferritin light subunit causes decrease of iron storage suggesting that iron is sequestered by the pathogen which requires it as a growth factor and for activation of its iron superoxide dismutases.
Identification of these and other proteins via DIGE analysis confirms the importance of this approach in understanding host-parasite interaction.

BSP237

Comparative expression profiling of gene subsets from three species of Leishmania

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Comparative analyses of the *Leishmania major*, *L. infantum* and *L. braziliensis* genomes show remarkable conservation of gene organisation and synteny, with ~98% of genes present in all 3 species. While expression levels of conserved parasite genes may differ considerably between and within species, the small number of species-specific sequences may play a critical role in disease outcome. It is also possible that parasite genotype plays only a minor role in determining disease phenotype.

Here, we describe comparative expression profiling of the 3 Leishmania species, focusing on (1) a subset of genes encoding amino acid repeats and (2) the genes that are differentially distributed between species. Expression profiling was performed using spotted oligo-arrays hybridised with lesion amastigote RNAs. Data analysis utilised Bioconductor, with verification by quantitative RT-PCR.
Initial analyses, comparing *L. major* and *L. infantum*, indicate that all species-specific genes are expressed at low levels while ~10% of genes common to all species show differential expression of >2-fold ($p>0.05$). Comparative analysis of *L. major* and *L. braziliensis* expression profiles is in progress.

BSP238

SL RNA overexpression leads to abnormal regulation of selected proteins and loss of virulence.

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The miniexon gene has a central role in the processing of polycistronic pre-mRNA of kinetoplastids. It is added to the 5' extremity of each mRNA, supplying the 5'-capped structure to the molecule. Here we show that no lesions were detected in animals infected with Leishmania transfectants overexpressing the miniexon array. Comparative transcriptome and proteome of an miniexon overexpressor we detected at least 20 proteins with abnormal patterns of expression and morphological changes had also be detected. Herein we propose that such changes interfere with the active proliferation of amastigotes within the mammalian host cell, which ultimately leads to the observed attenuation. The persistence of parasites in the host indicates that a stable line overexpressing the miniexon may be tested as live vaccine against leishmaniasis.

BSP241

Factors affecting the epidemiology of animal trypanosomiasis in Eastern province, Zambia

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A cross-sectional study on the prevalence of animal trypanosomiasis was conducted on 649 cattle, 811 goats, 58 sheep and 177 pigs in households distributed between the Luangwa valley and the Eastern Plateau in Mambwe District, Zambia. Animals were considered to be infected if either microscopic or PCR examination were positive for pathogenic trypanosomes. Trypanosomiasis prevalence was highest in cattle (28.4%) followed by pigs (21.5%), sheep (18.2%) and goats (9.2%). Trypanosomiasis

prevalence was higher in low altitude areas than areas of high altitude ($\chi 2$ =31.2, d.f. = 1, p = 0.001). Levels of infection in goats and particularly sheep demonstrated the importance of trypanosomiasis in small ruminants in this area; infected individuals had lower haemoglobin values than those that were negative. Prevalence of trypanosomiasis in particular livestock species also depended on the combinations kept within households. Small ruminants were more likely to be infected if cattle were also present.

BSP243

Trypanosomiasis in Ugandan black rats: more than just *Trypanosoma lewisi?*

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Trypanosomiasis is a well-known disease of man and livestock in Uganda with certain parasite species cycling through a variety of other local mammals. As part of a general parasitological survey of rodents, thick and thin Giemsa blood films were prepared finding prevalence of trypanosomiasis to be 60% and likely caused by *Trypanosoma lewisi*. Genomic DNA (gDNA) was extracted from a selection of 60 slides and a nested PCR targeting the ribosomal ITS-1 region was performed. In comparison to microscopy, the sensitivity and specificity of PCR detection methods were determined. Surprisingly, substantial length heterogeneity of ITS-1 products was observed and sequencing a selection of variants identified 98% sequence similarity with *T. brucei*, *T. vivax* and *T. evansi*. Owing to limited availability of gDNA, seeking absolute confirmation of these findings has proven difficult but it appears these rats may have harboured non-*T. lewisi* species likely perhaps as 'dead-end' hosts.

BSP244

Identification and characterization of species-specific genes that may influence Leishmania disease tropism

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Infection with different Leishmania species gives rise to a spectrum of disease in man. *L. infantum* and *L. major* usually cause visceral and cutaneous leishmaniasis respectively while *L. braziliensis* is the causative agent of mucocutaneous leishmaniasis. Parasite factors contributing to tropism and pathogenesis have not been fully evaluated. Comparative genomic analyses have identified species-specific genes that may influence disease outcome. One example is a Leishmania orthologue of the bacterial gene encoding cyclopropane fatty acyl phospholipid synthase (CFAS), present in the *L. infantum* and *L. braziliensis* genomes but not in *L. major*. These enzymes catalyze the cyclopropanation of unsaturated glycolipids in bacteria, a process important in host virulence and persistence in *Mycobacterium tuberculosis*. Leishmania CFAS is active

and membrane-localised in logarithmic phase *L. infantum* promastigotes. Ectopic expression indicates that CFAS is also functional in *L. major*. We are using *L. infantum* CFAS transgenic parasites to determine the role of this enzyme in visceral leishmaniasis.

BSP246

The colonisation of houses by silvatic *Rhodnius prolixus* in Venezuela.

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Rhodnius prolixus is the main vector of Chagas disease in Venezuela, where it colonises poor quality housing. Research has suggested that *R. prolixus* may also have a widespread silvatic distribution. Silvatic invasion potentially explains the persistence of domestic infestations despite four decades of vector control. However silvatic populations could also be Rhodnius robustus, a closely related species of minor epidemiological importance.
Using sequencing (mtcytb, D2) and microsatellite analysis (10 polymorphic markers) we investigated (1) the existence of silvatic R. prolixus and (2) if silvatic populations are genetically isolated.
We identified *R. prolixus* in palms and the colonisation of houses by silvatic bugs, with both ecotopes sharing 7 cytb haplotypes. Additionally, mitochondrial introgression was detected between *R. robustus* and *R. prolixus*. Microsatellite analysis revealed a lack of genetic structure between ecotopes (non-significant FST values), indicating gene flow. Our results demonstrate that silvatic populations of *R. prolixus* present a threat to the successful control of Chagas disease in Venezuela, requiring modified spraying and surveillance strategies.

BSP248

Trypanosome infections of tsetse in Serengeti National Park, Tanzania

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Tsetse flies transmit a number of trypanosome infections, including *Trypanosoma brucei rhodesiense*, the causative agent of sleeping sickness. Cases of sleeping sickness in tourists to Serengeti National Park, Tanzania have highlighted the importance of this disease as both a public health concern and a potential threat to the tourism industry. Further understanding of the epidemiology of trypanosomiasis in a complex ecosystem such as this is vital for effective disease management, and awareness of the prevalence of trypanosome infections in tsetse is particularly important in assessing and conveying risk of disease transmission. Tsetse trapped in Serengeti National Park during 2005 and

2006 were analysed using both dissection and molecular techniques. The prevalence of trypanosome infections was assessed and factors influencing prevalence considered.

BSP249

Population analysis of the *Leishmania donovani* complex in Sudan

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The life cycle of Leishmania is thought to be mainly asexual, with rarely recognised hybrids between species. However, with new molecular typing techniques it has been possible to study the population genetics in more detail and to recognise recombinant strains between closely related lineages. Data obtained from multilocus sequence and microsatellite typing have allowed investigation of the genetic structure of the *L. donovani* complex, the aetiological agents of visceral leishmaniasis, in Sudan. Results reveal evidence of intra-specific recombination. The implications of the population structure and of recombination in Leishmania, particularly for *L. donovani* in Sudan, are discussed.

BSP251

Identifying candidate genes for the regulation of the response to *Trypanosoma congolense* infection in mice.

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African cattle breeds differ significantly in their ability to survive low to moderate levels of challenge with *Trypanosoma congolense*. Similarly the survival times of inbred mouse strains vary substantially after infection. We have previously identified regions of the genome that regulate survival after infection in two crosses (AJ x C57BL/6 and Balb/c x C57BL/6). We have now used two strategies to reduce the size of the region that appears to be regulating survival. Firstly, congenic mice lines carrying defined regions of the C57BL/6 genome on an AJ background were developed to identify physical boundaries of the regions and confirm its effect. Secondly the response to infection has been mapped in an additional mouse strain (129J). The mapping data has been combined with haplotype maps to identify a 70kb high priority region containing just 12 strong candidates for the causative gene for resistance to trypanosomiasis in mice.

BSP254

Identification of conserved noncoding elements in Leishmania genomes and their potential role as protein-DNA/RNA interaction sites

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The completed genomes of *L. braziliensis*, *L. infantum* and *L. major* are important tools to improve comprehension of these pathogens biology. We conducted a computational analysis to identify sequence conservation in the flanking regions of annotated genes in the three Leishmania genomes in order to identify putative regulatory elements. We developed a pipeline to find these conserved noncoding sequences and to group them according to the function of their neighboring CDS using Gene Ontology Family categories. Seventy seven conserved sequences were found in 40 GO families and six of them were selected to be validated by another algorithm (MEME). Experimental analysis are in course to infer their role and it includes Electrophoresis Mobility Shift Assays. So far, EMSA results indicate that at least two of these elements are potential sites for protein-DNA/RNA interaction.

BSP257

Microsatellite genotyping of *Trypanosoma brucei rhodesiense* strains from Uganda that differ in pathology form distinct clusters that correlate with phenotype.

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44 *Trypanosoma brucei rhodesiense* strains, isolated from humans at eight sites in Uganda between 1988-1992, have exhibited two distinct clinical forms. Isoenzyme analysis has linked this apparent difference in virulence to different zymodemes, the phenotypes of which have been replicated in mice. Using 6 microsatellite markers, we have genotyped 15 of these samples and compared the results to other studied Ugandan strains (MacLean et al. J Infect Dis. 2007 196: 1620–1628) and have shown that although the genotypes cluster by clinical phenotype in both studies, the strains from the two studies do not cluster together. This is consistent with the hypothesis that there may be genetic differences in the parasites that are associated with differences in clinical phenotype. The presence of multiple clusters of less and more virulent parasites suggests there may have been gene flow between parasite populations or multiple origins of the virulence genotypes.

BSP261

Multilocus sequencing typing (MLST) of Paraguayan *Trypanosoma cruzi* reveals host associations and evidence of intralineage recombination.

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Genetic diversity and population structure were analysed for 114 isolates of *Trypanosoma cruzi* from Paraguay. Biological clones were initially characterised by mini exon, 18S rRNA, 24sα RNA PCR and isoenzyme electrophoresis. Six genes were sequenced for MLST, for which allelic phase was determined by allele specific PCR (with sequencing) and by haplotype reconstruction.
Lineage TCII predominated in Paraguay, with TCII b,c,d, and e all represented. Results confirmed TCIId and IIe were hybrids of TCIIb and IIc. There was low genetic diversity within and between TCIId and TCIIe, however there were SNP differences and population substructuring within TCIId, TCIIe and TCIIc. Lineages IId and IIe circulated in domestic cycles and IIc in silvatic cycles associated with the armadillo *Dasypus novemcinctus*. TCIId found in a silvatic specimen of the armadillo *Euphractus sexcinctus* suggests transfer from the domestic cycle to the sylvatic cycle or that IId may have originated in the silvatic cycle involving armadillos. Importantly, there was new evidence of genetic recombination within a *T. cruzi* lineage.

BSP265

Unravelling the mysteries of vector competence in the tsetse fly.

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The infamous tsetse is solely responsible for the spread of African trypanosomoses in humans and domestic livestock. Presence of this disease vector in sub-Saharan Africa limits agricultural productivity, exacerbates poverty and currently debilitates 350,000 people per annum. Since current treatment methods for human trypanosomiasis are abysmal, efforts to reduce disease prevalence have now shifted to vector control strategies. An investigation into the interactions between the trypanosome and the tsetse, and subsequent exploitation of tsetse-specific molecules involved in parasite transformation, growth and survival, will perhaps ultimately enable interference with disease transmission. Understanding the molecular mechanisms underlying vector competence is pivotal to identifying anti-trypanosome vaccine candidate antigens. We have identified a tsetse-specific immune responsive protein found within the fly midgut that appears to influence the vectorial competence of tsetse. Utilizing RNA interference in adult flies, knockdown of this gene significantly increased midgut trypanosome infection rates, thus effectively reversing the natural refractoriness of the tsetse to trypanosome infection.

BSP268

Characterization of complement lectin pathway in *Trypanosome cruzi*

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In this study we characterize the lectin pathway activation and resistance by *T. cruzi*. Fist, Kinetics of complement activation pathways with epimastigote showed 40% and 86% of lysis with NHS (12.5%) after 5 and 10 minutes, respectively. EGTA-treated NHS (10 mM) blocking classical and lectin pathway showed 26% and 44% of lysis, while EGTA-treated NHS and NHS pre-treated parasites, inhibiting classical but not lectin pathway, resulted in lysis around 46 - 80%, showing that parasite lysis was mainly by lectin pathway. Second, competition assay between carbohydrates and SNH showed 40 mM mannose inhibited 82.5% of parasite lysis, while galactose and glucose inhibited 13.1 % and 42.5%, respectively. Third, Far Western blotting revealed three major MBL acceptors (200, 70 and 40 kDa) present on the surface of *T. cruzi*. Finally, in vitro cleavage of C2 with recombinant MASP2 was inhibited with 7,5 μM CRIT-ed1 peptide. Furthermore 50 μM of CRIT-ed1 inhibited 40% parasite lysis.

BSP282

Contribution of species-specific genes to disease tropism in visceral leishmaniasis

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Different Leishmania species can give rise to distinct disease phenotypes. For example, *Leishmania major*, *L. braziliensis* and *L. infantum* are normally responsible for cutaneous, mucocutaneous and visceral forms of the disease, respectively. The genomes of these three Leishmania species have recently been published (Peacock et al., Nature Genetics, 39, 839-847 (2007) (doi:10.1038/ng2053)) and comparative analysis has identified a small subset of species-specific genes. We are investigating the hypothesis that disease tropism is influenced by these species-specific genes. One example is SEC14 cytosolic factor of L. infantum, a putative orthologue of the yeast phosphatidylinositol/phosphatidylcholine transfer protein Sec14p, which plays a key role in budding of secretory vesicles from the trans-Golgi network (TGN). Secretory pathway proteins could potentially influence parasite surface molecules and thus host-parasite interactions. We are investigating SEC14 and other species-specific genes as potential contributors to the development of visceral infection in mouse models.

BSP285

Phosphodiesterase inhibitors as potential chemotherapies for Human African Trypanosomiasis, and as a new tool in unravelling downstream effects of cAMP signalling in trypanosomes.

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Current chemotherapies for Trypanosomiasis are unsatisfactory: drug resistance and toxicity. There is an urgent need for a new class of compounds not cross-resistant to existing treatments.

Recently the trypanosomal phosphodiesterase (PDE) enzymes were validated as promising drug targets. We have screened a number of TbPDE inhibitors in vitro, showing excellent activities against bloodstream form trypanosomes: EC50 values in the low nanomolar range. The most potent compound, GJS-128, rapidly stimulated cAMP production in bloodstream trypanosomes. FACS analysis and DAPI staining indicate that, rather than killing the parasites outright, the inhibitors disrupt the cell cycle, resulting in blocked or incorrect cytokinesis. This suggests a role for cAMP signalling in this process. Crucially, a *T. brucei* cell line adapted to high levels of GJS-128 was not cross-resistant to diamidines and melaminophenyl arsenicals. Presence or absence of resistance to cAMP analogues is being tested and will indicate if the adaptation is the PDE enzyme or further downstream.

BSP288

The oxidative folding pathway in *T. brucei*

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The formation of correct disulphide bridges during protein folding, the oxidative folding pathway, is important in all cells. A number of features make trypanosomes particularly suitable for investigation of this pathway. First, there are only a limited number constituents in this pathway compared to mammalian cells. For example, the genome of *T. brucei* encodes five putative protein disulphide isomerases (TbPDIs) and a single endoplasmic reticulum oxidase 1 (TbEro 1). Second, there is an obvious endogenous substrate for the pathway, namely the variable surface glycoprotein (VSG). Despite huge variation in primary structure, all VSGs fold into the same structure, which is underpinned by a series of conserved disulphide bonds. We have observed that the oxidative pathway appears to be up-regulated in bloodstream forms relative to procyclic forms. This finding fits with the fact that the VSG contains multiple disulphides, whereas the major surface protein of procyclic forms, PARP, has no cysteine residues.

BSP296

Differential control of trypanosome Procyclin expression by a post-transcriptional regulatory factor, TbZFP3

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The regulation of gene expression in trypanosome parasites provides an extreme paradigm for post-transcriptional control in a eukaryote. Most of our current knowledge of such regulatory mechanisms in these organisms has been derived from the study of procyclin mRNAs, encoding the major surface antigens in the midgut of the parasite's tsetse-fly vector. Despite detailed analysis of the regulatory sequences over fifteen years, proteins controlling the post-transcriptional expression of procyclins have remained elusive. Here we describe the sequence-specific binding and differential regulation of procyclin mRNAs by the developmental regulator TbZFP3, a CCCH-class RNA binding protein. The association of TbZFP3 with distinct procyclin mRNA isoforms provides a mechanism for the complex regulation of these antigens during different phases of tsetse infection.

BSP299

Gene Targeting and Biochemical Characterization of Clan SB and SC Serine Proteases in *Leishmania spp*.

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Serine proteases have been implicated in key stages of the infectious lifecycle of kinetoplastid parasites. Treatment of *Leishmania donovani* with the broad serine protease inhibitor, Pefabloc, arrested replication in vitro. Over twenty distinct serine protease genes can be identified in the published *L. major* genome. Our initial biochemical and proteomics studies of *L. donovani* and *L. major* extracts confirmed the presence of an active clan SC protease, oligopeptidase B (OpdB). Previous work in other trypanosomatids has shown that this subfamily of enzymes plays a role in host-cell invasion and contributes to the pathogenesis of disease. Additionally we have identified an *L. donovani* clan SB subtilisin. We have begun functional characterization of these two proteases and knocked out both genes by gene targeting. Phenotypic analysis of these knockouts is underway. Both genes have been cloned and recombinant protein expression is underway in both Leishmania and heterologous systems. Initial investigations suggest that subtilisin null parasites have impaired promastigote to amastigote differentiation axenically.

BSP303
Target Assessment for Drug Discovery at Dundee
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Drug discovery is a lengthy, risky and expensive process. Pharmaceutical companies are largely uninterested in tackling neglected tropical diseases for obvious economic reasons. Many antiparasitic drugs that have been developed through "piggy-backing" of existing pharmaceutical products have little practical use in resource-poor settings and do not meet pre-defined therapeutic product profiles. Many discovery projects fail due to insufficient critical assessment of what makes a good molecular drug target. The criteria used to assess targets for entry into the discovery pipeline at Dundee (essentiality, assayability, druggability, the potential for toxicity and resistance) will be discussed1. Our progress towards identifying a preclinical candidate for the treatment of human African trypanosomiasis by 2011 will be presented.

1. Frearson, J.A., Wyatt, P.A., Gilbert, I.H. and Fairlamb, A.H. (2007) Target assessment for anti-parasitic drug discovery. Trends in Parasitology, 23: 589-595 .

BSP304
Honing in on *Trypanosoma brucei* spp.
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Owing to the importance of *T. b. gambiense* in causing disease, sequencing of the T.b.gambiense group 1 DAL972 strain was initiated. Current activities focus on manual annotation of the genome and identification of species-specific differences in coding and non-coding sequences with regards to the *T. brucei* 927 genome. Analysis also includes the development of a pipeline to identify single nucleotide polymorphisms (SNPs), insertions and deletions as well as genes under selection. In addition, the project aims to investigate the diversity of *T. brucei spp*. Using new sequencing technologies, parents and progeny of a *T. b. brucei* and *T. b. gambiense* group 2 cross are being sequenced, allowing the haplotypes of the parental strains to be defined and generating detailed SNP and haplotype maps. Such maps will contribute to population studies and will underpin future trait-association studies aimed at identifying genes associated with phenotypes differing between parental strains.

BSP305

Investigating the life cycle of avian trypanosomes by molecular phylogeny.

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Avian trypanosomes are heteroxenous parasites transmitted by bloodsucking Diptera. To elucidate host-parasite relationships of avian trypanosomes and their insect vectors, we performed RAPD analysis and SSU rDNA sequencing with 50 trypanosome isolates. According to the results of sequence analyses, it was possible to ascribe all isolates to three clades representing already known trypanosome species *Trypanosoma corvi*, *T. bennetti* and *T. avium*. Only *T. corvi* clade was monophyletic, with two groups supported by both methods. The other two major clades were polyphyletic due to RAPD analysis. The *T. avium* clade was splitted into several groups that contained mainly isolates from black flies and raptors which corresponded to our previous study. However, the sequence analysis revealed also distinct group of isolates from mosquitoes and insectivorous songbirds inside the *T. avium* clade. Interestingly, this group was also found in RAPD analysis as a separate clade. Our results show that avian trypanosomes are complex and most probably several cryptic species exist within them.

BSP311

Microsatellite analysis of a Gambian *T. vivax* population

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Trypanosoma vivax is an infective parasite with a host range including domestic breeds of cattle, horses and donkeys and is one of the causative agents of Nagana. With transmission by flies other than the tsetse possible the parasite can be found over much of sub-Saharan Africa in addition to much of the South American continent. We have identified eight *T. vivax* specific microsatellite markers and with them have investigated a field population of *T. vivax* collected from The Gambia. Our analysis of samples collected from donkeys, horses and cattle shows our markers to be polymorphic and the population structure to be non clonal, suggesting the occurrence of genetic exchange.

BSP315

Novel robust and inexpensive eukaryotic cell-free translation system based on *L. tarentolae* cell-extract

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L. tarentolae is a non-pathogenic protozoan that belongs to Trypanosomatidae family of Kinetoplastida order. To the unique features of these organisms belong RNA editing, arrangement of genes in tandem arrays and polycistronic transcription followed by trans-splicing. As a result of trans-splicing the capped 39 nt -long splice leader

sequence (SL) is linked to the 5' end of every monocistronic maturing mRNA. Based on the fact that every endogeneous mRNA of Leishmania bears the identical SL sequence on its 5'-terminus, anti- splice leader oligonucleotide (αSL) was employed to block translation of endogenous but not heterologous mRNAs in the novel cell-free protein expression system.

BSP317

Global metabolic responses of mice to *Trypanosoma brucei brucei* infection

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Treatment of trypanosomiasis depends on infection stage, and early diagnosis is crucial for effective management. The systemic host biochemical changes induced by infection that might relate to therapeutic outcome or enable biomarker discovery are largely unknown. Multivariate metabolic temporal responses of mice to *Trypanosoma brucei* brucei infection are characterized in this study, using high resolution nuclear magnetic resonance spectroscopic metabolic phenotyping in urine and plasma. Marked alterations in plasma metabolic profiles were detected already 1 day postinfection and the sum of biochemical changes indicated an increased glycolytic activity directly related to parasite metabolism, renal damage, which was supported by histopathological evidence and degradation of the host membrane as a response to parasitic rearrangements of GPI anchors.
A satellite-experiment, founded on a metabolic time trajectory of plasma, revealed that crossing of the blood-brain barrier already occurred after 4-7 days postinfection.

BSP319

High-yield recombinant protein expression system based on protozoan *Leishmania tarentolae*

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In our effort to develop an efficient eukaryotic protein expression system we chose to exploit unique organization of transcription/translation machinery of Trypanosomatidae protozoa. Using *Leishmania tarentolae* as a host we created an expression architecture utilizing transcriptional activity of T7 polymerases. The expression cassettes are delivered in the form of linear or circular episomes as linear or circular elements. We demonstrate using FACS analysis that linear elements are result in higher transformation efficiencies and in the greater homogeneity of the transformed population and inherited by the other mechanism as random segregation. The inducible expression of heterologous proteins in the system resulted in accumulation of

recombinant proteins in cytosol with the yields up to 10% of total cellular protein. We present expression and purification of several eukaryotic proteins using the above described system. Finally we adapted the system to the parallel format by performing cultivation and protein expression in 24 well blocks.

BSP325

Structure-activity relationship of nucleoside transporters in *Trypanosoma brucei*: subtype of P1 type adenosine transporters can be distinguished on the basis of quantitative models of substrate binding.

Mohammed Al-Salabi and Harry de Koning

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Studying purine transporters in these parasites is very useful to understand the biochemistry of these parasites as these transporters play essential roles in their nutrition at the different stages of their life-cycle. We here describe in great details the functional characterisation of Adenosine Transporter B (AT-B) [or Nucleoside Transporter 10 (NT10)] and Adenosine Transporter D (AT-D) or [Nucleoside Transporter 9 (NT9)] expressed in yeast, and their expression in different developmental stages in the life cycle of *T. brucei*. Our observations confirm that NT10/AT-B is a P1-type transporter expressed specifically in short-stumpy bloodstream forms. We also report a full characterisation of NT9/AT-D, which is also shown to be a P1-type transporter: it is predominantly a purine nucleoside transporter with at best a secondary capacity to transport nucleobases or pyrimidines. But NT9/AT-D displayed much higher affinity for adenosine than any other nucleoside transporter yet reported, with a Km of 68 ± 13 nM. This high affinity of NT9/AT-D appeared to be a result of the transporter binding adenosine through interactions with both the P1 and P2 substrate recognition motifs.

BSP329

Investigation of the association of HUS1 to telomeres and DNA amplification loci of *Leishmania major*.

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Expression of HUS1 in *L. major* confers resistance to the radiomimetic drug phleomycin, suggesting that the product of this gene is involved in end-joining DNA repair events. Since the HUS1 gene is encoded within the amplification prone H-locus we speculate on the involvement of HUS1 in amplicon formation. In other organisms, HUS1 forms a complex with Rad1 and Rad9, associates with telomeres and participates in the maintenance of chromosomal integrity. We have generated a LmHUS1-GFP transfectant to localize HUS1 product within the cell and to investigate its possible association with telomeres and telomerase activity, using FISH and imuno-localization experiments. Furthermore, we are currently testing if HUS1 interacts with repeated sequence elements that are believed to mediate H-region amplification.

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BSP348

***Trypanosoma cruzi* trans-sialidase-mediated bead endocytosis and vesicle recruitment**

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Trans-sialidase is virulence factor found in abundance on the surface of *Trypanosoma cruzi* and is thought to play an important role in cell invasion and parasite escape from the parasitophorus vacuole. The enzyme works by transferring sialic acid residues from its cell surface or from the host cell surface to an acceptor substrate which is vital for parasite survival. A recombinant version of this protein has been purified and the activity measured. Beads have been coated with both the active and inactive enzyme. The active enzyme mediates bead attachment and endocytosis. Laurdan microscopy demonstrates changes in the fluidity of the host cell membrane at the bead interphase. Attached beads also appear to cause an increase in GFP tubulin intensity and the formation of ring-like structures on the cytosolic side of this interface as well as the recruitment of early endosomes in a manner analagous to that observed with trypanosoma cruzi during cell invasion and supporting the role of trans-sialidase as one of the primary mediators of cell invasion by this organism.

BSP349

The role of trans-sialidase in cell invasion by American trypanosomes

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The American trypanosome *Trypanosoma cruzi* enter host cells by a mechanism that is G protein dependent and uniquely relies on parasite recruitment of lysosomes along a newly constructed microtubule scaffold. Several parasite surface determinants have been strongly implicated in this process and we have developed reductionist systems to test their respective contribution. Using these systems we show that one surface determinant in particular, the trans-sialidase, is strongly implicated in virulence and cell invasion. We report that 4 micron beads coated with trans-sialidase induce their own uptake by the non professional phagocyte cell line MDCK and that *T. rangeli*, a parasite which is not normally invasive of these cells, becomes so when transfected with a GFP chimera of the trans-sialidase. The processes by which bead and parasite enter this cell line being dependent on trans-sialidase activity and akin to the invasion mechanisms of .*T. cruzi*

DERMANYSSUS- BAYER ANIMAL HEALTH ABSTRACTS

BSP015

Effect of plant essential oils as acaricides against the poultry red mite, *Dermanyssus gallinae*, with special focus on exposure time.

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Essential oils from thyme and cade were effective acaricides against the poultry red mite, *Dermanyssus gallinae* (De Geer), a pest of layer hens, over 24 hours. When tested over periods less than 24 hours, thyme essential oil killed *D. gallinae* relatively quickly and so may make for an effective acaricide, even if the residual toxicity of this product is low (as suggested by work elsewhere). Cade was not as effective, suggesting it may hold less promise in *D. gallinae* management.

Both juniper leaf and black pepper essential oil were relatively ineffective at killing *D. gallinae* over 24 hours in previous work. The results suggest that the toxicity of these oils to mites will increase if mites are exposed to them for longer periods. Even after 96 hours however, these oils were still only able to kill some 30% of *D. gallinae* exposed to them at the concentration used (0.21 mg/cm^2), and are unlikely to make for effective acaricides.

BSP016

Development of a plant-based acaricide for the poultry red mite, *Dermanyssus gallinae.*

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Fifty plant essential oils were tested for their toxic effect on poultry red mite (*Dermanyssus gallinae* (De Geer)), a serious pest of layer hens. Twenty-four of these 50 essential oils were found to kill in excess of 75% of adult mites over a 24 hour period at 0.21mg/cm^2. Tests at lower concentrations showed that essential oils from cade, manuka and thyme were especially toxic.

The toxicity of selected essential oils was found to be stable at temperatures ranging from 15-29 °C, although results suggest that humidity and dust might influence the toxicity of at least some of the oils tested.

Around half of the selected essential oils tested were effective in preventing *D. gallinae* eggs from hatching, and at least as many juvenile mites were killed by exposure to selected essential oils as were adults.

From these results, it appears that certain plant essential oils may hold promise as acaricides for *D. gallinae*, being effective over a range of conditions and against all life stages of the mite.

BSP017

The influence of 'time since last blood meal' on the toxicity of essential oils against the poultry red mite, *Dermanyssus gallinae*.

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'Time since last blood meal' had a notable effect on how toxic a selection of plant essential oils were to the poultry red mite, *Dermanyssus gallinae* (De Geer), a serious pest of layer hens. In general, an increase in the toxicity of essential oils to mites occurred if they were deprived of the opportunity to feed for around three weeks, as opposed to when they had been more recently fed. This was consistent across all essential oils tested (thyme, palmarosa, caraway and juniper leaf).

As a control measure in *D. gallinae* management, essential oils may be especially effective if used to target mites that persist between flocks. These mites would have been starved after the removal of one flock, prior to the introduction of another.

In general, thyme and caraway essential oil were more toxic to *D. gallinae* than juniper leaf or palmarosa essential oil, and may make for better control products for this pest.

BSP048

***Dermanyssus gallinae* prevalence in laying hen units in the Apulia region, Italy.**

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A study on the prevalence of the poultry red mite, *Dermanyssus gallinae*, was performed from April to December 2007 in laying hen farms representing more than a thousand birds. A total of 58 farms were visited. A questionnaire was submitted to the farmers regarding the production and management systems they used and to investigate their perception of the problems related to infestation.

Forty-three farms (74.14%) were found to be infested. Farmers were generally aware of the red mites however they did not know the biology of *Dermanyssus gallinae* and the real consequences of the infestation; moreover, they use self-devised or inappropriate control measures. Due to frequent treatments with acaricides, the period time of observations, conflicting results obtained in some farms and the prevalence data registered might underestimate the situation. Further investigations in non-infested farms are needed to acquire the real prevalence in the investigated area.

BSP050

Evaluation of poultry red mite susceptibility, *Dermanyssus gallinae* (Acarina: Dermanyssidae), to some acaricides in Italy.

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In two selected Apulian farms, the susceptibility of a field population of *Dermanyssus gallinae* to the most used acaricides (carbaryl, amitraz and permethrin) was evaluated in triplicate by an adapted filter paper based bioassay using different concentrations (100X, 50X, 20X, 10X, 5X, 0X). After 24 h exposure, *D. gallinae* is susceptible to all concentrations of amitraz (100X, 50X, 20X, 10X, 5X) in both farms (A and B), partially (100X, 50X and 20X) and totally susceptible to permethrin in farm A and B, respectively ($p<0.05$). Furthermore, the poultry red mite was not susceptible to any concentration of carbaryl (100X, 50X, 20X, 10X, 5X) for both farms ($p<0.05$). The quite high percentage of survival showed by red mites exposed to carbaryl and permethrin suggests for a possible resistance of the Italian *Dermanyssus* populations to this molecule, whose investigation is still ongoing.

BSP153

Identification of a histamine release factor and its potential as a vaccine antigen for controlling *D. gallinae* infestation in commercial poultry houses.

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The blood-feeding poultry red mite *Dermanyssus gallinae* is the most economically important ectoparasite affecting commercial egg farming in Europe. Control of mite infestation is becoming increasingly difficult due to the withdrawal of acaricides; this has made alternative control strategies such as vaccination an attractive prospect.
Studies in ticks have demonstrated the feasibility of using vaccination with tick proteins (e.g. gut-associated, salivary and reproductive proteins) to control infestations. An orthologue of a conserved tick salivary protein, the histamine release factor (HRF) has been identified in *D. gallinae*. The predicted *D. gallinae* protein sequence (174aa) has a high percentage identity with several tick HRF orthologues ranging from 49.3% to 54.6%. Phylogenetically the *D. gallinae* HRF partitions with the tick HRF clade suggesting a shared linage and potentially a similar function.
A recombinant *D. gallinae* HRF protein has been purified and will be used to investigate the biological importance and function of HRF; the potential as a vaccine antigen will also be discussed.

BSP162

In vitro feeding assay to test protective effects of Poultry Red Mite antigens

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Dermanyssus gallinae, the poultry red mite (PRM) is a blood-feeding ectoparasite that infests many bird species. We are working towards identifying PRM antigens that could be used to generate protective immune responses against PRM. Whole PRM extract was solublised in PBS, Tween 20, Triton X100 and Urea sequentially giving four protein fractions. Five experimental groups of hens were used in a vaccination trial; four groups were vaccinated with each of the protein fractions in QuilA and a control group was vaccinated with adjuvant only. Booster injections were administered on weeks two and four. Eggs and blood samples were taken at weeks zero, four and six. Western blots were carried out using pre and post vaccination serum and aqueous extracts of yolk antibodies. Western blots revealed an antibody response against the injected proteins. Fresh chicken blood, supplemented with egg yolk extracts, was fed to conditioned mites in an in vitro feeding assay and repeated three times using all five groups in order to determine whether the antibodies have an anti-mite effect.

BSP228

***Dermanyssus gallinae* mites collected from pigeon nests and laying hens: a molecular study based on the ITS region**

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The complete internal transcribed spacer 1 (ITS1), 5.8S rDNA, and ITS2 regions of nuclear ribosomal DNA (including portions of the 3′ end of the 18S and 5′ start of the 28S) were used as tool for *Dermanyssus gallinae* identification. Individual samples of *Dermanyssus gallinae* from pigeon nests and laying hens from Southern Italy were PCR amplified (based on the complete ITS region) and sequenced to assess the utility of the ITS region for red mite identification and molecular phylogenetic studies. Comparative studies of sequences showed homogeneity at molecular levels among analysed samples and 98% identity with andalucian *Dermanyssus gallinae* available in the GenBank database (with slight differences in the ITS1 region in terms of point mutations). Results from this preliminary study suggest that the use of ITS region provides an efficient and reliable mean of identification.

BSP229

Dermatitis by *Dermanyssus gallinae* in humans living in urban environments, in Italy.

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This contribution reports seven cases of dermatitis caused by *Dermanyssus gallinae* in humans living in urban settings and highlights the zoonotic role of red mite in urban environment. In all the cases, it was constantly observed the presence of feral pigeons (*Columba livia*) and their abandoned nests, in close proximity to the infested rooms. *Dermanyssus gallinae* is the most common mite of feral pigeons and it can also colonizes domestic habitats and/or bite humans when their natural avian hosts are not available. These birds' growing presence in cities increases the possibility of human contact with red mites, which can be potential vectors /reservoirs of zoonotic agents.
Hence, this parasitosis can be considered as a problem of urban hygiene. As we observed, dermatologists often misdiagnose skin lesions. A close collaboration between physicians and veterinarians could be useful to manage correctly this epizoonosis.

BSP275

Predatory arthropods as biocontrol agents of *Dermanyssus gallinae*?

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As a first step to study biocontrol of *Dermanyssus gallinae* two species were selected for laboratory tests, a predatory mite *Hypoaspis miles*, and a predatory bug *Orius majusculus*. Both are currently used biocontrol agents in greenhouses. Predatory mite tests were performed in single cells where a female and a male of *H. miles* were fed merely on *D. gallinae* eggs. The consumed food and the egg production during the 25 days' feeding period were counted daily. In predatory bug tests 3rd instar nymphs were used and the food consumption was counted during the development to adult stage and one week thereafter. *H. miles* consumed 1.6 eggs or larvae per day and produced 1.5 eggs/day. *O. majusculus* developed from the 3rd nymph stage to adult in ten days and consumed 4.5, 10.8 and 76.8 eggs or larvae in the 3rd, 4th and 5th nymph stages, respectively. Adults consumed 17.5 eggs or larvae per day, and attacked also nymphs of *D. gallinae*. These figures encourage besides further studies on the above predators also screening for other potential arthropod predators.

BSP276

Phylogeny of *Dermanyssus Dugès*, 1834, definition of species limits, and preliminary assessment of host range.

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The genus Dermanyssus currently includes 23 hematophagous species found primarily on birds. A group of 14 species including *D. gallinae* exhibits intraspecifically high morphological variability, making the definition of species limits very difficult.
To examine and better define species boundaries, type material for most Dermanyssus species was obtained and extensive collections of wild bird nests were carried out. Morphological characters were coded for 20 Dermanyssus species and molecular data (COI, 16S, and ITS) was obtained for a subset of species.
Phylogenetic reconstruction of the morphological data set produced poorly resolved trees, whereas phylogenetic analysis of the combined morphological and molecular data for a subset of taxa produced a more resolved phylogenetic hypothesis. The results also challenge ideas on host specificity, because some species may have a narrower host range than expected. Finally, a phylogeographic approach to analyse populations of *D. gallinae* collected from poultry farms and wild birds is presented.

BSP277

Laboratory evaluation of the effect of inert dust formulations on the poultry red mite, *Dermanyssus gallinae*.

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In the EU-project SAFEHOUSE one objective is to develop alternative control methods against poultry red mites based on a combination of inert dusts and mite-pathogenic fungal isolates. Both components have some pathogenicity to the mites. However, the question is whether a combination of the two components might produce a synergistic effect as seen in other arthropods. As a first step, the effects of a range of inert dust formulations were evaluated in the laboratory at different doses and at different levels of air humidity. The results have shown major variations in the speed of kill but also in the repellency of the different formulations, and both parameters vary with the dose and the air humidity.

BSP278

Entomopathogenic fungi for control of arthropod pests in egg production facilities

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Beauveria bassiana and other species of entomopathogenic fungi are potential candidates for microbial control of major pests in egg layers, e.g. the poultry red mite (*Dermanyssus gallinae*), the housefly (*Musca domestica*) and the darkling beetle (*Alphitobius diaperinus*). We have selected an isolate of *B. bassiana* with high efficacy against all target pests in laboratory assays, and will review the existing information on the natural occurrence of these fungi in farms with confined animals and discuss the possibilities and constraints for exploitation of entomopathogenic fungi as control agents in egg production facilities.

BSP290

First identification of bacterial taxa associated with the hematophagous mite, *Dermanyssus gallinae* by 16S rDNA PCR amplification and TTGE fingerprint

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Dermanyssus gallinae has been involved in the transmission of a wide variety of pathogens. Although the mite may harbor both types of pathogens, nothing is known about its associated non-pathogenic bacterial community. To address this question, we examined the composition of bacterial communities in *D. gallinae* collected from standard poultry farms in France. Genetic fingerprints of bacterial populations were generated by temporal temperature gradient gel electrophoresis (TTGE) separation of individual polymerase chain reaction (PCR)-amplified 16S rRNA gene fragments, followed by DNA sequence analysis. Most of the sequences belonged to the Proteobacteria and Firmicute phyla with a majority of species corresponding to the enterobacteriales order and the *Staphylococcus* genus. Saprophytic species, opportunistic pathogens and pathogenic agents were identified. Endosymbionts were also present in the sub-dominant bacterial population.

BSP307

Dermanyssus gallinae: Acari parasite highly aggressive but still ignored in Morocco

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Infestations with Mesostigmata Acari are a major problem in humans and domesticated animals. Economic losses in poultry systems are nowadays obvious. In humans, dogs and horses this parasite is responsible for pruritus extremely difficult to cure. 56 farms (modern and traditional), a national park and two hospitals were visited. 75% of the traditional farms were infested compared to only 22.5% of modern farms. The study showed that children, farm workers, horses and dogs developed allergies possibly due to Dermanyssus acari. Pigeons, turtle doves and sparrows living on the roof of these two hospitals were highly infested.

BSP302
Bluetongue virus in 2007: It came, it saw, it conquered. What next?
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In 2007, bluetongue virus (BTV), a biting midge-borne virus of ruminants, appeared in the UK for the first time, following unprecedented outbreaks of the virus in northern Europe during 2006. I will initially give a short introduction to the virus and its epidemiology, then examine progress in implicating midge species in the transmission of the virus. Then I will discuss the various methods that can be employed in attempting to control the spread of the virus with an emphasis upon insect-control techniques currently available. Finally I will provide an overview of responses to BTV incursion, including those novel surveillance and control techniques being pioneered at IAH.

BSP327
Natural repellents from human hosts against biting insects
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Haematophagous insects show differential behavioural responses to odours from individual human hosts. Hosts that show low attractiveness to mosquitoes produce compounds that can interfere with host attraction. Several putative human-derived repellents were tested, as topically applied repellents, against several species of biting flies. Single compounds and mixtures were tested in dose response assays. Additionally, we investigated the potential of using the compounds as spatial repellents in a chemical dispenser, which maintains ratio integrity of the active compounds. Two of the human-derived compounds show effective topical and spatial repellency against mosquitoes and midges. Despite the high volatility of the active compounds, some persistence over time has been achieved. This study provides an insight into how biting insects respond to naturally produced human-derived repellent compounds, and how such compounds could be exploited as topically applied or spatial repellents.

BSP342
Risk factors for house entry by malaria vectors: a precursor to a trial of house screening against malaria in The Gambia
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In the pre-intervention year of a randomized controlled trial (RCT) investigating the protective effects of house screening against malaria-transmitting vectors, a multi-factorial risk factor analysis study was used to identify factors that influence mosquito house entry. Mosquitoes were sampled using CDC light traps in 976 houses, each on one night, in Farafenni town and surrounding villages during the malaria-transmission season in The Gambia. Catches from individual houses were both (a) left unadjusted and (b) adjusted relative to the number of mosquitoes caught in four sentinel houses that were operated nightly throughout the period, to allow for night-to-night variation. Houses were characterized by location, architecture, human occupancy and their mosquito control activities, and the number and type of domestic animals within the compound. The key findings of this work, as well as the design and progress of the RCT, will be discussed.

BSP314
The use of infrared thermography to study traumatic myiasis of sheep
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Traumatic myiasis, due to the development of fly larvae in wounds on the skin surface of vertebrate hosts or in natural body orifices, causes considerable pain and suffering. It can be difficult to detect the early stages of infestation by eye, especially in individuals in large flocks or herds in transit. At the site of infestations the skin temperature can be raised. The potential for infrared thermography to be used to detect such "hot spots" on sheep infested with larvae of the fleshfly *Wohlfahrtia magnifica* was studied in Hungary. Temperatures of healthy and infested tissues were recorded with FLIR Systems cameras. In general, the surface temperature of infested tissues or larvae in these tissues was about 1°C hotter than surrounding tissues and this could be detected from a distance. However, there was great variation and while infrared thermography will be a useful research tool for studying the pathology of traumatic myiasis, it is unlikely to have a significant application in detecting covert, larval infested wounds due to the complex thermal signature of sheep.

BSP343
New Approaches to Visceral Leishmaniasis Control in Latin America.
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Leishmania infantum the causative agent of visceral leishmaniasis is transmitted by the bite of infected female Lutzomyia longipalpis sandflies in Latin America. The disease causes an estimated 10 – 12 thousand deaths per annum in Brazil mostly among the urban and rural poor. Attempts to reduce disease incidence through vector and reservoir control is widely practiced, however despite intensive efforts the disease is increasing particularly in urban areas.
New approaches to vector control that can be widely applied would make a useful contribution to existing sandfly and disease control measures. One such approach may be to manipulate the chemical communication of mate seeking sandflies. We have synthesised a stable analogue of the sex pheromone of one of the members of the *L. longipalpis* species complex and shown in laboratory studies that it is as attractive as the real sex pheromone. Work is currently underway to test the feasibility of using this chemical in the field.

BSP320

Odour baits for malaria mosquitoes – elucidating the essential chemicals amongst a highly variable human population

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Malaria remains a highly prevalent disease in the tropics, transmitted by anopheline mosquitoes. Accurate assessment of epidemiological risk is hampered by lack of rapid and objective tools for estimating the adult vector population. Mosquitoes find their hosts mainly by olfactory cues, responding to odorants emitted by the host. Scientists have for many years worked on the identification of human odorants to mediate mosquito behaviour. In recent years we have studied human odorants that are attractive for the malaria mosquito *Anopheles gambiae s.s*. Significant and consistent differences in attractiveness between humans were found in the laboratory and (semi-) field. A blend of chemicals was significantly attractive in an olfactometer. Several of these chemicals also elicited attraction in a semi-field enclosure and in an outdoor situation. The paper will discuss the relevance of the research chain from the laboratory to the field, and how this can lead to the development of synthetic odour blends that mimic humans.

BSP321

Mosquito-pathogenic fungi and the development of rational, adult-based, control interventions

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Entomopathogenic fungi have been known to kill aquatic stages of mosquitoes but their use as a biological control tool against adults is very new. We present here the

rationale why delayed kill of adult female mosquito vectors is an important prerequisite to curb the development of resistance. In addition, we demonstrate that application of this tool can be cost-effective and is well-suited to be incorporated in Integrated Vector Management (IVM) programmes. Delivery and formulation research as well as novel methods to expose wild mosquitoes to the fungus (by using Ghanaian water storage pots) will be presented. Our previous field work (in Tanzania) has shown a 23% effective coverage and resulting decline of the EIR by nearly 75%. Recent data show that we can reach 39% coverage in laboratory experiments. Given such effects under field conditions, the EIR can be reduced by over 90%.

BSP334

Harmony between the sexes; auditory interactions between male and female Culex mosquitoes

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Mosquitoes produce sexually dimorphic wing beat frequencies, with up to twenty harmonics. We have shown previously that both male and female mosquitoes of the species *Toxorhyncites brevipalpis* respond to flight tones by altering their own wing beats. This report shows a similar type of auditory interaction for two species of blood-feeding mosquito of the genus *Culex*. The wing-beat frequencies of male-female pairs of *Culex* in tethered-flight were recorded. Each mosquito altered its own wing-beat frequency in response to the sound of the other, converging on the nearest shared harmonic frequency of their flight tones. The fundamental wing- beat frequency of the male is at least 1.5 times higher than that of the female and the closest shared harmonic can be > 1000 Hz, which exceeds the sensitive frequency ranges of both the mechanical tuning of their antennae and the neural tuning of their JO. It is possible, however, that mosquitoes detect the *difference* between their respective frequencies, usually < 20 Hz when they are close to synchrony. With respect to this, we measured behavioural responses from tethered *Culex* to low frequency (10 – 80 Hz), moderate level, acoustic stimulation.

BSP332

Chemical Ecology of the bedbug, *Cimex lectularius* (Cimicidae).

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Bedbugs which feed nocturnally upon the blood of human hosts have re-emerged as an important urban pest in developed countries. Bedbugs are known to emit alarm and aggregation pheromones. Although the alarm pheromone has been identified less is

known about the aggregation pheromone and associated behaviours. This study aims to understand the chemical ecology of bedbugs with the view to identify an aggregation pheromone.
Most available bioassays would be inappropriate as bedbugs are stressed by air flow. Preliminary experiments identified a suitable bioassay, the still-air Petri-dish olfactometer, results demonstrated that in the presence of a known stimulus there was a significant increase in bedbug activity. Further work with air entrainments, gas chromatography and electrophysiology will enable the identification of behaviourally active compounds which could be exploited to develop monitoring tools to detect bedbug infestations.

Late abstracts (addendum)

BSP350
Bluetongue vectors: A Northern Ireland Perspective
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The activity of bluetongue vectors (*Culicoides spp.)* in Northern Ireland was monitored at twelve sites using Onderstepoort suction-light traps. Trapping commenced in early October 2007 and a maximum of 8,884 adult *Culicoides spp*. were recorded at one site during a single evening trapping period. Representative species from Obsoletus and Pulicaris complexes were found at all sites, including C. chiopterus, *C. dewulfi*, *C. obsoletus*, *C. scoticus* and *C. pulicaris*. Adult midge activity declined throughout the latter part of the year and a bluetongue vector-free period was declared in late December 2007. During this period, and with no evidence of iatrogenic infection, a bluetongue outbreak occurred in Northern Ireland. A number of pregnant cattle were imported to a farm in Northern Ireland from the Netherlands. Post-importation tests indicated that some of the pregnant cattle had antibodies to the bluetongue virus but were not viraemic (RT-PCR negative). Two of the cattle subsequently gave birth to three calves that indicated bluetongue virus infection (RT-PCR positive) with one calf demonstrating viraemia. This is the first field evidence of transplacental transmission of bluetongue virus. Two further viraemic animals (one newly-calved heifer and one milking cow) were disclosed and circumstantial evidence suggests contact spread of the bluetongue virus with oral transmission being the most probable route of infection.

The following abstract is replacing BSP171
BSP351
Active state structures of Leishmania mexicana Pyruvate Kinase: structural templates for novel anti-trypanosome drugs.
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The tetrameric enzyme pyruvate kinase (PYK) is final enzyme in glycolysis, yielding ATP and pyruvate from ADP and phosphoenolpyruvate. The exact mechanistic action required for PYK to perform this reaction remains unclear, although snapshots of PYK in two conformations (T-state/inactive and R-state/active) have been captured by X-ray crystallography.
Here we report the preliminary analysis of two new X-ray structures of PYK from *Leishmania mexicana* (*Lm*PYK), which have been trapped in different conformations. These, together with the previously reported structure of *Lm*PYK in its inactive (T-state) conformation, allow comparisons of various different conformers of the same species of PYK. We have obtained crystals of *Lm*PYK with ammonium sulphate as precipitant, and shown that the structure of the homo-tetrameric enzyme corresponds

to a pseudo-active conformer with sulphate ions at the active and effector sites. *Lm*PYK has also been cocrystallized as a complex in its active (R-state) conformation with $Mg^{II}ATP$, oxalate, Mg^{2+} and K^{+}. The conformational changes observed show rotations of the $(\alpha/\beta)_8$-barrel A domains which act like cogwheels and provoke significant changes in the substrate binding pocket. These new structures provide insight into the various structural transitions experienced during catalysis and demonstrate potentially new drugable enzyme states for the development of novel anti-trypanosome drugs.

Cancelled abstracts

BSP064: Helen Taylor previously in session 5A (oral talk)
BSP171: Iain McNae et al (poster) replaced by **BSP351**
BSP265: Lee Haines et al (poster)

Change of Speakers

Orin Courtenay is now replacing Rupert Quinnell for Talk 3D2