Welcome to Edinburgh for BSP 2009

Dear Delegates,

It gives us great pleasure to welcome you to Edinburgh for the annual British Society for Parasitology Spring and Malaria meeting. We are delighted to be hosting delegates from many different countries around the world, including UK, and hope that you will all enjoy the opportunity to hear and discuss some interesting science and to catch up with old colleagues and meet some new ones.

We are very grateful to the many people who have helped to convene the different scientific sessions and to all our invited speakers enabling us to put on such a varied and interesting scientific programme. This includes a mini-symposium on "The changing faces of malaria"; several linked sessions on Apicomplexan parasites; a special symposium on *Neospora caninum* as well as sessions on Veterinary Parasitology; immunomodulation, genomics, gene silencing, live imaging and much more.

The plenary lecture this year will be presented by Prof Andrew Tait, Chair of Veterinary Parasitology at The University of Glasgow.

We hope you will enjoy your stay in Edinburgh and take part in some of the social activities in the programme including the Welcome reception on Sunday 5th April, a wine reception on Monday 6th April during the poster session and the Conference dinner and ceilidh being held at Dynamic Earth on 7th April.

We have also teamed up with the Edinburgh International Science Festival and there will be a talk on parasites at the science festival on the 8th April entitled: *The beasts within us: parasites* "http://www.sciencefestival.co.uk"

Finally we would like to express our gratitude to our many sponsors who have helped to fund invited speakers and to sponsor some of the symposia at the meeting.

We wish you a very enjoyable conference and a memorable visit to Edinburgh.

Very best wishes

John Jones (SCRI)
Lee Innes (Moredun Research Institute)
Richard Carter (University of Edinburgh)

SOCIAL EVENTS

Sunday 5th April	
18.00-20.00	Welcome reception drinks and finger buffet (Appleton Tower Concourse)
Monday 6th April	
17.30-20.00	Poster session with drinks and nibbles (Appleton Tower Concourse)
Tuesday 7th April	
19.00-0100	Conference Dinner and Ceilidh at Dynamic Earth (Holyrood Road)

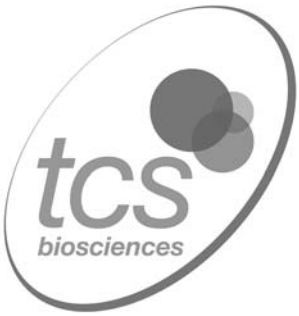
STUDENT PRIZES

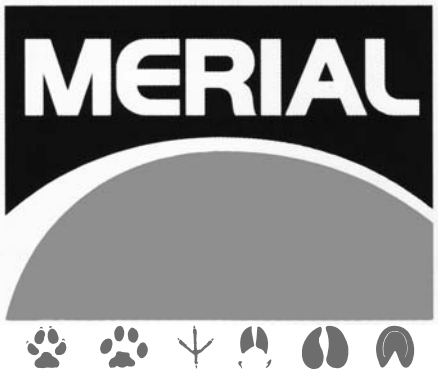
In addition to the BSP student prize competition there will also be additional prizes for the best student oral paper and best student poster paper in the Veterinary Parasitology section.

The Veterinary Parasitology prizes are being donated by Moredun Research Institute and Glasgow University Veterinary School.

Entrants for student prize competions are marked with an asterisk in the programme summary and poster summary tables.

The organisers would like to thank the following for their generous sponsorship of the 2009 BSP Spring & Malaria meeting:





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In addition, the following have taken trade stands at the meeting:

Bioline	VH Bio	Roche Applied Science
Royal society of Tropical Medicine and Hygiene	GATC Biotech	Fermentas Life Sciences
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TCS Biosciences	Elsevier	

BSP 2009 – Scientific Committee

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Lee Innes
Richard Carter
Paul Hunt
Sandie Cheesman
Joanne Thomson
James Brewer
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Alex Rowe
David Cavanagh
Alison Creasey
Petra Schneider
Hilary Hurd

PLEASE NOTE – SESSIONS ARE NOW STAGGERED OWING TO THE LARGE NUMBERS OF DELEGATES – TIMINGS ON THIS SUMMARY MAY THEREFORE NOT BE ACCURATE

BSP SPRING & MALARIA MEETING 2009. EDINBURGH, 5TH-8TH APRIL – PROGRAMME SUMMARY				
SUNDAY APRIL 5TH				
18.00 – 20.00	REGISTRATION, WLECOME RECEPTION & FINGER BUFFET (Appleton Tower Concourse)			
MONDAY APRIL 6TH				
09.00 – 10.30	1A: Mini Symposium – the changing faces of Malaria	1B: Parasites and the environment	1C: Veterinary Parasitology I: Chemotherapy and anthelminthic resistance	1D: Immunomodulation by helminths
10.30 – 11.30	Tea/Coffee break (staggered)			
11.00 – 12.30	2A: Malaria – evolutionary biology and ecology	2B: Apicomplexa I – gene expression	2C: Veterinary Parasitolgy II – immune response and vaccination	2D: Genetic manipulation and gene silencing in helminthes
12.30 – 14.00	Lunch break (Teviot Row)			
14.00 – 15.30	3A: Malaria – sexual development and transmission	3B: Apicomplexa II – population biology	3C: Ectoparasite control	3D: Helminth Genomics
15.30 – 16.00	Tea/Coffee break (staggered)			
16.00 – 17.30	4A: Malaria – vector biology	4B: Apicomplexa III – host-pathogen interactions	4C: Drug discovery for tropical diseases	4D: New sequencing technologies
17.30 – 20.00	POSTER SESSION WITH DRINKS RECEPTION (Appleton Tower Concourse)			
TUESDAY APRIL 7TH				
09.00 – 10.30	5A: Malaria – genetics, classical and genomic	5B: Neospora Symposium	5C: Immunomodulation by helminthes	5D: Helminth neurobiology *(n.b. this session starts at 8.45)*
10.30 – 11.00	Tea/Coffee break (staggered)			
11.00 – 12.30	6A: Malaria – pathogenesis	6B: Neospora Symposium	6C: Trypanosomes & Leishmania I – kinetoplastid parasite – vector interactions	6D: Helminth neurobiology
12.30 – 14.00	Lunch break (Teviot Row)			
14.00 – 15.30	Plenary Sessions (George Square Lecture Theatre)			
15.30 – 15.45	Comfort break			
15.45 – 17.00	BSP AGM (George Square Lecture Theatre)			
19.00	CONFERENCE DINNER (Dynamic Earth)			
WEDNESDAY APRIL 8TH				
09.00 – 10.30	8A: Malaria – vaccines	8B: Live parasite imaging	8C: Trypanosomes & Leishmania II	8D: Veterinary Parasitology III – epidemiology and population biology
10.30 – 11.00	Tea/Coffee break (staggered)			
11.00 – 12.30	9A: Antimalarial drugs – field and laboratory	9B: Live parasite imaging	9C: Open session	9D: Veterinary Parasitology IV
12.30 – 14.00	Lunch break (Teviot Row)			

Monday 6th April; 09.00 – 11.00							
Time	**Session 1A Mini Symposium: THE CHANGING FACES OF MALARIA.** Chair/Convenor: Alison Creasey	Time	**Session 1B PARASITES AND THE ENVIRONMENT**. Chair/Convenor: Huw Smith **Sponsored by DWQR and TCS Biosciences**	Time	**Session 1C VETERINARY PARASITOLOGY I – CHEMOTHERAPY AND ANTHELMINTHIC RESISTANCE** Convenor: Frank Jackson Chair: Philip Skuce **Sponsored by Merial**	Time	**Session 1D IMMUNOMODULATION BY HELMINTHS** Convenor: Rick Maizels Chair: David Pritchard
09.00	Colin Sutherland (LSHTM) **The global malaria situation in 2009 – what has changed? What else might soon change**	09.00		9.00		09.00	Grainger, J.R., McSorley, H.J., Harcus, Y.M., Filbey, K., Greenwood, E.J.D, Hewitson, J.P., Smith, K.A. and Maizels, R.M. (University of Edinburgh). **Helminth Immunoregulation through TGF-beta signalling**
09.30	Kevin Marsh (KEMRI Wellcome Programme) **Malaria in Africa: prospects for change**	09.30	John Moore (Belfast City Hospital) **Title to be confirmed**	09.30	Georg von Samson-Himmelstjerna, Janina Demeler, Stefan Pachnicke (University of Veterinary Medicine, Hannover) **Detection and mechanism of drug resistance in nematodes**	09.30	Sheila Donnelly, Sandra M. O'Neill, Mark W. Robinson & John P. Dalton. (University of Technology Sydney & Dublin City University) **Helminth 2-Cys Peroxiredoxin drives Th2 responses through a mechanism involving alternatively activated macrophages**
						09.45	David J. Dowling, Clare. M. Hamilton, Sheila Donnelly, James La Course, Peter M. Brophy John Dalton & Sandra. M. O'Neill. (Dublin City University, Aberystwyth University & University of Technology Sydney) **Helminth antigens induce distinct dendritic cell phenotype**

10.00	Nick Anstey (Menzies School of Health Research) **The changing face of malaria in the Asia-Pacific**	10.00	John Ellis, Damien Stark, Joel Barratt, Debbie Marriott & Jock Harkness (University of Technology, Sydney & St. Vincent's Hospital Sydney) ***Dientamoeba fragilis*: recent advances and its role as a human pathogen.**	10.00	Frank Jackson, Fiona Kenyon, Andy Greer, David Bartley, Philip Skuce, Alison Donnan, David McBean & Yvonne Bartley (Moredun Research Institute & Lincoln University) **Modern methods for the management of anthelmintic resistance in sheep**	10.00	Min Zhao, David Brown, Janis MacCallum and Lorna Proudfoot (Napier University). **Excretory-secretory (ES) products of *Nippostrongylus brasiliensis* L3 larvae inhibit LPS-induced inflammation**
		10.15	Rosely A. B. Nichols, Lisa Connelly, Huw V. Smith (Scottish Parasite Diagnostic Laboratory) ***Cryptosporidium* species and genotype diversity in UK waters**	10.15	David J. Bartley, J.Dupuy, M. Alvinerie, F. Jackson & A. Lespine (Moredun & INRA) **The influence of ketoconazole and pluronic 85 on the efficacy and pharmacokinetics of ivermectin in *Haemonchus contortus* infected lambs.**	10.15	*Ekta Bhardwaj, Kathryn. J. Else, Geoff Warhurst & Mike Rogan (University of Salford, Manchester University & Salford Royal Hospital) **Inflammatory Bowel Disease and *Trichuris muris***
		10.30	Lisa Connelly, Barbara.H. Craig, Josephine.M. Pemberton, Rosely A.B. Nichols & Huw V. Smith (Scottish Parasite Diagnostic Laboratory & University of Edinburgh) **Determining species and sub-genotypes of *Cryptosporidium* infecting St. Kilda Soay sheep**	10.30	*El-Abdellati A., De Graef J., Vercruysse J., Skuce P, Donnan A., Claerebout E., Geldhof P. (Ghent University & Moredun Research Institute) **Molecular analysis of the *GluClalpha3* gene in ivermectin-susceptible and ivermectin-resistant isolates of *Cooperia oncophora***		
		10.45	Marnie L. Brennan, Jonathan Wastling, Emily J. Brook & Robert M. Christley (University of Liverpool & Moredun Research Institute) **Molecular epidemiology of *Cryptosporidium* - can subgenotype variation be explained by contacts between cattle farms?**	10.45	Alison Dicker, John Gilleard, Philip Skuce, Alasdair Nisbet, David Bartley, Collette Britton & Frank Jackson (Moredun Research Institute & University of Glasgow) **Are P-glycoproteins involved in macrocyclic lactone resistance in *Teladorsagia circumcincta*?**		

*Presenters marked with an asterisk are entries for the student presentation competition

Monday 6th April; 11.00 – 12.30							
Time	**Session 2A**: **MALARIA: EVOLUTIONARY BIOLOGY AND ECOLOGY** Chair/Convenor: Petra Schneider	Time	**Session 2B: APICOMPLEXA I – GENE EXPRESSION** Chair/Convenor: Fiona Tomley	Time	**Session 2C: VETERINARY PARASITOLOGY II – IMMUNE RESPONSE AND VACCINATION** Convenor: Lee Innes Chair: Al Nisbet **Sponsored by Pfizer Animal Health**	Time	**Session 2D: GENETIC MANIPULATION AND GENE SILENCING IN HELMINTHS** Convenor: Angela Mousley Chair: Michael Kimber
11.00	Richard E.L. Paul (Institut Pasteur) ***Plasmodium falciparum* sexual strategies & human variability**	11.00		11.00		11.00	Kristine J. Kines, Victoria H. Mann, J. Gabriel Rinaldi, Sutas Suttiprapa & Paul J. Brindley (George Washington University Medical Center) **Transgenesis of schistosomes: approaches with retroviruses**
11.30	*Pollitt, L.C., Mideo, N. & Reece, S.E (University of Edinburgh). **Do rodent malaria parasites (*P. chabaudi*) change their investment into transmission stages in response to competition?**	11.30	Chris Newbold (John Radcliffe Hospital, Oxford)	11.30	Robin J. Flynn, Olwen Golden, Mary Sekiya and Grace Mulcahy (University College Dublin). ***Fasciola hepatica* infection in cattle – Prospects for Vaccination**	11.30	Lisa Pierson, Angela Mousley, Nikki J. Marks & Aaron G. Maule (Queen's University Belfast) **RNA interference in the tapeworm, *Moniezia expansa***
11.45	Petra Schneider (University of Edinburgh). **Virulence-dependent drug sensitivity: do dose, duration and type of treatment matter?**					11.45	*Matthias Lendner, Richard Lucius & Susanne Hartmann (Humboldt-Universität zu Berlin). **Pitfalls on the way to RNA interference in parasitic nematodes - The example of *Heligmosomoides polygyrus***
12.00	*Issa Lyimo & Heather Ferguson (University of Glasgow & Ifakara Health Institute) **Why does *Anopheles gambiae* specialize on humans?**	12.00	Jonathan Wastling. (University of Liverpool) **A proteomic perspective on gene expression in the Apicomplexa**	11.55	Smith W.D. (Moredun Research Institute) **Twists and turns en route to a vaccine for *Haemonchus contortus*.**	12.00	E. Cameron, A. Mousley, N.J. Marks, C. Lindsay & A.G. Maule (Queens University Belfast) **RNAi in different life stages of *Fasciola hepatica***

12.15	R Culleton, M Ndounga, FY Zeyrek, A Yadava, T Tsuboi, R Carter, K Tanabe (Nagasaki Univ, Centre de Etudes des Resources Vegetales, Harran Univ, Walter Reed Army Inst Res, Ehime Univ, Univ Edinburgh & Osaka Univ) **Evidence for the transmission of *Plasmodium vivax* in the Republic of Congo, West Africa**	12.15	*Eleanor Wong & Paul Horrocks (Keele University) **Application of the *bxb1* integrase system to investigate gene regulation in *Plasmodium falciparum***	12.15	Dick Schaap, R van Binsbergen, R Niessen, G Arts, L Sanders, G Chaka, M Mulumba, T Schetters (Intervet/Schering-Plough Animal Health & CTTBD) **Development of an ECF subunit vaccine**	12.15	*Buddhini Samarasinghe, David Knox* & Collette Britton (University of Glasgow and Moredun Research Institute) **Function and Regulation of *Haemonchus contortus* genes**
		12.30	Kalpana Lal, Elizabeth Bromley, Judith Helena Prieto, Sanya J Sanderson, Richard Oakes, John R Yates III, Jonathan M Wastling, Robert E Sinden, Fiona M Tomley (Imperial College London, Institute for Animal Health, The Scripps Research Institute & University of Liverpool) **Proteomic comparison of four *Eimeria tenella* life cycle stages; the merozoite, sporozoite, sporulated and unsporulated oocyts**	12.30	Damer P. Blake & Adrian L. Smith (Institute for Animal Health & University of Oxford) ***Eimeria maxima*: defining strain and stage specific immunity in the chicken**	12.30	Johnathan J. Dalzell, Steven McMaster, Michael J. Johnston, Colin C. Fleming & Aaron G. Maule (Queen's University Belfast & Agri-Food Biosciences Institute) **Non-nematode derived double stranded RNAs induce profound phenotypic changes in *Meloidogyne incognita* and *Globodera pallida* infective juveniles**
		12.45	Nadine Randle, Sanya Sanderson &Jonathan Wastling (University of Liverpool) **Proteomics of host-parasite interactions in *Cryptosporidium parvum***	12.45	Michael Stear, Darran Singleton and Louise Matthews (University of Glasgow). **MHC class II diversity and nematode infection**	12.45	*Johnathan J. Dalzell, Steven McMaster, Colin C. Fleming & Aaron G. Maule (Queen's University Belfast & Agri-Food Biosciences Institute) **siRNA-mediated gene silencing in *Globodera pallida* and *Meloidogyne incognita***

*Presenters marked with an asterisk are entries for the student presentation competition

Monday 6th April; 14.00 – 15.30							
Time	**Session 3A**: **MALARIA: SEXUAL DEVELOPMENT AND TRANSMISSION** Convenor: Joanne Thompson Chair: Rita Tewari	Time	**Session 3B: APICOMPLEXA II – POPULATION BIOLOGY** Chair/Convenor: Fiona Tomley	Time	**Session 3C: ECTOPARASITE CONTROL** Chair/Convenor: Olivier Sparagano	Time	**Session 3D: HELMINTH GENOMICS** Convenor: Christiane Hertz-Fowler Chair: Mark Viney **Sponsored by International Journal for Parasitology**
14.00	Louisa McRobert, Helen M. Taylor, Cathy J. Taylor, Wensheng Deng, Quinton L. Fivelman, Munira Grainger, Spencer D. Polley, Audrey Sicard, Anthony A. Holder & David A. Baker (London School of Hygiene & Tropical Medicine & Medical Research Council National Institute for Medical Research) **The *Plasmodium falciparum* cGMP-dependent protein kinase plays key roles in sexual development and schizogony**	14.00		14.00	*Bettina Schelkle, Loys Richards-Hobbs, Tracey A. King, Donna Snellgrove & Joanne Cable (Cardiff University & Waltham Centre for Pet Nutrition) Herbal treatments against gyrodactylids (Monogenea)	14.00	Klaus Brehm (University of Würzburg). **The *Echinococcus multilocularis* genome sequencing project – current status.**
				14.15	David R. George, Diane Holmes, Jonathan H. Guy & Olivier A.E. Sparagano (Newcastle University) **The Influence of dust and humidity on the toxicity of plant essential oils to poultry red mite (PRM)**		
14.30	*Arthur M. Talman, Judith H. Prietro, Sanjeev Krishna, Georges K. Christophides, John R. Yates III & Robert E. Sinden. (Imperial College, The Scripps Institute & University of London) **Proteomic analysis identifies a critical role for glycolysis in malaria male gamete motility.**	14.30	James W. Ajioka & L. David Sibley (University of Cambridge & Washington University School of Medicine) **The Population Structure of *Toxoplasma gondii***	14.30	*Schwarz, A., Helling, S.; Collin, N.; Teixeira, C.R.; Medrano-Mercado, N.; Johnston, V. (University of Aberdeen, Ruhr-University Bochum, LMVR, NIAID/NIH, UMSS, Bolivia, Universidade de Brasilia & Sanaria Inc) **Antibody responses to saliva of *Triatoma infestans*: Their potential as epidemiological tool for Chagas disease surveillance**	14.25	Matthew Berriman (Wellcome Trust Sanger Institute) **Genomics of parasitic helminths**
14.45	Andrew M. Blagborough, Yanjie Liu, Rita Tewari, William J. Snell, Oliver Billker & Robert E. Sinden (Imperial College London, University of Texas & University of Nottingham) **The conserved male gamete gene *HAP2* is essential for fertilisation of *Plasmodium berghei,* and is a promising transmission blocking vaccine candidate.**			14.45	David Harrington[1], Hatem Mohi el Din[1], Karen Robinson[2], Jonathan H. Guy[1] & Olivier A.E. Sparagano[1] (Newcastle University & University of Nottingham) **Immunization of laying hens with somatic antigens from poultry red mite (PRM)**	14.45	*Emma M. Sheils, Diane H. Massie and B. Connolly (University of Aberdeen) **The TGF-beta signalling pathway in the nematode *Trichinella spiralis***

15.00	*Medhat Ali &Hilary Hurd (Keele University) **Triggers inducing apoptosis-like death in *Plasmodium berghei* ookinetes**	15.00	F. Katzer, D. Ngugi, A.R. Walker, D.J. McKeever (Moredun Research Institute & Royal Veterinary College) **Genotypic Diversity, a Survival Strategy for the Apicomplexan Parasite *Theileria parva*.**			15.00	*Alan M. O'Connell and Darren R. Brooks (Salford University) **Isolation and Characterisation of Protease Genes from the Parasitic Nematode *Anisakis simplex***
15.15	Jonathan Mwangi & Lisa Ranford-Cartwright (University of Glasgow) **Analysis of *Plasmodium falciparum* Quantitative Trait Loci Determining Differential Infectivity to *Anopheles* mosquitoes**	15.15	*Martin Simuunza, William Weir, Brian Shiels & Andy Tait (University of Glasgow) **Population genetic analysis and sub-structuring in *Babesia bovis***			15.15	*Sophie Manuel, Gary Dillon, Alasdair Ivens, Alan Wilson (University of York & Fios Genomics Ltd) **Patterns of gene expression in *Schistosoma mansoni* larvae during infection of the human host.**
		15.30	*Joanna Rumsey, Simon Shayler, Daniel Read, Joanne Cable & Joanne Lello (Cardiff University) **Conflict in the Cockroach: Parasite interactions and resource use**				
		15.45	*Daland C. Herrmann, Nikola Pantchev, Majda Globokar Vrhovec, Franz J. Conraths, Gereon Schares (Friedrich-Loeffler-Institut & Vet Med Labor GmbH) **Molecular characterisation of *Toxoplasma gondii* in cats in Germany**				

*Presenters marked with an asterisk are entries for the student presentation competition

Monday 6th April; 16.00 – 17.30							
Time	**Session 4A: MALARIA: VECTOR BIOLOGY.** Chair/Convenor: Hilary Hurd	Time	**Session 4B: APICOMPLEXA III – HOST-PATHOGEN INTERACTIONS** Chair/Convenor: Lee Innes	Time	**Session 4C: DRUG DISCOVERY FOR TROPICAL DISEASES** Convenor: Ian Gilbert Chair: Paul Wyatt	Time	**Session 4D: NEW SEQUENCING TECHNOLOGIES** Chair/Convenor: Christiane Hertz-Fowler
16.00	Gerry F. Killeen **The new challenges of monitoring, evaluating and controlling Malaria in Africa**	16.00		16.00	Torreele E, Bourdin B, Bray M, Tweats D, Mazué D, Dormeyer M, Colombo P, Kaiser M, Brun R, Pécoul B (Drugs for Neglected Diseases *initiative* (DNDi), Accelera & Swiss Tropical Institute). **Fexinidazole: a new drug candidate for human African trypanosomiasis**	16.00	Neil Hall (Liverpool) **The application of second generation sequencing to parasite genomes**
16.30	Fred Aboagye-Antwi, Amadou Guindo, Amadou Sékou, Hilary Hurd, Mamadou Coulibaly, Sékou Traoré & Frédéric Tripet. (Keele University, & University of Bamako). ***Plasmosdium falciparum* infection and hydric stress affect survival in wild-caught *Anopheles gambiae* female mosquitoes**	16.30	Christopher A. Hunter (University of Pennsylvania). **The regulation of infection-induced inflammation**	16.25	Jeremy C. Mottram. (University of Glasgow) **How many drug targets do we need to fill the drug development pipeline?**	16.30	Axel Martinelli, Sujay Kumar, Urmi Trivedi, Pedro Cravo, Mark Blaxter & Paul Hunt (Universidade Nova de Lisboa & University of Edinburgh) **Genome-wide identification of mutations in *Plasmodium chabaudi* drug resistant clones**
16.45	*Abbasali Raz, Navid Dinparast Djadid, Catherin Bourgin, Maryam Okhovat & Sedigheh Zakeri (Pasteur institute of Iran, Institut Pasteur, Paris) **Molecular characterization of *An. stephensi* carboxypeptidase B (CPB) gene as a candidate for transmission blocking vaccine (TBV)**			16.45	Ruth Brenk, Alan Fairlamb, Mike Ferguson, Julie Frearson, Paul Wyatt & Ian H. Gilbert (University of Dundee). **Drug Discovery for Tropical Diseases**	16.45	Timothy J. Littlewood (The Natural History Museum) **Helminth mitogenomics – smaller, faster, cheaper. Better?**

17.00	Martha Betson, Musa Jawara & T. Samson Awolola. (LSHTM & Medical Research Council Laboratories, The Gambia) **Investigation of the susceptibility/resistance status of malaria vectors to insecticides used for malaria vector control in The Gambia**	17.00	Leigh Jones, Fiona Roberts, Andrew McKenzie, Fiona Henriquez, Craig Roberts & James Alexander (University of Strathclyde, Western Infirmary, Glasgow & MRC, Cambridge) **A role for IL-33 receptor signalling in protection against toxoplasmic encephalitis**	17.00	Kelly L. Johnston, Bo Wu, Ana Guimarães, Louise Ford, Barton E. Slatko & Mark J. Taylor (Liverpool School of Tropical Medicine & New England Biolabs Incorporated) **A-WOL Drug Discovery: bacterial lipoprotein biosynthesis as a target for antifilarial drugs**	17.00	Roz Laing, Steven Laing, Debra Woods, Matt Berriman & John Gilleard (University of Glasgow, Pfizer Animal Health, The Sanger Institute & University of Calgary) **The cytochrome P450 family in the parasitic nematode *Haemonchus contortus***
17.15	*S.N. Emami, H. Vatandoost & M.A. Oshaghi (University of Glasgow & Tehran University) **Molecular assay for species identification of An.culicifacies sibling species in southeast Iran**	17.15	*Sam Mason, Rupert Quinnell & Judith Smith (University of Leeds) **Benign vertical transmission of *Toxoplasma gondii* to lambs**	17.15	Gareth D. Westrop, Roderick A.M. Williams, Rachel L. Clark, Simon P. Mackay & Graham H. Coombs (University of Strathclyde), **Cysteine synthase as a drug target in *Leishmania* and *Trichomonas vaginalis***	17.15	James D. Hilley, Jonathan M. Wilkes, Pawel Herzyk, Deborah F. Smith, Paul M. Kaye & Jeremy C. Mottram (University of Glasgow, University of York & Hull York Medical School) **2nd generation sequencing of the *Leishmania donovani* genome - insights into the mechanism of *Leishmania* disease tropism**
		17.30	Farah M. Barakat, Vincent McDonald & Daniel S. Korbel (Barts and the London School of Medicine) **Interferon-γ-dependent innate immunity against *Cryptosporidium parvum* infection in mice operates in the absence of natural killer (NK) cells**			17.30	S. Decuypere, L. Zheng, R.A. Scheltema, S. Rijal, J-C. Dujardin, R. Breitling, D.G. Watson, G.H. Coombs (University of Glasgow, University of Strathclyde, Koirala Institute of Health Sciences, Institute of Tropical Medicine Antwerp & University of Groningen) **Application of metabolomic technologies to unravel the biochemical basis of phenotypic diversity in parasite populations.**
		17.45	*Heshborne Sheltone Tindih, Bruno Maria Goddeeris , Dirk Geysen, Jan Naessens (International Livestock Research Institute, Institute of Tropical Medicine & Katholieke University of Leuven) ***Theileria parva* isolates of different virulence infect different T lymphocyte subpopulations**				

*Presenters marked with an asterisk are entries for the student presentation competition

17.30 – 20.00: Poster session (Drinks provided). Appleton Tower Concourse.

Tuesday 7th April; 09.00 – 10.30							
Time	**Session 5A: MALARIA: GENETICS – CLASSICAL AND GENOMIC.** Chair/Convenor: Sandra Cheesman	Time	**Session 5B: NEOSPORA SYMPOSIUM - I** Convenor: Lee Innes Chairs: Lee Innes & Jens Mattsson **Sponsored by Trends in Parasitology/Cell Press and The Creative Science Company**	Time	**Session 5C: IMMUNOMODULATION BY HELMINTHS II** Chair/Convenor: Rick Maizels **Sponsored by Elsevier**	Time	**Session 5D: HELMINTH NEUROBIOLOGY 1** Chair/Convenor: Angela Mousley
09.00	Paul Hunt (University of Edinburgh) **Understanding drug resistance using integrated genetic and genomic strategies**	09.00		09.00	David Pritchard (University of Nottingham) **The costs and potential benefits of hookworm infection**	09.00	
		09.15	David Buxton, **The pathogenenesis of neosporosis – an overview.**			09.15	Mario de Bono **Title to be confirmed**
09.30	Kevin K.A. Tetteh, L.B. Stewart, L.I. Ochola, A.A. Ngwa, A.W. Thomas, K. Marsh, G.D. Weedall & D.J. Conway (London School of Hygiene & Tropical Medicine, MRC Laboratories The Gambia, KEMRI Centre for Geographic Medicine Research, Biomedical Primate Research Centre, Instituto Gulbenkian de Ciência) **Prospective identification of Malaria parasite antigen genes under balancing selection**			09.30	Shona Wilson, Frances M. Jones, Joseph K. Mwatha, Gachuhi Kimani, Mark Booth, H. Curtis Kariuki, Eric Muchiri, Birgitte Vennervald & David W. Dunne. (University of Cambridge, Kenya Medical Research Institute, Kenyan Ministry of Health & University of Copenhagen) **The immunoepidemiology of childhood hepatosplenomegaly associated with *S. mansoni* infection and chronic exposure to malaria**		
09.45	Elaine O'Mahony, Richard Carter, Kathryn Degnan and Sandie Cheesman (University of Edinburgh) **Does the genetic make up of the murine host affect which parasite antigens are targeted by strain-specific protective immunity against the rodent malaria parasite *Plasmodium chabaudi*?**	09.45	David J. P. Ferguson (Oxford University) **Should *Neospora caninum* be considered a coccidian parasite?**	09.45	CM. Fitzsimmons, KF. Hoffmann, JM. Fitzpatrick, IW. Chalmers, FM. Jones, H. Goodwin, BJ. Vennervald, G. Kimani, JK. Mwatha, NB. Kabatereine & DW. Dunne. (University of Cambridge, Aberystwyth University, University of Copenhagen, KMRI & Ugandan Ministry of Health). **IgE responses to allergen-like molecules from different life-stages of *Schistosoma mansoni* provide a possible explanation for the slow development of human immunity**	09.45	N. Warnock, C.L. Moffett, N.J. Marks, G.R. Mair, A.G. Maule & A. Mousley (Queens University Belfast) **FMRFamide-like peptide expression and function in the free-living nematode, *Panagrellus redivivus***

10.00	*G. Humphreys & L. Ranford-Cartwright (University of Glasgow) **Factors affecting *Plasmodium falciparum* sporozoite production in *Anopheles* mosquitoes**	10.00	J.M. Wastling, A.J. Trees, A. Pain, A. Sohal, H. Prieto, R. Norton & S.M. Latham (University of Liverpool & Wellcome Trust Sanger Institute) **Sequencing and annotation of the genome of *Neospora caninum***	10.00	Sheena Cruickshank, Matthew Deschoolmeester, Richard Grencis & Kathryn Else (University of Manchester). **Rapid Dendritic Cell Mobilisation to the Large Intestinal Epithelium is Associated with Resistance to *Trichuris muris* Infection**	10.00	*Hayley Bennett, Sally Williamson, Samantha McCavera, Tracey Williams, Alan Robertson & Adrian Wolstenholme (University of Bath, Pfizer Animal Health, Iowa State University) **A novel nicotinic ACh receptor subunit, ACR-26, from *Ascaris suum*.**
10.15	David Arnot, Adam Pedersen & Dominique Bengtsson (University of Copenhagen) **Fluorescence in situ hybridisation as a tool to analyse *P. falciparum* PfEMP1 gene expression through the cell cycle: FISHing for clues at the single cell level**	10.15	Walter Basso, Susann Schares, Daland C. Herrmann, Nikola Pantchev, Majda Globokar Vrhovec, Franz J. Conraths & Gereon Schares (Federal Research Institute for Animal Health, UNLP, CONICET & Vet Med Labor GmbH) **Microsatellite analysis of *Neospora caninum* from bovine foetuses and dogs in Germany**	10.15	Ida Friberg, Joseph Jackson, Jerzy Behnke & Janette Bradley (Nottingham University & University of Liverpool) **Immunoregulation in wild mammals: associations between Toll-like receptor (TLR) function and individual infection status**	10.15	Louise E. Atkinson, Paul McVeigh, Nikki J. Marks, Michael J. Kimber, Tim A. Day, Betty A. Eipper, Richard E. Mains & Aaron G. Maule (Queen's University Belfast, Iowa State University, University of Connecticut) **Localisation and functional characterisation of peptidyl-α-hydroxyglycine α-amidating lyase, a novel *Schistosoma mansoni* amidating enzyme**
		10.30	Sarwat Al-Qassab, Michael Reichel & John Ellis (University of Technology Sydney). **Repetitive sequences and multiplex DNA typing of *Neospora caninum***			10.30	S.M. WILLIAMSON, A. ROBERTSON , R. MARTIN , T. WILLIAMS , D. WOODS , D.B. SATTELLE , A.J. WOLSTENHOLME. (University of Bath, MRC Functional Genetics Unit, Oxford, Iowa State University, Pfizer Animal Health) **The nicotinic acetylcholine receptors of *Ascaris suum***
		10.45	Thomas Dijkstra, Chris Bartels, and Willem Wouda (GD-Animal Health Service) **Abortion pattern in cattle herds after natural postnatal infection with *Neospora caninum*.**			10.45	Michael J. Kimber, Laura Sayegh, Fouad El-Shehabi, Chuanzhe Song, Debra J. Woods, Tim A. Day, Paula Ribeiro (Iowa State University, McGill University, Pfizer Animal Health). **Identification of an *Ascaris* G Protein-Coupled Acetylcholine Receptor With Atypical Muscarinic Pharmacology**

*Presenters marked with an asterisk are entries for the student presentation competition

Tuesday 7th April; 11.00 – 12.30							
Time	**Session 6A: MALARIA: PATHOGENESIS** Chair/Convenor: Sandra Cheesman	Time	**Session 6B: NEOSPORA SYMPOSIUM - II** Convenor: Lee Innes Chairs: Lee Innes and Jens Mattsson **Sponsored by Trends in Parasitology/Cell Press and The Creative Science Company**	Time	**Session 6C: TRYPANOSOMES & LEISHMAINIA I: KINETOPLASTID PARASITE-VECTOR INTERACTIONS** Convenor: Karen Grant Chair: Paul Bates	Time	**Session 6D: HELMINTH NEUROBIOLOGY II** Convenor: Angela Mousley
11.00	Katie Hughes, Giancarlo Biagini & Alister Craig (Liverpool School of Tropical Medicine) **Modulation of adhesive processes in *P. falciparum* cytoadherence**	11.00		11.00	Rod J. Dillon (Liverpool School of Tropical Medicine) **The Phlebotomine sand fly response to gut infection by Leishmania.**	11.00	
		11.15				11.15	Alan Robertson, Sreekanth Putachary, Samuel Buxton, Cheryl Clark, Saurabh Verma, Sally Williamson, Adrian Wolstenholme & Richard Martin (Iowa State University, University of Bath, Oregon Health & Science University) **Channels, contraction and cholinomimetics in nematodes**
11.30	Morten A. Nielsen, Vera Valadão Pinto, Mafalda Resende, Madeleine Dahlbäck, Pernille Andersen, Sisse B Ditlev, Silas Bruun, Thor G. Theander & Ali Salanti (University of Copenhagen & Copenhagen University Hospital) **Induction of adhesion-inhibitory antibodies using single domains of VAR2CSA**	11.30	Mélanie Loobuyck, Jenny Frössling, Ann Lindberg & Camilla Björkman (Swedish University of Agricultural Sciences, National Veterinary Institute, Sweden & Wageningen University) **Prevalence and spatial distribution of *Neospora caninum* in a population of beef cattle**	11.30	R. Wilson, M. D. Bates, A. Svarovska, L. Jecna, R. J. Dillon, P. Volf & P. A. Bates (Liverpool School of Tropical Medicine & Charles University, Prague) **Stage-specific adhesion of *Leishmania* promastigotes to sand fly midguts assessed using a novel binding assay**		
11.45	*Pongsak Khunrae, Judith M.D. Philip and Matthew K. Higgins (University of Cambridge) **Distinct chondroitin sulphate binding sites on DBL domains important in placental malaria**	11.45	Bartels, C.J.M., Berends, I.M.G.A., Dijkstra, Th., Wouda, W. (Animal Health Service) **Simulating control strategies for *Neospora caninum* infection in Dutch dairy herds**.	11.45	Frédéric Tripet, Simon Clegg, Dia-Eldin Elnaiem & Richard Ward (Keele University & LMVR/NIAID/NIH). **Cooperative Blood-feeding explains feeding aggregations in Phlebotomine Sandflies**	11.45	Cheryl C. Clark, Sreekanth Puttachary, Alan P. Robertson & Richard J. Martin. (Iowa State University). **A role for RYRs in the response to levamisole in *Ascaris suum*: Turner**

12.00	*Tina Dobrilovic & Lars Hviid (University of Copenhagen and Rigshospitalet) **A robust and quantitative *in vitro* assay of CSA-specific adhesion of *Plasmodium falciparum*-infected erythrocytes**	12.00	Anne Rosbottom, Helen Gibney, Anja Kipar, Robert Smith, Catherine Hartley, Alexander Trees & Diana Williams (University of Liverpool). **Recrudescence of *Neospora caninum* in persistently infected, pregnant cattle is associated with an increase in maternal cytokine expression in the placenta but limited placental and foetal pathology**.	12.00	M. Yeo, I. Mauricio, J. Sadlova, P. Volf, R. Baleela, S.Fitzpatrick, D. Sabatini-Doto, M.D. Lewis & M. A. Miles*. (London School of Hygiene and Tropical Medicine & Charles University in Prague) **Recombination in *Leishmania***	12.00	Lynda Devine, Angela Mousley, George Allen, Nikki J. Marks, Aaron G. Maule & John Nelson (Queens University Belfast) **Development of Surface Plasmon Resonance platforms to deorphanise helminth neuropeptide receptors**
12.15	*Casper Hempel, Fatima El-Assaad, Valéry Combes, Angeles Sanchez-Perez, Jørgen Kurtzhals, Jean-Marie Mathys & Georges E. R. Grau (University of Sydney, Royal Prince Alfred Hospital, Copenhagen University Hospital & University of Copenhagen) **Expression of microRNAs involved in inflammation and endothelial activation in experimental cerebral malaria**	12.15	Marugán-Hernández V, Álvarez-García G, Risco-Castillo V, Fernández-García A, Regidor-Cerrillo J & Ortega-Mora LM SALUVET (Complutense University of Madrid) **Identification of novel bradyzoite and tachyzoite stage specific proteins by Ettan 2D-DIGE**	12.15	Lori Peacock, Vanessa Ferris, Mick Bailey & Wendy Gibson (University of Bristol, Bristol) **F1 crosses, backcrosses and intraclonal mating in *Trypanosoma brucei***	12.15	*Vijayaraghava TS Rao, Salma Z Siddiqui, Roger K Prichard, and Sean G Forrester (McGill University & University of Ontario Institute of Technology) **HcGGR3: Characterisation of a novel ligand-gated chloride channel (LGCC) subunit in *Haemonchus contortus***
		12.30	P.M. Bartley, S.E. Wright, S.W. Maley, D. Buxton, M. Nath & E.A. Innes (Moredun Research Institute & BIOSS) **The development of immune responses in Balb/c mice following inoculation with attenuated or virulent *Neospora caninum* tachyzoites**			12.30	Paul McVeigh, Gunnar R. Mair, Ekaterina Novozhilova, Nikki J. Marks, Tim A. Day & Aaron G. Maule (Queens University Belfast, Institute of Molecular Medicine Lisbon, Iowa State University) **Discovery of multiple neuropeptide families in phylum Platyhelminthes**
		12.45	Regidor-Cerrillo J.,, Gómez-Bautista M., del Pozo I., Jiménez-Ruiz E., Aduriz G., Ortega-Mora L.M. & SALUVET, M. (Complutense University of Madrid & NEIKER-Tecnalia). **Influence of intra-species variability of *Neospora caninum* in the outcome of infection in a pregnant BALB/c mice model.**			12.45	Mostafa Zamanian, Michael J. Kimber, Paul McVeigh, Aaron G. Maule & Tim A. Day, (Iowa State University & Queen's University Belfast) **G protein-coupled receptors in two platyhelminths: *Schistosoma mansoni* and *Schmidtea mediterannea***.

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Tuesday 7th April; 14.00 – 15.30	
SESSION 7: PLENARY SESSIONS	
GEORGE SQUARE LECTURE THEATRE	
Chair: Prof Graham Coombs	
14.00	**PLENARY LECTURE: PROF ANDY TAIT** **Parasite Genetics: past achievements and future prospects in the genomic era**
14.45	**WRIGHT MEDAL LECTURE: Dr TIM LITTLEWOOD** **Tree hugging for parasite apologists**

15.30 – 15.45 Comfort break

Tuesday 7th April; 15.45 - 17.00
GEORGE SQUARE LECTURE THEATRE
BSP AGM

Wednesday 8th April; 09.00 – 10.30							
Time	**Session 8A: MALARIA: IMMUNOLOGY/VACCINES** Convenor: David Cavanagh	Time	**Session 8B: LIVE PARASITE IMAGING** Chairs/ Convenors: Joanne Thomson & James Brewer **Sponsored by Lonza Cologne AG, Zeiss and Leica**	Time	**Session 8C: TRYPANOSOMES & LEISHMANIA II** Convenor: Karen Grant Chair: Jeremy Mottram	Time	**Session 8D: VETERINARY PARASITOLOGY III – EPIDEMIOLOGY AND POPULATION BIOLOGY** Chair/Convenor: Frank Katzer
09.00	Eleanor M. Riley. (London School of Hygiene & Tropical Medicine) **Immunoregulation during Malaria Infections**	09.00	David J. P. Ferguson (Oxford University) **The evolving role of light and electron microscopy in parasitology research with particular reference to the Apicomplexa**	09.00	Annette Macleod (University of Glasgow). **The application of next generation sequencing technology to the study of the genome and transcriptome *of T. brucei gambiense.***	09.00	Robin Beech, Gary Saunders, Kate Mungall, Matt Berriman & John Gilleard (McGill University, University of Glasgow, Sanger Centre & University of Calgary) ***Haemonchus contortus* genome as a population genetic resource**
						09.15	Charlotte G S Burgess, Yvonne Gordon, Libby Redman, Fiona Whitelaw, John S Gilleard, Andrew Tait & Frank Jackson (Moredun Research Institute, University of Glasgow & Calgary). **The initial findings of a survey to examine the species of ovine nematodes present on UK farms**
09.30	Anne Teirlinck, Matthew McCall, Meta Roestenberg, Adrian Luty, André Van de Ven, Rob Hermsen & Robert Sauerwein. (Radboud University Nijmegen Medical Centre) **Cellular immune responses in human volunteers protected against malaria by repeated sporozoite inoculation under chloroquine prophylaxis**	09.30	*Ross A. Paveley, Sarah A. Aynsley, Joseph D. Turner & Adrian P. Mountford (University of York) **A novel method to visualise the interaction between schistosome larvae and cells of the innate immune system.**	09.30	Ahmed, H. A.; Picozzi, K.; Eisler, M. and Welburn, S. C. (Centre of Tropical Veterinary Medicine, Edinburgh University). **Materials and tools for the molecular diagnosis of African trypanosomes in cattle blood samples**	09.30	Libby Redman, Charlotte Burgess, Fiona Whitelaw, Yvonne Bartley, Frank Jackson Andy Tait and John Gilleard (University of Newcastle pon Tyne, Moredun Research Institute, University of Glasgow & Calgary). **Population genetics of parasitic nematodes of UK sheep**
09.45	Simon J. Draper, A.D. Douglas, S. Biswas, M.D.J. Dicks, L. Siani, G. Perretta, A. Taglioni, E.J. Remarque, S.C. Gilbert & A.V.S. Hill. (Oxford University). **Combined viral vector and protein vaccine regimes induce simultaneous high-titre antibodies and T cells against blood-stage malaria antigens in mice and rhesus macaques**.	09.45	Joseph Turner, Mark Coles, Alan Wilson & Adrian Mountford (University of York) **Dynamics of T lymphocyte foci surrounding maturing *Schistosoma mansoni* eggs in the small intestine: a multi-photon laser scanning microscope study**	09.45	*John von Freyend S., Rosenqvist H., Wiese M (University of Strathclyde). **Analysis of the LmxMPK4 signalling cascade of *Leishmania mexicana***	09.45	*McCann, C.M., Baylis, M., & Williams, D.J.L. (University of Liverpool) **The use of a GIS to identify risk factors for liver fluke infection in dairy herds in England and Wales**

10.00	Roberta Spilotri, David Arnot and David Cavanagh (University of Edinburgh) **Recombinant measles virus as a vector for malaria vaccines**	10.00	Toni Aebischer (University of Edinburgh) **Imaging *Leishmania spp* infections in vitro and in vivo: Introducing and reviewing the toolbox**	10.00	*Daniela Tonn, Graham Coombs & Jeremy Mottram (University of Glasgow & University of Strathclyde). **Intracellular trafficking of potential virulence factors in *Leishmania major***	10.00	*Arnaud Bataille Andrew A. Cunningham & Simon J. Goodman (ZSL & University of Leeds) **Importance of vector population structure and history for disease emergence**
10.15	Ruth A. Corrigan and J. Alexandra Rowe (University of Edinburgh) **Strain variation in induction of IFN-gamma by *Plasmodium falciparum***	10.15	Christopher A. Hunter (University of Pennsylvania) **Imaging the immune response to *Toxoplamsa gondii***	10.15	Roderick A.M. Williams, L. Tetley, Jeremy C. Mottram & Graham H. Coombs (University of Strathclyde & University of Glasgow). **The characterization of the individual ATG4 isoforms of *Leishmania major***	10.15	Hamish E.G. McWilliam, Alasdair J. Nisbet, Samantha M.J. Dowdall, Jane E. Hodgkinson, Jacqueline B. Matthews (Moredun Research Institute & University of Liverpool) **Identification and characterisation of a potential immunodiagnostic marker for larval cyathostominosis**

*Presenters marked with an asterisk are entries for the student presentation competition

Wednesday 8th April; 11.00 – 12.30							
Time	**Session 9A: ANTIMALARIAL DRUGS – FIELD AND LABORATORY** Convenor: Paul Hunt Chair: Pedro Cravo **Sponsored by Dafra Pharma International**	Time	**Session 9B: LIVE PARASITE IMAGING** Convenor: Joanne Thomson & James Brewer **Sponsored by Nikon and Leica**	Time	**Session 9C: OPEN (SCHISTOSOMES AND CESTODES)**	Time	**Session 9D: VETERINARY PARASITOLOGY IV** Convenor: Lee Innes **Sponsored by Intervet/Schering Plough**
11.00	Harald Noedl, Medical University of Vienna, Austria **A new emerging disease: artemisinin-resistant malaria**	11.00	James Brewer, Owain Millington, Fabrizio Ortolano, Hilary Carswell, Pasquale Maffia & Paul Garside..(University of Strathclyde) **Imaging Infection and Immune Responses in vivo.**	11.00	*Michael French, Thomas Churcher, Jimmy Kihara, Joanne Webster, Maria-Gloria Basáñez (Imperial College, & Kenya Medical Research Institute) **Mathematical models for schistosomiasis transmission dynamics and control in sub-Saharan Africa: lessons from Kenya and Uganda**	11.00	I.M. Sutherland & D.M. Leathwick (The Hopkirk Institute) **Sustainable worm controlstrategies – a New Zealand perspective.**
		11.20	Freddy Frischknecht (Heidelberg) **Imaging motile *Plasmodium* sporozoites**	11.15	*Kate M. Mitchell, Francisca Mutapi & Mark E.J. Woolhouse (University of Edinburgh) **Explaining the slow development of protective immunity against human schistosomes: mathematical modelling of the threshold hypothesis**		
11.30	*Nahla Gadalla, Ishag Adam, Salah Eldin El-Zaki, Izdihar Mukhtar, Amal Gadalla, Sahar Bashir, David Warhurst, Badria Babiker, Colin Sutherland (London School of Hygiene & Tropical Medicine, University of Khartoum, Tropical Medicine Research Institute, Ministry of Health, Khartoum) **Genetic mechanisms of drug resistance in Sudanese patients following artemether-lumefantrine treatment**			11.30	Bonnie Webster, David Rollinson, Russell Stothard, Tine Huyse, Mohmoudane Seye, Djibril Faye & Oumar Diaw (Natural History Museum, Katholieke Universiteit Leuven, Institute of Tropical Medicine & Institut Sénégalais de Recherches Agricoles **Transmission dynamics and genetic epidemiology of *S. mansoni* and *S. haematobium* in Northern, Senegal**	11.30	Robert E.B. Hanna, Ian Fairweather, Gerard P. Brennan, Emma Toner, Maeve McConville, Ailish M.Flanagan, Laura Shaw, Hillary Edgar & Shirley McConnel (AFBINI & Queens University Belfast) ***Fasciola hepatica*: histological changes in the reproductive organs following treatment *in vivo* with triclabendazole**

11.45	*Sofia Borges, Paul Hunt, Axel Martinelli, Richard Fawcett, Alison Creasey & Pedro Cravo (CMDT/IHMT Lisbon & University of Edinburgh) **Duplication of the plasmodial multi-drug resistance 1 gene is selected by mefloquine, artemisinin and lumefantrine from a complex parasite genetic background**	11.50	Ute Frevert (NYU School of Medicine) ***Plasmodium* Live – Stalking the Secrets of Malaria**	11.45	Marthe H.R. Ludtmann, David Rollinson & Anthony J. Walker (Kingston University & The Natural History Museum) **Protein kinase C signalling during miracidium to mother sporocyst development in *Schistosoma mansoni***	11.45	Leigh Jones, Panagiotis Sakkas, Jos Houdijk, David Knox & Ilias Kyriazakis (SAC, Moredun Research Institute & University of Thessaly). **Nutritional manipulation of periparturient immunity to parasites**
12.00	Richard Pearce & Cally Roper. London School of Hygiene and Tropical Medicine, **Multiple origins and regional dispersal of resistant *dhps* in African *Plasmodium falciparum* malaria.**			12.00	Anthony J, Bodell, Russell Richardson, Philip Craig, Eberhard Zehyle, and Mike Rogan (University of Salford & AMREF). **Pilot Study: Molecular Cloning, Expression and Potential Recognition of Three *Echinococcus granulosus* Recombinant Proteins Using Defined Human Sera**	12.00	Stewart T.G. Burgess, David Frew, Francesca Nunn, Andy Greer, Craig Watkins & John Huntley (Moredun Research Institute) **Sheep scab – An integrated genomic approach to the host-parasite interaction**
12.15	Katarzyna Modrzynska & Paul Hunt (University of Edinburgh) **Fitness cost of chloroquine resistance in *Plasmodium chabaudi***			12.15	Alice Tembo, Maria-Claudia Guezala, Hugo Garcia, Helen Bradshaw & Philip S. Craig (University of Salford & School of Veterinary Medicine, Lima). **Detection of human taeniosis in a coproantigen ELISA**	12.15	Joana A Carvalho, Gabriel A. Monteiro, Jorge Atouguia, Duarte Miguel F. Prazeres & Jean Rodgers (Instituto Superior Técnico, Lisboa, University of Glasgow & Universidade Nova de Lisboa) **The challenges of developing a DNA vaccine for African Trypanosomiasis**

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Monday 6th April; 17.30 – 20.00
POSTER SESSION
Appleton Tower Concourse.

MALARIA	
P1	Christian W. Wang, Pamela A. Magistrado, Morten A. Nielsen, Thor G. Theander and Thomas Lavstsen (University of Copenhagen and Copenhagen University Hospital) Preferential transcription of conserved *rif* genes in two phenotypically distinct *Plasmodium falciparum* parasite lines
P2	Ashfaq Ghumra and J Alexandra Rowe (University of Edinburgh) Developing rosette disrupting antibodies to *Plasmodium falciparum* erythrocyte membrane protein 1 (PfEMP1)
P3	*Karen Grocock, T. J. Lamb, J. Langhorne, J. E. Allen and A. L. Graham (University of Edinburgh; University of Reading, NIMR) Antibody cross-reactivity in malaria-nematode co-infection
P4	Roberta Spilotri, David Arnot and David Cavanagh (University of Edinburgh) Recombinant measles virus as a vector for malaria vaccines
P5	*Carol Hunja, Sandra Cheesman and Richard Carter (University of Edinburgh) Haplotype analysis of *Plasmodium falciparum* to determine the genetic relatedness and dispersal of these parasites in endemic locations in Kenya
P6	*Karen Russell, Eleanor Wong, Richard Emes and Paul Horrocks (Keele University) Developing a rationale-based model for *in silico* searches of *cis*-acting sequences in *Plasmodium falciparum*
P7	Janet Storm & Sylke Müller (University of Glasgow) Phospho*enol*pyruvate carboxylase, an enzyme involved in carbon dioxide fixation in *Plasmodium falciparum*
P8	*Louise Rodrigues, Gisela Henriques, Sofia Borges, Paul Hunt and Pedro Cravo (University of Edinburgh) Malaria parasites can develop resistance to Artemisinin Combination Therapy (ACT)
P9	Mohammad S. Rana A. Tanveerand Ammara H. Tahir . D.G. (Health Services,Lahore; University of the Punjab, Lahore) Survival probability of *Plasmodium falciparum* against chloroquine in Punjab, Pakistan
P10	*Annie Z. Tremp and Johannes T. Dessens (London School of Hygiene and Tropical Medicine) Functional characterization of PbIMC1h, a putative membrane-skeleton protein of *Plasmodium berghei*
P11	*Ricardo S. Ramiro and Sarah E. Reece (University of Edinburgh) Mating under attack: is transmission blocking immunity gender-specific during *Plasmodium berghei* fertilization?
P12	Amani Kheir , Davis Nwakanma , Yagut Akbarov Salma Al-Saai , Aisha Al-Gazali, Göte Swedberg Hamza A. Babiker (Uppsala University, Sultan Qaboos University; Medical Research Council Laboratories, The Gambia) Transmission of *Plasmodium falciparum dhfr* haplotypes in the Gambia
P13	N.J.C. Okolie (Imo State University, Nigeria) Seropositivity of malaria parasite and syphilis among prospective blood donors in Federal Medical Centre Owerri, Nigeria
P14	*Jaime R. Adame-Gallegos, Jianguo Shi, Richard McIntosh, Tim Smith, Anthony A. Holder and Richard J. Pleass (University of Nottingham; National Institute for Medical Research, London) Investigating the role of antibodies and their Fc-receptors in immunity to malaria.
P15	*Lisa Ranford-Cartwight and Sharron Meaden (University of Glasgow) Genetic Determinants of Sex Ratio in *Plasmodium falciparum*
P16	Dominique Bengtsson, Adam Pedersen and David Arnot (University of Copenhagen) Confocal fluorescence microscopy with locked RNA (LNA) oligonucleotide probes for in situ DNA and RNA hybridisations to *P. falciparum* chromosomes in human red blood cells: FISHing for genetic rearrangements at the limits of detection and optical resolution
P17	Kirk Rockett on behalf of the MalariaGEN consortium. (Wellcome Trust Centre for Human Genetics, University of Oxford) A Resource for Genome-wide Studies of Severe Malaria

P18	Rachel Hallett, on behalf of MALACTRES Consortium (London School of Hygiene and Tropical Medicine) MALACTRES: a new EU-funded consortium to tackle multi-drug resistance in malaria under combination therapy
P19	Emily K. Forbes, Simon J. Draper, Sumi Biswas, Sarah C. Gilbert and Adrian V. S. Hill. (University of Oxford) Development of viral vector combination vaccine strategies targeting both pre-erythrocytic- and blood-stage malaria.
P20	Leyla Akman-Anderson, Michael George, Channe D. Gowda and Shirley Luckhart (University of Oxford; University of California at Davis; Pennsylvania State University) More than just a gut feeling: Transcriptional changes triggered by *Plasmodium falciparum* glycosylphosphatidylinositols in *Anopheles gambiae* midgut
P21	Paul Bedingfield, Deborah Cowen and Glenn McConkey (University of Leeds) Inhibiting Malarial Dihydroorotate Dehydrogenase
P22	*Katarzyna Modrzynska, Axel Martinelli, Alison Creasey and Paul Hunt (University of Edinburgh; Nova Universidade de Lisboa) Genetic determinants of chloroquine resistance in *Plasmodium chabaudi*
P23	*Sohini Sanyal and Thomas J. Templeton (Weill Medical College of Cornell University) Characterization of the 2TM hypervariable gene families in *Plasmodium falciparum*
P24	Solabomi A. Ogun, Rita Tewari, Ellen Knuepfer, Steven A. Howell and Anthony A. Holder (National Institute for Medical Research, London University of Nottingham,) Analysis and characterization of a parasite line lacking the gene coding for a member of the py235 gene family
P25	*R. Armson, A. Blagborough, K. Lal and R.E. Sinden (Imperial College London) Evaluation of a family of multi-domain proteins of the rodent malarial parasite *Plasmodium berghei*; are they effective targets for blocking transmission?
P26	*Bamidele A Iwalokun, A. Ogunledun, Senapon O Iwalokun and Patrick U Agomo (Nigerian Institute of Medical Research) Analysis of ferrodynamics in relation to nitric oxide metabolism in Nigerian children with asymptomatic malaria
P27	Mònica Arman and J Alexandra Rowe (University of Edinburgh) Comparison of *var* gene transcription patterns between platelet-mediated clumping and non-clumping *Plasmodium falciparum* parasites
P28	Fiona Kenyon, Andy Greer, David Bartley, Alison Donnan, David McBean, Yvonne Bartley, Charlotte Burgess and Frank Jackson (Moredun Research Institute; Lincoln University,New Zealand) Liveweight gain as a marker for targeted selective treatments in lambs
P29	A. Afonso, Z. Neto, H Castro, AM Tomás and V Do Rosário (CMDT-LA, Portugal; IBMC/IBET-LA, Portugal) Studies on accelerated resistance to multiple drug resistance (ARMD) in different *Plasmodium chabaudi* clones with atovaquone and amodiaquine.
P30	N. Moazami, S. Zakeri, A. Sheighi Nejad and A. Ramezani (Iranian Research Organization for Research and Technology; Pasteur Institute of IRAN.) Screening and identification of bioactive compound for prevention and treatment of Malaria.
P31	K Dhanasarnsombut, Alison Creasey and David Cavanagh (University of Edinburgh) MSP-1 Block1/Block2 hybrid: a vaccine candidate against *Plasmodium falciparum*
P32	*A Reyes-Sandoval; S. Gilbert; S. Colloca; L Siani; R Cortese; A Nicosia and AVS Hill (University of Oxford; Okairòs, Rome, Italy) Development of a malaria liver-stage vaccine based on simian adenoviral vectors and MVA expressing *P. falciparum* ME-TRAP
P33	Emma J. Dawes, Shijie Zhuang, Robert E. Sinden and María-Gloria Basáñez (Imperial College London) The temporal dynamics of *Plasmodium* density through the sporogonic cycle within *Anopheles* mosquitoes
P34	Cristian Koepfli, Ivo Mueller, Jutta Marfurt and Ingrid Felger (Swiss Tropical Institute; PNG Institute of Medical Research, Papua New Guinea) Evaluation of *Plasmodium vivax* genotyping markers for molecular monitoring in clinical trials
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P36	Saber Gholizadeh, Navid Dinparast Djadid, Hamid Reza Basseri, Sedigheh Zakeri and Hossein Ladoni (Pasteur Institute of Iran (PII); Tehran University of Medical Science) Molecular identification and characterization of WARP in temperate and tropical *Plasmodium vivax* isolates

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P38	Maryam Shahrabi, Shadi R. Motmaen, Mandana Afsharpad, Navid D. Djadid, Ahmad Raeis and Sedigheh Zakeri (Pasteur Institute of Iran (PII); Khatam University; Center for Diseases Management and Control, Tehran, Genetic analysis of antifolates resistance associated genes, (*dhfr and dhps*) in *Plasmodium falciparum* and *P. vivax* isolates from Iran
P39	Bakri Y. M. Nour, Albadawi Abdelbagi Talha**,** Giancarlo Majori, Carlo Severini, Michela Menegon, Walter H. Wernesdorfer Sayed M. Elbushra and Ahmed A. Mohamadani (University of Gezira, Sudan; Department of Parasitology Instiuto Superiore di Sanita, Italy; Medical University Vienna) Monitoring *Plasmodium Falciparum* Sensitivity to Chloroquine After Three Years Of Chloroquine Withdrawal From Sudan Malaria Treatment Policy in Gezira State – Central Sudan

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P42	Claire McArthur, Ailie Robinson, Jane Hodgkinson, Jacqui Matthews (Moredun Research Institute; University of Liverpool; University of Edinburgh Investigating macrocyclic lactone resistance in cyathostomin populations using the larval migration assay
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P44	*Ab Aziz Rosilah, Michael Godard, Alan Walker, Chris Williams, Miran Aprahamian and Darren Brooks (University of Salford; CEFAS, Lowestoft; Environment Agency, UK) Occurrence of the swimbladder nematode *Anguillicola crassus* in European Eels (*Anguilla anguilla*) within the United Kingdom
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P51	Susana Pina, Teresa Barandela, Maria João Santos, Fernanda Russell-Pinto and Pedro Rodrigues (Abel Salazar Institute for the Biomedical Sciences, University of Porto, Portugal) Identification and description of *Bucephalus minimus* (Digenea: Bucephalidae) life cycle in Portugal: morphological, histopathological and molecular data
P52	Susana Pina, Jessica Tajdari, Fernanda Russell-Pinto and Pedro Rodrigues (Abel Salazar Institute for the Biomedical Sciences) Morphological and molecular studies on life cycle stages of *Diphtherostomum brusinae* (Digenea: Zoogonidae) from northern Portugal
P53	Alexa Brett Roberts, Dave Knox and Collette Britton (University of Glasgow Vet School;Moredun Research Institute, Edinburgh) Expression of parasitic nematode enzymes using *Caenorhabditis elegans*
P54	Brian Boag, Isabella Cattadori, Peter Hudson, Joanne Lello (University of Glasgow; The Pennsylvania State University; Cardiff University) The possible impact of climate change on the composition of parasite communities

P55	Seona Birrell, Huw Smith, Jo Peet and Steve Kippin (Stobhill Hospital, Glasgow; LGC Ltd Teddington) Analysis of Trends in the Performance of Water Laboratories Participating in the Inter-Laboratory Cryptosporidium Proficiency Testing Scheme (CRYPTS) 1. Microscope Slides.
P56	Seona Birrell, Huw Smith, Jo Peet and Steve Kippin (Stobhill Hospital, Glasgow; LGC Ltd Teddington) Analysis of Trends in the Performance of Water Laboratories Participating in the Inter-Laboratory Cryptosporidium Proficiency Testing Scheme (CRYPTS). 2. Filters.
P57	Seona Birrell, Huw Smith, Jo Peet and Steve Kippin (Stobhill Hospital, Glasgow; LGC Ltd Teddington) Analysis of Trends in the Performance of Water Laboratories Participating in the Inter-Laboratory Cryptosporidium Proficiency Testing Scheme (CRYPTS) 3. Suspensions.
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P59	Hamish E.G. McWilliam, Alasdair J. Nisbet, Samantha M.J. Dowdall, Jane E. Hodgkinson and Jacqueline B. Matthews (Moredun Research Institute; University of Liverpool; University of Edinburgh) Identification and characterisation of a potential immunodiagnostic marker for larval cyathostominosis
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P66	Laura M. Jones, Elise Cameron, Neil N. Mackintosh, Russ M. Morphew, Samirah Perally, E. James LaCourse, Angela Mousley and Peter M. Brophy (University of Liverpool; Aberystwyth University; Liverpool School of Tropical Medicine; Queens University) The use of RNAi to understand the role of fatty acid binding protein in the metabolism and resistance to triclabendazole in *Fasciola hepatica*
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P154	Federica Giordani, A. Sutherland, David W. Boykin, David Jones and Mike P. Barrett (University of Glasgow; Georgia State University, USA; Philipps University Germany) LED fluorescence microscopy for diagnosis of human African trypanosomiasis
P155	Caesar Lubaba, Sue Welburn, Rafal Bola, Crawford Revie and Mark Eisler (University of Edinburgh; Strathclyde University, Glasgow) Cattle activity analysis using motion sensors in rural Zambia
P156	*Ayodele Majekodunmi, Alexandra Shaw, Sue Welburn (University of Edinburgh; A P Consultants, Andover) Religious violence, natural resource conflict and the epidemiology of African animal trypanosomiasis
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ABSTRACTS OF PAPERS

Session 1A THE CHANGING FACES OF MALARIA.
Chair/Convenor: Alison Creasey

M1 The global malaria situation in 2009 – what has changed? What else might soon change?
Colin Sutherland
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At a time when global eradication of malaria is again being considered as a feasible objective the current malaria situation will be presented in broad overview, considering all species that infect humans. Three specific questions of interest will then be considered:
Will current approaches to malaria diagnostics improve our estimates of the global number of malaria cases?
Will new integrated control strategies sustainably reduce the prevalence of infection in Africa, or will reduced burdens of morbidity in the short-term be followed by long-term rebound in the number of symptomatic cases?
How will new interventions aimed at *Plasmodium falciparum,* particularly ACTs and the RTS,S vaccine, affect morbidity due to other parasite species, particularly *P. ovale* in Africa?

M2 Malaria in Africa: prospects for change
Kevin Marsh
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Malaria has historically been a major public health problem in Africa. Over the period from the 1970s to the beginning of the new century the malaria situation in Africa was not simply static but actually deteriorating, with estimates that mortality due to malaria may have doubled in many areas as chloroquine resistance spread.
Over the last seven years there has been an unprecedented expansion in International attention and funding for malaria control. There has been a major focus on increasing coverage of impregnated bed nets and on the supply of effective anti malarial drugs. The success of these initiatives is variable, with some countries showing rapid progress whilst in others there has been little change.
More recently it has become clear that in some areas of Africa there have been dramatic reductions in the incidence of malaria. Whilst it is a natural assumption that this must be related to the general increase in control activities, the actual relationship between any particular set of activities and the epidemiological changes is not yet clear.
I will present an overview of the malaria situation in Africa, focusing on evidence for a changing epidemiology in some areas and on remaining problems. I will examine possible future scenarios and the potential role of different approaches to malaria control in Africa.

M3 The changing face of malaria in the Asia-Pacific
Nick Anstey
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Over the last few decades a major increase in the malaria-exposed population in South and Southeast Asia has resulted in the region contributing approximately 25% of the global burden of *Plasmodium falciparum* (~130 million cases per year). In addition *Plasmodium vivax* accounts for up to 390 million cases per year.
Although successful introduction of artemisinin combination therapy has reduced malaria incidence in some regions, the emergence of artemisinin-resistant *P. falciparum* in western Cambodia poses a major threat to malaria control.
Chloroquine-resistant *P. vivax* is widespread in New Guinea and is increasingly being recognised in other parts of Asia. In the regions with the highest levels of chloroquine resistance, (Indonesian Papua and PNG), *P. vivax* infection is associated with severe and fatal malaria, particularly severe anaemia in infants and under-fives.
P. knowlesii is more widespread than first appreciated and increasingly recognised as a cause of severe and fatal malaria. The presence of a monkey host poses significant challenges to its control.
Eradication is now a medium-long term goal of a number of malaria control programmes in the region. As the incidence of falciparum malaria falls, *P. vivax* will cause an increasing proportion of malaria. Eradication will require an integrated strategy that tackles emerging drug resistant parasites and the prevention of *P. vivax* relapse.

Session 2A EVOLUTIONARY BIOLOGY AND ECOLOGY
Chair/Convenor: Petra Schneider

M4 *Plasmodium falciparum* sexual strategies and human variability

Richard E.L. Paul
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Organismal characteristics influencing sexual reproduction are under very strong selection pressure for the obvious reason that it is the determinant step in generating fitness. The extent to which malaria parasites adhere to predictions, based on solid evolutionary theory, in their overall gametocyte production as well as differential allocation of male and female gametocytes reveals the parasite's potential weakspots. There is incontrovertible evidence that *Plasmodium* conforms to general theories of sex allocation, which is being increasingly substantiated for *Plasmodium falciparum.* The overall epidemiological significance of parasite sexual strategies is increasingly pointing towards transmission from reservoir chronic and asymptomatic rather than symptomatic infections. Recent analyses have revealed that there is heritable human genetic variation in the tendency to carry gametocytes but only for asymptomatic infections. Is it thus not likely that the major selective pressures shaping parasite sexual strategies concern its capacity to transmit to ensure survival rather than simply to maximise the number of new infections? Gametocyte presence, however, is not an absolute proxy for transmission. Exposure-dependent acquisition of immunity defines who best infects mosquitoes: seemingly those age groups that have the lowest rate of recovery from infection. Adaptive production of gametocytes in these age groups would increase the parasite's R_0. The immunological basis to infection duration might best be explored through analysis of gametocyte production and pinpoint who is the reservoir of infection and why.

M5 Do rodent malaria parasites (*P. chabaudi*) change their investment into transmission stages in response to competition?

L.C. Pollitt, N. Mideo and S. E. Reece
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While in the vertebrate host malaria parasites must invest in the production of both asexual forms which establish and maintain the infection within the host and gametocytes which provide the potential for transmission. This means that like all sexually reproducing organisms, malaria parasites have to split their investment between growth (asexuals) and reproduction (gametocytes), in the best way in order to maximise their fitness. Evolutionary theory predicts that the optimal investment strategy will alter according to the type of infection the parasites are in. More specifically we expect that when competing with unrelated strains parasites will lower their relative investment in the production of gametocytes. Our data support this prediction revealing that by reducing relative investment in gametocytes, parasites may be investing more in survival when in the presence of competing parasite strains.

M6 Virulence-dependent drug sensitivity: do dose, duration and type of treatment matter?

Petra Schneider
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During pyrimethamine treatment, virulent *Plasmodium chabaudi* parasites have a survival advantage compared to genetically similar avirulent parasites. This survival advantage increased with increasing drug doses, whilst the higher transmission potential of virulent parasites was maintained or increased. This virulence-dependent drug sensitivity may result in drug-induced selection of more virulent parasites. We investigate how treatment regimes and type of drug affect the magnitude of survival and transmission benefits of virulence.
Mice were infected with either virulent or avirulent *P. chabaudi* parasites and treated with placebo, low, medium or high doses of artemisinin or pyrimethamine during one or four days. Parasite dynamics were monitored using quantitative PCR. We compare survival and transmission potential between virulent and avirulent parasites and how it varies with type of drug, dose and duration of treatment.
It is predicted that chemotherapy that does not eliminate all parasites and completely prevents transmission, will select for parasites with traits that are beneficial in the face of drug treatment. We show that virulent parasites have a fitness advantage over avirulent parasites when treated with both pyrimethamine and artemisinin, but the magnitude varies with dose and duration of treatment. This suggests that, if drug-induced selection of increased virulence occurs, it would happen across various treatment regimes but could be minimised by selecting the most appropriate treatment regime (cure patient and minimise risk of future infections).

M7 Why does *Anopheles gambiae* specialize on humans?

Issa Lyimo and Heather Ferguson
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Here we conducted experimental investigation of the fitness of the African malaria vector *An. gambiae* after feeding on preferred and non-preferred hosts in a bid to explain why this vector specializes on humans.
An experimental hut was constructed within a semi-field system at the Ifakara Health Institute, Tanzania. During each night of experiments, one vertebrate host of either human, cow,goat, dog or chicken was placed in the hut at dusk (6 replicates per host treatment) along with 200 insectary reared female *An. gambiae* mosquitoes. Mosquitoes were allowed to forage freely until morning when they were recaptured, and their feeding success determined.
The feeding success of *An. gambiae* varied significantly between host species ($F_{6,21}$ = 2.95, P = 0.03), with dogs being fed on most successfully. The size of blood meals taken by *An. gambiae s.s* also varied significantly between host species ($F_{6,1861}$=148.73, P<0.01) and between individuals of the same host species ($F_{21,\ 1861}$=83.60, P<0.01). Post hoc comparison indicated that mosquitoes feeding on humans (without a net), acquired more than 2 times as much as blood as those feeding on any other host type. The use of bednets by humans had significantly reduced both survival of the mosquitoes and the amount of bloodmeal ingested by them. Therefore, the specialisation of *An.gambiae* on humans may be driven by the efficiency of blood acquisition.

M8 Evidence for the transmission of *Plasmodium vivax* in the Republic of Congo, West Africa

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Plasmodium vivax is not thought to be transmitted in West Africa due the fixation of the Duffy negative phentoype in the local population, a condition which confers complete resistance against the parasite. There have been, however, sporadic accounts of its presence in this area, most of which concern the infection of Duffy positive travelers. To investigate whether transmission of *P. vivax* may occur in this region, antibodies specific to *P. vivax* pre-erythrocytic stage antigens were measured from individuals in the Republic of Congo. ELISA analysis of blood samples from 415 individuals resident in the town of Ponte-Noire, revealed 25 individuals with elevated antibodies against a chimeric PvCS *protein*, a *P. vivax* specific pre-erythrocytic antigen. Additionally, there was a correlation between antibody response to PvCS and PvMSP1, an erythrocytic stage protein. All ELISA positive individuals were Duffy negative, and had never travelled outside the country. This suggests that transmission of *P. vivax* is maintained in a population with very high levels of Duffy negativity.

Session 3A: MALARIA: SEXUAL DEVELOPMENT AND TRANSMISSION
Convenor: Joanne Thompson; Chair: Rita Tewari

M9 The *Plasmodium falciparum* cGMP-dependent protein kinase plays key roles in sexual development and schizogony

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The life-cycle of the malaria parasite is complex with distinct phases occurring in the human and mosquito. There is a surprising lack of information about how progression of the life-cycle is controlled. By analogy with other systems, it is likely that differentiation is regulated by intracellular signalling cascades involving specific phosphorylation/dephosphorylation events. Following an early report in the literature suggesting a role for the parasite cGMP signalling pathway in male gametogenesis (exflagellation), our recent work has investigated the role of the *P. falciparum* cGMP-dependent protein kinase (PfPKG) in the parasite life-cycle. We have used specific inhibitors of apicomplexan PKG in conjunction with transgenic parasites expressing an inhibitor-insensitive PfPKG to provide direct evidence of a role for this kinase in *P. falciparum* gametogenesis. Furthermore, we have used this approach recently to elucidate a central role for PfPKG in the late events of *P. falciparum* blood stage schizogony. Discovery of essential functions for PfPKG in asexual and sexual development suggest that it may be a good target for new anti-malarial drugs.

M10 Proteomic analysis identifies a critical role for glycolysis in malaria male gamete motility.

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Transmission of malarial parasites from the vertebrate host to the mosquito vector is uniquely mediated by the sexual stages of development. Generation of gametes from the intraerythrocytic, developmentally-arrested, progenitor gametocytes occurs within the blood meal of the female mosquito. We describe the first proteomic analysis of Plasmodium berghei microgametes, which are amongst the simplest of eukaryotic cells, composed solely of an axoneme and a nucleus, both enveloped by a plasma membrane. We, further, determine glycolysis to be the major metabolic activity, identify and locate the hexose transporter to the gamete plasma membrane; and demonstrate that microgamete motility can be suppressed effectively by inhibitors of this transporter and of the glycolytic pathway.

M11 The conserved male gamete gene *HAP2* is essential for fertilisation of *Plasmodium berghei,* and is a promising transmission blocking vaccine candidate.

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Fertilisation in the rodent malarial parasite, *Plasmodium berghei*, is a complex process involving the emergence of eight flagellate male gametes from each male microgametocyte, and subsequent fertilisation of an immotile female macrogamete. Previous studies have demonstrated that the *Plasmodium*-specific male gamete surface protein *P48/45* is a transmission-blocking vaccine candidate. Gene disruption studies in *P.berghei* and complimentary studies on the green alga *Chlamydomonas* have demonstrated that a conserved male gamete sterility gene, *HAP2*, is essential for fusion. Genetic disruption of the *HAP2* locus reveals that fertilisation, and therefore mosquito transmission, of the parasite is prevented, however, *hap2* KO male gametes still retain the ability to form tight pre-fusion membrane attachments with females. Heterologous expression of the *P.berghei* HAP2 protein, and subsequent immunisation, has yielded anti-sera that reacts specifically against recombinant HAP2, and the native protein on the male gamete. Additionally, anti-HAP2 sera reduces *in vitro* formation of ookinetes by up to 81%, and, by standard membrane feeding assay, appears to reduce oocyst burden within the mosquito host significantly. These results indicate that HAP2 should be examined as a target for a transmission blocking vaccine.

M12 Triggers inducing apoptosis-like death in *Plasmodium berghei* ookinetes

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Apoptosis-like cell death has been reported in some protozoan parasites, including *Plasmodium falciparum* and *Plasmodium berghei*. *In vitro* apoptotic ookinetes of *P. berghei* clearly exhibit features of mammalian apoptosis including; chromatin condensation, activated caspases-like molecules that bind caspase substrates and phosphatidylserine translocation. Our objective is to identify extrinsic triggers that induce ookinete-apoptosis. Nitric oxide (NO) and reactive oxygen species (ROS) were potential candidates. Three NO donors, S-nitrosoglutathione, sodium nitroprusside and S-nitroso-N-acetyl-DL-penicillamine were tested. Significant increases in ookinetes expressing markers of apoptosis occurred at specific concentrations of all of these NO donors, especially with 100 µM sodium nitroprusside after only 1 hour. Treating ookinetes with the ROS donors 3, 4-dihydroxy-L-phenylalanin (L-DOPA), and hydrogen peroxide (H_2O_2) did not induce apoptosis-like death but caused death attributed to necrosis. Other potential apoptosis-inducers such as tumour necrosis factor, interferon gamma and chloroquine are being investigated. In the mosquito, inducible NOS (iNOS) is involved in NO production. Previous work has shown that *NOS* gene transcription is upregulated within hours of *Plasmodium* infection in *anopheline mosquitoes*. We report a comparison of the *NOS* transcription profile in *P. berghei*-infected *A. stephensi* and *A. gambiae* and the relationship between *NOS* upregulation and apoptosis induction in ookinetes. Finally we describe attempts to knock-down *NOS* using siRNAs to demonstrate the putative link between NO production and apoptosis induction in parasite stages in the midgut.

M13 Analysis of *Plasmodium falciparum* Quantitative Trait Loci determining differential infectivity to *Anopheles* mosquitoes

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Malaria parasite lines differ in their prevalence and intensity of mosquito infection. Our aim is to determine the number of parasite genetic loci that contribute to infectivity differences, and to locate them on a genetic map. We have identified two genetically distinct clones of *P. falciparum* (denoted 3D7 and HB3) that differ in their ability to establish mature oocyst infections in *Anopheles gambiae* that are natural vectors of human malaria. Progeny clones have been generated from a genetic cross between 3D7 and HB3, and microsatellite markers have been used to generate a genetic map. Here we present phenotyping data of independent recombinant progeny clones that demonstrate segregation of the parental infection phenotypes (infection prevalence and intensity) in the progeny. This formally demonstrates that the parasite competence trait is a heritable phenotype and that it can be discriminated into two components (prevalence and intensity of infection). The presence of intermediate phenotypes amongst the (haploid) progeny suggests the involvement of several genetic loci. We are therefore using a quantitative trait loci (QTL) approach to map the contributing loci.

Session 4A: MALARIA. VECTOR BIOLOGY
Chair/Convenor: Hilary Hurd

M14 The new challenges of monitoring, evaluating and controlling malaria in Africa

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Following a generation of neglect, de facto malaria control is becoming an increasingly common activity across Africa as the availability of insecticide-treated nets, indoor residual spraying, artemisinin-based combination therapy and rapid diagnostic tests slowly increases. As the presence of such interventions becomes the rule rather than the exception, this forces a new set of questions upon malaria specialists that challenge established, often sterotyped views of how malaria is transmitted in Africa and how best to control it. In modestly endemic areas where local elimination is feasible with existing control tools, how do we document and sustain it? In more typical situations where full coverage of existing from line tools fall short of eliminating local transmission, how do we monitor and manage that remaining transmission and burden. What new tools do we need to go further and actually achieve elimination? What forms of resistance and behavioural adaptation should we expect from parasites and vectors that will undermine these gains, how can we monitor these and how should our interventions strategies respond? Most importantly, how do we ensure that African countries have the expert personnel to address these questions and attractive career paths to retain them in roles as researchers, technical advisers and implementers?

M15 *Plasmosdium falciparum* infection and hydric stress affect survival in wild-caught *Anopheles gambiae* female mosquitoes

Fred Aboagye-Antwi[1], Amadou Guindo[2], Amadou Sékou[2], Hilary Hurd[1], Mamadou Coulibaly[2], Sékou Traoré[2] & Frédéric Tripet[1]
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Whether *Plasmodium falciparum*, the agent of human malaria responsible for over 2 million deaths per year, causes fitness costs in its mosquito vectors is a burning question that has not yet been adequately resolved but it has critical implications for the natural selection of parasite-resistance mechanisms. There is mounting evidence that susceptibility to the parasite may be determined by a limited number of loci. Understanding the evolutionary forces that determine the frequency of susceptibility and refractory alleles is critical for understanding malaria transmission dynamics.
Here we show that in wild-caught bloodfed *A. gambiae* females from an endemic area in Mali, West Africa, plasmodium falciparum infection strongly reduces survival. Fitness costs could be linked to oocyst but not sporozoite development. The negative effects of infection on survival were much stronger when female mosquitoes were simultaneously submitted to hydric stress. No significant differences in prevalence of infection nor in infection-induced mortality were found between the M and S molecular forms of *A. gambiae*. Our results are relevant to vector control strategies aiming at boosting naturally occurring refractoriness or spreading natural or foreign genes for refractoriness using genetic drive systems in vector populations.

M16 Molecular characterization of *An. stephensi* carboxypeptidase B (CPB) gene as a candidate for transmission blocking vaccine (TBV)

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An. stephensi is the main vector of human malaria parasite (plasmodium) from north of Africa to the east of the Asia. Plasmodiums are ingested when the mosquito takes blood on an infected person. So, the first interaction site between parasite and insect is the mosquito midgut.
Carboxypeptidases are exopeptidases that remove a single amino acid residue from the C-terminus of proteins. Most of the studies on TBV have focused on parasite antigens that expressed in the mosquito midgut. In a study that was performed by Bourgin *et.al* on *An. gambiae*, they discovered that CPB can be a good candidate for TBV, because anti-CPB could inhibit parasite asexual stage development in *An. gambiae*. In current study, CPB as a candidate for TBV was characterized in *An. stephensi* collected from malaria epidemic region bordering Iran-Pakistan. Sequence characterization of cpb was performed by 3´and 5´ RACE techniques. The out coming results showed 88% identity between cpb genes in *An. stephensi* and *An. gambiae*. Further, cloning and expression of this protein in *An. stephensi* and a non-vector *Anopheles* species has been performed, which will be discussed in details.
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M17 Investigation of the susceptibility/resistance status of malaria vectors to insecticides used for malaria vector control in The Gambia

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Insecticide resistance has arisen in *Anopheles* malaria vectors in many areas and presents a major challenge to vector control. In this study a cross-sectional survey was undertaken of the current levels of insecticide resistance in *Anopheles* in The Gambia. *Anopheles* larvae were collected at six malaria surveillance sites: Brikama, Essau, Farafenni, Mansa Konko, Kuntaur and Basse. Larvae were reared to adulthood, identified to species and tested for susceptibility to DDT and pyrethroids. All mosquitoes sampled belonged to the *An. gambiae* s.l. complex. 26% were *An. gambiae* s.s., 54% were *An. arabiensis* and 20% were An. melas. Resistance to DDT and potential resistance to permethrin and deltamethrin were detected in *Anopheles* from Essau. Mosquitoes from Farafenni showed potential resistance to all three insecticides. No evidence of resistance was found at other surveyed sites. This is the current country-wide survey of insecticide resistance in Anopheles in The Gambia. It provides baseline information on resistance levels before the Gambian health authority embarks on its planned indoor residual spraying (IRS) campaign using DDT. The presence of DDT resistance in Essau and Farafenni indicates that careful monitoring of insecticide resistance in Anopheles mosquitoes should accompany the IRS.

M18 Molecular assay for species identification of *Anopheles culicifacies* sibling species in southeast Iran.

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Anopheles culicifacies Giles is a complex of five sibling species, provisionally designated as species A, B, C, D and E. Species A and B are the main vectors of malaria in Iran. Typically these species are identified by polytene chromosome examination. The present study investigated whether the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method can be used to differentiate the members of this complex. *A. culicifacies* were collected from Iranshahr, Sistan and Baluchestan province in Iran. The complete ITS2 region along with part of the 5.8S and 28S rDNA sequences (507- 560bp) and the mitochondrial cytochrome oxidase II (640 bp) were amplified and digested with different restriction endonucleases. The Alu I digest of the COII amplicon and Rsa I digest of the ITS2 amplicon could distinguish two categories: one including species A and D, and another including species B, C and E. Further Dde I digestion of the COII amplicon made it possible to distinguish species E from species B and C within the latter category. The PCR-RFLP techniques developed in this study can thus be applied to areas where species A and B, and species B and E are sympatric, and help resolve their relative contribution to malaria transmission in this region.

Session 5A: MALARIA: GENETICS – CLASSICAL AND GENOMIC.
Chair/Convenor: Sandra Cheesman

M19 Understanding drug resistance using integrated genetic and genomic strategies.
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Strategies and technologies now exist to identify the genetic basis of drug resistance rapidly, even before resistance arises in the field. These powerful tools have been used to identify specific mutations underlying the *in vivo* resistance of *P. chabaudi* malaria parasites to chloroquine, mefloquine and artemisinin. The genetic strategy - Linkage Group Selection - employs genetic crosses and quantitative genetic markers to scan the whole genome for signatures of drug selection. The genomic strategy uses Solexa short-read genome re-sequencing to generate an inventory of specific mutations arising within a congenic lineage of naturally selected drug-resistant mutants. When combined, these approaches unambiguously define the mutations underlying *in vivo* resistance phenotypes. In these ways we have defined mutations in two transporters which confer chloroquine resistance and a duplication and translocation event (involving *mdr1*) underlying mefloquine resistance. Artemisinin resistance is conferred by mutations in a de-ubiquitinating enzyme. Data such as these are likely to suggest new directions in understanding the mode of action of a drug and the way in which a parasite responds to drug treatment. They suggest approaches for understanding the genetic basis and evolution of drug resistance in human malarias, *P. falciparum* and *P. vivax*. These experimental studies could contribute to the optimisation of drug combinations and drug sequences if integrated into the development of new drugs.

M20 Prospective identification of malaria parasite antigen genes under balancing selection
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Analyses of *Plasmodium falciparum* polymorphic antigen genes show concordance between two independent indices of balancing selection: positive Tajima's D indices (excess of intermediate frequency alleles) and positive skew in MacDonald-Kreitman (MK) tests (excess of coding versus non-coding polymorphisms compared with fixed differences from the closely related P. reichenowi). The antigens positive in both tests are important targets of immunity and regarded as prime vaccine candidates (e.g. eba175 and ama1).
To identify additional antigens under balancing selection, 26 genes known or predicted to encode merozoite surface-associated proteins were studied in a panel of 14 *P. falciparum* isolates (plus *P. reichenowi*).
This study shows that a small proportion of surface-exposed merozoite protein genes have significant signatures predicting them to be targets of protective acquired immunity, encouraging intensive analysis of the few that do and sensitive genome wide approaches to identifying the remainder.

M21 Does the genetic make up of the murine host affect which parasite antigens are targeted by strain-specific protective immunity against the rodent malaria parasite *Plasmodium chabaudi*?
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We have identified a narrow locus on chromosome 8 of the rodent malaria parasite *P.chabaudi* that encodes the target antigen of strain-specific protective immunity (SSPI), the Merozoite Surface Protein 1 (MSP-1). To identify this locus, we used a powerful molecular genetic approach called Linkage Group Selection (LGS). This involved crossing genetically distinct *P. chabaudi* strains (e.g. ASxCB or AJxCB) and selecting their progeny in AJ, AS or CB immune mice. In these LGS studies, the MSP-1 locus was strongly selected in AJ-, AS- and CB- immunised CBA/Ca female mice. The purpose of the present study is to investigate (i) if SSPI is a general feature of *P. chabaudi* infections in murine hosts with a different genetic make-up and (ii) if SSPI does occur in such mice, whether MSP-1 or different parasite antigens are targeted by SSPI. We conducted SSPI experiments in C57BL/6 female mice (as opposed to CBA/Ca females) and data from these experiments will be presented in this paper.

M22 Factors affecting *Plasmodium falciparum* sporozoite production in *Anopheles* mosquitoes
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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 genetic or environmental factors which influence sporozoite production within a single oocyst.
Our aims were to study the variation in sporozoite numbers (i) from different clones (genotypes), and (ii) in oocysts derived from self-fertilisation events and cross-fertilisation events. In addition we have investigated the influence of environmental factors such as mosquito size and infection density on sporozoite numbers.

M23 Fluorescence in situ hybridisation as a tool to analyse *Plasmodium falciparum* PfEMP1 gene expression through the cell cycle: FISHing for clues at the single cell level
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Certain *P.falciparum* genes, notably the PfEMP1/var genes, belong to large multigene families whose transcription, translation and transport are controlled to ensure that a single parasitized erythrocyte displays only one PfEMP1 adhesion antigen on the host cell membrane. One level of control is that chromatin marking via histone modifications appears to operates to silence the expression of all var promoters, except that of the promoter controlling the expression of the 'selected\' variant- the allelic exclusion or mutually exclusive expression hypothesis. Another layer of control is that the PfEMP1 genes analysed to date appear to be to be activated in situ, without any chromosomal rearrangements but with intra-genomic repositioning of the activated gene in distinct spatial domains associated with the periphery of the parasite nucleus.
We have used genome and chromosomal analysis and confocal laser scanning immunofluorescence microscopy of in situ DNA and RNA hybridisation with oligonucleotide probes (DNA and RNA FISH) to analyse the transcription and translation of PfEMP1 genes through the development of parasites in synchronised in vitro cultures. Our objective is to test, at the single cell level, the various predictions of the currently dominant hypothesis that PfEMP1 proteins are expressed in a mutually exclusive fashion, via chromosomal intra-nuclear repositioning, without genetic translocations.

Session 6A: MALARIA: PATHOGENESIS
Chair/Convenor: Sandra Cheesman

M24 Modulation of adhesive processes in *Plasmodium falciparum* cytoadherence.
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Binding of infected erythrocytes to endothelium is one of a number of interactions that have been associated with pathology in *P. falciparum* infection. This is a complex process that involves a wide range of host receptors and, principally, the parasite ligand PfEMP1. Although the primary adhesion process has been well described, the mechanism by which this results in severe disease is poorly understood. As part of a programme aimed at alleviating severe disease we have investigated the modulation of cytoadherence by a range of mechanisms.

M25 Induction of adhesion-inhibitory antibodies using single domains of VAR2CSA
Morten A. Nielsen, Vera Valadão Pinto, Mafalda Resende, Madeleine Dahlbäck, Pernille Andersen, Sisse B Ditlev, Silas Bruun, Thor G. Theander and Ali Salanti
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In endemic areas pregnancy associated malaria (PAM) is an important cause of maternal anaemia, stillbirth and delivery of low birth weight children. The syndrome is precipitated by the accumulation of *Plasmodium falciparum* infected erythrocytes (IE) in the placenta mediated through an interaction between a parasite protein expressed

on erythrocytes named VAR2CSA and chondroitin sulphate A (CSA) on syncytiotrophoblasts. VAR2CSA is a large polymorphic protein consisting of six Duffy-Binding-like (DBL) domains and with current constraints on recombinant protein production it is not possible to produce entire VAR2CSA proteins. Thus, the challenge for vaccine development is to define smaller parts of the molecule, which induce antibodies that inhibit CSA binding of IE. In this study, we show that antibodies against specific single domains can inhibit parasite binding, possibly interfering with the quaternary structure of VAR2CSA. These domains are prospective vaccine candidates to protect against malaria during pregnancy.

M26 Distinct chondroitin sulphate binding sites on DBL domains important in placental malaria

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Severe malaria during pregnancy is associated with accumulation of parasite-infected erythrocytes on the placenta surface. VAR2CSA protein on the infected erythrocyte binds specifically to placental chondroitin sulphate proteoglycan (CSPG) and contains multiple CSPG-binding domains. As VAR2CSA is a major therapeutic target, a stereochemical understanding of its interaction with CSPG will provide useful information for development of vaccines and small molecule inhibitors to target malaria of pregnancy. Here we present crystal structures of the DBL3X and DBL6ε domains. We confirmed through surface plasmon resonance analysis that these domains bind to placental CSPG and used binding studies, together with mutagenesis, to map the CSPG binding sites. We show that DBL3X binds to CSPG through a sulphate-binding pocket and positively charged patch. This surface forms when a disordered loop becomes ordered on sulphate binding. In contrast, DBL6ε binds CSPG through a positively charged surface patch located to the side of the second subdomain. We also show that, in contrast to infected erythrocytes, these domains bind all tested glycosaminoglycan carbohydrates, with greatest affinity for ligands with high sulphation and negative charge. This suggests that VAR2CSA does not consist of a series of independent CSPG-specific binding domains. Instead these findings support a model in which individual domains contribute different, non-specific, surface features that come together in the intact protein to create a specific CSPG binding site.

M27 A robust and quantitative *in vitro* assay of CSA-specific adhesion of *Plasmodium falciparum*-infected erythrocytes

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Parasite-encoded, clonally variant surface antigens (VSA) on the surface of *P. falciparum*-infected erythrocytes (IEs) allow their adhesion to a number of vascular host receptors.
Pregnancy-associated malaria (PAM), which is a major cause of mother-offspring morbidity and mortality in areas with stable transmission of *P. falciparum* parasites, is caused by IEs that can sequester selectively in the placental intervillous space because they express particular pregnancy-associated VSA (VSA_{PAM}) with specificity for chondroitin sulphate A (CSA).
Antibody-mediated interference with CSA-specific IE adhesion in the placenta appears central to acquired immunological protection from PAM. A standardised and reproducible *in vitro* assay of specific IE adhesion to CSA is therefore a research priority, in particular if the assay is quantitative and can be implemented in laboratories with limited facilities.
We have developed a modified version of the standard, manual Petri dish assay used in most studies so far. Our assay overcomes problems with patchy IE adhesion and low reproducibility caused by low affinity of CSA for plastic surfaces and bias-prone manual assay read-out. It does not require advanced equipment for removal of non-adhering erythrocytes, and it does not quantify adhesion by using radio-labelled IEs. The assay is characterised by uniform CSA coating that allows non-patchy IE adhesion, which can be easily and objectively quantified and documented using a microscope-mounted digital camera and object recognition software.

M28 Expression of microRNAs involved in inflammation and endothelial activation in experimental cerebral malaria

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Cerebral malaria (CM) is a serious complication to untreated malaria and accounts for substantial mortality worldwide. CM is characterised by dysregulated immune responses but its pathogenesis remains incompletely understood. Recently, small non-coding RNAs, named microRNAs (miRNAs), have emerged as gene expression regulators that play an important role in immune functions and inflammation. The expression of miRNAs has not been studied in CM, therefore we aimed to elucidate the expression of selected miRNAs. Using quantitative PCR, we analysed the relative expression of selected miRNAs in the brain and in the heart. Organs were harvested from *Plasmodium berghei* ANKA infected CBA mice at the time of CM onset. let-7, miR-24, miR-26a, miR-126, miR-146, miR-150 and miR-155 were significantly down-regulated in brains of infected mice, compared to those of uninfected animals. In contrast, no significant change in the relative expression levels of these miRNAs was detected in the heart tissue; an organ with no pathology during acute malaria. Taken together, our findings suggest a role for miRNAs in the immunopathogenesis of murine CM. These results prompt further exploration of miRNAs and their therapeutic potential for improving CM outcome.

Session 8A: MALARIA: IMMUNOLOGY/VACCINES
Convenor: David Cavanagh

M29 Immunoregulation during Malaria Infections

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Failure to establish an appropriate balance between pro-and anti-inflammatory immune responses is believed to contribute to the pathogenesis of severe malaria. IL-10 and TGF-β are known to be important components of the regulatory response, but determining the cellular source of these cytokines – and the role of different populations of regulatory T cells – has proved controversial.
In murine models of acute malaria infection we observed no role for classical $Foxp3^+$ regulatory T cells (Tregs) but found that adaptive IL-10-producing, $CD4^+$ T cells (which are $CD25^-$, $Foxp3^-$, and $CD127^-$ and do not produce Th1, Th2 or Th17 associated cytokines) are able to down-regulate pro-inflammatory responses; but, in so doing, these IL-10 producing cells impede parasite clearance.
In studies of malaria-exposed human populations in West Africa we have observed that, in healthy individuals, a transient increase in Th-1 responses during the malaria transmission season is balanced by a commensurate Treg response, ensuring that immune homeostasis is rigorously maintained. By contrast, although this balance is transiently disturbed in children with acute clinical malaria, Tregs are insufficient to regulate acute malarial inflammation but may limit antigen-specific memory responses. Importantly, we identified a population of $FOXP3^-$, effector memory, $CD4^+$ T cells which co-produce IL-10 and IFN-□ and are more prevalent in children with uncomplicated malaria than in those with severe disease. These IL-10 producing effector T cells may be the true regulators of acute malarial inflammation.
These studies were funded by the Wellcome Trust and the UK Medical Research Council.

M30 Cellular immune responses in human volunteers protected against malaria by repeated sporozoite inoculation under chloroquine prophylaxis

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Introduction: Repeated inoculation of healthy malaria-naïve adult volunteers with intact sporozoites under chloroquine (CQ) prophylaxis induces complete sterile protection against subsequent patent challenge. Here we have explored the specificity and longevity of cellular immune responses induced in these volunteers by immunisation and challenge.
Methods: Peripheral blood mononuclear cells were collected from volunteers prior to immunisation and prior to, during & post-challenge. These cells were re-stimulated *ex vivo* with whole sporozoites, schizonts-infected erythrocytes (PfRBC) or stage-specific antigens. Enumeration and phenotypical characterisation of cellular responses was performed by intracellular cytokine staining and flow-cytometry.
Results: Sporozoite inoculation under CQ prophylaxis induced modest cellular responses to sporozoites and robust anti-PfRBC responses. Challenge infection further boosted cellular responses in these protected volunteers. In unprotected naive control volunteers, challenge infection induced equally robust anti-PfRBC, but not anti-sporozoite, responses. Cellular responses in both groups were long-lived, being still readily detectable at 14 months post-challenge.

Conclusions: Both pre-erythrocytic and blood-stage cellular responses are induced in sporozoite-immunised volunteers and may contribute to protection against challenge. Interestingly, a single patent malaria infection appears sufficient to induce equally robust and long-lived cellular responses to blood-stage parasites in previously naive volunteers, although it remains unknown whether these are subsequently protective.

M31 Combined viral vector and protein vaccine regimes induce simultaneous high-titre antibodies and T cells against blood-stage malaria antigens in mice and rhesus macaques.

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Protein-in-adjuvant formulations and viral-vectored vaccines encoding blood-stage malaria antigens have both shown protective efficacy in rodent malaria models and *in vitro* assays against *Plasmodium falciparum*. Antibodies are central to protective efficacy against blood-stage malaria, but T cell responses against some classical 'blood-stage' antigens can also have a protective effect against liver-stage parasites. However to date, no subunit vaccine strategy alone has generated demonstrable high-level efficacy against blood-stage infection in clinical trials.
The induction of potent T and B cell effector and memory populations is likely to be essential to achieve immediate and sustained protective responses. The combination of protein and viral vector vaccines is one strategy that stands a significant chance of success in achieving this goal. We show that in mice using the PfMSP1 antigen and in rhesus macaques using the PfAMA1 antigen that regimes combining both subunit vaccine technologies can achieve simultaneous antibody and T cell responses which equal, or in some cases surpass, the best achievable immune responses induced by either strategy alone. These data further encourage the clinical development of both vaccine technology platforms in an attempt to induce maximal immunogenicity against blood-stage malaria antigens in humans.

M32 Recombinant measles virus as a vector for malaria vaccines

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Polymorphic regions of some *Plasmodium falciparum* merozoite antigens have been shown to be immunogenic and the target of strain-specific immune responses. These responses are associated with protection from clinical malaria episodes in malaria-exposed cohorts of African children. The Block 2 region of the major merozoite surface protein (MSP-1) is such a target. Block 2 is highly polymorphic, containing repetitive tripeptide sequences in many parasite serotypes, and is a member of a family of intrinsically unstructured protein domains. Despite the high level of sequence diversity, we have been able to design, construct and express synthetic recombinant polypeptides encompassing the majority of sequence and antigenic polymorphism in this protein domain. One major problem with malaria vaccine development is the difficulty in promoting immunogenicity of subunit vaccines expressed as proteins to be delivered with adjuvants. The recombinant measles virus platform, based on the attenuated and well-characterised Edmonston-Zagreb strain of the human paramyxovirus has been used in this study to express the MSP-1 Block 2 hybrid as a GPI anchored protein in MRC5 cells. The protein is expressed in measles infected cells at high levels, is recognised by sera against all serotypes of Block 2, and is presently undergoing immunogenicity testing in transgenic mice.

M33 Strain variation in induction of IFN-gamma by *Plasmodium falciparum*

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Previous work has highlighted the importance of early IFN-gamma production by the human host for malaria resolution and immunity to clinical symptoms. There is known to be variation between donors in the amount of IFN-gamma produced in response to *Plasmodium falciparum*, and this heterogeneity has an impact on clinical outcome. Little attention has been paid to the possibility that there could also be variation in the immunogenicity of *P. falciparum* isolates.
Our preliminary experiments confirmed the donor variation in IFN-gamma responses to individual laboratory strains, but also suggested parasite strain-dependent effects on the IFN-gamma response of a single donor. To specifically address whether there is strain variation in immunogenicity, PBMCs from a single donor were

incubated with 4 laboratory strains of *P. falciparum* (HB3, PAR, R29, and TM284) at a range of physiological parasitaemias. IFN-gamma production was assessed by ELISA after 24 hours.
Across 11 donors, PAR always elicited the highest IFN-gamma response and TM284 the lowest. HB3 and R29 provided moderate stimulation. Using a multiplexed bead assay, we found that the inflammatory cytokines GM-CSF, IL1-beta, IL-6 and TNF-alpha followed the same strain-dependent pattern as IFN-gamma. Production of anti-inflammatory cytokines TGF-beta and IL-10 was not associated with a low inflammatory response.
Future work will aim to clarify immunogenicity of field isolates and association with clinical disease manifestation.

Session 9A: ANTIMALARIAL DRUGS – FIELD AND LABORATORY
Convenor: Paul Hunt Chair: Pedro Cravo

M34 A new emerging disease: artemisinin-resistant malaria

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Since 2001, more than 56 countries throughout the world have adopted ACTs as first or second line therapy for *Plasmodium falciparum* malaria. Artemisinins have become the most essential class of antimalarials, their impact comparable only to that of chloroquine in the mid 20th century. Spreading artemisinin resistance could therefore have a devastating impact on malaria control efforts throughout the world. The concept of artemisinin resistance has been a contentious one for many years, with some authorities suggesting that it was unlikely to arise in the first place. However, recent data indicate that the first cases of genuine artemisinin resistance have already emerged in western Cambodia. We may already be losing artemisinins in selected parts of the world. Our data showing individual parasite isolates resistant to high doses of artemisinins, prolonged parasite clearance times, and reduced in vitro drug response indicate that this phenomenon is as yet limited to a relatively small proportion of the parasite isolates and probably also to a relatively small area in Southeast Asia. Once it starts spreading, resistance to artemisinin derivatives, currently the most essential antimalarial drugs, could very well be the most devastating event in the history of malaria control in the 21st century. Artemisinin-resistant malaria is a new emerging disease that will require new treatment and control strategies to limit the impact and spread of resistance to the rest of the malaria-endemic world.

M35 *Genetic mechanisms of d*rug resistance in Sudanese patients following artemether-lumefantrine treatment

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Plasmodium falciparum causes considerable morbidity and mortality in sub Saharan Africa.
Development of drug resistance to antimalarial drugs has posed a great challenge in the control of malaria in endemic areas.
Artemisinin based combination therapy (ACT) has been deployed within the last five years in many malaria endemic areas with the rational for combination therapy being to delay development of resistance.
Artemether-lumefantrine (AL) is one of the few co-formulated ACTs available in tropical Africa. However, treatment failure following administration of this drug has been reported in the field.
Studies on the genetic determinants of resistance to AL have identified a selection of *pfmdr*-1 alleles in the failure cases. An earlier study reported association with the *pfcrt* gene. Reports of association with *pfmdr*-1 amplification have also been made.
In this study we investigate the possible association of *pfmdr*-1 alleles, *pfcrt* alleles and *pfmdr*-1 amplification in treatment response to artemether-lumefantrine in clinical samples collected from Sudanese patients within 28 days of treatment. The study was conducted in a low and seasonal transmission setting. In this setting all age groups are at risk of acquiring infection and developing symptoms.
The results of this study will be discussed in the presentation.

M36 Duplication of the plasmodial multi-drug resistance 1 gene is selected by mefloquine, artemisinin and lumefantrine from a complex parasite genetic background

Sofia Borges[1], Paul Hunt[2], Axel Martinelli[1], Richard Fawcett[2], Alison Creasey[2] & Pedro Cravo[1]
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Due to high levels of drug resistance in *Plasmodium falciparum*, the identification of genes underlying resistance to novel treatment policies, such as Artemisinin Combination Therapies (ACTs) is urgently needed. Here, we describe two innovative and powerful resources, Linkage Group Selection (LGS) and Solexa whole genome re-sequencing, as genetics and genomics tools respectively, to investigate the genetic basis of mefloquine, artemisinin and lumefantrine resistance in the rodent malaria *P. chabaudi*. We analyse a genetic cross between a mefloquine resistant mutant AS-15MF and a genetically distinct sensitive clone AJ, by LGS and identify the relevant mutations using Solexa genome re-sequencing. Mefloquine, artemisinin and lumefantrine selected parasites with duplication of a segment of chromosome 12 which contains the multi-drug resistance 1 gene (*mdr1*). Thus, the role of *mdr1* duplication in multi-drug resistance is rigorously demonstrated for the first time, highlighting the potential limitations of ACT use. Additionally, we identify a single mutation, unique to AS-15MF, in a gene encoding a putative lysine decarboxylase, which does not segregate with mefloquine responses in cloned progeny of a genetic cross, suggesting that it may play a compensatory role to resistance.

M37 Multiple origins and regional dispersal of resistant *dhps* in African *Plasmodium falciparum* malaria.

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Although the molecular basis of resistance to common antimalarial drugs is well known, a geographic description of the emergence and dispersal of resistance mutations across Africa has not been attempted. To that end we have characterised the evolutionary origins of antifolate resistance mutations in the dihydropteroate synthase (*dhps)* gene and mapped their contemporary distribution. We used microsatellite polymorphism flanking the *dhps* gene to determine which resistance alleles shared common ancestry and found 5 major lineages each of which had a unique geographical distribution. The extent to which allelic lineages were shared among 20 African *P. falciparum* populations revealed the existence of 5 major geographic regions within which parasite populations were well mixed with the same resistance lineages common to all sites within them. The most marked differentiation was between east and west African *P. falciparum* where resistance alleles were of different ancestry and also carried different resistance mutations. Resistant *dhps* has emerged independently in multiple sites in Africa during the past 10-20 years. Our data show the molecular basis of resistance in east and west Africa is different, and this is likely to translate into differing antifolate sensitivity. We have also demonstrated that the dispersal patterns of resistance lineages give unique insights into recent parasite migration patterns.

M38 Fitness cost of chloroquine resistance in *Plasmodium chabaudi*

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Resistance to antimicrobial drugs may incur a biological cost for malaria parasites. Recent field data indicates that this may be the case for chloroquine resistance. In Malawi, for example, the frequency of resistant alleles of *pfcrt* in the population decreased substantially after the withdrawal of the drug. This gives hope that with time chloroquine may regain some of its effectiveness and be reintroduced again. Because direct *in vivo* experiments necessary to confirm the existence of fitness costs are difficult to perform using human malaria parasites, we have exploited the rodent model.
Here we compare the relative growth of two congenic strains of rodent malaria parasite *Plasmodium chabaudi* of different level of chloroquine resistance. AS-30CQ exhibits higher level of chloroquine resistance than its progenitor AS-3CQ. We have grown these parasites alone and in competition with each other, under different drug concentrations. In single infections, the AS-3CQ grew faster and achieved higher parasitemias in the absence of CQ, but was suppressed by lower CQ concentrations than AS-30CQ. In mixed infections we used a known mutation to show that the two strains established an equilibrium that was stable throughout the infection. The proportions of the two parasites depended upon drug concentration - in the absence of drug and at low doses, AS-3CQ achieved a higher proportion than AS-30CQ. These data support the existence of a trade-off between high-level chloroquine resistance and growth in mouse infections.

Session 1B PARASITES AND THE ENVIRONMENT.
Convenor: Huw Smith

S1 John Moore

S2 *Dientamoeba fragilis*: recent advances and its role as a human pathogen
John Ellis[1], Damien Stark[2], Joel Barratt[1,2], Debbie Marriott[2] and Jock Harkness[2]
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Dientamoeba fragilis is an unusual protozoan parasite and very little is known about it. First described by Jepps and Dobell (1918) as a binucleate amoeba, this parasite was ignored as a cause of disease until recently. *Dientamoeba fragilis* is a "neglected cause of diarrhoea and dysentery" in people, with clinical signs associated with infection being diarrhoea, abdominal pain and overall looseness of stools. Long term, chronic infection is common unless treated. The life cycle of *D. fragilis* is unknown, but transmission is probably via the faecal/oral route. Recent data suggests it may be a zoonotic infection (involving pigs) and studies indicates that drug treatment, which eliminates the organism from patients, results in improvement of clinical signs.
Definitive diagnosis is based on prompt fixation and permanent staining of faecal samples, in vitro culture or PCR. In Australia, the prevalence of this organism varies from 0.4% to 16.8% of patients studied. The potential for misdiagnosis of dientamoebiasis as irritable bowel syndrome has resulted in calls for clinical laboratories to more actively test for this organism. Since testing procedures are not performed in most laboratories the true prevalence of *D. fragilis* in humans is underreported.

S3 *Cryptosporidium* species and genotype diversity in UK waters
Rosely A.B. Nichols, Lisa Connelly and Huw V. Smith
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The genus *Cryptosporidium* comprises 20 species and > 40 genotypes that cannot be discriminated morphologically. *C. parvum*, a zoonotic species, and *C. hominis*, are the major species causing human disease. *C. meleagridis*, *C. felis* and *C. canis*. *C. suis, C. muris, C. andersoni-like* genotypes, and *Cryptosporidium* monkey, cervine, chipmunk I, rabbit and skunk genotypes also infect humans. Identification of environmental *Cryptosporidium* isolates is essential for understanding parasite transmission cycle(s) and for assessing risk to public health, particularly in cases of water contamination, but oocysts normally occur in low abundance (<10) in water samples.
We retrieved FITC–mAb and DAPI stained oocysts previously fixed on slides, and extracted oocyst DNA for PCR amplification using a maximised freeze-thaw protocol. PCR-RFLP and PCR-sequencing, at two 18S rRNA loci, were used to determine species / genotypes. One 18S locus was consistently more sensitive in our hands. Of 146 slides analysed, 68% yielded amplicons. Of 99 samples genotyped, 29.3% were mixed *Cryptosporidium* species, 23.2% *C. andersoni*, 5% *C. hominis*, 4% *C. parvum*, 1% *C. ryanae*, 9% *C. parvum* or *C. hominis*, 19.2% unknown genotypes, 6% *Cryptosporidium* cervine, 1% rabbit and 1% goose genotypes, indicating the diversity of species / genotypes present in UK drinking and raw waters.

S4 Determining species and sub-genotypes of Cryptosporidium infecting St Kilda Soay sheep
Lisa Connelly[1], Barbara H. Craig[2], Josephine M. Pemberton[2], Rosely A.B. Nichols[1] and Huw V. Smith[1]
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We report the species / genotypes / sub-genotypes of Cryptosporidium infecting feral Soay sheep (Ovis aries) population on Hirta, St Kilda, over two years of varying host population density. Oocyst abundance was low in the majority of infections, and immunomagnetic separation (IMS) was required to concentrate oocysts. Cryptosporidium DNA was extracted by freeze-thawing IMS bead-oocyst complexes, and species / genotypes identified following nested-PCR-RFLP / PCR-sequencing at two Cryptosporidium 18S rRNA loci. C. parvum positive isolates were subtyped using two nested-PCR assays that amplify the Cryptosporidium GP60 gene. Of 255 faecal samples tested were 28.9% were C. parvum, 11.1% Cryptosporidium cervine genotype, 11.4% C. bovis, 2% C. ryanae (mixture with C. bovis) and possibly C. andersoni (4.2%). There is genetic variation in the Hirta C. parvum population. GP60 heterogeneity was observed in eleven sequenced amplicons, 6 of which represent previously unknown sub-genotypes. Further confirmatory analyses are being performed. The feral Soay sheep population of Hirta have a high prevalence and diversity of Cryptosporidium species / genotypes / sub-genotypes.

S5 Molecular epidemiology of *Cryptosporidium* - can subgenotype variation be explained by contacts between cattle farms?

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In this study we sought to test the hypothesis that the genetic diversity and population structure of *Cryptosporidium* in cattle may, in part, be explained by the pattern of contacts between farms. Parasites isolated from calves of unknown disease status were subgenotyped using five micro/minisatellites. Subgenotypic diversity was then compared with contact network data collected from the farms. Fifty-five out of 215 samples were found to be *Cryptosporidium* positive; 46 amplified across all five micro/minisatellite loci. Twenty-nine multilocus genotypes (MLGs) were found with six occurring more than once, highlighting considerable diversity. Some of the genetic variability could be explained by contact types between farms; the use of dealers (P=0.004) and markets (P=0.04) by producers appeared to promote diversity. Farms with closed herds had significantly fewer MLGs (P=0.09) than farms with open herds. The number of MLGs found per farm appeared to be somewhat related to farmers\' attitudes towards biosecurity; farmers who thought the preventive practices 'very useful\' had fewer MLGs than those who thought the practices 'not very useful\' (P=0.09). By using a combination of approaches, it is possible to understand further how pathogens are transmitted and potentially, controlled.

Session 1C VETERINARY PARASITOLOGY I– CHEMOTHERAPY AND ANTHELMINTHIC RESISTANCE. Convenor: Frank Jackson; Chair: Philip Skuce

S6 Detection and mechanism of drug resistance in nematodes

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The efficient use of anthelmintics in grazing animals is essential for the success of parasite management practices. However, the further increasing prevalence of resistant populations of major parasitic nematode species represents a significant threat for worm control efforts. Using benzimidazole resistance as an example, several investigations have shown that resistance remains on farms where it has developed even when BZs were not applied on the particular farm for many years. Therefore, amongst attempts to reduce the selection for drug resistance in the field, for example by targeted selective treatment instead of whole flock treatments, it is compulsory to detect the development of resistance as early as possible to allow adequate adoption of control programmes. In vivo, in vitro and molecular tests for the assessment of anthelmintic resistance have been developed with varying applicability for the different major drug classes and parasite species. Several of these tests have been evaluated and standardized in a collaborative effort by a number of international partners within the EU-funded project PARASOL.
The understanding of the mechanism of resistance supports the development of resistance tests but also may contribute to the identification of new drugs. Drug target directed or specific and unspecific, e.g. drug efflux based, mechanisms of resistance were found to occur in nematodes. Experimental selection of resistance can either be achieved by repeated subtherapeutic treatment or mutagenesis.

S7 Modern methods for the management of anthelmintic resistance in sheep

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Anthelmintic resistance is a worldwide phenomenon with resistance being reported to all of the current anthelmintic families. The consensus amongst veterinary parasitologists is that the key to reducing the rate of development of anthelmintic resistance lies in the ability to maintain an unexposed parasite population (often referred to as a population *in refugia*). A variety of different infection, morbidity and production parameters have been tested in the field as a means of maintaining parasite susceptibility by exploiting the population *in refugia*. Faecal egg count monitoring appears to provide the most useful marker for targeting treatments (TT) at the herd level. The first useful indicator for treating affected individuals using a targeted selective treatment (TST) approach used the FAMACHA© system. In this system, a morbidity marker (anaemia) arising from *Haemonchus*

infections is used to direct individual anthelmintic treatments. Subsequently other production parameters including milk and liveweight production have also been used as TST indicators.
Field studies within the EU sponsored PARASOL project involving 5 European and 2 African research groups have examined a range of different, regionally appropriate TT and/or TST markers. These studies have confirmed the value of this approach in reducing anthelmintic usage whilst maintaining acceptable levels of production and most importantly susceptible parasite phenotypes.

S8 The influence of ketoconazole and pluronic 85 on the efficacy and pharmacokinetics of ivermectin in Haemonchus contortus infected lambs

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Non-specific mechanisms of resistance such as the ATP binding cassette proteins play an important role in xenobiotic clearance in ovine gastro-intestinal nematodes. The aim of this trial was to assess the possibility of increasing drug bioavailability in the host whilst reducing nematode drug clearance, thereby improving treatment efficacy. Thirty-six lambs were infected with 5000 multiple resistant *Haemonchus contortus* L_3 and separated in to six groups (n=6); ivermectin alone (IVM; 0.2mg/kg body-weight), ketoconazole alone (KET; 10mg/kg BW), pluronic 85 alone (P85; 4mg/kg BW), IVM+KET, IVM+P85 or untreated control. Ivermectin treatments were single administrations on day 28 post-infection (PI) for all appropriate groups, whereas the KET and P85 treatments were both administered as five separate doses on days 26-30 PI inclusively.
Concomitant administration of KET or P85 with IVM induced increases in plasma and tissue concentrations of IVM in treated animals, resulting in a two-fold increase in the area under the time-concentration curve (AUC, $p<0.05$). Faecal egg counts and worm burdens of the IVM+KET and IVM+P85 groups were lower than the untreated, KET and P85 control animals.
The lack of any significant shift in treatment efficacy as a consequence of increased IVM bioavailability within the host is interesting and merits further study.

S9 Molecular analysis of the *GluClalpha3* gene in ivermectin-susceptible and ivermectin-resistant isolates of *Cooperia oncophora*

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Macrocyclic lactone resistance is an emerging problem for the control of gastrointestinal nematodes of cattle such as *Cooperia oncophora*. Although there is still a poor understanding of the molecular basis of macrocyclic lactone resistance, it is clear that macrocyclic lactones exert their activity in *C. oncophora* and other nematodes by binding to the glutamate-gated chlorine channel GluClalpha3. The L256F mutation in the *GluClalpha3* gene of *C. oncophora*, which was shown to be associated with ivermectin-resistance in a UK field isolate, was not found in resistant field and lab isolates from Belgium. However, a significant difference in *GluClalpha3* allele frequencies was observed between the ivermectin-susceptible isolate and the ivermectin-resistant lab isolate, suggesting that the *GluClalpha3* gene is under selection by ivermectin treatment. Analysis of the full length sequence of the *GluClalpha3* in the two resistant *C. oncophora* isolates showed no specific amino acid changes associated with resistance. In contrast, there was actually a higher sequence variability in the susceptible isolate compared to both the resistant *C. oncophora* isolates. At the moment we are investigating the transcription levels of the *GluClalpha3* gene and possible splice-variants and how these splice-variants fit in the genomic organisation of the *GluClalpha3* gene.

S10 Are P-glycoproteins involved in macrocyclic lactone resistance in *Teladorsagia circumcincta*?

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Teladorsagia circumcincta is the most important gastrointestinal nematode parasite of sheep in the UK. It is also the principal resistant genus, with increasing reports of multidrug resistance, including resistance to macrocyclic lactone anthelmintics, most notably ivermectin. Resistance is thought to come about either by genetic mutations in the drug's binding site and/or changes in gene expression of the parasite's drug handling mechanisms.

Previous inhibitor studies with *T. circumcincta* have implicated P-glycoproteins (Pgps), a large family of xenobiotic drug pumps, in the ivermectin resistant phenotype. We have identified eleven novel Pgp gene sequences from *T. circumcincta*. Real-Time PCR analysis reveals that some of these exhibit an altered gene expression pattern between susceptible and resistant parasite isolates. Single nucleotide polymorphisms (SNPs) have also been identified within some of these genes. These genetic changes require further validation but may provide a lead in the search for molecular markers for ivermectin resistance.

Session 1D IMMUNOMODULATION BY HELMINTHS
Convenor: Rick Maizels; Chair: David Pritchard

S11 Helminth Immunoregulation through TGF-beta signalling

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Regulatory T cell populations expand in many helminth infections, including *Heligmosomoides polygyrus*, which establishes chronically in the intestine of mice. We have found that molecules secreted by adult worms, termed HES (*H. polygyrus* Excretory-Secretory products) increase the proportion of CD4+ T cells expressing Foxp3 (the regulatory T cell master transcription factor). Expansion was dependent on co-incident TCR ligation, did not require APC, and was lost on heat treatment of HES. Importantly, by adding HES to GFP-ve T cells from a GFP-Foxp3 reporter mouse, de novo induction of Foxp3 in naive non-regulatory precursors was demonstrated. HES was found to act in a manner similar to mammalian TGF-beta, through the TGF-beta receptor, with susceptiblity to inhibitors of TGF-beta signalling. Antibody to mammalian TGF-beta did not interfere with the ability of HES to drive Foxp3, while abolishing all activity of TGF-beta itself. Thus HES contains a functional homologue of TGF-beta able to ligate and signal through the TGFbeta receptor. While antibodies to host TGF-beta did not affect parasite worm loads, the signalling inhibitor (which blocks downstream effects of both host and parasite ligands) significantly reduced parasite numbers. Hence, this parasite has evolved to mimic Treg induction by TGF-beta, in order to accentuate Treg activity which is now implicated in the persistence of many chronic helminth parasite infections.

S12 Helminth 2-Cys Peroxiredoxin drives Th2 responses through a mechanism involving alternatively activated macrophages

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During helminth infections alternatively activated macrophages (AAMacs) are key to promoting Th2 responses, and suppressing Th1-driven inflammatory pathology. Th2 cytokines IL-4 and/or IL-13 are believed to be important in the induction and activation of AAMacs. We show using murine models for the helminth infections caused by *Fasciola hepatica* (Fh) and *Schistosoma mansoni* (Sm) that a secreted antioxidant, peroxiredoxin (Prx), induces alternative activation of macrophages. These macrophages, enhanced the secretion of IL-4, IL-5 and IL-13 from naïve $CD4^+$ T-cells. Administration of recombinant FhPrx and SmPrx to wild-type and $IL\text{-}4^{-/-}$ and $IL\text{-}13^{-/-}$ mice induced the production of AAMacs. In addition, Prx stimulated the expression of markers of AAMacs *in vitro*, using a different pathway compared to IL-4 activation. The immunomodulatory property of Prx is not due to its anti-oxidant activity as an inactive recombinant variant, with active site Cys residues replaced by Gly, could also induce AAMacs. We propose that Prx activates macrophages as an initial step in the induction of Th2 responses by helminth parasites and thereby is a novel pathogen-associated molecular pattern.

S13 Helminth antigens induce distinct dendritic cell phenotype

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Two antigens derived from the helminth parasite *Fasciola hepatica*, a recombinant Cathepsin (CL) L1 and a recombinant sigma Class Glutathione S-transferase (GST), have been found to induce a dendritic cell (DCs) phenotype that differs from the archetypal or "classically" DC maturation induced by LPS. While these helminth antigens are biochemically and functionally different, they both induce a subset of DCs defined by the expression of interleukin (IL)-6, IL-12p40 and the cell surface marker CD40. Similarly to LPS maturation, this induction was also found to be TLR4 dependent. Cellular signalling, functional characterisation and the T cell priming ability of these DCs were also investigated. There is growing interest in the host's ability to recognise helminth derived antigens through TLRs, but also for the parasite-derived molecules to modify the host's immune response in a TLR dependent manner. The data present should contribute to further our knowledge of host-parasite interactions.

S14 Excretory-secretory (ES) products of *Nippostrongylus brasiliensis* L3 larvae inhibit LPS-induced inflammation

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A few studies have demonstrated a lack of TNF-α production and limited recruitment of neutrophils in the rat lungs after *Nippostrongylus brasiliensis* infection. The anti-inflammatory properties of parasitic helminths have been largely linked to their excretory-secretory (ES) products. In rats, our previous work observed that instillation of *N.brasiliensis* L3 larvae ES (L3 ES) can inhibit the recruitment of neutrophils by approximately 45% on the background of LPS induced inflammation. Similar reduction of recruited neutrophils was obtained in this study. This was associated with the significant inhibition of gene transcription of the key adhesion molecule, ICAM-1, and a major chemoattractant, MIP-2, in bronchoalveolar lavage (BAL) cells by L3 ES, determined by quantitative real-time PCR. The LPS-stimulated gene transcription of pro-inflammatory cytokines including TNF-α and IL-1β were both significantly reduced by L3 ES. Inducible nitric oxide synthase (iNOS), which catalyses the formation of nitric oxide (NO), was normally elevated in classically activated macrophages. The inhibition of iNOS expression by L3 ES may suggest the phenotype change of the activated macrophages. The general reduction of pro-inflammatory molecule gene transcription observed in this study implies the inhibition of LPS-stimulated inflammation by L3 ES. Using the rat alveolar macrophage cell line NR8383, the inhibition of TNF-α production was strongly associated with a >3kD concentrate of L3 ES.

S15 Inflammatory Bowel Disease and *Trichuris muris*

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Inflammatory bowel disease (IBD) is a group of idiopathic chronic relapsing inflammatory conditions of the gastrointestinal tract. The precise etiology of IBD is unknown, although it is clearly multifactorial. Prevalence of IBD is far more common in developed countries as compared to the developing world, where infection with intestinal parasites is common. Evidence suggests that therapeutic administration of helminth can reduce the symptoms of IBD in both animal models and man. The mechanisms by which helminths interact with the gut immune system in IBD-susceptible hosts are poorly understood. To address this we have investigated the interaction between *Trichuris muris* and the *mdr1a (-/-)* mouse model, which lacks the epithelial barrier protein, p-glycoprotein and slowly develops a spontaneous colitis in the presence of a normal gut flora. Our aim was to investigate (i) whether mdr1a gene deletion, which makes the host susceptible to colitis, alters host susceptibility to *T.muris* infection and (ii) the effects of *T. muris* infection on the development of colitis in *mdr1a-/-* mice. The results show that the *mdr1a -/-* mice were unable to expel *T.muris* and had a higher worm burden at day 19 after infection compared to controls. Interestingly, there was also evidence that T. muris infection may accelerate the development of gut inflammation in the *mdr1a-/-* mice.

Session 2B: APICOMPLEXA I – GENE EXPRESSION
Convenor/Chair: Fiona Tomley

S16 Chris Newbold

S17 A proteomic perspective on gene expression in the Apicomplexa
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The genome sequences that are now available for both parasites and their hosts contain potentially all the information required to understand the complete host-parasite relationship. In practice, the tools needed to translate this information into biological understanding are seriously deficient. Here we discuss how proteomics can be used alongside other functional genomic tools to help understand fundamental biological questions such as the mechanisms of host-cell invasion and the modification of host-cell function which sustains successful parasitism. Crucially, we show how proteomics questions the relationship between transcription and protein expression, helping us better to interpret events at the host-parasite interface. Using examples from the Apicomplexa, we also discuss how proteome data can be used to inform not just gene expression in parasites, but also gene annotation. We describe how comprehensive proteomic analysis can be used to validate and modify gene models aswell as suggest alternative models.

S18 Application of the *bxb1* integrase system to investigate gene regulation in *Plasmodium falciparum*
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The distinct morphological appearance of *P.falciparum* developmental stages as well as a necessity to rapidly adapt to diverse environmental niches has always implied that an extensive programme of developmentally-linked gene expression underpins this parasite's complex life cycle. Following the publication of the parasite's genome, definitive evidence for such a programme was provided by high-throughput microarray and proteomic analyses. How different molecular mechanisms of control integrate to provide for coordinated temporal control of gene expression is still poorly understood. Reporter gene assays based on transient and stable episomal transfection offer us the means to explore these mechanisms *in vivo*, yet are hampered by the lack of chromatin assembly and concatermerised plasmids, respectively.
Here we describe the application of the *bxb1*-integrase system to develop homogeneous populations of parasites all bearing a single integrated copy of a *Pfpcna* reporter construct. We have thus far established (i) the absolute and temporal activity of *Pfpcna* flanking sequences are retained in the reporter construct (ii) employs the same transcription start and stop sites as the endogenous gene (iii) deletion of 5' and 3' sequences appear to affect absolute but not temporal activity and (iv) repression of transcription in ring-stage parasites cannot be released via histone-hyperacetylation alone. Our data indicates we have a GM parasite model that is fit for purpose in dissecting the mechanisms that control coordinated gene expression.

S19 Proteomic comparison of four Eimeria tenella life cycle stages; the merozoite, sporozoite, sporulated and unsporulated oocyts
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We report the proteomes of four life cycle stages of the Apicomplexan parasite *Eimeria tenella*. 1535 proteins were identified from merozoites, 848 from sporozoites, 630 and 698 from the unsporulated and sporulated oocyst stages respectively, by 2D gel LC-MS/MS or MudPIT shotgun proteomics. 'Glideosome' proteins are found in the invasive, merozoite and sporozoite and unsporulated oocyst stages. Refractile body proteins are present in the sporozoite, sporulated and unsporulated oocysts but absent in the merozoite. This suggests these described proteins are synthesised early in preparation for use in the sporozoite or perform additional roles in the early oocyst. Merozoites appear to commit more protein to transcription and protein synthesis, consistent with preparation for massive replication during schizogony. Merozoites utilise almost three times as many peptides in oxidative phosphorylation than the other stages, consistent with a metabolic shift mobilising greater energy

production. LC-MS/MS identifies individual proteins in multiple spots on 2D gels which suggests these proteins are post-translationally modified for example by glycosylation, phosphorylation or proteolytic cleavage.

S20 Proteomics of host-parasite interactions in *Cryptosporidium parvum*

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Our previous work has determined the proteome of excysted Cryptosporidium parvum sporozoites using a variety of proteomic approaches (Sanderson et al. 2008 Proteomics: 8, 1398). A total of 1237 proteins were found to be expressed in this life-stage, representing approximately 30% of the total predicted proteome of C. parvum. Further analysis of these data revealed that many of the proteins were homologues of known apical organelle proteins in other Apicomplexa. A large number of the proteins identified were uncharacterised mucins and surface antigens. In addition, there were numerous proteins containing interesting domains that are suggestive of protein-protein interactions. We believe that many of these proteins may have potential roles in facilitating the invasion of the parasite and its subsequent establishment within the host cell. In order to address this we are taking a proteomics and bioinformatics based approach to characterise the sub-proteomes of the apical complex and the excretory/secretory proteins of C. parvum. This information will confirm the apical location of these proteins and further our understanding of the invasion biology of the parasite and its establishment within the host cell.

Session 2C: VETERINARY PARASITOLOGY II – IMMUNE RESPONSE AND VACCINATION
Convenor: Lee Innes; Chair: Al Nisbet

S21 *Fasciola hepatica* infection in cattle – Prospects for Vaccination

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The liver fluke, *Fasciola hepatica*, including Ireland. Recent abbatoir surveys suggest that at least 65% of cattle in Ireland are infected. In some areas (South America, Egypt, Iran) it is also responsible for very significant zoonotic disease. Our group and others have shown that cattle develop a strongly skewed Th2 response to infection, but that this response does not protect from re-infection. Experimental vaccination using adjuvants that promote a Th1 response, on the other hand, can deliver partial protection. Animals infected with *F. hepatica* have an altered response to some bacterial infections. We undertook to investigate the effects on disease diagnosis, immune responsiveness and disease progression of a co-infection of *F. hepatica* in animals infected with a low dose of *Mycobacterium bovis*. Peripheral blood mononuclear cells (PBMC) from co-infected animals produced lower levels of IFN-g in response to restimulation *in vitro* with mycobacterial antigens than did animals infected with *M. bovis* alone. In contrast, levels of the type-2 cytokine IL-4 and regulatory cytokine TGF-b were higher. In response to restimulation with *F. hepatica* specific antigen, PBMC of co-infected animals produced lower levels of IL-4 and TGF-b than animals infected with the helminth only. Responsiveness to the single comparative intradermal skin test (SCITT) was reduced in co-infected animals. Collectively, these results provide evidence of downregulation of Th2/cellular immune responsiveness to *M. bovis* in animals co-infected with *F. hepatica*. Paradoxically, there was also some evidence of reduced bacterial load in co-infected animals.

S22 Twists and turns en route to a vaccine for *Haemonchus contortus*.

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Haemonchus contortus is the most important nematode parasite of sheep in the world but drug resistance is widespread. Substantial protection can be achieved by vaccination with native H11 or H-gal-GP, two glycoprotein enzyme complexes located on the parasite intestinal membranes. H11 is a family of four aminopeptidases whilst H-gal-GP is composed of four metallo-, two aspartyl, and at least one cysteine protease. All these enzymes are presumed to be involved in the digestion of the parasite blood meal. Neither antigen complex protects if it is denatured, nor do recombinant versions of any of the subcomponents, even when used as cocktails.
Recent 3D reconstructions of H-gal-GP from electron microscopy data has revealed a complex novel structure with two-fold symmetry of approximately 27 x 16 x 14 nm. Three holes in the structure open into an internal

chamber in which we speculate haemoglobin and other protein substrates are digested. We hypothesise that protective vaccine antibodies bind around these chamber entrances impeding substrate ingress and leading to starvation and death of the parasite.
It is highly unlikely that the quaternary structure of native H-gal-GP required for protection can be reproduced with current recombinant DNA methods. Moreover, recent trials indicate that it may be possible to make a commercially viable vaccine from native antigen.

S23 Development of an ECF subunit vaccine

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East Coast Fever (ECF) is an economically important cattle disease in Africa and is caused by *Theileria parva*, a tick transmitted protozoan. The disease is a lympho-proliferative disorder that typically results in death 2-4 weeks after infection. ECF kills over 1 million cattle in 11 African nations each year and causes losses of at least $200 million. At present only an \"infection-treatment\" vaccination is being used, which can induce solid immunity in approximately 80-85% of animals, but is too labor intensive and expensive to be widely implemented.
The only identified *T. parva* vaccine candidate antigen is p67, a sporozoite surface protein. P67 expression was optimized in a baculovirus expression system, and we previously reported successful protection with this recombinant product in a vaccination/challenge trial with cattle in Kenya. We now present the further development of this product. In a recent dose-finding trial, it was shown that young calves have a level of innate resistance to *T. parva* infection compared to larger calves, which was reflected in reduced mortality. Vaccination of the highly sensitive older calves reduced mortality by >70% and surviving calves showed less clinical signs. For these trials a novel ECF scoring method was developed to follow clinical disease. The new scoring method is based on haematocrit values and piroplasm parasitaemia and is much more practical in the field.

S24 *Eimeria maxima*: defining strain and stage specific immunity in the chicken

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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 many protozoan parasites progress has been slow. The identification of antigens capable of stimulating protective immune responses in the host has proven difficult and for the *Eimeria* species the relevant lifecycle stages are yet to be definitively defined. Using immunologically distinct *Eimeria maxima* strains and a population-based genetic mapping approach we have identified six distinct loci associated with susceptibility to strain-specific immune selection. Quantification of *E. maxima* replication within the naïve and immune host has revealed the first 24 hours (including the first invasive and asexual stages of the eimerian lifecycle) to be key during homologous secondary infection. Quantification of T cell and associated fluctuations in immune mediators will be described as will the persistence of polymorphic genetic markers associated with susceptibility to strain-specific immune selection in hybrid parasite populations under immune selection.

S25 MHC class II diversity and nematode infection

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Major histocompatibility complex class II alleles are associated with resistance to nematode infection. In sheep, the MHC class II region contains the strongest known quantitative trait locus (qtl) for resistance to the nematode *Teladorsagia circumcincta.* However the differences among alleles have only been estimated for one locus – *DRB1.* There are no estimates of effect sizes for the other class II loci. As part of a study on the influence of other MHC loci, we have examined the extent of polymorphism at the major class II loci. The number and frequency of alleles varies across breeds and among flocks within the same breed. Within the Scottish Blackface breed the number of alleles at a given locus varies from less than 10 to more than 30*; DRB1* is the most polymorphic locus. Among breeds, the number of alleles at *DRB1* varies from less than 10 to more than 30. Nematodes may be more important drivers of MHC class II diversity than the more commonly invoked microbes, because unlike most microbes, their host-parasite coevolution extends over millions of years. In addition, heterozygotes at the ovine *DRB1* locus have lower egg counts than homozygotes and this heterozygote advantage could be an important factor in maintaining MHC class II polymorphism.

Session 2D: GENETIC MANIPULATION AND GENE SILENCING IN HELMINTHS
Convenor: Angela Mousley; Chair: Michael Kimber

S26 Transgenesis of schistosomes: approaches with retroviruses
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Retrovirus mediated transduction offers a means to establish transgenic lines of schistosomes, to elucidate schistosome gene function and expression, and to advance functional genomics approaches for these parasites. We investigated the utility of the Moloney murine leukemia retrovirus (MLV) and HIV-1 lentivirus pseudotyped with vesicular stomatitis virus glycoprotein (VSVG) for the transduction of *Schistosoma mansoni* and delivery of reporter transgenes into schistosome chromosomes. Schistosomules were exposed to virions of VSVG-pseudotyped retrovirus/ lentivirus after which genomic DNA was extracted from the transduced schistosomes. Southern hybridization analysis indicated the presence of proviral retrovirus in the transduced schistosomes. Fragments of the retroviral transgenes and flanking schistosome sequences recovered using anchored PCR-based approaches demonstrated definitively that somatic transgenesis of schistosome chromosomes had taken place and, moreover, revealed widespread retrovirus integration into schistosome chromosomes. Reporter luciferase transgene activity driven by the schistosome actin gene promoter was expressed in the tissues of transduced schistosomules and adult schistosomes. Furthermore, retroviral transgenes were transmitted vertically in snails to cercariae following infection of snails with retrovirus transduced miracidia. These findings indicate the utility of VSVG-pseudotyped retroviruses for transgenesis of schistosomes, herald a tractable pathway forward towards germline transgenesis and functional genomics of parasitic helminths.

S27 RNA interference in the tapeworm, *Moniezia expansa*
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Cestodes of livestock pose a significant burden on the agricultural sector and resistance to anthelmintic treatments is now emerging, e.g. to benzimidazoles in *Moniezia expansa.* This demonstrates the need for novel mode-of-action chemotherapeutics to treat tapeworm infections of livestock. To facilitate this, we need new drug targets from cestodes and neuropeptide signalling pathways could provide target discovery opportunities. RNA interference (RNAi) is an accepted method of probing gene function via post-transcriptional gene knockdown and facilitating the identification/validation of novel drug targets. RNAi has been demonstrated in turbellarians, one monogenean and the trematodes *Schistosoma mansoni* and *Fasciola hepatica*, with most success being documented in schistosomes. At this time, RNAi has not been documented in cestodes. This study aims to develop RNAi protocols in the cyclophylidean cestode *M. expansa.* Here we report attempts to silence both neuronally expressed neuropeptide F (NPF) and the more widely expressed actin in *M. expansa* adults. The knockdown of *Mx-npf* transcript levels was inconsistent and appeared mostly refractory to RNAi following the delivery of dsRNAs by soaking and/or electroporation. However, *mx-actin* RNAi was profound, with a marked, specific and significant reduction in *mx-actin* transcript levels following silencing. Further investigations on actin-RNAi worms revealed a significant decrease in actin levels and that muscle contraction was compromised, confirming the presence of a functional RNAi pathway in this cestode.

S28 Pitfalls on the way to RNA interference in parasitic nematodes - The example of *Heligmosomoides polygyrus*
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RNA interference (RNAi) is used as powerful tool of reverse genetics in multiple organisms. First discovered in the free living nematode *Caenorhabditis elegans* it was supposed to be a promising tool for parasitic nematodes. To elicit the potential of RNAi in the parasitic nematode *Heligmosomoides polygyrus*, we used different methods to applique double-stranded RNA (dsRNA) of tropomyosin to various life stages. Feeding of dsRNA expressing bacteria to L1 and L2 stages did neither result in phenotypical changes nor in reduced transcript levels of tropomyosin. In contrast, *C. elegans* elicited the expected phenotype, such as body morphology defects. Electroporation of small interfering RNA (siRNA) failed in L1 but could penetrate into the cuticle of adults. However, no systemic spread was observed. Soaking of adults in dsRNA led to worms showing alterations, such

as disrupted guts and ovaries, but these alterations were not reflected on transcriptional levels of tropomyosin. Searching for orthologue proteins involved in the RNAi pathway of *C. elegans* revealed the lack of proteins essential for uptake and systemic spreading in *Brugia malayi* and *Haemonchus contortus*, whereas a majority of proteins involved in dsRNA processing/amplification and mRNA regulation are present. Thus, our results indicate that functional gene studies using RNAi in *H. polygyrus* are limited, possibly due to an insufficiency in the protein machinery necessary for dsRNA uptake and spreading.

S29 RNAi in different life stages of *Fasciola hepatica*

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Fasciola hepatica is responsible for causing fasciolosis in ruminants and is emerging as an important zoonotic disease. Currently, Triclabendazole (TCBZ) is the only drug available that has significant efficacy against both the adult and the pathogenic juvenile stages. However, with TCBZ-resistance well established, the development and application of novel control measures are an imperative. RNA interference (RNAi) provides a reverse genetic platform which facilitates investigations into gene function, providing opportunities for drug target identification and validation. The key fluke virulence proteins, cathepsins L (FheCL) and B (FheCB) are amongst the most highly expressed proteins within *F. hepatica*, have been proposed to play key roles in all life stages and have been proposed as leading vaccine candidates. Our previous work has shown that FheCL and FheCB RNAi compromises gut penetration in the newly excysted juvenile stage. Here, through the modification of existing protocols, we have extended the application of RNAi in liver fluke to the adult stage, with evidence for specific reductions in the levels of cathepsin and cathepsin transcript. Further, preliminary data from metacercariae reveal phenotypic consequences following exposure to cathepsin double stranded RNAs. The efficacy of siRNAs has also been examined. These efforts have revealed functional RNAi pathways in multiple life stages of *F. hepatica* and further highlight FheCL and FheCB as promising control targets.

S30 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 *Caenorhabditis elegans* but has proven to be less effective in parasitic nematodes. However we have obtained successful knockdown for some *H. contortus* genes. We are currently testing different genes and dsRNA delivery methods. 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 different genes by RNAi to establish a pattern for successful knockdown. We are also looking at different ways to deliver dsRNA to test for enhancement of the effect.

S31 Non-nematode derived double stranded RNAs induce profound phenotypic changes in *Meloidogyne incognita* and *Globodera pallida* infective juveniles

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Although initial accounts of RNAi-based reverse genetics in parasitic nematodes revealed a picture of specific and reliable gene silencing, subsequent datasets exposed pronounced discrepencies in the efficacy of application and reproducibility among different species. The animal parasite community first questioned the validity of such an approach to gene function analysis, due to the evident unreliability of published accounts and inconsistent RNAi effects. By contrast, similar endeavours to establish RNAi protocols in plant parasitic nematodes (PPNs) have been considerably more successful. However, the control of gene silencing experiments in both sets of parasitic nematodes remains notoriously inconsistent, and in an effort to increase the rigour with which we examined the specificity of gene silencing in PPNs we have uncovered a serious issue with the application of negative controls. We demonstrate the induction of an undocumented inhibitory phenotype presented by both *M. incognita* and *G. pallida* J2s on exposure to varying amounts of non-nematode dsRNA; as little as 0.1 mg/ml in M. incognita. We observe this phenomenon consistently, and believe that it highlights a significant deficiency in our knowledge and awareness of the variables inherent to RNAi-based investigations in nematode parasites.

S32 siRNA-mediated gene silencing in *Globodera pallida* and *Meloidogyne incognita*

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The *in vitro* analysis of gene function through RNAi-based reverse genetics in plant parasitic nematodes remains inexplicably reliant on the use of long double-stranded RNA (dsRNA) silencing triggers; a practice inherently disadvantageous due to the introduction of superfluous dsRNA sequence, increasing chances of aberrant or off-target gene silencing through interactions between nascent siRNAs and non-cognate mRNA targets. Here we report, for the first time, robust gene-specific knockdown of FMRFamide-like peptide (flp) transcripts, using discrete 21 bp small interfering RNAs (siRNAs) in the potato cyst nematode *Globodera pallida*, and the root knot nematode *Meloidogyne incognita*. Both knockdown at the transcript level through RT-PCR and qRT-PCR analysis, and functional data derived from migration assay, indicate that siRNAs targeting certain areas of FLP transcripts are effective and potent in the silencing of gene function. We also reveal profound variation in the efficacy of individual siRNAs, highlighting the need for much rigour in the interpretation of post-silencing datasets. In addition, we present what we believe to be the first lethal RNAi-based target and protocol combination in *M. incognita*, through siRNA-mediated silencing of Drosha (a nuclear RNAse III enzyme concerned with the excision of pri-miRNAs) in eggs, along with a simple method of manipulating siRNA activity through micromanagement of siRNA thermodynamic profile.

Session 3B: APICOMPLEXA II – POPULATION BIOLOGY
Convenor: Fiona Tomley

S33 The Population Structure of *Toxoplasma gondii*

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Toxoplasma gondii is one of the most successful parasites as it infects virtually all warm-blooded animals including man. Although *T. gondii* infection is relatively benign under most circumstances, it is a serious opportunistic pathogen in man (particularly in the immunocompromised) and some strain/host combinations result in fatal disease. *T. gondii* has a striking clonal population structure composed of three predominant lineages/haplogroups in North America and Europe. These lineages appear to be descendant from a single original genetic cross and are related by only a couple of subsequent crosses. In these regions, the clonality is associated with a specific monomorphic version of Chromosome 1a that appears to have driven a selective genetic sweep within the past 10,000 years. The South American strains diverged from the North America/Europe strains about 1-2 million years ago. Again the lineages appear to be descendant from a very limited number of genetic crosses, but include genetic polymorphisms not found in North America and Europe. Selection and/or population isolation has resulted in higher diversity in South America, but the introgression of the monomorphic Chromosome 1a has resulted in the selection for highly clonal lineages. Modeling of population structure shows that collectively across continents, the strains are derived from four original genotypes where only a few crosses are required to explain the existing 11 haplogroups. Current evidence suggests that the population structure of *T. gondii* is shaped by clonal expansion driven by infrequent recombination events and selective genetic sweeps.

S34 Genotypic Diversity, a Survival Strategy for the Apicomplexan Parasite *Theileria parva*

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The tick-borne protozoan parasite *Theileria parva* causes East Coast fever, a severe lymphoproliferative disease of cattle and is a major constraint to the improvement of livestock in eastern, central and southern Africa. The protective cytotoxic T lymphocyte response against this pathogen is tightly focused on a limited number of polymorphic schizont antigen epitopes. In individual animals only one or two predominant antigens are recognized and which antigen is seen is dependent on the MHC class I phenotype of the animal. Although genetic diversity has been described for *T. parva*, little was known about how this diversity was generated and its function. To address these questions a genome wide molecular marker set was developed, consisting of 94 satellite markers and PCR-defined size polymorphisms in five transcribed genes. These markers allowed the

characterization of diversity of existing parasite stabilates, changes of parasite populations during tick passage and the effect of a protective immune response on parasite transmission. The results showed that extensive recombination of *T. parva* parasites occurs in ticks which increases genetic diversity at a population level. Some of these new recombinant genotypes, when infecting immune cattle are likely to evade the tightly focused immune response and thus result in the survival of the parasite population.

S35 Population genetic analysis and sub-structuring in *Babesia bovis*

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Babesia bovis, transmitted by *Rhipicephalus* (*Boophilus) microplus*, is one of the causes of bovine babesiosis, an economically important disease of cattle in tropical and subtropical countries. Using the recently published genome of the parasite, we developed a panel of eight genetic markers and proceeded to investigate the population structure and diversity of the parasite using isolates from Zambia and Turkey. Population genetic analysis revealed high levels of genetic diversity with geographical sub-structuring quantified using F_{ST} values. Linkage disequilibrium was observed when isolates from all countries were treated as one population, but when isolates from Zambia were analysed separately linkage equilibrium was observed. The Turkish isolates were sub-structured, containing two genetically distinct sub-groups, one of which was in linkage equilibrium. Preliminary results of the Zambian study suggest that a sub-set of the parasite population is responsible for the westward spread of babesiosis into the previously disease-free central region of the country. Overall, these results indicate fundamental biological differences between *B. bovis* populations both within and between countries and these differences may have implications for the development of vaccines against the disease.

S36 Conflict in the Cockroach: Parasite interactions and resource use

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Parasites compete with hosts for resources and this competition can drive changes in respective life histories. The presence of multiple parasites within the hosts could provide further selective pressure for these changes. We examined resource storage (lipid levels) in the German cockroach, *Blattella germanica* and examined how this resource altered in hosts infected with a protozoan gut-parasite *Gregarina blattarum.* We found female cockroaches had higher lipid stores than males (after correcting for body weight). Host *G. blattarum* intensity was correlated with a substantial reduction in lipid levels. In a second series of experiments the fecundity of the entomopathogenic nematode *Steinernema carpocapsae* was measured in singly infected versus co-infected hosts. The output of infective larvae was lower in male than female cockroaches in both infection groups. Hosts co-infected with *G. blattarum* produced significantly fewer *S. carpocapsae* larvae than singly infected hosts. We have shown that competition for resources exists between the host and parasites. Further, we have demonstrated clear evidence that both parasites exert selective pressures upon the host and upon one another; as *S. carpocapsae* infection results in both the death of the host and of any gregarines infecting it and *G. blattarum* reduces host nutrient stores and *S. carpocapsae* fecundity. These clear selection pressures mean that this host / multi-parasite system is ideal for future co-evolutionary dynamic research.

S37 Molecular characterisation of *Toxoplasma gondii* in cats in Germany

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Toxoplasmosis is a parasitic disease caused by the protozoan parasite *Toxoplasma gondii*. Its population structure is highly clonal with three (I, II, and III) lineages (clonotypes). Despite a sexual phase in its lifecycle, recombinant genotypes are rarely found between the lineages. It still remains to be established whether the strain type affects the disease outcome in humans. There is paucity of information regarding the variety of *T. gondii* lineages occurring in Germany. Oocysts from infected cats were collected and analyzed for *T. gondii*. Of all samples analysed, fourty-four were found to be PCR-positive for *T. gondii* which were genotyped by PCR-RFLP using independent, unlinked markers for SAG2, SAG3, GRA6, BTUB, c22-8, c29-2, L358, PK1 and Apico. The majority of isolates were found to be of genotype II while a few other *T. gondii* positive isolates were of atypical genotypes or shown not to amplify PCR-products for most of the markers. Furthermore, *T. gondii* oocysts were used to infect IFN-γ knockout mice to isolate tachyzoites that were propagated in cell culture. In-vitro growth studies were conducted to further characterise the virulence of isolates for infected cells.

Session 3C: ECTOPARASITE CONTROL
Convenor/Chair: Olivier Sparagano

S38 Herbal treatments against gyrodactylids (*Monogenea*)

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Gyrodactylus infections cause high economic losses in fish aquaculture and threaten natural Atlantic salmon (*Salmo salar*) populations. The treatment of gyrodactylidosis is problematic due to (1) partial efficacy, at best; (2) toxicity to host, environment and/or humans; (3) increasing acquired resistance by *Gyrodactylus* species; and (4) difficulties with large scale application. Hence, there is interest to find alternatives. With the recent resurgence of natural products, botanics have been trialled against various diseases as they are thought to be less detrimental for non-target organisms. In our laboratory several botanics have been tested on gyrodactylids using guppies (*Poecilia reticulata*) infected with *Gyrodactylus turnbulli*. Compound X and its active components appeared to reduce parasite burdens of fish when applying a high dose initially, however, when fish were exposed to only the subsequent low dose efficacy was reduced. Other compounds, such as Pimafix (active ingredient West Indian Bay Leaf) and Melafix (Tea Tree Oil), marketed for antifungal and antibacterial properties, respectively, show varying results. Pimafix is not effective, whereas Melafix prevented population growth of gyrodactylids on guppies co-infected with various other parasites. This supports Steverding *et al*.'s (2005) study and our own results in experiments using tea tree oil. However, further research on all compounds is needed including experiments on different host-parasite combinations, fish behaviour and ecological effects.

S39 The influence of dust and humidity on the toxicity of plant essential oils to poultry red mite (PRM)

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Pest resistance and product withdrawal are driving the search for alternatives to synthetic chemicals for managing the poultry red mite (PRM), a pest of layer hens. Several plant essential oils may hold promise as PRM acaricides, but their efficacy may be affected by dust or humidity (George et al. 2009).
This laboratory project examined the influence of three levels of dust and humidity (after George et al. 2009, but with an additional 'intermediate' level of both and in a factorial design) on the effectiveness of two essential oils; clove bud and cinnamon bark, as acaricides for PRM. Oils were used (with a no-oil control) at predetermined LC_{50} levels.
Results confirmed that the essential oils of clove bud and cinnamon bark are toxic to PRM, and that mortality of mites exposed to these oils is increased when humidity and dust levels are high. There was no significant interaction between humidity and dust effecting mite mortality, suggesting that the effect of one might over-ride that of the other.
Reassuringly, any complicated interactions between dust and humidity (that might be difficult to predict and control in poultry houses) should not influence the effectiveness of essential oil-based products against PRM.
George *et al.* (2009). Med Vet Entomol, *in press*.

S40 Antibody responses to saliva of *Triatoma infestans*: Their potential as epidemiological tool for Chagas disease surveillance

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The 'Southern Cone Initiative' was highly successful in significantly reducing populations of *Triatoma infestans*, the main vector of Chagas disease in Latin America. New methologies are required to detect re-emerging *T. infestans* populations at an early stage after control programmes have finished. This study analysed the antibody response of chickens and guinea pigs to the saliva of *T. infestans*. Highly immunogenic antigens (14, 21, 26 kDa) were recognized as soon as two days after the first exposure to bug bites by all chicken sera and a 79 kDa protein by all guinea pig sera. Out of four identified proteins by mass spectrometry, a 14.6 kDa antigen was expressed and tested against animal sera from laboratory studies and from free-living hosts of *T. infestans* from

Bolivia. Cross reactivity experiments with salivary proteins of other haematophagous species confirmed the usefulness of the recombinant protein not only as an epidemiological marker for the detection of low-level infestation of *T. infestans* but also for other triatomines.

S41 Immunization of laying hens with somatic antigens from poultry red mite (PRM)

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Alternative control methods for PRM in egg laying poultry are needed in light of increasing reports of acaricide resistance. In this study, two groups of laying hens were immunized at 0 and 21 days with adjuvant and either saline or proteins extracted from PRM (DGE). Antibody response to immunization was determined by ELISA and western blotting using immunoglobulins (Igs) extracted from egg yolk. DGE efficacy was measured using a PRM *in vitro* feeding assay and blood spiked with extracted IgY.
DGE immunization of hens resulted in a significant IgY response compared to controls with peaks in IgY titres on day 19 and 43. There was no significant difference in IgM response between treatments. A number of proteins were identified by western blotting using IgY antibodies from DGE immunized birds, most prominently at 40 and 230 kDa. Protein analysis confirmed the identity of tropomyosin, whilst other proteins showed high sequence homology with myosin and actin from other arachnid and insect species. Immunization of hens with DGE resulted in a 50.6 % increase in mite mortality ($P < 0.001$) 17 hours after feeding when tested in the PRM feeding model. Data in this study demonstrate that somatic antigens from *D. gallinae* can be used to stimulate a protective immune response in laying hens.

Session 3D: HELMINTH GENOMICS
Convenor: Christiane Hertz-Fowler; Chair: Mark Viney

S42 The *Echinococcus multilocularis* genome sequencing project – current status.

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The larval stage of the fox-tapeworm *Echinococcus multilocularis* is the causative agent of aleveolar echinococcosis, one of the most dangerous parasitoses of the Northern Hemisphere. In contrast to other prominent Taenid cestodes such as *E. granulosus* or *Taenia solium*, *E. multilocularis* larvae can be easily kept in the laboratory and are accessible to in vitro cultivation methods. One particularly useful in vitro cultivation system, by which metacestode larvae can be completely re-constituted from totipotent *Echinococcus* stem cells, has recently been used by us for the stable introduction of foreign DNA into the *E. multilocularis* genome. Since *E. multilocularis* is thus the first cestode for which methods of genetic manipulation are available, we have, in cooperation with the Sanger Centre Pathogen Sequencing Unit, initiated a whole genome sequencing project for this model cestode. Using chromosomal DNA from the parasite's protoscolex larval stage as a source, followed by capillary sequencing as well as 454 and SOLEXA techniques, the latest assembly yielded a total scaffold length of 106 Mb, contained in around 900 scaffolds with an N50 contig size of 1.6 Mb. In this presentation I will discuss several features of the *E. multilocularis* genome in comparison with known data from the *Schistosoma mansoni* and *Schmidtea mediterranea* genome projects. Furthermore, I will present data on viral integration sites for recombinant gene transfer and the structure of cell-cell communication and signalling mechanisms.

S43 Genomics of parasitic helminths

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Infectious and pathogenic diseases account for almost one quarter of global disease incidence. Of these, parasitic worm infections are perhaps the most neglected and account for more than a billion infections worldwide. The availability of complete genome sequence data from both parasites and their mammalian hosts provides a unique opportunity to take a genomic approach to understanding parasite biology. In fields such as malaria, the availability of genome sequences has had a pronounced enabling effect. Now, with the advent of cheaper sequencing technologies, de novo sequencing of parasitic worm genomes is possible and is catapulting a struggling and neglected field into the genomic era. The helminth genomes initiative at the Wellcome Trust Sanger Institute is aiming to produce reference genomes from a wide phylogenetic spectrum of parasitic worms. Currently genomes for blood flukes, tapeworms, hookworms, giant round worms and threadworms are in

progress. Although, high levels of polymorphism and the lack of inbred lines remain serious problems, steps are being taken to decrease the complexity of the assembly task and produce high quality sequences within which genes can be found. To help with the latter, new sequencing technologies are having a profound effect; deep sequencing of the transcriptome with short reads allows precise definition of gene structures, transcribed non-coding regions and alternative splicing.

S44 The TGF-beta signalling pathway in the nematode *Trichinella spiralis*

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The recent release of genome sequence information is revolutionising the study of helminth parasites by providing important datasets for comparative genomics, allowing us to analyse signalling pathways that regulate nematode parasite development. Much of our current knowledge of nematode signalling pathways is based on the study of the free-living model *Caenorhabditis elegans.* The recent availability of the draft genome sequence of *Trichinella spiralis* has presented an opportunity to study signalling pathways of this parasitic nematode. We have undertaken an analysis of TGF-beta signalling pathways in *T. spiralis*. To date we have identified five genes encoding TGF-beta-like ligands, three of which belong to the BMP subfamily and are closely related to the vertebrate BMP2/4, BMP5/8 and BMP3 proteins. The remaining two proteins belong to the TGF-beta/activin subfamily. Only one member (DAF-7) of this subfamily has been identified in other nematode species, including *C. elegans* and there is some support for grouping one of the *T. spiralis* proteins with the Daf-7-like proteins. In contrast, the second protein shares significant amino acid identity with myostatin, a protein involved in regulating muscle mass in adult vertebrates. In addition, we have identified five putative TGF-beta pathway receptor kinases, three type I and two type II receptors, two receptor SMADs and a single co-SMAD. Data on the identification, analysis and expression of these proteins will be presented.

S45 Isolation and characterisation of protease genes from the parasitic nematode *Anisakis simplex*

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Nematode proteases have been shown to be involved in numerous biological processes and are attractive drug targets in parasites. *A. simplex* is an ascaridian nematode of marine mammals that is responsible for anisakiasis in humans. With a view to further understanding the roles of peptidases in the biology of *A. simplex*, this study has focussed on isolating and characterising three protease genes; 1), a cytosolic non-specific dipeptidase (CNDP), 2), a matrix metalloprotease and 3), the puromycin-sensitive aminopeptidase (*pam-1*). Briefly, a PCR strategy was used to isolate the full length CNDP and MMP genes and an almost full length *pam-1* gene from cDNA prepared from both larval and adult stages of the parasite. A differential expression pattern was acquired for all genes, showing that the larval proteases are expressed at higher levels than in adults. Expression analysis of *pam-1* has been carried out in the model nematode *C. elegans* using transgenic gfp expressing lines and also a PAM-1 specific antibody. Further translational studies of the CNDP and the MMP are planned in *C.elegans* to assist understanding of the biological roles of these proteases in nematodes.

S46 Patterns of gene expression in *Schistosoma mansoni* larvae during infection of the human host

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Larval schistosomes infect the human host by penetration of unbroken skin before gaining access to a blood vessel and beginning their intravascular life. Some of the proteins involved in this process have been identified, but much remains to be discovered. We have compared the patterns of gene expression in the intramolluscan germ ball (embryonic cercaria), infective cercaria, and *in vitro* cultured day 3 schistosomulum (skin stage larva), stages that represent the transition from the snail host, through fresh water, to the potentially hostile environment of mammalian skin. A novel custom high-density oligonucleotide array was made by Roche-NimbleGen, comprising probes designed using a compilation of all the *S. mansoni* gene predictions and ESTs available at GeneDB.org. Three biological replicates of double stranded cDNA from each stage were labelled and hybridised to the array. Transcripts that are significantly up- or down-regulated will be described, and hypotheses presented about the roles of the proteins they encode in this important snail-water-mammal transition.

Session 4B: APICOMPLEXA III – HOST-PATHOGEN INTERACTIONS
Convenor: Lee Innes

S47 The regulation of infection-induced inflammation

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Multiple mechanisms, including Tregs and a growing list of cytokines, control the balance between protective and pathological inflammatory responses. Studies with *Toxoplasma gondii* have shown that although T cells are required to control this pathogen these lymphocytes can also cause severe pathology. This provides an experimental system to study the factors that limit T cell activity and early reports identified IL-10 as essential to prevent immune hyper-reactivity during toxoplasmosis. Another example is IL-27, a cytokine originally ascribed pro-inflammatory activities, but IL-27R-/- mice infected with *T. gondii* develop a lethal CD4+ T cell mediated inflammatory response. Studies from this laboratory revealed that IL-27 antagonizes the production of the T cell growth factor IL-2 and can inhibit Th1, Th2 and Th17 responses. More recently, IL-27 (and its cousin IL-6) has been recognized as an important inducer of T cell production of IL-10 and that these events are mediated through STAT1 and STAT3. At present it is unclear how many of the suppressive effects of IL-27 are due to its ability to induce IL-10 and the relative contribution of IL-6 and IL-27 to the levels of IL-10 produced during different stages of toxoplasmosis is uncertain. While there is good evidence that there is a CD4+ (non-Treg) population that produces “protective” IL-10 our preliminary data suggest a model in which IL-6 and IL-27 regulate the production of IL-10 in distinct T cell subsets. This has been further complicated by the observation that IL-27 prevents Treg expansion and IL-27 transgenic mice develop an inflammatory condition characterized by systemic levels of cytokines and which may be related to a lack of Treg.

S48 A role for IL-33 receptor signalling in protection against toxoplasmic encephalitis

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T1/ST2 is an immunoregulatory protein of the IL-1 receptor family and has recently been reported as being a component of the IL-33 receptor. IL-33 is a newly described cytokine known to amplify the Th2 response and reduce the production of Th1 cytokines. The function of T1/ST2 during *Toxoplasma gondii* infection is as yet undescribed. Given the requirement for a balanced type 1/type 2 response for effective control of parasite number and immunopathology during infection with *T. gondii*, it is likely that T1/ST2 may play a part in aiding this process. Accordingly, we have shown that T1/ST2 mRNA transcripts are upregulated in the brains of *T. gondii* infected mice. Mice deficient in T1/ST2 demonstrated increased susceptibility to infection with *T. gondii* that correlated with both increased pathology and greater parasite burdens in the brains. Real time PCR analysis of cerebral cytokine levels revealed increased mRNA levels of iNOS, IFN-γ and TNF-α. These effects were independent of changes in IL-10 production. We provide the first evidence of a specific role for IL-33 receptor signalling in the brain as well as highlighting the requirement of this mechanism in limiting infection of an intracellular parasite.

S49 Benign vertical transmission of *Toxoplasma gondii* to lambs

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The relative importance of horizontal and vertical *T. gondii* transmission to lambs is under debate since the introduction of PCR screening. I report a titration which showed that the B1 PCR target was more repeatable than the P30, 5\'SAG2, 3\'SAG2 and SAG3 targets when parasite DNA was scarce, but that it remained subject to ascertainment bias. I standardised a B1-PCR protocol for screening the umbilical cords of viable and non-viable lambs and the internal tissues of non-viable lambs. *T. gondii* DNA, indicating prenatal exposure, was detectable in non-viables (4/55 (7.3%) Charollais, 0/16 (0%) Swaledale) and in viables (13/243 (5.3%) Charollais, 29/264 (11.0%) Swaledale). For the same viable lambs at age four months, seroprevalence was 50/411 (12.2%) in Charollais and 10/329 (3.0%) in Swaledale. PCR positivity did not predict seropositivity for individual lambs or for litters. Nevertheless my data was consistent with frequent benign vertical transmission to Charollais lambs. Seropositive Charollais were aggregated in litters (P<0.001) and the seropositive proportion of the litter was highest when maiden ewes had triplet lambs (logistic regression; P=0.004 for Parity:LitterSize

interaction). This confirmed previous reports of age-related vertical transmission and it identified prolificacy as another influence. However in contrast to those reports, vertical transmission was often benign. I extended that conclusion by seeking impacts of T. gondii on the gestational success of ewes and on the growth of lambs, but detecting none.

S50 Interferon-γ-dependent innate immunity against *Cryptosporidium parvum* infection in mice operates in the absence of natural killer (NK) cells

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Cryptosporidium parvum infects enterocytes and is commonly the cause of the diarrhoeal disease cryptosporidiosis. Immunocompromised mice that lack T and B lymphocytes partially control infection in part through interferon (IFN)-γ activity. NK cells are the main source of IFN-γ in innate immunity, but a protective role against *Cryptosporidium* has not been established.

An investigation was made of the role of NK cells in innate immunity to *C. parvum* employing $Rag2^{-/-}$ mice that lack T and B cells and $Rag2^{-/-}\gamma c^{-/-}$ mice that lack T, B and NK cells.
Adult mice developed chronic infections that increased in intensity at a faster rate in $Rag^{-/-}\gamma c^{-/-}$ than in $Rag2^{-/-}$ mice and the $Rag^{-/-}\gamma c^{-/-}$ mice died after several weeks. Neonatal mice initially developed acute infections that were heavier in $Rag^{-/-}\gamma c^{-/-}$ mice, but both strains survived this initial phase of infection. Surprisingly, significant levels of intestinal IFN-γ mRNA were expressed in neonates of both strains. Furthermore, infections were exacerbated in both strains after anti-IFN-γ-neutralising antibody treatment.
These results confirm that innate immunity to *C. parvum* involves IFN-γ and demonstrates a protective role for NK cells. However, the findings with $Rag2^{-/-}\gamma c^{-/-}$ mice also indicate that a cell type other than NK cells is a significant source of IFN-γ.

S51 *Theileria parva* isolates of different virulence infect different T lymphocyte subpopulations

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Theileria parva is an intracellular protozoan parasite infecting bovine lymphocytes. *T. parva* isolates is thought to vary in virulence. *T. parva* Chitongo (Tpc) presents a lower virulence than *T.p* Muguga (Tpm). Using identical numbers of infective sporozoites, Tpc induced less mortality in vivo and a delayed in vitro transformation of lymphocytes
Comparison of infections on PBMC, purified $CD4^+$ and γδ-T cells indicated that all cell populations were infected with Tpm, while infected cells were only observed in the PBMC population with Tpc. Further analysis of Tpm infected PBMC revealed CD4, CD8 and γδ T cell phenotypes, while Tpc infections were only observed in CD8 phenotype, suggesting that Tpc only infects CD8 lineage of lymphocytes. Binding of sporozites on PBMC as detected by flow cytometry and monoclonal antibody (anti-sporozoite p67 antigen), revealed binding of the Tpm, but not Tpc sporozoites to PBMC. We have now established that *T. parva* Chitongo do bind infects and transform CD8 T cells.
The low virulence of Tpc might be due to a lower number of target cells leading to low infection rates, or a lower pathogenicity of infected host CD8 lymphocytes.

Session 4C: DRUG DISCOVERY FOR TROPICAL DISEASES
Convenor: Ian Gilbert; Chair: Paul Wyatt

S52 Fexinidazole : a new drug candidate for human African trypanosomiasis

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Through an extensive compound mining effort to explore new and old nitroimidazoles as drug leads against human African trypanosomiasis (HAT), DNDi has identified fexinidazole as a promising candidate for stage 2 HAT. Fexinidazole is a 5-nitroimidazole that was in preclinical development as a broad-spectrum anti-protozoal by Hoechst in the 1980s. Extensive profiling over the past 2 years demonstrated that fexinidazole exhibits *in vitro*

and *in vivo* activity against African trypanosomes. Oral administration of fexinidazole cures mouse models of acute and chronic infection. ADME and PK studies show that fexinidazole is well absorbed and readily distributes throughout the body, including the brain. It is rapidly metabolized, resulting in the formation of 2 active metabolites which account for most of the pharmacological activity. Regulatory toxicology studies (including safety pharmacology and 4-week repeated-dose toxicokinetics in rat and dog) have shown that fexinidazole is well tolerated, with no issues identified. While fexinidazole, like many nitroheterocycles, is mutagenic in the Ames test, it is not genotoxic to mammalian cells *in vitro* or *in vivo*. Taken together, fexinidazole is a promising candidate for development as a new oral treatment for both stages of HAT. Worldwide, fexinidazole is the only drug candidate entering clinical development for HAT.

S53 How many drug targets do we need to fill the drug development pipeline?

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There is a perceived shortage of well validated drug targets ready to enter the drug development pipeline for Human African trypanosomiasis or leishmaniaisis. This is not too surprising as most investigators are interested in the biology of the parasite, with target validation and assay development a by-product of hypothesis driven research (and therefore receiving a relatively low priority in research activities). *In silico* database mining of the trypanosome and *Leishmania* genomes, coupled to expert knowledge via community participation, has identified a list of potential drug targets. A major challenge for the drug development research community is how to carry out large scale validation of these potential drug targets and how to progress them through the drug development pipeline. A flowchart showing major processes, outputs and decision points for an RNAi-based *Trypanosoma brucei* target validation project is proposed. This will involve assessment of targets for essentiality using RNAi in vitro and in vivo, the expression and purification of recombinant protein from those genes shown to be essential and the development of enzyme assays suitable for high throughput screening. Data gained from studies in *Trypanosoma brucei* can be used to direct target validation studies in *Leishmania*. Examples of target validation studies with protein kinase and peptidase genes will be presented.

S54 Drug Discovery for Tropical Diseases

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There is an urgent need for the development of new drugs for tropical diseases, yet limited resources available for this. In Dundee we have recently set up a Drug Discovery Unit with a major focus on the development of new drugs for tropical diseases and in particular human African trypanosomiasis. There is a high attrition rate in drug discovery, so we are using a portfolio-based approach. We describe here the process of selection of molecular targets for screening that we have adopted, together with the selection of compound libraries for screening.

S55 A-WOL Drug Discovery: bacterial lipoprotein biosynthesis as a target for antifilarial drugs

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Lymphatic filariasis and onchocerciasis are debilitating diseases caused by parasitic filarial nematodes, which harbour an essential bacterial endosymbiont, *Wolbachia*. Doxycycline is an effective macrofilaricidal agent but is unsuitable for use in mass drug administration (MDA). The Anti-*Wolbachia* (A-WOL) Consortium aims to identify novel anti-*Wolbachia* drugs or combinations that are suitable for use in MDA. A cell-based assay has been developed for high throughput screening and has been used to screen over 1000 tetracycline derivatives and the complete human pharmacopeia (~2600 drugs) delivering 166 hits with improved activity over doxycycline. Target discovery has identified lipoprotein biosynthesis as a potential target. Globomycin targets the signal peptidase II enzyme (LspA) of the biosynthetic pathway. A putative LspA gene has been identified from the *Wolbachia* genome and its functionality was verified using complementation. In the cell-based assay globomycin depleted *Wolbachia* numbers and inhibited lipoprotein biosynthesis. Treatment of *Brugia malayi* adult females *in vitro* resulted in reduced motility and viability of parasites. These experiments validate lipoprotein biosynthesis as a target and identify globomycin as a novel class of antibiotic with anti-*Wolbachia* and anti-filarial activity.

S56 Cysteine synthase as a drug target in *Leishmania* and *Trichomonas vaginalis*.
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We have characterised the enzymes involved in cysteine biosynthesis in two protozoan pathogens: *Leishmania major* and *Trichomonas vaginalis*. In *Leishmania*, cysteine is a precursor of glutathione that conjugates with spermidine to form trypanathione - which has a key role in redox homeostasis and antioxidant defence. *Leishmania* has two pathways for cysteine biosynthesis, a *de novo* pathway involving serine acetyltransferase (SAT) and cysteine synthase (CS) and the reverse transsulfuration (RTS) pathway that converts homocysteine to cysteine. *Trichomonas vaginalis* does not produce glutathione but has relatively high levels of cysteine, which is thought to function as the main intracellular redox buffer. *Trichomonas* lacks the enzymes of the RTS pathway but uses CS for cysteine biosynthesis and has a pathway for cysteine catabolism involving mercaptopyruvate sulfurtransferase (MST). Our analyses indicate that CS and MST are regulated to maintain cysteine homeostasis.
The CSs of *Leishmania* and *Trichomonas* differ significantly, including in substrate preferences. Comparison of the CSs show differences in kinetic parameters and in their ability to form a bienzyme complex with SAT. CS is absent from humans and thus could be a viable drug target in both parasites. The possibilities of obtaining specific inhibitors as drugs, including via the use of virtual screening methods, will be discussed.

Session 4D: NEW SEQUENCING TECHNOLOGIES
Convenor/Chair: Christiane Hertz-Fowler

S57 The Application of Second Generation Sequencing to Parasite Genomes
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The field of genome sequencing has changed dramatically over the last few years with the advent of second-generation sequencing. This technology, which allows massively parallel random DNA sequencing, has enabled the analysis entire genomes in a few weeks at a fraction of the cost of traditional methods. As a result, it is now possible not only to sequence a more species than before but also full genome sequencing can be used to assay diversity within a species. In this talk, I will give examples of how we have employed different platforms to look at diversity within *Trypanosoma brucei* and *Entamoeba histolytica* in order to understand the genetic basis of virulence. I will also present examples from other systems to show how this powerful technology can be used to simplify forward genetics and transcriptomics in order to directly identify gene function.

S58 Genome-wide identification of mutations in Plasmodium chabaudi drug resistant clones
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The rodent malarial parasite, *Plasmodium chabaudi* has been extensively used to study the genetic basis of drug resistance. A congenic lineage of drug-resistant mutants resistant to pyrimethamine, chloroquine, mefloquine and artemisinin derivatives has been selected by the *in vivo* passage of parasites in the presence of these drugs. We have used short-read whole genome re-sequencing based upon the Illumina Solexa platform to identify all the mutations arising within this lineage. The original sensitive clone and 2 other drug-resistant clones were sequenced at between 25- and 80-fold coverage. Solexa single-end reads were mapped onto the Wellcome Trust Sanger Institute genome database using the MAQ software suite and custom scripts were used to identify single nucleotide substitutions, insertion/deletion events and gene copy number variation. Dideoxy sequencing was applied to confirm the accuracy of the approach and to verify the origin of crucial mutations within the lineage.
Our data complements genome-wide scans for signatures of drug selection to specify the critical mutations underlying drug resistance phenotypes.

S59 Helminth mitogenomics – smaller, faster, cheaper. Better?

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The characterization of complete mitochondrial genomes (mtDNAs) is gaining popularity in order to address a number of questions in parasitology, especially now that their amplification, through longPCR ,and sequencing is becoming easier. Using examples from recent studies on nematodes, cestodes and digeneans I explore (i) ways of assessing and capturing maximal information content of genes and genomes for use in population studies and as diagnostic markers, (ii) the utility of mtDNAs in systematics and phylogenetics, and (iii) recent and future developments in high throughput sequencing that might provide complete mtDNAs as ultimate barcodes.

S60 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 worldwide and resistance to the anthelmintics necessary for their control is becoming widespread.
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. The aim of this project is to characterise the CYP gene family in *Haemonchus contortus* with a view to investigating roles in drug resistance.
We have annotated supercontigs containing 95 fragments of partial gene sequence and are using these as tags to assay gene expression by real-time PCR. 68 out of the 95 CYP tags were found to be constitutively expressed in L3 larvae or adults. Expression levels of the CYP tags have been compared between life cycle stages, sexes, tissues, and after anthelmintic and other drug-exposures, using both susceptible and resistant isolates.
We are also applying new massively parallel sequencing technologies to assay the *H. contortus* transcriptome at the whole genome level, in order to confirm our CYP annotations, assemble full length genes, and compare CYP expression. It will also provide a more global approach to investigate drug-associated changes in gene expression in the parasite.

S61 2nd generation sequencing of the *Leishmania donovani* genome - insights into the mechanism of *Leishmania* disease tropism

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The leishmaniases are a spectrum of diseases caused by species of the vector borne parasite *Leishmania*. There are three main forms of the disease – cutaneous (CL), mucocutaneous (MCL) and visceral (VL) and these are normally associated with particular *Leishmania* species. VL is caused by species of the *L. donovani* complex, where parasites can disseminate away from the bite site of the sandfly vector and target visceral organs such as spleen and liver. Comparison of the *L. major*, *L. braziliensis* and *L. infantum* genomes has revealed that there are just 26 apparently unique *L. infantum* genes (a species of the *L. donovani* complex). We hypothesise that some of these genes are required for VL disease tropism. Illumina2 sequencing of the genome of a closely related species (*L. donovani*) to rule out the possibility that these genes are strain-specific, has identified 128,000 SNPs compared with *L. infantum* (45,000 within coding sequences) and that 25 of the 26 genes are also intact, supporting the idea that they are required for *L. donovani*-specific processes, including dissemination and targeting the viscera.

S62 Application of metabolomic technologies to unravel the biochemical basis of phenotypic diversity in parasite populations.

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Metabolomics represents the latest 'omic' technology and deals with the identification and quantitative measurement of all metabolites that act in the biochemical network of a biological system. Since the metabolome (downstream of transcriptome and proteome) is considered the closest correlate to the phenotype, it is expected that application of metabolomic technologies will dramatically improve our understanding of the biochemical basis underlying the phenotypic diversity observed in parasite populations. In this study we carried out metabolome-wide comparison of multiple drug-sensitive and -resistant *Leishmania donovani* isolates using ultra high resolution Fourier Transform mass spectrometry (LTQ Orbitrap). The results of this study demonstrate how revealing the diversity on the whole metabolome level in a natural *Leishmania* population can significantly contribute to (i) distinguishing the different phenotypes present in a population, (ii) giving a global overview of all factors involved in drug resistance by highlighting specific metabolic pathways, and (iii) enhancing our understanding of parasite flexibility. The used methodology, from sample preparation to identification of metabolic signatures of drug-resistant parasites, will be presented and discussed in the context of future applications for parasite research.

Session 5B: NEOSPORA SYMPOSIUM - I
Convenor: Lee Innes; Chairs: Lee Innes and Jens Mattsson

S63 The pathogenenesis of neosporosis – an overview.

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In cattle *Neospora caninum* has particular significance as a cause of abortion while in dogs, although clinical disease is also of importance, their role as the parasite's definitive host, capable of producing oocysts and therebye spreading infection, is of greater significance. Disease may occur in other species sporadically and in horses clinical illness has been associated with the closely related *Neospora hughesi*.
To establish an infection *Neospora* penetrates and multiplies in a host's cells to release further organisms to invade and destroy more cells and so cause greater damage. In the absence of an effective immune response the parasite continues to multiply causing progressively more cell death until the animal dies. More commonly however, parasite multiplication is controlled although the host remains persistently infected. The maintenance or failure of this dynamic equilibrium dictates the incidence of clinical disease.
Pregnancy may influence this balance. Recrudescence of a persistent infection and a subsequent parasitaemia in a pregnant cow can allow *Neospora* to invade the placenta and then the fetus. Fetal survival depends upon the nature of any maternal placental inflammation, the extent of placental necrosis or fetal damage or a combination of all three, which are all also influenced by fetal age. An understanding of the factors that rule the dynamic equilibrium between *Neospora* and its host is essential for the development of control strategies.

S64 Should *Neospora caninum* be considered a coccidian parasite?

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From reviewing the history of *Toxoplasma gondii* and the discovery of faecal transmission there were many difficulties in identifying it as a coccidian parasite. Initially the isosporan oocysts seen in cat faeces were considered as resulting from a contaminant infection. Once these "oocyst-like" structures were identified as the infectious form, they were initially termed the "new cyst" of *Toxoplasma*. Due to the revolutionary nature of this discovery, it was only when typical coccidian developmental stages were observed in the cat intestine that it was felt appropriate to term them oocyst. From comparison with previously describe isosporan species infecting cats the oocysts of *T. gondii* were similar to the small form those identified as *Isospora bigemini.* From the early part of the 20th centaury there was not thought to be any difference in the species of Coccida infecting both dogs and cats and both species were reported to produce *I. bigemina* oocysts. The question is, are *the I. bigemina* oocysts

in fact oocysts of *Neospora caninum* or *Hamondia.* However to the best of my knowledge the coccidian stages have never been observed in the intestine of the dog. The close relationship between *Toxoplasma* and *Neospora* would confirm the classification. Why is it so difficult to infect the definitive host? Could it be that the specific stains known at present have lost the ability to readily infect dogs or is *Neospora* evolving away from its coccidian ancestry?

S65 Sequencing and annotation of the genome of *Neospora caninum*

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We present the first sequence and annotation of the genome of *Neospora caninum*. Over 900,000 reads were obtained from the NC-Liverpool isolate giving an estimated 8.2x coverage of the 62MB genome. Sequence was assembled using PHARP into 1,114 contigs with a N50 of 200 kb (). A total of 14 pseudo chromosomes were predicted for *Neospora* by PROMER alignment with *T. gondii*. Gene finding tools using Jigsaw found an initial 5,591 putative protein coding genes and this first pass semi-automated annotated sequence is available in GeneDB (). The mean length of each gene was 6,182 bp, containing on average, 7.28 introns and with a mean intergenic distance of 11,036 bp. Gene prediction was supported by mapping over 21,000 ESTs back to the sequence. In addition, 9,000 peptides obtained from proteomic analysis of Neospora tachyzoites were used to refine the annotation. We also report on preliminary comparative genomic analysis with T. gondii, suggesting high synteny between the two genomes.

S66 Microsatellite analysis of *Neospora caninum* from bovine foetuses and dogs in Germany

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Neospora caninum infection is an important cause of bovine abortion. The aim of the present study was to compare *N. caninum* DNA derived from aborted bovine foetuses and from oocysts from naturally infected dogs using a microsatellite-based typing technique. Nested-PCR techniques were developed for the sensitive and specific amplification of regions in the *N. caninum* genome which contain microsatellites. Amplification products were analysed by length determination using capillary electrophoresis or by direct sequencing. Substantial genetic diversity was observed and in most cases individual microsatellite patterns were present. However, identical microsatellite patterns were observed among foetuses collected during epidemic abortion outbreaks, foetuses of the same herd in consecutive years and foetuses from different herds from the same region. All canine *N. caninum* oocyst isolates had individual microsatellite patterns except for those of two dogs. Microsatellite analysis may allow the typing of *N. caninum* from clinical samples without need of culturing the parasite. The technique may prove useful for molecular-epidemiological studies.

S67 Repetitive sequences and multiplex DNA typing of *Neospora caninum*

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Genetic diversity of *Neospora caninum* was investigated through a study of repetitive sequences. Mini and microsatellite DNA were characterised in the genome of *N. caninum* by PCR and DNA sequencing and a wide range of isolates sourced from around the world were compared. Based on observed polymorphisms detected at these loci amongst isolates, a multiplex PCR was developed and validated for multilocus strain typing using three microsatellites and three minisatellites of *N. caninum*. The results show that a multiplex PCR can unambiguously identify cultured isolates of *N. caninum* based on the DNA profile generated. All isolates studied were genetically diverse, although the three New Zealand isolates studied were identical in genotype.
Two additional uses of the multiplex PCR technology are described. In the first example, multiplex PCR was able to distinguish amongst strains of *N. caninum* used as either live vaccine or challenge strains in animal vaccination experiments. In the second example, multiplex PCR was used to genotype *N. caninum* DNA found in natural infections of animals. The multiplex PCR is therefore a rapid, highly informative and sensitive method for

assessing diversity within *N. caninum* and should prove to be a valuable tool in future studies in the epidemiology of *N. caninum*.

S68 Abortion pattern in cattle herds after natural postnatal infection with *Neospora caninum*.
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Three dairy problem herds with evidence of a point source infection with *N. caninum* during a limited period were selected for this study. In one herd, the infected age-group consisted of young cattle, which had seronegative dams. In one herd, the infected age-groups consisted of older cattle, which had seronegative daughters, indicating that the dams had been infected after the birth of their seronegative offspring. In one herd, the postnatal infection of the adult age-group had occurred during the dry-period. Insemination dates and birth dates of all cattle were retrieved from the Dutch Identification and Registration system. The period of infection was compared with dates of insemination and abortion. Cows infected before and after insemination aborted in 45% and 33%, respectively. None of three cows infected in late pregnancy aborted.

Session 5C: IMMUNOMODULATION BY HELMINTHS II
Convenor/Chair: Rick Maizels

S69 The costs and potential benefits of hookworm infection
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Infections with *Necator americanus* are clearly detrimental to human health, yet epidemiological studies continue to demonstrate a potential benefit of infection in patients with allergic disease. Hookworms and other nematode infections may also protect against the development of autoimmune diseases.
In order to address this paradox, where a parasite is subject to role reversal, a number of placebo - controlled clinical trials have been commissioned, to determine in the first instance whether regulated infection with *Necator americanus* is safe.
The first study is now complete, and data will be reported to support the belief that hookworm infection of tolerable intensity induced a natural immunological phenotype, had no adverse effects on lung function, and did not potentiate anti-allergen IgE responses.
With these data in mind, plans for future trials will be presented, in allergy and autoimmune diseases, beginning in patients with multiple sclerosis.
This work was supported by The Wellcome Trust

S70 The immunoepidemiology of childhood hepatosplenomegaly associated with *S. mansoni* infection and chronic exposure to malaria
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Evidence from community based epidemiological studies suggests that there is dissociation between peak prevalence of presentation with *S. mansoni* associated hepatosplenomegaly and ultrasound detectable periportal fibrosis, with the presence and extent of hepatosplenomegaly peaking in an age group younger – older children and adolescents – than the age group in which the prevalence of periportal fibrosis peaks – adults in their fourth and fifth decades of life. The peak in non-periportal fibrosis associated hepatosplenomegaly corresponds with the peak in *S. mansoni* infection intensities, and significant associations between the two have been reported. This has led to the proposal that non-periportal fibrosis associated hepatosplenomegaly is due to an inflammatory process. It does appear, however, that the aetiology of this childhood hepatosplenomegaly is not as simple as *S. mansoni* infection alone. Evidence suggests that when malaria is co-endemic, children have exacerbated hepatosplenomegaly. Here, we present evidence that when co-exposed to the two parasites, Th2 responses to SEA – those associated with periportal fibrosis – are down-regulated and pro-inflammatory responses are up-regulated and that hepatosplenomegaly is associated with pro-inflammatory responses.

S71 IgE responses to allergen-like molecules from different life-stages of *Schistosoma mansoni* provide a possible explanation for the slow development of human immunity

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Human immunity to *Schistosoma mansoni* occurs in endemic areas, but develops so slowly that it is only really evident in adults. This protective response is associated with anti-parasite IgE and appears to be directed against incoming larvae. The dominant IgE-antigen is Sm22.6, founding member of the tegument-allergen-like (TAL) family. We compared developmental expression of TAL family members, Sm22.6/TAL1, Sm21.7/TAL2 and Sm20.8/TAL3 and a novel example SmTAL4, using a new *S mansoni*-DNA-microarray. SmTAL4 is only expressed in skin-stage larvae and thus an anti-SmTAL4 response could be protective. We have expressed SmTAL4 as a recombinant protein and measured levels of SmTAL4-binding-IgE in 50 infected Ugandan men and in a separate population of 199 infected men and boys. We demonstrate IgE binding to SmTAL4, but show that this is due to cross-reactivity with adult-specific protein, Sm20.8/TAL3. IgE binding to SmTAL4 was absent in young children but increasing common with age. We propose that the gradual accumulation of adult-larval-IgE cross-reactivity could explain the remarkably slow development of human immunity.

S72 Rapid dendritic cell mobilisation to the large intestinal epithelium is associated with resistance to *Trichuris muris* infection

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We investigated the innate response of colonic DCs to *Trichuris muris* in mice that are inherently resistant or susceptible to infection. One day post- infection, there was a significant increase in the number of immature colonic DCs in resistant but not susceptible mice. This increase was sustained at day 7 post-infection in resistant mice when the majority of the DCs were mature. There was no increase in DC numbers in susceptible mice until day 13 post-infection. In resistant mice, most colonic DCs were located in or adjacent to the epithelium post-infection. There were also marked differences in the expression of colonic epithelial chemokines in resistant mice and susceptible mice. Resistant mice had significantly increased levels of epithelium-derived CCL2, CCL3, CCL5 and CCL20 compared with susceptible mice. Furthermore, neutralizing CCL5 and CCL20 in resistant mice prevented DC recruitment. This study provides clear evidence of differences in the kinetics of the DC response in hosts that will be resistant and susceptible to infection. DC responses in the colon correlate to resistance to infection in the large intestine. Differences in the production of DC chemotactic chemokines by colonic epithelial cells in response to infection in resistant versus susceptible mice may underlie the different kinetics of the DC response.

S73 Immunoregulation in wild mammals: associations between Toll-like receptor (TLR) function and individual infection status

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Immunomodulatory influences by parasites acting at the level of innate immunity may have consequences for systemic immunoregulation and the dynamics of coinfection. Studies on natural host-parasite systems allow a unique insight into “real-world” immunological variation in outbred vertebrate populations exposed to multiple environmental stressors and a diversity of parasites and pathogens. Here we update and extend an existing survey of innate immune response profiles and infection variables in *Apodemus sylvaticus* at Cotgrave Forest, Nottinghamshire (to date based on 200 wood mice sampled in Spring-Autumn 2007 and 2008). Using newly developed *A. sylvaticus*-specific quantitative real-time PCR (Q-PCR) reagents we measured Toll-like receptor (TLR-2, -4, -9) and cytokine (IL10, TNF-α) mRNA accumulations in cultured splenocytes stimulated with a panel of defined TLR agonists. TNF-α mRNA measurements were corroborated by ELISA analyses of protein levels in culture supernatants. Variation in TLR expression and innate regulatory (IL10) and pro-inflammatory (TNF-α) responses were analysed with respect to individual host infection status. In each host we surveyed selected microparasites and the entire macroparasite community, either by microscopy (*Eimeria* spp. cestodes, digeneans, nematodes, mites, ticks, fleas, lice) or PCR-based diagnosis (murine gammaherpes virus 68). Results develop our earlier findings that innate immune response profiles are differentially influenced by members of the macro- and microparasite community.

Session 5D: HELMINTH NEUROBIOLOGY 1
Convenor/Chair: Angela Mousley

S74 Mario de Bono

S75 FMRFamide-like peptide expression and function in the free-living nematode, *Panagrellus redivivus*
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FMRFamide-like peptides (FLPs) are the largest and most diverse family of invertebrate neuropeptides peaking in numbers and complexity within the phylum Nematoda. Current bioinformatic data indicates that nematodes possess at least 32 *flp* genes and that the encoded peptides are, most commonly, structurally conserved between nematode species. FLPs are widely expressed within the nematode nervous system and have been shown to be potent modulators of muscle activity with specific roles in locomotion, feeding and reproduction. *Panagrellus redivivus* is a free-living nematode that can be easily manipulated under laboratory conditions and thus provides a useful model with which to probe the neuropeptidergic system. Two *flp*-genes (*flp*-11, *flp*-18) have been identified in *P. redivivus* through PCR analysis, and the peptides encoded on *flp*-11 have been biochemically characterised. This study uses *in situ* hybridisation and immunofluoresence methodologies to describe the expression of *flp*-11 in *P. redivivus*. To investigate *flp-11* function, this study describes (i) attempts to establish an RNA interference platform in *P. redivivus* and (ii) the effects of the *flp*-11 encoding peptides (AMRNALVRFamide, AAGMRNALVRFamide and NGAPFVRFamide) on behavioural parameters using a variety of muscle-based bioassays. Results facilitated clade-specific comparative analyses of *flp*-gene expression and function between *P. redivivus* (clade IV) and *Caenorhabditis elegans* (clade V).

S76 A novel nicotinic ACh receptor subunit, ACR-26, from *Ascaris suum.*
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Parasitic nematodes are important aetiological agents of disease, infecting humans, livestock and companion animals. Nicotinic acetylcholine receptors (nAChRs) are significant drug targets for parasitic nematodes, with pyrantel and levamisole being notable examples, and a new class of drugs, the amino-acetonitrile derivatives, currently in development. Anthelmintic resistance is a growing problem for helminth chemotherapy, so there is an increasing need to identify drug targets and understand resistance development.
We have found a novel nAChR subunit gene, *acr-26*, that is conserved in several evolutionary distinct parasitic species, including *Brugia malayi, Ascaris suum* and *Haemonchus contortus,* but, to date, not in any free-living or plant parasitic species. An antibody for the *A. suum* ACR-26 subunit was produced. We demonstrated that it recognised ACR-26 specifically by expressing an epitope-tagged version of the subunit in mammalian cells and showing that the immunofluorescence produced by the anti-ACR-26 antibody completely overlapped with that of the epitope antibody. When the antibody was applied to *A. suum tissue,* specific immunofluorescence was observed on muscle cells from the head region of the worm, but not from the body wall.
Sequence data similarities with other nAChR subunits, such as the nematode ACR-16 and the mammalian α7 and α9 subunits, and computer modelling predicted that ACR-26 was capable of forming a homomeric receptor. We injected cRNA encoding ACR-26 into *Xenopus* oocytes and observed that a novel receptor was expressed in these cells, forming cation channels sensitive to acetylcholine and nicotine. We are continuing the functional characterisation of these receptors.

S77 Localisation and functional characterisation of peptidyl-α-hydroxyglycine α-amidating lyase, a novel *Schistosoma mansoni* amidating enzyme
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In the majority of eukaryotes the neuropeptide amidating enzymes PHM (peptidylglycine α-hydroxylating monooxygenase) and PAL (peptidyl-α-hydroxyglycine α-amidating lyase) are expressed as separate domains on the bifunctional protein PAM (peptidylglycine α-amidating monooxygenase). Previous work has characterised

Schistosoma mansoni PHM, suggesting that SmPHM and SmPAL are expressed as separate monofunctional proteins. In the present study post-genomic tools have been employed to elucidate the role of the novel SmPAL and the previously reported monooxygenase SmPHM. Whole mount *in situ* hybridisation (WISH) has demonstrated the expression of *Sm-pal-1* mRNA transcript in the central nervous system (CNS) of adult *S. mansoni*. Functional expression of *Sm-pal-1* established that SmPAL is a monofunctional, catalytically active, efficiently secreted amidating enzyme, with functional characteristics analogous to other eukaryotic amidating enzymes. RNA interference (RNAi) of *Sm-phm-1* and *Sm-pal-1*, with dsRNA delivery by either electroporation or soaking, had variable success in larval schistosomules. Electroporation-based delivery of dsRNAs did not induce measurable gene silencing in either transcript, while extended dsRNA-soaking procedures induced gene silencing of *Sm-phm-1* (≤89 %) and *Sm-pal-1* (≤90 %) in one third of experiments. The fundamental role of SmPAL in neuropeptide maturation provides drug target appeal.

S78 The nicotinic acetylcholine receptors of *Ascaris suum*

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Nicotinic acetylcholine receptors (nAChRs) are important targets of the anthelmintic drugs levamisole and pyrantel, and more recently, the AADs. Despite their importance as drug targets in parasites, most molecular studies of nematode nAChRs have previously been carried out using *C. elegans.* Using a bioinformatics approach applied to the genomes of *Brugia malayi* and *Trichinella spiralis*, we have demonstrated that these parasites have very few nAChR genes compared to *C. elegans*, but certain components of the levamisole-sensitive neuromuscular nAChR (*unc-38, unc-29* and *unc-63*) are well conserved. We have cloned cDNAs encoding UNC-29 and UNC-38 from *A. suum;* antibody labelling shows that *Ascaris* UNC-38 and UNC-29 co-localise on the muscle cell membrane.
We have co-expressed *Ascaris* UNC-38 and UNC-29 in *Xenopus* oocytes to form functional levamisole-gated ion channels, the first successful heterologous expression of a parasite
nAChR. We have also shown that changing the receptor stoichiometry produces receptor populations with different pharmacological properties: a 3:2 UNC-38:UNC-29 stoichiometry is more sensitive to nicotine and oxantel, whereas a 2:3 UNC-38:UNC-29 stoichiometry is more sensitive to levamisole and pyrantel. The pharmacology of these receptors resembles the L- and N- subtypes observed in native *A. suum* muscle cell membranes.

S79 Identification of an *Ascaris* G Protein-Coupled Acetylcholine Receptor With Atypical Muscarinic Pharmacology.

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Acetylcholine (ACh) mediates its effects in nematodes through interaction with both ligand-gated ion channels (LGICs) and G protein-coupled receptors (GPCRs). The structure, pharmacology and physiological importance of nematode ACh LGICs have been appreciably elucidated, however, less is understood about ACh GPCRs, termed GARs (G protein-linked ACh receptors). What we do know is based on *C. elegans* as no GARs have been characterized from parasitic species. Here we clone a putative GAR from the pig gastrointestinal nematode *Ascaris suum* with high structural homology to the *C. elegans* receptor GAR-1. Our GPCR, dubbed AsGAR-1, is alternatively spliced and expressed in the head and tail of adult worms. ACh activated heterologously expressed AsGAR-1 but the receptor was not activated by other small neurotransmitters. Some, but not all, classical muscarinic agonists tested were AsGAR-1 agonists. AsGAR-1 activation by ACh was partially antagonized by atropine but pirenzepine and scopolamine were largely ineffective. Certain biogenic amine GPCR antagonists also blocked AsGAR-1. Our conclusion is that *Ascaris* possesses G protein-coupled ACh receptors and that although they have some sequence homology to vertebrate muscarinic receptors, their pharmacology is atypically muscarinic.

Session 6B: NEOSPORA SYMPOSIUM - II
Convenor: Lee Innes; Chairs: Lee Innes and Jens Mattsson

S80 Prevalence and spatial distribution of *Neospora caninum* in a population of beef cattle
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The aim of this study was to estimate the national prevalence of *N. caninum* infection in Swedish beef cattle and to investigate any geographical patterns of the infection. The investigation was based on a serological survey comprising blood samples collected from 2,754 animals in 2,130 herds. The overall seroprevalence was 2.8% (CI: 2.18-3.44) which is similar to the prevalence in Swedish dairy cattle. Positive samples were found exclusively in the southern half of Sweden, the prevalence being higher in the South-East than in other parts of the country. Spatial autocorrelation was assessed using the Moran\'s I test and cluster detection was performed using LISA- and spatial scan statistics. The analyses identified clusters of high prevalence in the southern part of Sweden, in the counties of Kronoberg and Skåne. This was not expected as this high risk area corresponds largely to the area of lower risk for dairy cattle. Because beef and dairy cattle are distinct populations in Sweden, with limited breeding contacts, the different spatial trend among the two management systems supports the hypothesis that the parasite is predominantly transmitted by endogenous transplacental infection, in this country.

S81 Simulating control strategies for *Neospora caninum* infection in Dutch dairy herds.
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The middle-long term (2009-2020) prevalence and economic losses of *Neospora caninum* in dairy herds were simulated under current conditions and compared with simulated effects of optional control strategies. A dynamic stochastic model with three state-transitions (susceptible (S; ‹ 3% infection), low infection (Ilow; <15% infection) and high infection (Ihigh: ≥ 15% infection)) was build. This distinction was based upon previous studies where increased abortion problems were related with ≥15% within-herd prevalence. The results of the epidemiological module were input for the economical module. The costs consisted of direct losses due to infection and costs for control strategies. Simulated control strategies focused on 1) testing of animals that were newly introduced into the herd, 2) annual bulk milk testing (indicating Ihigh state) to raise awareness of herd owners and 3) measures to separate farm dogs from cattle. Various levels (25%, 50% or 75%) of risk reduction by these dog measures (tethering of dog, nurturing, no raw meat and safe disposal of dog faeces) were assumed. In the current situation, 21% of herds were susceptible, 63% had status Ilow and 16% status Ihigh. The average annual economic losses were €7.2 million of which 72% was attributed by Ihigh herds. Strategy 3 was most effective. Prevalence of Ihigh reduced to 4% in 2020 and annual economic losses reduced by 33.6%.

S82 Recrudescence of *Neospora caninum* in persistently infected, pregnant cattle is associated with an increase in maternal cytokine expression in the placenta but limited placental and foetal pathology.
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Eight cattle, naturally, persistently infected with *Neospora caninum* were artificially inseminated and their antibody response measured to detect when, during pregnancy, parasite recrudescence occurred. Cows were euthanized within two weeks of parasite recrudescence. All ten foetuses (including two sets of twins) showed evidence of *N. caninum* infection either by PCR, histology or immunohistology, but all ten were alive immediately before the dams were killed. There was evidence of *N. caninum* infection in the placentas of all eight cows but necrosis was mild and focal. The cytokines, interferon-gamma, interleukin (IL) 4, 10, 12, 2 and tumour necrosis factor- alpha, were quantified in the maternal caruncle by quantitative reverse-transcriptase polymerase chain reaction. There was a highly significant increase in IFN-γ and IL4 and modest but significant increases in levels of IL10, IL12 and TNF-α mRNA. These data suggest that a strong maternal immune response occurs in the placental of *N. caninum* infected cattle but that this was not associated with foetal death.

S83 Identification of novel bradyzoite and tachyzoite stage specific proteins by Ettan 2D-DIGE

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Neospora caninum bradyzoite stage seems to play an important role in evading the immune response and establishing the persistence of infection. Thus the identification of bradyzoite proteins may lead to a better knowledge of tachyzoite-bradyzoite convertion mechanisms and improve diagnosis and vaccination. Most known *Neospora* proteins are shared by both parasite stages, and only two bradyzoite stage specific antigens have been described -NcSAG4 and NcBSR4-, due to the difficulty in obtaining bradyzoites. In the present work, a comparative proteomic study of *N. caninum* tachyzoites and bradyzoites (mostly intermediate bradyzoites) was accomplished for the first time by applying the powerful Ettan 2-D - DIGE technology followed by MS analysis. A total of 72 differentially expressed spots were visualized (relative abundance 1.5, $p < 0.05$ en T-Test). Fifty three spots were increased in bradyzoites, whereas 19 spots did in tachyzoites. In addition the data obtained from MALDI/TOF and MS/MS analyses were used to search for protein candidates in NCBI and Swiss PROT/ TrEMBL databases. MS analysis raised 21 peptide sequences and revealed homologues that are common to *Toxoplasma gondii*. Up to date we have identified 3 novel proteins differentially expressed in the bradyzoite stage: fructose-1,6-bisphosphate aldolase, enolase 1 and glyceraldehyde-3-phosphate dehydrogenase. On the other hand the ribosomal phosphoprotein P0 was differentially expressed in tachyzoites.

S84 The development of immune responses in Balb/c mice following inoculation with attenuated or virulent *Neospora caninum* tachyzoites

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Balb/c mice were inoculated intraperitoneally (i.p.) with either $5x10^6$ live virulent (group 1) or $5x10^6$ live attenuated (group 2) tachyzoites, or vero cells (group 3). Animals were sacrificed at 0, 14, 28 and 42 days post-inoculation (p.i.), with the remaining mice receiving a lethal challenge on day 48 p.i. Serum, spleen and brain samples were collected post mortem to examine humoral and cell mediated immune (CMI) responses as well as pathological lesions and to quantify parasite loads. On day 14 p.i. group 2 demonstrated significantly ($P<0.001$) lower levels of morbidity and weight loss, while also showing significantly ($P<0.01$) higher levels of splenocyte proliferation and IFN-γ production ($P=0.003$), compared to group 1. Histology of brain samples showed milder lesions and a lower incidence of positive immunohistochemistry, demonstrating tachyzoites and tissue cysts, and significantly ($P=0.015$) lower burdens of parasite DNA in group 2 compared to group 1. No mice from group 1 survived beyond day 24 p.i. so it was not possible to perform group analyses after day 14 p.i. However, samples from group 2 were collected at days 28 and 42 p.i. and analysed for humoral and CMI responses. Group 3 mice only demonstrated parasite-specific CMI and PCR following the challenge inoculation.

S85 Influence of intra-species variability of *Neospora caninum* in the outcome of infection in a pregnant BALB/c mice model.

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Previous assays in pregnant cattle have demonstrated the influence of different host factors and timing of infection in the outcome of neosporosis, although the implication of biological characteristics of isolate has been poorly investigated. For this reason, in this work we investigated the pathogenicity and vertical transmission variability of Nc-Liv and nine Spanish *N. caninum* isolates using a pregnant mice model. For it, BALB/c mice were subcutaneously inoculated with 2x106 tachyzoites at day 7 of pregnancy and morbidity/mortality in both, dams and offspring, and the transmission of infection to the progeny were evaluated. Clinical signs of neosporosis were only exhibited in dams infected with Nc-Liv and three of the Spanish isolates (Nc-Spain 4H, Nc-Spain 5H and Nc-Spain 7), and a variable number of affected neonates were also observed in the litters from all *Neospora*-infected groups. Neonatal mortality rate varied from less than 2% (Nc-Spain 8) to 100% (Nc-Liv, Nc-Spain 4H). Moreover, vertical transmission rate (parasite detection by PCR in progeny) oscillated from 50% to 98% according to the inoculated *N. caninum* isolate. These results confirm biological diversity among *N. caninum* isolates and its influence in the outcome of *N. caninum* infection during pregnancy, at least in this mice model.

Session 6C: TRYPANOSOMES and LEISHMAINIA I: KINETOPLASTID PARASITE-VECTOR INTERACTIONS
Convenor: Karen Grant; Chair: Paul Bates

S87 The Phlebotomine sand fly response to gut infection by Leishmania.
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Progress in our knowledge of Phlebotomine sand fly interactions with the Leishmania parasite population in the gut has been fragmented and piecemeal over the years. Much of that progress has relied on our knowledge of the parasite rather than the insect. The diminutive size of the insect, difficulty in rearing the flies and a lack of sequence data have been some of the factors hampering progress. Methods for rearing the flies have been refined over the years so that we can now rear thousands of flies if required.
Biotechnological advances including those in genomics, proteomics and metabolomics mean that the amount of biological material is no longer a big issue. Following a slow start in the acquisition of DNA sequence data for sand flies we expect a rapid increase in data in the near future. The second phase in genome sequencing of the two most well researched species *Lutzomyia longipalpis* and *Phlebotomus papatasi* has begun.
We have used the results from a whole insect cDNA microarray for *L.longipalpis* to inform and develop biologically relevant questions about aspects of the interaction between the kinetoplastid parasite and its insect host. The careful application of the RNAi technique for gene knockdown, that we developed for use in sand flies, has enabled us to start identifiying some of the key molecular components of the sand flies response to Leishmania.

S88 Stage-specific adhesion of *Leishmania* promastigotes to sand fly midguts assessed using a novel binding assay
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Binding of promastigotes to the midgut epithelium is regarded as an important, possibly essential, part of the life cycle in the sand fly vector, enabling the parasites to persist beyond the initial bloodmeal phase and establish the infection. However, the precise nature of the promastigote stage(s) that mediate binding is not fully understood. To address this issue we have developed a gut binding assay in which two promastigote populations labelled with different fluorescent dyes compete for binding to dissected sand fly midguts. Binding of procyclic, nectomonad, leptomonad and metacyclic promastigotes of *Leishmania infantum* and *Le. Mexicana* to *Lutzomyia longipalpis* midguts has been investigated. The results show that procyclic and metacyclic promastigotes do not bind to the midgut epithelium in significant numbers, whereas both nectomonad and leptomonad promastigotes both bind strongly and in similar numbers. These data confirm that gut binding is stage-regulated and is restricted to certain life cycle stages. In addition, the competitive binding of different *Leishmania* species were studied in several sand fly species. These results have implications for understanding the roles of promastigote surface glycoconjugates such as lipophosphoglycan in mediating midgut attachment.

S89 Cooperative Blood-feeding explains feeding aggregations in Phlebotomine Sandflies
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Given the importance that the evolution of cooperation bears in evolutionary biology and social sciences, extensive theoretical work has focused on identifying conditions that promote cooperation among unrelated individuals despite potential cheating, non-cooperating individuals. In insects, cooperative or altruistic interactions typically occur amongst related individuals and are explained by kin selection. Here we provide evidence that in *Lutzomia longipalpis*, a small biting fly and an important vector of leishmaniasis in the New world, cooperative bloodfeeding in groups of unrelated individuals results in a strong decrease in saliva expenditure. Feeding in groups significantly affected the timing and duration of the flies' bloodmeal and resulted in greatly enhanced egg production. The benefits of feeding aggregations were particularly strong when flies fed on older hosts suggesting that flies were better able to overcome the stronger immune response of pre-sensitized hosts. Our results demonstrate unequivocally that, in *L. longipalpis*, feeding cooperatively maximizes the effects of salivary components injected into hosts to facilitate blood intake and to counteract the host immune

response. As a result, cooperating sandflies enjoy enormous fitness gains. This constitutes the first functional explanation for feeding aggregations in hematophagous insects and a rare example of cooperation in a non-social insects species. The evolution of cooperative group feeding in sandflies may have important implications for the epidemiology of leishmaniasis.

S90 Recombination in Leishmania

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Leishmania are considered to have predominantly clonal life cycles and population structures. Nevertheless, several putative interspecies and intraspecies hybrids have been described using molecular typing techniques, including multilocus sequence typing (MLST) and multilocus microsatellite typing (MLMT).
To clarify the presence of genetic recombination in natural *Leishmania* populations we analyzed cloned strains by MLST and/or MLMT. The populations studied were *L. donovani* from Sudan, the *L. braziliensis* complex from South America, and Old World *L. tropica*. We find evidence of both interspecies and intraspecies recombination events, at different levels according to the population concerned.
For *Trypanosoma cruzi*, the experimental use of transgenic parasites proved that T. cruzi has an extant capacity for genetic hybridization. Accordingly, we have generated transgenic *Leishmania* expressing either red or green fluorescent protein in conjunction with a selectable drug resistance marker (either hygromycin B or neomycin). Crossing experiments were performed, in which selected pairs of strains were co-passaged through the sand fly vector. Organisms expressing red or green fluorescence were visualised. Strikingly, in some crosses both red and green (yellow) fluorescence co-localised within individual parasites, indicating the presence of genetic hybrids, amenable to re-isolation and genetic characterisation. This is a powerful approach to the detailed analysis of inter and intraspecific interactions and mechanisms of genetic recombination in *Leishmania* .

S91 F1 crosses, backcrosses and intraclonal mating in *Trypanosoma brucei*

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Trypanosoma brucei undergoes genetic exchange when two different strains 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. We have used this system to investigate F1 and back crosses as well as intraclonal mating. Other lower eukaryotes avoid intraclonal crossing by only permitting strains of different mating types to cross, but it is not known what factors control compatibility in trypanosomes. All 12 F1 and back crosses attempted produced hybrid progeny, with both diploid and polyploid hybrid clones identified. There is thus no simple system of mating types in *T. brucei*. Hybrids were also demonstrated in the single strain transmissions, confirming that intraclonal mating is possible, but without the requirement for outcrossing trypanosomes reported previously. This means that it is no longer possible to assume that strains necessarily remain as genetic clones following fly transmission. Taken together, our results suggest that genetic exchange is a normal part of the transmission cycle of *T. brucei* rather than a rare event.

Session 6D: HELMINTH NEUROBIOLOGY II
Convenor: Angela Mousley

S92 Channels, contraction and cholinomimetics in nematodes

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Muscle contraction, and therefore movement, in nematodes requires the presence of several ion-channel types on the muscle cell surface. These include nicotinic acetylcholine receptors, calcium selective channels and potassium selective channels. In addition the muscle contains several other types of channel e.g. Ca-dependent large conductance chloride channels, peptide gated chloride channels and probably many more. These channels have been exploited therapeutically by compounds such as pyrantel, levamisole and emodepside which are believed to act on nAChRs and potassium channels in nematodes. Many of the channels found on nematodes have orthologs in the vertebrate host. However, differences in pharmacological properties have been exploited to develop effective anthelmintics e.g. the cholinomimectics. We are interested in characterizing these channels pharmacologically and electrophysiolgically to three main ends: 1) a greater understanding of nematode neurobiology, 2) increasing efficacy of existing therapeutic compounds and 3) identifying potential target sites for novel therapeutic compounds. Here we review some of the recent findings on the properties of some of these channels and how they are modulated.
Supported by NIH RO1 A1047194

S93 A role for RYRs in the response to levamisole in *Ascaris suum*: Turner
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Levamisole has long been used as an anthelmintic and resistance is now a serious problem. Levamisole is believed to act by gating nicotinic non-selective cation channels in muscle membrane of nematode parasites. We have continued to investigate actions of levamisole using a muscle strip assay and electrophysiological techniques with the pig nematode parasite, *Ascaris suum*. We find that there are added complexities to effects of levamisole that need to be considered when thinking about its mode of action and mechanisms of resistance. In our contraction assays we examined effects of extracellular calcium, ryanodine, dantroline, AF2 and caffeine on levamisole induced contractions. We found that: extracellular calcium was necessary for contraction; 1μM AF2 potentiated contraction; 1-30mM caffeine inhibited contraction; 1-1000nM ryanodine inhibited but did not abolish contraction; and that 100μM dantroline had a selective effect on the AF2 potentiation of levamisole contraction. Electrophysiological experiments also revealed a secondary depolarization phase in the levamisole response that was increased by AF2 pre-treatment but inhibited by ryanodine. The experiments illustrate the requirement for extracellular calcium and a role for RYRs in the response to levamisole. The experiments suggest that RYRs should be examined further as potential sites of action for novel therapeutic agents and also for a role in levamisole resistance.
Supported by NIH R01AI047194

S94 Development of Surface Plasmon Resonance platforms to deorphanise helminth neuropeptide receptors
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Nematodes have extremely complex neuropeptide signalling networks as is evidenced by the *C. elegans* genome which encodes over 250 distinct neuropeptides. The largest family of these (~70) is the FMRFamide-like peptides (FLPs). FLP signalling pathways are highly conserved across the nematode clades and between free-living and parasite species. FLPs are known to modulate many fundamental aspects of worm biology including locomotion, feeding, reproduction and sensory perception. Despite publication of the *C. elegans* genome more than 10 years ago, pairing of putative receptors and their ligands remains difficult. Indeed, few *C. elegans* FLP receptors have been characterised; such that the distinct receptors for some of the most abundant and potent FLPs remain undiscovered. The BIAcore 3000, which utilizes Surface Plasmon Resonance technology, has been employed in this study to facilitate the development of novel platforms to aid FLP receptor deorphanisation. Techniques have been optimised for (i) surface immobilisation of specific FLPs to biosensor chips (ii) receptor solubilisation from membrane extracts of nematode (*Panagrellus redivivus*), and FLP receptor-expressing cells and (iii) receptor recovery. Preliminary data indicate successful BIAcore binding of FLP receptor-expressing cell extracts to FLP-coated chips, and methods to recover and identify the captured receptor are ongoing. This holds potential as a novel approach to receptor deorphanisation that would aid drug target identification in helminths.

S95 HcGGR3: Characterisation of a novel ligand-gated chloride channel (LGCC) subunit in *Haemonchus contortus*

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HcGGR3 is a gene which encodes a novel LGCC subunit. Protein sequence analysis indicates that this channel is anion selective and possesses all the signature motifs of a chloride channel subunit. Analysis of the cDNA sequence shows putative microRNA interaction site which could be important in relation to developmental expression of this subunit. qRT-PCR analysis of *HcGGR3* shows that it is differentially expressed among the various life-stages and the rank order of expression was eggs > adult female > larvae > adult male. In addition, *HcGGR3* is significantly down regulated in macrocyclic lactone (ML) selected laboratory strains of *H. contortus*. We also found a single nucleotide polymorphism in the 3' UTR that appears to be associated with ML selection. Immunolocalization of this subunit in adult worms has revealed that in females, the localization is distinctly punctate around the cervical papillae and in males, expression was observed around the cervical papillae and possibly some sheath cells. Electrophysiological analysis of this protein expressed in *Xenopus laevis* oocytes revealed that it forms a homomeric channel that responds primarily to dopamine. This subunit could have a possible role in mechanosensation.

S96 Discovery of multiple neuropeptide families in phylum Platyhelminthes

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Available evidence shows that short amidated neuropeptides are widespread and have important functions within the nervous systems of all flatworms (phylum Platyhelminthes) examined, and could therefore represent a starting point for new lead drug compounds with which to combat parasitic helminth infections. However, rigorous exploration of the flatworm peptide signalling repertoire has been hindered by scant genomic data, so that only a handful of endogenous peptides have been characterised. Through BLAST (basic local alignment search tool) trawls of both expressed sequence tags and genomic resources, we have identified 97 neuropeptides on 61 precursors from ten flatworm species. Most of these (52 predicted peptides on 15 precursors) are completely novel, and are apparently restricted to flatworms; the remainder comprise nine recognised peptide families including FMRFamide-like (FLPs), neuropeptide F (NPF)-like, myomodulin-like, buccalin-like and neuropeptide FF (NPFF)-like peptides; notably, the latter have only previously been reported in vertebrates. Several of the novel peptides have been localised immunocytochemically to the nervous systems of *Schistosoma mansoni* and other species. Our dataset provides a springboard for investigation of the functional biology and "druggability" of neuropeptides in flatworms, simultaneously launching flatworm neurobiology into the post-genomic era.

S97 G protein-coupled receptors in two platyhelminths: *Schistosoma mansoni* and *Schmidtea mediterannea*.

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The G protein-coupled receptor (GPCR) superfamily constitutes one of the most expansive protein families in the Metazoa. These cell-surface receptors are a fertile source of drug targets; 40-60% of pharmaceuticals target GPCRs. At present, there exists no comprehensive study of GPCRs within the phylum platyhelminthes. The recent availability of both the *S. mansoni* and *S. mediterranea* genomic assemblies provide resources and the basis for an in silico approach to the accumulation of undiscovered and potentially novel GPCR sequences in both species. Hidden Markov Models (HMMs) and phylogenetic methods were used to classify receptors at the family plane, while more sensitive Support Vector Machine (SVM)-based methods were used for sub-family classification. We report that both species contain members belonging to each of the five major GRAFS families

thought to be present in the Bilateria. Over 80% of the GPCRs present in both genomes are from the *Rhodopsin* family, which is a typical percentage. Within the *Rhodopsin* family, the largest two subfamilies are alpha amine GPCRs and the beta subfamily that contains neuropeptide receptors. Outside of the large *Rhodopsin* family, the genome reveals representatives from each of the smaller families of GPCRs: *Glutamate* family, *Frizzled* family, and the *Secretin/Adhesion* family.

SESSION 7: PLENARY SESSIONS
Chair: Prof Graham Coombs

PLENARY LECTURE: **Parasite Genomics: Past achievements and future prospects in the genomic area**
Prof Andy Tait
Glasgow Biomedical Research Centre, 120 University Place, Glasgow G12 8TA, Scotland, UK

WRIGHT MEDAL LECTURE: **Tree Hugging for parasite apologists**
Dr Tim Littlewood
Natural History Museum, London SW7 5BD

Session 8B: LIVE PARASITE IMAGING
Convenor/Chairs: Joanne Thomson and James Brewer

S98 The evolving role of light and electron microscopy in parasitology research with particular reference to the Apicomplexa
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Light and electron microscopy was fundamental to the characterisation of the majority of protozoan parasites. Ultrastructural observations of the apical organelles associated with the infectious stages of an apparently diverse group of parasites (*Plasmodium, Eimeria, Toxoplasma, Cryptosporidium*) resulted in the formation of a new phylum - the Apicomplexa. Studies on these parasites will be used to illustrate the changing, but still vital, role to be played by light and electron microscopy (combined with immunocytochemistry) in furthering our understanding of the molecular knowledge gained from recent genomic and proteomic studies. It is in relating this new molecular information to the parasite as a whole that there is a role for immuno-light and electron microscopy. For example, the identification of proteins associated with the apical organelles, which can then be related to their role in cell invasion and colonisation. It also has a major role to play in evaluating the expression and localisation of specific proteins within developmental stages that are resistant to *in vitro* culture. While molecular studies are important, it is also vital to confirm any *in vitro* observations with parasite development *in vivo*. It is in this role that microscopy still has an important part to play in apicomplexan research.

S99 A novel method to visualise the interaction between schistosome larvae and cells of the innate immune system
Ross A. Paveley, Sarah A. Aynsley, Joseph D. Turner and Adrian P. Mountford
Department of Biology, University of York, York, UK

Schistosoma mansoni cercariae have been shown to modulate the host immune response during infection of the skin. Using a novel technique, live cercariae were labelled with the fluorescent tracer, Carboxyfluorescein Succinimidyl Ester (CFDA-SE). Fluorescent dye bound predominantly to parasite molecules located within the pre- and post-acetabular glands which are then released upon infection of the skin and act of the host immune response. Using an *in vitro* model of macrophage activation, CFSE-labelled material released by cercariae was phagocytosed by and activated co-cultured thioglycollate-elicited macrophages and bone marrow derived macrophages. CFSE labelled material taken up by macrophages was initially targeted into early EEA-1^+ phagosomes, which matured, to LAMP-1^+ phagolysosomes. CFDA-SE labelled cercariae were observed to infect through the pinnae of mice and released material was determined to interact with macrophages and dendritic cells in the skin. When tested *in vitro* dendritic cells had a higher activation level than the macrophages suggesting different roles for both cells types. The mannose receptor found on the surface of macrophages belongs to a group of pathogen recognition receptors called C-type lectins. The addition of mannan or D (+)

mannose and studies using mannose receptor deficient mice showed a decrease in uptake of CFSE-labelled excretory/secretory molecules from cercariae which suggest a role for the mannose receptor.

S100 Dynamics of T lymphocyte foci surrounding maturing *Schistosoma mansoni* eggs in the small intestine: a multi-photon laser scanning microscope study

Joseph D. Turner, Mark Coles, Alan Wilson and Adrian P. Mountford
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A prerequisite of the *S. mansoni* life cycle is the passage of eggs from mesenteric venules into the environment. A role for an intact lymphocyte response in egg excretion has been proposed. We utilised DsRed CD2+ reporter mice (>90% CD3+) to study the incidence of T cells surrounding eggs within mesenteric venules over 28 days from the onset of egg deposition. Intestinal vasculature was visualised with fluorescein-conjugated anti-PECAM1 and tomato lectin. Eggs were detected by autofluoresence. One micrometer interval, 640 μM2 field of view stacks of 40 μM depth were captured using a Zeiss LSM510 NLO meta (chameleon laser, 870 nm). DsRed expressing cells within a 110 μM radius of each egg and mid-point egg areas were quantified using Volocity 4.3.2 software. This study has revealed that the occurrence and intensity of a CD2+ inflammatory foci is positively associated with the maturation status of eggs and that progression of infection selectively influences the intensity of CD2+ foci around mature but not immature egg stages. Furthermore, the vast majority of mature eggs visualized were still contained within venules, suggesting extravasation is rapidly succeeded by transit into the lumen. Our data suggests that factors associated with egg development provoke a local T cell response and supports a role for T cells in mediating intestinal egg egress.

S101 Imaging *Leishmania spp* infections in vitro and in vivo: Introducing and reviewing the toolbox

Toni Aebischer
Marie Curie Team Pathogen Habitats, Institute of Immunology and Infection Research, University of Edinburgh

Imaging infections in whole organisms in real time and at high resolution is on the agenda of many infectious disease researchers. Scientists studying *Leishmania* infections are no exception. Fluorescence based approaches have the greatest appeal in this field because of the possibility to monitor several analytes at the same time. Here, I will review and comment on the tools that have been developed for *Leishmania* over the past two decades to aspire towards this goal. I will first re-visit enabling techniques, such as transgenesis to introduce genes encoding fluorescent proteins, to discuss important features of frequently used expression systems for *Leishmania* spp. Next, the current state of live fluorescent Leishmania imaging will be presented by reference to recently published work from this field. Finally, additional applications of live colours for investigating *Leishmania* host interactions will be presented and integrated into an outlook of what seems to be a bright future.

S102 Imaging the immune response to *Toxoplamsa gondii*

Christopher A. Hunter
University of Pennsylvania, Philadelphia USA.

Toxoplasma gondii is a protozoan parasite that can infect a wide range of hosts including humans. Infection with *T. gondii* is potentially life threatening in immuno-compromised individuals and it can be detrimental during pregnancy often leading to abortion of the fetus. Dendritic cells are thought to play a vital role in the development of protective immunity to *Toxoplasma gondii* through their ability to produce immunological signals such as cytokines and also process and present parasite derived peptides to T cells. However little is known about the actual interactions between these cell types in an intact organ, such as the lymph node, during infection. Using the technology of live imaging by 2 photon microcopy we have identified a very early window of time during infection (36 hours) when dendritic cells and T cells make sustained contacts with one another which could be crucial for the generation of protective responses. We also show that substantial changes are induced in the lymph node micro-architecture as a result of infection, which in turn could have effects on immune responses to secondary pathogens. Understanding the interaction between these immune cells in vivo during active infection would help in the design of better strategies to develop protective immune responses against this pathogen in otherwise immuno-compromised individuals.

Session 8C: TRYPANOSOMES and LEISHMANIA II
Convenor: Karen Grant; Chair: Jeremy Mottram

S103 The application of next generation sequencing technology to the study of the genome and transcriptome *of T. brucei gambiense*
Annette MacLeod
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The introduction of new high throughput sequencing technologies is beginning to revolutionize genetic and biological research allowing affordable genome-wide analysis to be carried out in within individual institutions, within the confines of modest grant budgets, rather than in large sequencing centres. The next generation sequencing has not only been used for genome analysis; very recently, it has been successfully applied to the analysis of the transcriptome of a number of organisms. Here I will describe the application of massively parallel sequencing technology to the study of the genome and transcriptome of the human pathogen, *T. brucei gambiense*, the causative agent of African sleeping sickness.

S104 Materials and tools for the molecular diagnosis of African trypanosomes in cattle blood samples
H.A. Ahmed, K. Picozzi, M. Eisler and S.C. Welburn
Centre of Tropical Veterinary Medicine, Edinburgh University, EH25 9RG, UK

Diagnosis is an essential element in the management of disease, both at the level of individual patient care and at the level of population disease-control. Diagnostic tests must be sufficiently robust for field applications; with the cost appropriate for those communities affected by the disease. Molecular testing is now firmly established in the routine diagnostic decisions for infectious diseases, however, this diagnosis requires the appropriate infield preparation of samples to ensure that sufficient amounts of genomic material are available. In this presentation, the validation of FTA cards is discussed, with the introduction of a new approach of pre-lysing the blood samples prior to application on the matrix. These methods were compared with DNA extraction prepared with-out the used electricity, to validate the best method of in field sample preparation. Different PCR reactions are currently used for the diagnosis of trypanosome species; in the current study, these reactions have been evaluated with discussing the advantages and drawbacks of each reaction.
We are interested to discover if other peptidases are secreted and whether they also act as virulence factors. To identify secreted peptidases, we have been investigating the intracellular localisation of several candidates, including a Bem46-like serine peptidase of the Clan SC family.
Apart from secretion of virulence factors, rapid protein turnover, e. g. in the lysosome, is also important for the infectivity of *Leishmania*. To investigate lysosome structure and function in *L. major*, two potential lysosomal membrane proteins, a LAMP-like protein and a CLN3-like protein, have been identified. The location and role of these proteins are being investigated in more detail, using GFP-tagged proteins, fluorescence microscopy and the generation of mutant parasites.

S105 Analysis of the LmxMPK4 signalling cascade of *Leishmania mexicana*
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We have shown that the mitogen-activated protein (MAP) kinase LmxMPK4 is essential in *Leishmania mexicana* promastigotes and amastigotes and therefore poses a potential target for anti-leishmanial drugs. A prerequisite for successful drug screening using recombinant MAP kinases is the ability to produce an activated enzyme. Here, we will discuss the identification of a protein kinase enabling us to achieve *in vitro* activation of LmxMPK4. To investigate the biological function of LmxMPK4 we generated an inhibitor-sensitised version of the kinase. We could prove the specificity of the inhibitor for the engineered kinase *in vivo*. Treatment of mutant promastigotes led to a reversible growth arrest, which was not due to a distinctive block in cell cycle control. Phenotypic analysis of these cells is currently under way.

S106 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 by releasing virulence factors.
The cysteine peptidase CPB has been shown to be released by the parasite and act as such a virulence factor. CPB is released through the flagellar pocket while being trafficked to the lysosome, or through direct secretion.

S107 The characterization of the individual ATG4 isoforms of *Leishmania major*

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Autophagy occurs naturally during differentiation of the life cycle stages of the protozoan parasite *Leishmania.* The hydrolytic activities of ATG4 on the small ubiquitin-like modifier ATG8 is considered crucial for autophagy. *Leishmania* has two ATG4s that selectively cleave the parasite's ATG8 paralogues, which suggested that the roles of ATG4.1 and ATG4.2 are distinct but each may be essential. Here we show that deletion of the genes encoding ATG4.1 in *L. major* results in mutant promastigotes that are viable but form autophagosomes poorly and have a markedly reduced ability to infect macrophages and mice successfully. Mutants lacking the *ATG4.2* gene similarly are viable, although promastigotes accumulate autophagosomes and undigested materials within the cytoplasm. These cells failed to undergo metacyclogenesis, transformed poorly to amastigotes and the latter were larger than their wild type counterparts and also contained abundant autophagosome-like structures. The roles of both *Leishmania* ATG4s for an efficient infection of macrophages by promastigotes, their functions in the cell's autophagic pathway and the implications for inhibiting the autophagic pathway of the parasite by targeting ATG4 will be discussed.

Session 8D: VETERINARY PARASITOLOGY III – EPIDEMIOLOGY AND POPULATION BIOLOGY
Convenor: Frank Katzer

S108 *Haemonchus contortus* Genome as a Population Genetic Resource

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Parasitic nematodes are thought to contain extensive DNA sequence variation that explains rapid development of anthelmintic resistance. Characterizing this variation could increase our knowledge of its underlying genetic mechanisms. The genome sequence of *Haemonchus contortus* is derived from a mix of several individual parasites as well as one single male. We have used this resource to investigate the nature and extent of sequence variability in the H. contortus genome. Comparing randomly chosen sequence reads, nucleotide diversity was estimated at 0.022 for both mixed and single worms. The similarity Overlapping BAC sequences of 50 kb to 130 kb found regions of near identity extending up to 6 kb, surrounded by sequence whose divergence ranges from 1% to more than 40%. Variation is found predominantly within predicted non-coding sequence. Insertion-deletions range from single nucleotides up to almost 5 kb. Several insertions have similarity to reverse transcriptase, suggesting these may represent retrotransposons as well as other regions with characteristics of transposons and repetitive sequence. All longer indels lie outside of coding regions, either within introns or in intergenic regions. Overall, the pattern of variation looks quite similar to that seen in *Drosophila* and other invertebrates.

S109 The initial findings of a survey to examine the species of ovine nematodes present on UK farms

Charlotte G.S. Burgess[1], Yvonne Gordon[1], Libby Redman[2], Fiona Whitelaw[2], John S. Gilleard[3], Andrew Tait[2] and Frank Jackson[1]

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A survey of ovine nematodes present on UK farms was carried out with the long term aim of identifying species present and to examine the genetic diversity of populations from around the UK with regard to the molecular basis of anthelmintic resistance. Around 200 farms from all over the UK were asked to participate by providing 20 faecal samples in the spring from ewes and in the summer from lambs. Faecal samples from individual farms were pooled, with each animal contributing the same unit weight of faeces. Nematode eggs were extracted and cultured to the first larval stage (L1). The L1's were archived in ethanol and DNA was extracted from an aliquot of 1000 L1's from each farm. PCR analysis was carried out to determine the presence of the main gastrointestinal nematode species. Preliminary analyses show that *Teladorsagia circumcincta* was present on every farm. *Haemonchus contortus* was found on 53% of farms in England, 38% of farms in Wales and 27% of farms in Scotland.

S110 Population genetics of parasitic nematodes of UK sheep

Libby Redman[1], Charlotte Burgess[2], Fiona Whitelaw[1], Yvonne Bartley[2], Frank Jackson[2], Andy Tait[1] and John S. Gilleard[3]

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UK populations of sheep parasites have been sampled to assess species diversity and the genetic differentiation of *Haemonchus contortus* and *Teladorsagia circumcincta*. Faeces were collected from ewes and lambs through 2008 present on 100 farms across the UK. Using a species-specific PCR assay based on the ITS gene the prevalence of a range of commonly-found species was assessed. *T. circumcincta* was present in all samples containing helminth eggs whereas *H. contortus* was found to be present in 57% of farms, with a bias of these being located in the South of England. The population differentiation of the 2 species was estimated using panels of microsatellites: a panel of 5 *T. circumcincta* microsats and a panel of 15 *H. contortus* microsatellites. From the trace profiles produced from bulk worm lysates of 1000 worms the allele frequencies were estimated from the area under the allele peaks. Fst analysis using estimated allele frequency data revealed that *T. circcumcincta* possessed very little genetic differentiation whereas there were high levels of genetic differentiation in *H. contortus* populations.

S111 The use of a GIS to identify risk factors for liver fluke infection in dairy herds in England and Wales

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Fasciolosis has a major impact on animal health and productivity worldwide. The apparent recent increased prevalence in the UK is likely to be associated with phenomena related to climate change. The prevalence of infection in dairy herds in England and Wales was estimated using a *Fasciola hepatica* specific bulk tank milk ELISA in the winter of 2006-7. Data layers of environmental and climatic variables were used to construct a Geographical Information System (GIS) of the study area and values extracted for each herd and Postcode Area (PCA).

Best-fit multivariate models using contemporary and 5-year (2001-2005) climatic variables and environmental and farm specific data were obtained to explain the prevalence of infection at the farm and at the PCA levels. Model fitting was greater at the PCA compared to the farm level. The main factors associated with fasciolosis in dairy herds were the intensity of summer and autumn rainfall, summer temperatures and the presence of sheep on the farms. These predictive spatial models may be used as tools to assist decision making on treatment of stock at the farm rather than at the regional level, as is the case presently.

S112 Importance of vector population structure and history for disease emergence

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The study of the mechanisms by which disease vectors colonize and adapt to novel environments is critical to predict and react to the emergence and spread of vector-borne diseases. Here we focus on mosquito species of the Galapagos archipelago, using population genetic and phylogenetic data to understand their historical and current population dynamics. We show that two mosquito species found in the archipelago have very different historical and contemporary evolutionary histories: one species, *Aedes taeniorhynchus*, naturally colonized the archipelago 200,000yrs ago and is now found widely in the islands, having adapted and spread to a range of different habitats. It has also changed its feeding-behaviour and frequently feeds on reptiles in addition to mammals, unlike the continental progenitor populations. These properties potentially make *Aedes taeniorhynchus* a key bridge-vector in the archipelago for any new invading mosquito borne diseases. In contrast, we show that *Culex quinquefasciatus,* a major vector of diseases such as West Nile virus and avian malaria, has been introduced on multiple occasions since 1985 via human transportation networks and that its distribution and movement in the archipelago depend greatly on human activities. These two species might play an important role in the introduction and spread of new diseases in the Galapagos archipelago.

S113 Identification and characterisation of a potential immunodiagnostic marker for larval cyathostominosis

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Parasitic nematodes of the group *Cyathostominae* are an important cause of equine disease. This group consists of approximately 50 species which all have a similar life cycle, which involves the encystment of parasitic larval stages in the large intestinal wall. Large numbers of developing larvae can emerge synchronously, damaging the intestinal mucosa and result in a pathogenic condition known as larval cyathostominosis. A diagnostic tool which estimates larval burden would allow targeted treatment and may delay drug resistance; however such a test has yet to be developed. We report the identification of a larval protein that shows promise for utility as a diagnostic antigen for larval cyathostomin burdens. This antigen was identified via immunoscreening of a cyathostomin mixed-larval stage cDNA library. Recombinant antigen was expressed in *E. coli* and was shown to be immunogenic, displaying low cross-reactivity to proteins present in other equine helminths. Transcription of the antigen is restricted to mucosal larval stages, and the protein was localised to the nematode gut. The equivalent gene encoding this antigen was identified in ten separate cyathostomin species, indicating its ubiquity. This study indicates the promise of this antigen as a diagnostic marker for larval cyathostominosis.

Session 9B: LIVE PARASITE IMAGING
Convenor: Joanne Thomson and James Brewer

S114 Imaging Infection and Immune Responses in vivo

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Much of what we understand about the anatomy and architecture of the immune system was revealed through exquisite experiments performed in the 1950s to 70s. These studies identified the role that anatomy played in a number of fundamental immunological phenomena including recirculation, induction of immune priming or tolerance and the interactions of T and B cells. The recent resurgence of interest in the role of immune architecture and anatomy in basic immunological and infectious phenomena is almost entirely due to technological developments in identifying and tracking cells and parasites in vivo, not least through the ability to do this dynamically, in real time through the application of multiphoton microscopy. Here I will outline the background to our own studies applying multiphoton microscopy to analysis of immune priming and tolerance including studies in the context of malaria infection. This work provides a visual insight into host/parasite interactions and highlight the importance of early cellular dialogue in the development of adaptive immune responses.

S115 Imaging motile Plasmodium sporozoites

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Plasodium sporozoites develop in parasitic cysts at the midgut wall of infected mosquitoes. They accumulate in salivary glands and are injected by the Anopheles mosquito into the skin of a host from where they progress to the liver. There, they invade hepatocytes to develop into thousands of merozoites which invade red blood cells eventually causing malaria.
Sporozoites as well as merozoites and ookinetes need to be motile to penetrate through tissue barriers and invade their respective host cells. We are interested in the molecular and biophysical basis of this motility and entertain a number of different optical approaches to investigate motile sporozoites. Reflection interference contrast microscopy showed that sporozoites adhere at specific regions with the substrate. Turnover of these adhesion sites regulates motility in an actin dependent way. Traction force microscopy also revealed the unequal actin-dependent distribution of traction forces along the sporozoite during motility suggesting that the first role of dynamic actin is to mediate adhesion turnover of the parasite.

S116 *Plasmodium* Live – Stalking the Secrets of Malaria

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Intravital imaging has provided an entirely new concept of the basic biology of *Plasmodium* in the mammalian host. After entering dermal capillaries at the mosquito bite site, sporozoites travel with the bloodstream to the liver, pass through Kupffer cells into the parenchyma, traverse several hepatocytes, and finally settle down to produce thousands of merozoites. In contrast to sporozoites, merozoites are highly susceptible to phagocytosis and must avoid contact with Kupffer cells on their way out of the liver. This is accomplished by formation of merosomes, large bags of merozoites enveloped in hepatocyte membrane. The majority of these merosomes exits the liver intact, survives the turbulent passage through the right heart, and is arrested in the lungs. Here, the merosomal membrane eventually disintegrates thus liberating merozoites into the pulmonary microcirculation. Severe malaria is associated with the sequestration of infected red blood cells (iRBCs) to the microvasculature of the brain. iRBCs adhering to the vascular endothelium alter the blood rheology and attract inflammatory cells thus triggering a cascade that ultimately leads to occlusion of cerebral capillaries.
By closely reflecting the intricate metabolic, hemodynamic, and inflammatory conditions in the intact organism, intravital microscopy can help unravel the full dimension of the cellular events that govern a *Plasmodium* infection within the complex architecture of internal organs.
The work was supported by NIH grants RO1 AI51656 and NCRR-019288 and NSF grant 9977430.

Session 9C: OPEN (SCHISTOSOMES AND CESTODES)

S117 Mathematical models for schistosomiasis transmission dynamics and control in sub-Saharan Africa: lessons from Kenya and Uganda

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Large-scale anthelmintic campaigns, such as the Schistosomiasis Control Initiative, provide an invaluable opportunity to parameterize mathematical models by fitting them to empirical baseline and intervention data with important theoretical and applied implications. Age-structured models (e.g. EpiSchisto) were fitted by maximum likelihood to detailed (mean and individual) data from Kenya and Uganda, describing infection profiles with host age and time after various treatment rounds with praziquantel, taking into account parasite overdispersion. Tailoring models to the baseline level of endemicity in a given area provided the most robust modelling results. In low transmission areas, reducing treatment frequency to biennially may be considered an effective option in resource-constrained settings. In high transmission areas, continued yearly treatment may be required in order to keep morbidity suppressed. The change in the degree of parasite overdispersion under chemotherapy was investigated; it was established that although overdispersion is a function of infection intensity, a single

relationship encapsulating these changes can be usefully incorporated into the models. Individual-based models capturing inherent heterogeneities of helminth infections are being constructed and will be discussed. These will help identify individuals predisposed to morbidity, and who may be missed if only a population-based modelling approach is used for monitoring and evaluation.

S118 Explaining the slow development of protective immunity against human schistosomes: mathematical modelling of the threshold hypothesis

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Studies of schistosomiasis in human populations suggest that protective immunity develops gradually. Specific antibody responses to Schistosoma haematobium in two Zimbabwean communities show clear changes in antibody subclasses occurring at around the age that infection intensity begins to decline. One hypothesis for the slow development of protective immunity is that individuals must experience a threshold level of antigen (either currently or cumulatively over time) in order to stimulate a protective immune response which can control infection.
We are taking a quantitative approach to explore this hypothesis, using mathematical models which describe the interactions between schistosome worms and specific antibody responses within individuals in endemic populations, to see whether the proposed mechanism can delay the development of protective antibody in these models. The patterns of infection and antibody predicted by the models are compared with those seen in Zimbabwean field data.
Deterministic models of homogeneously-exposed populations show that either type of threshold can delay the development of protective concomitant immunity and reproduce age-intensity curves consistent with field data. We are now developing stochastic individual-based models of the threshold hypothesis which allow heterogeneity in individual rates of exposure to infection, to determine whether these models can reproduce the distributions and co-distributions of infection intensity and antibody levels observed in the field.

S119 Transmission dynamics and genetic epidemiology of *S. mansoni* and *S. haematobium* in Northern 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 groups of children from 2 villages Nder and Temeye in 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 then 6 and 12 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 a twelve-month period. The data provide surprising insights into treatment strategies, transmission dynamics and the genetic diversity of these schistosomes from each village.

S120 Protein kinase C signalling during miracidium to mother sporocyst development in *Schistosoma mansoni*

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For schistosomes, transformation of the miracidium to mother sporocyst within a compatible molluscan host requires considerable physiological and morphological change by the parasite. To begin to establish the importance of kinase-mediated signal transduction to this developmental process, the phosphorylation (activation) of protein kinase C (PKC) in larval stages of *Schistosoma mansoni* undergoing *in vitro* transformation

was explored. Miracidia freshly-hatched from eggs possessed a phosphorylated 78 kDa PKC, activation was attenuated when miracidia were incubated with the PKC inhibitor GF109203X. During the initial 24 h transformation, PKC phosphorylation was sustained, whereas after 31 and 48 h, times coinciding with the post-miracidium to mother sporocyst transition, phosphorylation was reduced by 72% and 86%, respectively. Confocal microscopy of miracidia revealed phosphorylated PKC associated with the neural mass, excretory vesicle, tegument, ciliated plates, terebratorium and germinal cells; in larvae undergoing transformation for 31 h, phosphorylated PKC was only occasionally detected, being present in regions likely corresponding to the ridge cyton. Inhibition of PKC in miracidia resulted in accelerated transformation, particularly to the postmiracidium stage; miracidial plates were also shed more rapidly. These results highlight the dynamic nature of PKC signalling during *S. mansoni* postembryonic development and suggest that activated PKC restricts the transformation of *S. mansoni* miracidia into mother sporocysts.

S121 Pilot Study: Molecular Cloning, Expression and Potential Recognition of Three *Echinococcus granulosus* Recombinant Proteins Using Defined Human Sera

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Ultrasound based classification of hydatid cyst morphology can relay some valuable information with regard to the natural history of cystic development, from a mature fertile and viable cyst, to a lesion which is degenerate and non-viable. However, accurate assessment of viability based on ultrasound alone is not possible. Much work up to now has focussed on using *Echinococcus* antigens for diagnostic purposes whilst little work seems to have been done in relation to using these proteins as potential indicators of disease progression or regression. The coding regions for *Echinococcus* fatty acid binding protein (EgFABP1), thioredoxin peroxidase (EgTPx) and heatshock protein 70 (EgHSP70) genes were obtained from a protoscolex cDNA library and cloned into the *Nde*1 and *Bam*H1 restriction enzyme sites of the pET19b expression vector and subsequently expressed as His-tagged fusion proteins in *E. coli* BL21 (DE3) host cells. The recombinant proteins were analysed by SDS-PAGE and probed with a pool of defined sera in Western blot. A small sample of patient sera of different cyst morphologies (CE1-CE4) was then tested with the antigens by ELISA. Results will be discussed with relevance to location of the parasite antigens within the cyst and their likelihood of exposure to the host.

S122 Detection of human taeniosis in a coproantigen ELISA

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Taenia solium and *Taenia saginata* infections are global parasitic cestode zoonoses with both public health and economic significance. An antigen-capture ELISA employing both anti-somatic and anti-ES polyclonal antibodies (hybrid assay) for the detection of coproantigens was developed to discriminate specific taeniosis infections due to *T. saginata* or *T. solium.* A panel of 56 human faecal samples comprising *Taenia* negative volunteers (n=4), *T. saginata* (n=23), *T. solium* (n=27) and *T. asiatica* (n=2) were tested and compared with a genus (*Taenia*) specific coproantigen ELISA based entirely on anti-somatic antibodies which does not discriminate the three human *Taenia* parasites. The results of a *T. saginata* (hybrid) coproantigen assay showed sensitivity, specificity and Youden's index (J) of 91.3%, 93.9% and 0.852 respectively. Similarly, a *T. solium* (hybrid) coproantigen assay revealed 92.6% sensitivity, 100% specificity and 0.926 (J). The data suggest that the two assays have potential as epidemiological tools for more specific monitoring of taeniosis especially in co-endemic human populations.

Session 9D: VETERINARY PARASITOLOGY IV
Convenor: Lee Innes

S123 Sustainable worm control strategies- a New Zealand perspective

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Livestock productivity in the New Zealand pastoral sectors is heavily reliant on the effectiveness of anthelmintic drenches. The sustainability of worm control, however, has become increasingly threatened by the development

of resistance in worm populations. Recent research reports and information from a large-scale national survey indicate that resistance is now common on New Zealand farms. Field-based research is focused on the identification and mitigation of major risk factors for resistance such as pre-lamb ewe drenching and drenching onto clean pasture. Current research is being conducted into the practical application of refugia to slowing the development of resistance, and in the impact of highly-effective drenches (in this case combination products) on the selection and population dynamics of resistant worm populations. Furthermore, the development of worm control programs which are designed to utilise these mitigating practices in concert are proposed to offer the greatest potential for sustainable worm control. Complementary laboratory-based research involves the development of a package of diagnostic tools to assist in sustainable worm control. Improved marker-assisted selection of a range of genotypes, including productive and parasite-resistant animals has been demonstrated in the laboratory and on-farm, while the utility of tools to determine the spectrum and severity of parasitism, as well as the presence of resistance alleles, are being assessed.

S124 *Fasciola hepatica*: histological changes in the reproductive organs following treatment *in vivo* with triclabendazole

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Experimentally-infected sheep harbouring 12 week-old triclabendazole (TCBZ)-sensitive or triclabendazole-resistant *Fasciola hepatica* isolates were treated with therapeutic doses of triclabendazole (10 mg/kg) and killed 1 to 4 days later. The flukes collected from the bile ducts were fixed with formalin and wax-embedded. Histological sections of the reproductive structures were examined. In the TCBZ-sensitive individuals, but not in the TCBZ-resistant flukes, changes were evident in the testes, uterus, vitelline follicles, ovary and Mehlis gland from 24hrs post-treatment, and these developed progressively throughout the study period. The lesions seen were attributed to inhibition of mitosis, inhibition of protein synthesis and induction of apoptosis. Histological techniques may facilitate early and accurate screening for drug resistance in fluke populations.

S125 Nutritional manipulation of periparturient immunity to parasites

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Many mammals exhibit periparturient relaxation of immunity (PPRI) to gastrointestinal nematodes culminating in increased worm burdens and worm egg excretion. Small ruminant studies suggest that PPRI may have a nutritional basis, as this effect on host resistance is considerably augmented when protein supply is scarce. To investigate the immunological mechanisms underlying this phenomenon, a *Nippostrongylus brasiliensis* lactating rat re-infection model has been developed. We have previously shown that the reduced intake displayed by lactating rats fed a low protein diet resulted in an augmented PPRI compared to their high protein counterparts. This effect on parasite burden correlated strongly with reduced mucosal mast cell accumulation and goblet cell concentrations. We have recently shown through restrictedly feeding one of three levels of protein at one of two levels of energy, that the earlier observed nutritional sensitivity of worm burdens and mucosal mast cell concentration is related to variation in protein and not energy intake. Further analysis of cytokines, local and systemic antibodies is pending for this study. Since host responses to protein are by definition responses to amino acids, our future work aims to assess the role of specific amino acids on immune responses to gastrointestinal parasite infection during the periparturient period, particularly those key to mast cell function.

S126 Sheep scab – An integrated genomic approach to the host-parasite interaction

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Sheep scab is a highly contagious ectoparasitic disease of sheep caused by the mite *Psoroptes ovis* (*P. ovis*). It is an important welfare issue due to the disease symptoms which include intense pruritis and severe exudative dermatitis. Host response to infection is directed against mite secretory/excretory products and is typical of an immediate hypersensitivity reaction. Sheep scab is traditionally controlled by chemical intervention however there are concerns over chemical residues in meat and their effect on human health and the environment. There

is also an emerging problem of resistance to the chemicals used in treatment. Vaccine candidates have been identified by fractionating mite proteins, however efficacy is low (despite further fractionation) and isolation of the specific proteins involved is difficult. To further improve the development of a sheep scab vaccine we must gain a better understanding of the host-parasite relationship. Sheep scab is an excellent model with which to achieve this as we have access to both host and parasite material. In order to interrogate the host-parasite interaction we are utilising an integrated genomic approach, combining an in-house *P.ovis* cDNA microarray with host-specific microarrays. We believe that these resources will provide us with a greater understanding of the underlying biology of sheep scab infection and will lead to the identification of potential vaccine candidates and biomarkers.

S127 The challenges of developing a DNA vaccine for African Trypanosomiasis

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African trypanosomiasis is an emergent disease with significant social and economical impact. Available drug regimens are limited and highly toxic therefore development of a vaccine would be extremely advantageous. Ten DNA vaccine prototypes were constructed encoding ISG and TSA genes from *T.brucei,* linked to different targeting sequences directing the protein to the major antigen processing pathways: (1) LAMP-1 targets the protein to the lysosomes (pISGlamp, pTSAlamp); (2) E1A directs the protein to the ER (pe1aISG, pe1aTSA) (3) secretion signal promotes protein secretion (pscISG, pscTSA) (4) E1A and LAMP combined addresses the protein to the lysosomes (pe1aISGlamp, pe1aTSAlamp). Semi-quantitative RT-PCR analyses demonstrated that a higher number of cytokines were expressed following pe1aISG, pISGlamp, pe1aISGlamp and pTSAlamp immunisation, including IL-4 and IL-10 expression. This finding may be relevant in therapeutic vaccination approaches.
FACS analyses showed that spleens from infected mice, immunised with pe1aISGlamp, contained a significantly higher percentage of $CD4^+$-cells compared to $CD8^+$-cells. This was not found following immunisation with the other vaccines or controls. Changing this balance could be beneficial since $CD8^+$-cells are associated with increased disease susceptibility. These results provide encouraging results for the potential development of a trypanosomiasis vaccine.

ABSTRACTS OF POSTERS

MALARIA – POSTERS

P1 Preferential transcription of conserved *rif* genes in two phenotypically distinct *Plasmodium falciparum* parasite lines

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Plasmodium falciparum variant surface antigens (VSA) are targets of protective immunity to malaria. *Plasmodium falciparum* erythrocyte membrane protein 1 (PfEMP1) and repetitive interspersed family (RIFIN) proteins are encoded by the two variable multigene families, *var* and *rif* genes, respectively. Whereas PfEMP1s are known to mediate cytoadhesion, the function of RIFINs is unknown. The sequence diversity and organisation of *rif* genes of the *P. falciparum* clones 3D7, HB3, DD2, and IT/FCR3 were investigated using a tree-building method which allowed sub-grouping of RIFINs into distinct groups. Two novel *rif* gene groups, *rifA1* and *rifA2*, containing inter-genomic conserved *rif* genes, were identified. All *rifA1* genes were orientated head-to-head with a neighbouring Group A *var* gene whereas *rifA2* was present in all parasite genomes as a single copy gene with a unique 5´ untranslated region. *Rif* transcript levels were determined in two different parasite lines, 3D7-Lib and NF54-VAR2CSA, expressing VSA associated with severe malaria in children and pregnant women, respectively. The 3D7-Lib showed high transcript levels of Group A *var* and neighbouring*rif* genes, whereas *rifA2* was found highly transcribed in the VAR2CSA-expressing parasite line. In addition, two *rif* genes were found transcribed at early and late intra-erythrocyte stages independently of *var* gene transcription. *Rif* genes are organised in groups and inter-genomic conserved gene families, suggesting that RIFIN sub-groups may have different functional capacities. This conclusion is experimentally supported by group-specific *rif* transcription in parasites with different VSA and PfEMP1 expression phenotypes.

P2 Developing rosette disrupting antibodies to *Plasmodium falciparum* erythrocyte membrane protein 1 (PfEMP1)

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Plasmodium falciparum erythrocyte membrane protein 1 (PfEMP1) is a parasite-derived variant antigen expressed on the surface of infected erythrocytes. PfEMP1 consists of Duffy binding-like (DBL) domains and cysteine-rich interdomain regions (CIDR) and is a parasite ligand involved in rosetting, the binding of infected erythrocytes to non-infected erythrocytes. Rosette-mediating PfEMP1 variants bind to a variety of host receptors on uninfected erythrocytes. The study of recombinant PfEMP1 domains has shown that DBL1□ (R29var1 variant, from parasite clone R29) binds to complement receptor 1, DBL2□ (R29var1) binds to heparin and DBL4□ (TM284var1 variant, from parasite clone TM284) binds to IgM. This study aims to elucidate whether rosetting ligands from multiple parasite isolates such as TM284 and R29 show antigenic cross-reactivity. The study involves the production of functional recombinant DBL domains and subsequent immunization of chickens to produce anti-PfEMP1 antibodies. The ability of these antibodies to bind recombinant/native PfEMP1 and their influence on host-parasite interaction is the focus of the study. The work presented here aims to characterise antibodies to DBL4□ of the TM284var1 variant. Future work will focus on making antibodies to DBL2□ (R29var1) and DBL1□ (R29var1) to provide further information on cross-reactivity amongst strains. This information will be important in evaluating the feasibility of developing a vaccine based on PfEMP1 to raise antibodies that inhibit rosetting with activity against multiple parasite strains.

P3 Antibody cross-reactivity in malaria-nematode co-infection

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Utilising a murine model of malaria-helminth co-infection we analysed antibody responses of mice singly- or co-infected with *Plasmodium chabaudi chabaudi* and/or *Nippostrongylus brasiliensis*. We observed sizeable titres of

cross-reactive antibodies that recognised antigens from both pathogens. These cross-reactive responses were not apparent in control mice but were observed in infected mice irrespective of whether crude parasite antigen preparations or purified recombinant proteins were used in ELISA.
To determine the relative strength of cross-reactive versus antigen-specific responses we calculated antibody titre from a serial dilution of serum. We further examined whether cross-reactive responses were targeted toward carbohydrate or protein moieties by treating the parasite antigens with periodate, thus disrupting carbohydrate epitopes by oxidising carbohydrates to aldehydes. Periodate treatment affected both antigen-specific and cross-reactive responses.
Cross-reactive responses, as described in this and other co-infection systems, raise the interesting question of whether production of cross-reactive antibodies might be the optimal response for a host faced with infection by an unpredictable range of parasites, where maintaining some degree of polyclonality may confer a broader spectrum of protection.

P4 Recombinant measles virus as a vector for malaria vaccines

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Polymorphic regions of some *Plasmodium falciparum* merozoite antigens have been shown to be immunogenic and the target of strain-specific immune responses. These responses are associated with protection from clinical malaria episodes in malaria-exposed cohorts of African children. The Block 2 region of the major merozoite surface protein (MSP-1) is such a target. Block 2 is highly polymorphic, containing repetitive tripeptide sequences in many parasite serotypes, and is a member of a family of intrinsically unstructured protein domains. Despite the high level of sequence diversity, we have been able to design, construct and express synthetic recombinant polypeptides encompassing the majority of sequence and antigenic polymorphism in this protein domain. One major problem with malaria vaccine development is the difficulty in promoting immunogenicity of subunit vaccines expressed as proteins to be delivered with adjuvants. The recombinant measles virus platform, based on the attenuated and well-characterised Edmonston-Zagreb strain of the human paramyxovirus has been used in this study to express the MSP-1 Block 2 hybrid as a GPI anchored protein in MRC5 cells. The protein is expressed in measles infected cells at high levels, is recognised by sera against all serotypes of Block 2, and is presently undergoing immunogenicity testing in transgenic mice.

P5 Haplotype analysis of *Plasmodium falciparum* to determine the genetic relatedness and dispersal of these parasites in endemic locations in Kenya

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Plasmodium falciparum remains a global burden with an estimated 300 million cases annually of which around 1 million end fatally. The parasites are transmitted by the female *Anopheles* mosquito as it takes a blood meal. *P. falciparum* is haploid in the human host; in the mosquito's midgut, sexual reproduction occurs giving rise to progeny with novel genotypes. Mixed genotype infections occur in nature with highest incidences observed in areas of most intense malaria transmission. This study was devised to determine how malaria parasites are dispersed over time and space by measuring their genetic relatedness. This will be done by the identification of haplotypes of the parasites in natural infections using single nucleotide polymorphisms (SNPs) as genetic markers. Examination of these haplotypes will provide information on how parasites are related and distributed within a locality. Since parasite mixtures are common in natural malarial infections, a method is needed that is able to distinguish parasite genotypes in mixed infections. We have used Pyrosequencing™ to quantify parasites carrying specific alleles (SNPs) present in parasite mixtures and to assign them haplotypes. The method is being applied to the analysis of parasites in natural infections of *P. falciparum* from Kenya.

P6 Developing a rationale-based model for *in silico* searches of *cis*-acting sequences in *Plasmodium falciparum*

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Intraerythrocytic development of *P.falciparum* is marked by a significant programme of developmentally-linked gene expression, with evidence indicating that cofunctional genes are often cotranscribed. A role for *cis*-acting sequences in directing absolute and temporal promoter activity has been established, although these studies

have had limited scope. The last 2 years have seen significant advances in the application of post-genomic technologies in characterising molecular mechanisms that impart temporal control on gene expression. *In silico* searches for *cis*-acting motifs has revealed an apparent enrichment of some motifs in coregulated genes. However, this approach has limitations, primarily in selecting appropriate flanking sequences to be searched. We have generated a reductive model to identify (i) the amount of untranslated sequence present in *P.falciparum* mRNA and (ii) how intergenic space is organised to accommodate this large UTR. Our data indicates; (i) irrespective of the size, structure, orientation and timing of gene expression, a mean of c. 1.4kb of UTR is present and (ii) that intergenic space is not randomly organised and that depending upon whether two promoters, one promoter/one terminator or two terminators are present – an approximate 3:2:1 space ratio exists. This latter observation extends from *P. falciparum* to include other *Plasmodium* spp., apicomplexan parasites and unicellular organisms that share compact genomes. These data are key for our future plans to search for informative *cis*-acting sequences.

P7 Phospho*enol*pyruvate carboxylase, an enzyme involved in carbon dioxide fixation in *Plasmodium falciparum*

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Carbon dioxide is essential for the intraerythrocytic development of *Plasmodium,* presumably because it plays a role in the parasite's metabolism. CO_2 fixation with phospho*enol*pyruvate, a product of glycolysis, is catalysed by phospho*enol*pyruvate carboxylase (PEPC) or phospho*enol*pyruvate carboxykinase, which supply the cytosol with oxaloacetate. PEPC occurs in plants, bacteria and some protozoa but is absent in mammals, and could therefore be an ideal target for antimalaria chemotherapy. Its role in plants is well understood and it is primarily involved in replenishing C_4-dicarboxylic acids that are used for energy metabolism and biosynthetic processes. In mammalian cells, the generation of oxaloacetate in the mitochondrion is achieved through pyruvate carboxylase, which is one of the most important anaplerotic reactions. This enzyme however, does not exist in *Plasmodium* and it is possible that cytosolic PEPC indirectly replaces this function by generating oxaloacetate, which is subsequently reduced to malate by a cytosolic, NADH-dependent malate dehydrogenase. Malate is transferred into the mitochondrion to fuel TCA cycle activity or to provide reducing equivalents for malate:ubiquinone oxidoreductase, another unique protein present in these parasites. To assess whether *P. falciparum* PEPC is essential for parasite survival a reverse genetics approach was used. We were unable to disrupt the PEPC gene; however, the gene locus was targeted by a knock-in control construct. Further attempts to disrupt the function of PEPC will be described and its potential role as an essential metabolic enzyme discussed.

P8 Malaria parasites can develop resistance to Artemisinin Combination Therapy (ACT)

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The malaria parasite *Plasmodium falciparum* has developed resistance to almost all drugs available for its treatment. The only exceptions are the Artemisinin (ART) derivatives which are usually administered in combination with other drugs (ACT). In most malaria-endemic areas resistance to the "artemisinin partner drug" is already established. Using the rodent model *Plasmodium chabaudi*, we aimed to generate parasites resistant to a commonly used version of ACT, Artesunate-Mefloquine (ATN-MF) starting from a MF-resistant clone, exposed to stepwise increasing concentrations of ATN. After thirty-six sub-inoculations in the presence of ATN, a drug-resistant parasite was selected and cloned. This clone, denoted AS-MFATN, was not only selectively more resistant to ATN but was also able to survive in the presence of both drugs when administered together. These results clearly indicate that ACT-resistant parasites can arise through drug pressure, highlighting the worrying possibility of resistance in natural populations of *P. falciparum*. The biological dynamics and genetics of resistance are currently under investigation.

P9 Survival probability of *Plasmodium falciparum* against chloroquine in Punjab, Pakistan

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5952 persons screened during the malaria non transmission season (November-January) in five districts of Punjab, Pakistan from 2000-05, showed malaria incidence (%) of 3.10 for *Plasmodium vivax* and 23.67 for *P.*

falciparum. Out of 1409 (23.67%) positive cases of *P. falciparum* 404 subject with uncomplicated *falciparum* malaria were tested on day 1,2,3,7,14,21 & 28 against chloroquine by *in vivo* technique showed 35.4 % resistance with 31.2% RI, 4.2% RII and zero R 111. Two variables were found important predictors of treatment failure; a young age and a high parasitaemia count (>6000/μl) at day 0. Parasite density /μl showed positive correlation with the resistance and highest resistance (53.8%) was associated with parasitaemia /μl of 6000 or above. The treatment failure (resistance) was found 19%, 36 %, 20% and 25% in first, 2nd, 3rd and 4th week respectively. The significant predictors of parasite survival were found age and parasitaemia. The survival of parasite was higher (58%) among positive cases than negative cases (18%) at day 3. Keeping in view these findings the adoption of combination therapy as first line treatment for uncomplicated *falciparum* malaria is safely suggested.

P10 Functional characterization of PbIMC1h, a putative membrane-skeleton protein of *Plasmodium berghei*

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Membrane skeletons are cytoskeletal elements that have important roles in cell development, shape and structural integrity. Malaria parasites encode a conserved family of putative membrane skeleton proteins related to articulins. Two members of this family, PbIMC1a and PbIMC1b, were previously found to be involved in cell shape, mechanical strength, motility and infectivity of *Plasmodium berghei* sporozoites and ookinetes, respectively. A third member of this protein family, PbIMC1h, is currently being characterized. We have generated genetically modified parasites that express PbIMC1h tagged with green fluorescent protein and show that it is targeted to the pellicle of both ookinetes and sporozoites. We also show that PbIMC1h-deficient ookinetes and sporozoites display abnormal cell shape, decreased mechanical strength and reduced infectivity. These findings are consistent with a membrane skeletal role of PbIMC1h and provide experimental support for the view that membrane skeletons form an integrate part of the pellicle of apicomplexan zoites and function to provide rigidity to the pellicular membrane complex. We are currently further characterizing PbIMC1h, as well as investigating the function of the other PbIMC1 family members and their potential interactions.

P11 Mating under attack: is transmission blocking immunity gender-specific during *Plasmodium berghei* fertilization?

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To transmit to new hosts, malaria and related blood parasites must reproduce sexually inside a dipteran vector. Within 15 to 30 minutes of being taken up in a vector's bloodmeal, male and female gametocytes are activated to produce gametes and fertilisation occurs. A variety of host-derived immune factors (including cytokines, nitric oxide and antibodies) are able to reduce or even block transmission and levels of these immune factors vary widely throughout infections.
Recent theoretical and experimental work suggests that the gametocyte sex ratio (proportion of males) within the host is correlated with the degradation of the parasite's environment caused by anaemia and circulating immune factors which could reduce the capacity of male gametocytes to form gametes and/or limit the ability of male and female gametes to interact.
However, this assumes that males are disproportionately affected, relative to females, by host-derived factors that reduce fertility. We tested this by analysing the mating ability of males and females in fertilization assays in which the parasites were incubated with nitric oxide and tumour necrosis factor-α; factors involved in transmission blocking immunity.
Our results can help explain the evolution of reproductive strategies in malaria parasites. Understanding the ecological factors shaping the mating strategies of these parasites may provide insights into their control.

P12 Transmission of *Plasmodium falciparum dhfr* haplotypes in the Gambia

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Background: Sulfadoxine-pyrimethamine (SP) is a common partner of artemisinin-based combination therapy (ACT) in Africa. A high-level pyrimethamine resistance *P. falciparum* lineage with triple *dhfr* mutations (51I, 59R, 108N) prevails across Africa, however, additional minority lineages of this genotype were also seen. This genotype transmits readily to mosquito following SP treatment; however, it is not known whether different lineages of this genotype vary in their transmission capacity. Methods. Alleles of *dhfr*, microsatellites flanking *dhfr* and *MSP-1* were typed among *P. falciparum* infected children prior to SP-treatment, and infected mosquitoes fed on blood collected post-treatment. Results: Sixteen *dhfr* haplotypes existed among infected children, 2 carried double mutations (51I,108N) while 14 harboured triple mutations. However, only 9 haplotypes with triple mutations transmitted to mosquitoes. A single triple mutant *dhfr* haplotype (haplotype-11) predominated among children (42%) and mosquitoes (60%).
Conclusions: The major triple *dhfr* haplotypes in the Gambia, which exhibited substantial transmission advantage following SP-treatment, has great similarity to those in other African countries. This agrees with the hypothesis of migration of a high-level pyrimethamine resistance lineage across Africa. However, presence of multiple triple mutant haplotypes, and evidence of cross-mating between them, signifies the role of local evolution.

P13 Seropositivity of malaria parasite and syphilis among prospective blood donors in Federal Medical Centre Owerri, Nigeria

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Several infections have been shown to be associated with blood transmission. The present study was undertaken to assess the sero-prevalence rate of malaria parasites and syphilis among one hundred and twenty prospective blood donors seen between the months of January and March,2008 at the Federal Medical Centre Owerri. The subjects were randomly selected from donors who visited the hospital within the period for the purpose of donating blood and screened for seropositivity to malaria parasite and siphilis using anti-malaria monoclonal antibody rapid diagnostic test (RPD) method and Veneral Disease Research Laboratories test (VDRL) kit respectively. Male donors had a higher sero-prevalence rate of malaria parasite (54.16% out of the total malaria prevalence rate of 61.66%) and syphilis (3.34% out of the overall sero-prevalence rate of 4.16% for syphilis) than the females, the sex-related prevalence being statistically different ($P<0.05$). A weak negative correlation existed between infection with malaria parasite and those of syphilis. (correlation coefficient: 0.167311). The study indicates a high risk of transmission of malaria parasite and low risk of transmission of syphilis to susceptible recipients (non-immune and immuno-compromised). Routine screening of blood donors for malaria parasites and siphilis is recommended in Federal Medical Centre Owerri.

P14 Investigating the role of antibodies and their Fc-receptors in immunity to malaria.

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Malaria affects millions of people every year throughout the world. Due to increasing levels of resistance to common drugs used for treatment, new approaches are urgently sought. Antibodies play a major role in immune protection against infection with the parasite; therefore, they may be
used to develop new therapies to fight the disease. Activatory/Inhibitory ratios have been used to determine the efficacy of the various IgG subclasses at killing tumours.
Using an innovative *in vivo* double transgenic model that resembles *Plasmodium falciparum* infection in humans, we are generating a panel of recombinant mouse IgG1, IgG2a, IgG2b and IgG3 antibodies targeting and identical epitope on *Plasmodium falciparum* MSP1-19. We are investigating the properties of this unique panel of epitope-matched mouse IgG subclasses in wild type, FcR-knockout, and Fc-transgenic animals using *Plasmodium berghei* transgenic for PfMSP1-19, thereby allowing us to determine the activatory/inhibitory ratios for each subclass with respect to their receptors. Similar studies are also being conducted with a panel of mouse mAbs directed against *Plasmodium yoelii.* We will present data on progress to date.

P15 Genetic Determinants of Sex Ratio in *Plasmodium falciparum*

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The life-cycle of *Plasmodium falciparum* alternates between a human host and an Anopheline mosquito. *Plasmodium* replicates asexually in the human host, but in order to leave the human host and infect a mosquito, *Plasmodium* must switch to sexual reproduction for the production of male and female gametocytes. These sexual stages are essential for malaria transmission. There is little understanding with regards to how sex is determined in *Plasmodium*, but the control is not chromosomal as *P. falciparum* is haploid in the human host and can give rise to both sexes (Downs, 1947). The *P. falciparum* sex ratio (defined as the proportion of male gametocytes) tends to be female-biased and could be influenced by environmental stimuli, such as the immune state of the host or the presence of other *P. falciparum* clones, as well as genetic programming. We will present evidence to suggest that the sex ratio is a heritable trait whereby the progeny of a cross between two genetically distinct *P. falciparum* clones inherit the sex ratio exhibited by one of the two parents of the cross.

P16 Confocal fluorescence microscopy with locked RNA (LNA) oligonucleotide probes for in situ DNA and RNA hybridisations to *P. falciparum* chromosomes in human red blood cells: FISHing for genetic rearrangements at the limits of detection and optical resolution

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FISH (fluorescence in situ hybridisation) is a widely used cell biological technique for studying gene location and expression in cells and tissues. Most eukaryotic cells are, microscopically speaking, biggish, with chromosomes in nuclei that are >10µm diameter. The problem for malaria FISHing is that rbcs are small (7-8µm diameter) and the parasite nucleus within an infected erythrocyte is around 1µm diameter. Given 14 chromosomes within pre-mitotic parasite nuclei, and diffraction limiting optical resolution to a few hundred nanometres, discerning the relative movements of telomeres, centromeres and genes in the *P. falciparum* nucleus is a challenging task.
As the parasite develops the parasite enters S phase and its unusual crypto-mitotic replications, where the DNA divides but the nuclear membrane does not. Chromosomally, a degree of organised chaos reigns during schizogony, as 14 chromosomes replicate themselves 4-5 times, resulting in up to 448 chromosomes, still within a single nuclear envelope. Approximately 16-32 distinct nuclear bodies become visible, at which point the nuclear envelope finally subdivides and segmentation into individual daughter merozoites is apparent.
To understand the genome biology we have visualized telomeric sequences and single copy genes during intra-erythrocytic schizogony using DNA FISH-based gene localisation and analysed of specific gene transcription via RNA FISH. To improve detection and resolution of intra-nuclear gene and chromosome localisations we have used bi-cyclic RNA probes and increased hybridisation temperatures for enhanced stringency of detection of such short, specific and permeable detection probes. We have also developed colorimetric methods which permit enhanced detection and double fluorescence staining.

P17 A Resource for Genome-wide Studies of Severe Malaria

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MalariaGEN brings together research groups with different projects and scientific objectives to work together on large-scale investigations that depend on samples, data and expertise from multiple investigators.
One of MalariaGEN's key questions is to ask why, in regions where every individual is repeatedly exposed to malaria parasites, do some people become severely ill or die from the infection, while others show little or no sign of disease?
By combining large-scale epidemiological studies with state-of-the-art genomic technology, we hope to find places in the human genome where small differences can have a big impact on the way the immune system responds to malaria. Our partners from 21 Institutions, that includes
15 malaria-endemic countries, are pooling resources of DNA samples and clinical data to build large datasets enabling genetic analyses to be undertaken with confidence.
Before large-scale experiments are undertaken, all samples are processed using consistent methods for storage, genotyping and collection of clinical data.

Our consortial project 1, which is aimed at a genome-wide association in severe malaria, currently comprises in excess of 12,000 cases and 12,000 controls. We have now generated baseline data for several key malaria polymorphisms on all samples and will discuss some of the characteristics and initial finding from this study.

P18 MALACTRES: a new EU-funded consortium to tackle multi-drug resistance in malaria under combination therapy

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Improved diagnostic tools to support the clinical suspicion of malaria in resource poor settings are urgently needed. Furthermore, malaria treatment is shifting from older drugs, which have lost their effectiveness due to resistance, to artemisinin-based combination therapies (ACTs). There is a growing risk that ACTs will also eventually fail due to the emergence of resistance. This process could be exacerbated by the over-treatment of fever cases with ACTs, in the absence of a reliable laboratory diagnosis.
The MALACTRES project has received €2.8 million from the EU to address both challenges in a new multidisciplinary consortium. Our aim is to assess specific genetic markers for ACT resistance and to develop innovative, rapid and simple diagnostics for malaria.
The work now underway comprises two complementary strands:

1. Use of parasite DNA and RNA from ACT-treated individuals to identify genetic markers for selective changes induced by ACT action.
2. Development and validation of simple tests in new formats, applicable in the field, for these markers and for already established markers of parasite resistance to ACT partner drugs.

The work is a blend of clinical field work, laboratory research and test development and details of current and planned studies will be presented. A number of our consortium members will be present at the BSP meeting and would welcome your attendance at our poster.

P19 Development of viral vector combination vaccine strategies targeting both pre-erythrocytic- and blood-stage malaria.

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The malaria field as a whole recognises that the first highly efficacious malaria vaccine will likely require a multi-antigen and/or multi-stage subunit vaccine against *Plasmodium falciparum*.
Recombinant viral vectors administered in heterologous prime-boost regimes have been developed as approaches to target both the liver- and blood-stages of malaria infection. The most promising regimes involve priming with replication-defective adenoviruses (Ads) and boosting with the poxvirus vector modified virus Ankara (MVA) eight weeks later. This induces remarkably strong T cell and antibody responses in animal models, and a Phase Ia clinical trial of this approach using the ME-TRAP pre-erythrocytic antigen is currently in progress at Oxford University. We are evaluating mixtures of vectored vaccines as a potentially highly effective approach to generating maximal pre-erythrocytic and blood-stage protection. This work employs viral vaccines targeting CSP and MSP-1 in the *P. yoelii* mouse model of malaria. Serum antibody responses, as well as the quantity and quality of T cell responses in the blood, spleen and liver, have been assessed in mice immunised with each component vaccine alone and in a mixture. Challenge studies will aim to assess protective efficacy in the mouse model, before translating these finding directly into studies using *P. falciparum* vaccine candidates expressing ME-TRAP and PfMSP-1 and/or AMA-1.

P20 More than just a gut feeling: Transcriptional changes triggered by *Plasmodium falciparum* glycosylphosphatidylinositols in *Anopheles gambiae* midgut

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Several innate immune responses against malaria parasites are shared between mosquitoes and mammals (Luckhart *et al*., PNAS, 1998). In order to understand how anti-parasite responses are regulated in mosquitoes, we have utilized Plasmodium-associated molecules that are known to induce immune responses in mammals, such as malaria toxin, glycosylphosphatidylinositol (GPI) and malaria pigment, hemozoin (Akman-Anderson *et*

al., Infection and Immunity, 2007, Vol. 75, No. 8, p. 4012-4019). Using gene array technology, we have recently identified several pathways in *Anopheles gambiae* midgut that are perturbed by Plasmodium falciparum GPIs (PfGPIs) at different time points after bloodfeeding. Our results support earlier experiments that show PfGPIs signal through mitogen-activated protein kinases (MAPKs). Expression data obtained via microarrays are currently being used as a guide for selection of targets for further molecular analyses.

P21 Inhibiting Malarial Dihydroorotate Dehydrogenase

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De novo pyrimidine biosynthesis is one of the leading antimalarial targets as *Plasmodium* cannot salvage pyrimidines vital for DNA and RNA synthesis but rely solely on the *de novo* biosynthesis pathway. Humans conversely can synthesise and salvage pyrimidines. Indeed, *Plasmodium falciparum* dihydroorotate dehydrogenase (PfDHODH), the fourth enzyme in the de novo synthetic pathway, has been shown to be vital for parasite survival in culture and potent inhibitors of the human enzyme are not toxic (McRobert *et al*. 2002; Painter *et al*. 2007; Breedveld *et al. 2000*). This enzyme catalyses conversion of the intermediary compound dihydroorotate to orotate and is the sole enzyme of the pathway located in the mitochondria. Several high potency inhibitors have been identified (Heikilla *et al. 2007*, Phillips *et al. 2008*). We continue our studies with an ever growing number of compounds that inhibit PfDHODH in vitro and with some that are also active against parasites. The nature of drug development means that a large number of active inhibitors are required as the chances of any inhibitor making it as far as a drug are very slim. Here a number of compounds have been designed as potential inhibitors of PfDHODH using a combination of traditional medicinal chemistry and *in silico* design. These have been synthesised and tested against recombinant PfDHODH and the human enzyme to obtain information on activity and specificity. These results, combined with mutation analysis of PfDHODH provide new insights to the nature of the inhibitor binding site.

P22 Genetic determinants of chloroquine resistance in *Plasmodium chabaudi*

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The evolution, genetic basis and mechanism of chloroquine resistance have been intensively studied. Genetic analysis has revealed that mutations in the gene encoding the chloroquine resistance transporter (*pfcrt*) are the major determinants of chloroquine resistance in *Plasmodium falciparum*. This transmembrane protein is thought to facilitate the export of chloroquine from the digestive vacuole. However, there are still unanswered questions concerning details of the mechanism of chloroquine action and resistance. For instance, other genetic factors influencing the drug response in *P. falciparum* remain unidentified, and the cause of resistance in another important human parasite *Plasmodium vivax* are not known.
Here we have used the congenic *P. chabaudi* lineage of multi-drug resistant mutants to study the origins of chloroquine resistance in rodent malaria. Using a novel approach combining Linkage Group Selection and Solexa sequencing we identified three loci underlying CQR that are selected sequentially by increasing drug concentrations, and 3 mutations contained therein. The genes defined include orthologues of PFF1430c on chromosome 11 and PFB0675w on chr3 that both encode transporters in the membrane of the digestive vacuole, and PFA0220w on chr2 that encodes a de-ubiquitinating enzyme. The identities, predicted locations and functions of the gene mutations define new experimental approaches that may contribute to our understanding of chloroquine resistance in human malaria parasites.

P23 Characterization of the 2TM hypervariable gene families in *Plasmodium falciparum*

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The human malaria parasite, *Plasmodium falciparum*, possesses a broad repertoire of proteins that are trafficked to the erythrocyte cytoplasm or surface, including the large families of predicted two transmembrane proteins (2TM), the Rifins, Stevors, and Pfmc-2TMs. Orthologs of 2TM proteins are not conserved across the Plasmodium genus; however, all species have amplified families of topologically similar 2TM proteins which universally possess a Pexel/HT erythrocyte trafficking motif. Thus across the Plasmodium genus the 2TM proteins likely participate in creating conserved parasite-derived modifications to the infected erythrocyte, such

as the new permeability pathways, rather than lineage-specific modifications such as the cytoadherent knobs of *P. falciparum*. The breadth of the 2TM family rules out targeted gene disruption, and for this reason we are investigating 2TM protein function utilizing parasite lines that have reduced 2TM protein levels using a blasticidin titration method. Solute permeability studies on these 2TM down-regulated transgenic lines show decreased sorbitol lysis in comparison with wildtype NF54 parasites, indicating that 2TMs may be involved in the formation or regulation of erythrocyte solute channels. We are currently investigating the topology of the 2TM proteins within the erythrocyte membrane and present the results of studies on the cellular localization of 2TM proteins which are epitope-tagged at the carboxy or amino- termini, or within the hypervariable loop predicted to be exposed on the erythrocyte surface.

P24 Analysis and characterization of a parasite line lacking the gene coding for a member of the py235 gene family

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A subset of the proteins expressed by members of the *py235* gene family of *Plasmodium yoelii*, bind to the surface of erythrocytes and are recognised by two protective monoclonal antibodies (mAbs). A role has been proposed for these proteins in parasite recognition and invasion of these erythrocytes. A parasite line has been made in which the gene encoding one of these proteins, Py235EBP-1 (Py235 Erythrocyte Binding Protein 1) has been knocked out. We have characterised this parasite line and compared it to the wild type to establish the effect of deleting the *py235EBP-1* gene on growth in vivo and the expression of other family members. A switch in the Py235 family members expressed in the knock-out line was detected. Using mass spectrometry, it has been established that the two mAbs 25.77 and 25.37, recognise different PY235 proteins, indicating that mAb25.77 and mAb25.37 recognise discrete epitopes. Additionally, these two antibodies recognise distinct PY235 family members in the same merozoite.

P25 Evaluation of a family of multi-domain proteins of the rodent malarial parasite *Plasmodium berghei*; are they effective targets for blocking transmission?

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A family of LCCL / lectin adhesive–like proteins (LAPs) have been described as surface / secreted proteins of *Plasmodium* gametocyte and have essential roles in sporogonic development of the parasite. They have therefore been included among potential targets to block parasite transmission from the mosquito to the vertebrate host. It is thought that the proteins, characterised by LCCL and / or lectin domains may interact with parasite or mosquito molecules, and a role in immunomodulation has been hypothesised. In the rodent malarial parasite *P. berghei*, individual disruption of five of the six *lap* genes generates mutant parasites capable of forming oocysts but which fail to produce sporozoites. It is suggested that they play a role during development form the gametocyte to ookinete, with gene disruption resulting in failure to undergo cytokinesis in the oocyst. Exact functions and mechanisms, however, are yet to be resolved. Attempts have been made to generate antisera against regions of the *Pb*LAP family, providing a means for studying localisation and performing transmission blocking assays. Presented here are results from such studies in which no antibody-mediated blockade in parasite development is detected.

P26 Analysis of ferrodynamics in relation to nitric oxide metabolism in Nigerian children with asymptomatic malaria

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Background: Studies have shown that asymptomatic falciparum malaria sustained by higher nitric oxide level and alterations in iron homeostasis are crucial in the development of chronic anaemia in African children. This study investigated the levels and interactions of plasma nitric oxide metabolites and iron parameters in asymptomatic malarial children.

Methodology: With the exclusion of clinical malaria, a total of 108 consented and apparently healthy children (mean age of 4.6 years, 59.3% males) from Lagos were included in the study. Plasma levels of nitrate plus nitrite (NOx), arginine and iron parameters were determined by standard methods. Data were analyzed statistically.
Results: Asymptomatic malaria (380 – 1930 parasites/□L), with a prevalence rate of 67.6% accounted for 11 (84.6%) of 13 anaemia cases (Hb<10g/dL) seen (P<0.05).
Compared with healthy control, significant (P<0.05) reductions in plasma levels of TIBC (328.4±8.5 vs. 301.4±3.9 □g/dL), % transferrin saturation, (30.4±0.9 vs. 27.5±0.5 %) and arginine (62.3±2.1 vs. 47.1±2.2 □mole/L) were found in asymptomatic malarial children.
Anaemia in the healthy control was characterized by lower ferritin level, suggesting iron deficiency.
NOx correlated inversely with arginine and more iron parameters in the anaemic subgroup.
Conclusion: Our results indicate that elevated nitric oxide level may mask the contribution of iron deficiency to the development of chronic anaemia in Nigerian children with asymptomatic malaria.

P27 Comparison of *var* gene transcription patterns between platelet-mediated clumping and non-clumping *Plasmodium falciparum* parasites

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Platelet-mediated clumping of *Plasmodium falciparum*-infected erythrocytes (IEs) has been associated with severe malaria and high parasitemia. Although three platelet surface receptors have been involved in clumping (CD36, gC1qR/HABP1/p32, and CD62P), nothing is yet known about the parasite proteins that mediate binding. In order to investigate the role of PfEMP1 in clumping, we developed a new method for positively (+) and negatively (-) selecting *P. falciparum* parasites using magnetic beads coated with anti-platelet CD62P antibody to isolate platelet-mediated clumps. We then investigated *var* gene transcription profiles in (+) and (–) selected parasites (clone HB3) to determine whether specific PfEMP1 variants are transcribed by clumping parasites (there are approximately 60 *var* genes per haploid genome encoding 60 different PfEMP1 variants). *Var* gene transcription was analysed by reverse transcriptase-PCR using degenerate primers against the DBLalpha domain and sequencing of cloned PCR products. Results suggest that multiple *var* genes are transcribed in both (+) (75% of IEs forming clumps in the presence of platelets) and (-) (3% of IEs in clumps) HB3 populations, with no single *var* gene related to clumping. Moreover, the HB3 (-) population showed almost the same pattern of transcribed *var* genes as the (+) one, suggesting that another unidentified parasite protein might be necessary for clumping (which is missing in the (-) culture).

P28 Liveweight gain as a marker for targeted selective treatments in lambs

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The development of anthelmintic resistance in ovine gastrointestinal nematodes now threatens the sustainability of small ruminant production systems. One approach that may prolong the efficacy of current anthelmintics is the maintenance of a parasite population in refugia through the use of targeted selective treatments (TSTs). In this approach treatment is only given to those animals that are most likely to benefit.
Replicated field trials, conducted in lambs over 3 years at Moredun, have compared a TST approach with 3 other commonly used anthelmintic treatment regimes; i.e. treatments given neo-suppressively (NST), prophylactically (SPT) or metaphylactically/therapeutically (MT). A decision support system, utilising production efficiency, has been developed and used to identify individuals requiring treatment. The TST, NST and SPT groups performed similarly but the MT group had daily liveweight gains of around 20 % lower than these groups. For each treatment given to the TST lambs, the NST, SPT and MT lambs received 1.9, 1.1 and 0.9 treatments respectively. Faecal egg count reduction tests show that efficacy was maintained in all groups apart from the NST group where efficacy declined each year to reach a low of 43% during the final season.
These studies suggest that the use of a TST approach may usefully prolong the efficacy of current anthelmintics.

P29 Studies on accelerated resistance to multiple drug resistance (ARMD) in different *Plasmodium chabaudi* clones with atovaquone and amodiaquine.

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Malaria control is limited by widespread occurrence of parasites resistant to antimalarial drugs. Current drug therapies include combinations with drugs with different mechanisms of action and structurally unrelated. Failure to antimalarial agents can arise rapidly as seen in rodent models and in the wild with *P. falciparum*. This ability of malaria parasites to readily develop resistance to structurally and mechanistically unrelated compounds, with accumulated mutations, represents a powerful strategy of survival and is designated accelerated resistance to multiple drugs (ARMD), which genetic basis is largely unknown.
We are estimating the ARMD phenotype in malaria parasites, using a rodent model of malaria, *P. chabaudi.*
For this we used a number of available genetically distinct clones obtained by sequential treatment over a number of years, and thus presenting different profiles of drug sensitivities, now subjected in separate, to atovaquone and amodiaquine pressure.
Though previous data would suggest that the clone already resistant to multiple drugs would be the one must prompt to show decreased sensitivity to a new drug, we observed that for amodiaquine but not to atovaquone. Here, it was a clone possessing a phenotype of resistance to only 2 drugs, which quickly developed resistance. Possible mechanisms for this experimental finding will be discussed.
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P30 Screening and identification of bioactive compound for prevention and treatment of Malaria.

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Malaria control strategies fall into three broad categories,
Vector control, vaccines and therapeutics. We are slowly losing the war against malaria, insecticide programs have been hampered by the emergence of resistance to DDT and other insecticides.
Several promising malaria vaccines have failed to protect against infection at levels high enough to warrant further development.
The prokaryotic protein presumably act selectively against malaria parasites because of their action against prokaryotic like plasmodial organelles known as Apicoplast , which seem to have Cyanobacterial origins and are related to algal plastids, recently identified potential selective target for antimicrobial drugs include components of type 2 fatty acid biosynthesis
It has been possible to develop an algorithm to define a set of rules predicting the Apicoplast targeted protein.
We are introduce new and innovative approaches, in order to identify and analyzed possible parasites derived analogue from marine organisms, involved in sequestration of *Plasmodium falciparum* as candidate for treatment and prevention of Malaria,
In the course of screening of bioactive molecule we are identified four active product that inhibited the proliferation of *P. falciparum* K1 in infected red blood cells. Their activities on apicoplast function identified by electron microscope and will be presented in the conference.

P31 MSP-1 Block1/Block2 hybrid: a vaccine candidate against *Plasmodium falciparum*

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The polymorphic N-terminal Block 2 of the *Plasmodium falciparum* Merozoite Surface Protein 1 (MSP-1) is being developed as a potential malaria vaccine candidate. This region has been support by field studies for the production of protective antibodies against clinical malaria. (Conway et al, 2000; Cavanagh et al, 2004)
However, design of an effective vaccine to a polymorphic molecule, presented a big challenge. In this study, a synthetic MSP-1 Block 2 hybrid construct based on all the polymorphic variants found in natural *P. falciparum* isolates and conserved Block 1 of MSP-1 was expressed as soluble protein in *E. coli* and purified by heating and anion exchange chromatography. This antigen has been shown to be remarkably heat-stable and immunisation of this protein into animals has been shown elicit antibodies against all three Block2 serotypes. Specific antibodies have shown functional inhibition against parasites in vitro. We have shown that production and purification of this candidate antigen is scalable to 12L with no loss of immunogenicity.

P32 Development of a malaria liver-stage vaccine based on simian adenoviral vectors and MVA expressing *P. falciparum* ME-TRAP

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Simian adenoviral vectors (SAds) are currently being developed as malaria vaccines. We have used a mouse model of liver-stage malaria to test potency and protective efficacy elicited by the SAds AdCh63, AdC7, AdC9 and AdC6 containing the ME-TRAP transgene and were compared to MVA, FP9 and the widely used human serotype AdH5, using multi-parameter flow cytometry to assess T-cell memory generation and multifunctional T-cell responses.
Upon a single inoculation, the SAds elicited outstanding T-cell frequencies that in the cases of some SAds outperformed AdH5. High levels of sterile protection were achieved by most Ad vectors and a boost with MVA elicited long-lasting sterile protection for at least 6 months after the boost. Ad vectors induced multifunctional $CD8^+$ T-cell responses (co-expressing IFN□, TNF□ and IL-2) and the percentage of triple positive cells increased upon an MVA boost compared to a single prime. Phenotypic analysis of $CD8^+$ T cells revealed that Ad vectors alone or in prime-boost regimes elicit a predominant CD8 Tem phenotype that generate potent liver T-cell responses that could be crucial to protect against malaria.
Our data demonstrate the potential of adenoviral vectors as future malaria vaccines for humans.

P33 The temporal dynamics of *Plasmodium* density through the sporogonic cycle within *Anopheles* mosquitoes

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Renewed calls for malaria elimination make it essential to understand the role of parasite density on malaria transmission. Particularly, it is crucial to determine the effect of parasite density on the temporal dynamics of the developmental stages within the mosquito. This will enable development of mathematical models describing the sporogonic cycle of *Plasmodium* within the vector using a parasite density framework, helping to evaluate the impact of transmission blocking strategies. Data was generated in a series of three experiments, in which cages of *Anopheles stephensi* mosquitoes were fed on blood infected with a range of *Plasmodium berghei* (fluorescently marked) ookinete densities (ranging from 50 to 2,000 ookinetes per µl) to determine patterns of oocyst and salivary gland sporozoite abundance over time post-infection. Every 24 to 48 hours after membrane feeding, samples of 20 surviving mosquitoes were dissected and the number of established oocysts and salivary gland sporozoites counted. The results were used to parameterize a compartmental model (comprising differential equations for ookinetes, oocysts, and sporozoites) and quantify the rates of progression between the developmental stages. Results indicate that the magnitude of these transition rates depends on parasite density, suggesting facilitation preceding limitation from ookinetes to oocysts, and therefore have implications for our understanding of the impact of transmission blocking strategies on malaria transmission.

P34 Evaluation of *Plasmodium vivax* genotyping markers for molecular monitoring in clinical trials

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Many antimalarial interventions are accompanied by molecular monitoring of parasite infections and a number of molecular typing techniques based on different polymorphic marker genes are applied. Here, we present a genotyping technique that provides a fast and precise approach to study *Plasmodium vivax* infection dynamics where individual clones must be followed over time. The method was tested with samples from an in vivo drug efficacy study.
PCR fragments were sized by capillary electrophoresis to determine the extent of size polymorphism of 9 potential genetic markers (5 genes of merozoite surface proteins (*msp*) and 4 microsatellites) in 93-108 *P. vivax* positive blood samples from 3 villages in Papua New Guinea.
The two microsatellites MS16 and Pv3.27 showed the greatest diversity in the study area with 66 and 31 different alleles found, followed by two fragments of *msp1* and two other microsatellites. *msp3α*, *msp4,* and *msp5* revealed limited polymorphism. Performance of coding regions and microsatellites was compared.
Even the most diverse markers showed allele frequencies of up to 6 or 13%. To reduce the theoretical probability of superinfection with parasites having the same haplotype as present on baseline we propose to combine at least two markers for genotyping individual *P. vivax* infections. Multiplex PCR protocols allow the analysis of these markers in parallel.

P35 Application of real-time quantitative PCR (qPCR) in anti-malarial drug trial

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Slide microscopy remains the standard method for malaria diagnosis and determination of parasite density. However, in clinical trials where thousands of slides are usually generated, microscopy is considered slow, labour intensive and unreliable for detecting low-grade infections. We compared real-time quantitative PCR (qPCR) assay with microscopy for malaria diagnosis in 1077 hospital patients and subsequently evaluated the performance of both methods in an efficacy trial of two anti-malarial drugs. Blood samples collected from 106 study patients at 8-hourly intervals over a 3-day period (~1060 blood samples) were analysed by qPCR amplification of *Plasmodium falciparum* 18S rDNA and microscopy. Agreement between microscopic and qPCR diagnosis (Kappa=0.86; 95% CI = 0.83-0.90) as well as concordance of parasite density estimates (rho_c=0.97; 95% CI= 0.96-0.97) was very high. However, estimates of parasite clearance time in the clinical trial cohort differed between the two methods with 96% (76/79) by microscopy and 83% (66/79) by qPCR clearing their infections 48 hours post-treatment. Low-grade parasitaemia (<20 parasites-µl) was detectable by qPCR but not by microscopy in 16% (12/74) of the patients by day 3 post-treatment. These results suggest that applying a sensitive parasite detection method could lead to a more precise determination of the relative efficacies of anti-malarial drugs in clinical trials.

P36 Molecular identification and characterization of WARP in temperate and tropical *Plasmodium vivax* isolates

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Many years after changing eradication strategy of malaria to control, today more attention is focused on eradication of all types of malaria. However, malaria elimination in affected areas with different epidemiological conditions urges the need for considering new strategies in vaccine development. *Plasmodium vivax* was neglected, but vivax malaria can become lethal in a similar way to severe falciparum malaria. *P. vivax* is the main cause of malaria in Iran that is transmitting in north and south of Iran by different Anopheline vectors. In present study, *warp* gene amplified from northern and southern Iranian *P. vivax* isolates by using designed specific primers in order to define the extend of its polymorphism and/or conserved nature as a candidate Transmission Blocking Vaccine (TBV). The results showed *pvwarp* is conserved in Iranian *P vivax* isolates (98-100% similarity). Highly conserved nature of this gene in different field isolates support the concept that ookinete secreted protein (WARP) may constitute a general class of malaria TBV candidates. Further advances on cloning and expression of this protein that have been carried out in our laboratory will be discussed.

P37 Prevalence of malaria and mosquito vectors in Depalpur, District Okara Punjab Pakistan

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The present survey was conducted to determine the prevalence of malaria and mosquito vectors in selected four villages of Tehsil Depalpur, District Okara where malaria is endemic from the last few years. Blood samples (116 from June 07 to August 07) were collected by PCD from four private clinics and a common Basic Health Unit (BHU) related to these villages. Prevalence was determined across various variables such as village residence, season, age and gender. Our data indicated 26% prevalence of malaria with respect to Slide Positivity Rate (SPR) and 22% using Enzyme Linked Immuno-Sorbent Assay (ELISA). In addition 18% cases were male positive and 8% were female positive. Children (43%) were more susceptible as compared to adults (22%). In general prevalence of malaria was greater (41%) in August as compared to June and July (22% and 23% respectively). Among these, 98% cases were found suffering from malaria due to *Plasmodium vivax* and 2% due to *P. falciparum*, indicating *P. vivax* was predominantly present in these months. Entomological survey was conducted from September 06 to April 07. Total 726 mosquitoes (indoor) belonging to three different species were collected. The most common was *Culex qunquifasciatus* (74.9%) while among the malaria vectors *Anopheles stephensi* was more (13.6%) as compared to *An. culicifacies* (11.4%). The role of *An. stephensi* in rural areas of Punjab, Pakistan needs to be determined.

P38 Genetic analysis of antifolates resistance associated genes, (*dhfr and dhps*) in *Plasmodium falciparum* and *P. vivax* isolates from Iran

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Molecular study has shown that resistance to sulfadoxine pyrimethamine (SP) in both *P. falciparum* and *P. vivax* isolates are associated with point mutations in *dhfr* and *dhps* genes. Therefore, detection of mutations in clinical isolates is very important in mapping of resistance and monitoring of malaria control program. In the present study, nested PCR/RFLP was applied to detect polymorphisms previously shown to be associated with SP resistance in 136 and 150 *P. falciparum* and *P. vivax* clinical isolates, respectively. Double mutations at codons 58 (20%) and 117 (30%) which are strongly associated with pyrimethamine resistance in *P. vivax* isolates was detected in 10% of our isolates. However, 99% of examined *P. falciparum* isolates carried parasites with double mutant alleles of *pfdhfr (*C59R + S108N) and 52% of samples carried parasites with triple mutant alleles of *pfdhfr and pfdhps* (C59R + S108N + A437G). The present data suggest that the increasing mutations in *pfdhf, pfdhps* and *pvdhfr* might be alarming in development of *P. falciparum* and *P. vivax* resistance to SP in Iran.

P39 Monitoring *Plasmodium Falciparum* Sensitivity to Chloroquine After Three Years Of Chloroquine Withdrawal From Sudan Malaria Treatment Policy in Gezira State – Central Sudan

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This study aims to monitor the level of chloroquine (CQ) resistance of *P. falciparum* parasite in Central Sudan after three years withdrawal from malaria treatment policy, using *in vitro* test and molecular markers. In November to December 2007, this study was carried out at three health centers in Wad Medani-Central Sudan, The standard WHO *in vitro* micro-test was performed on 70 *P. falciparum* isolates and 100 blood spot of infected patients with falciparum malaria were investigated for CQ molecular markers using Real Time –PCR..
The result showed that 45/70 *P. falciparum* isolates produced successful schizont growth, of which 25/45 (55.6%) were i*n vitro* sensitive to CQ, 17/45 (38.8%) showed low resistance level and 3/45 (6.6%) were marked *in vitro* resistant to CQ. The DNA was extracted from 100 samples, and were investigated for the prevalence of the targeted mutation (*Pfcrt* and *Pfmdr1*) , screening of the *Pfcrt* by Real Time –PCR revealed 63/100 (63%) carried mutant allele K76T, and 37/100 (37%) having the wild type and the screening of the mutation of the *Pfmdr1* revealed that 46/100 (46%) carried mutant allele Y86N, while 49/100 (49%) having the wild type and 5/100 (5%) having the mixed alleles *Pfmdr 1* (wild and mutant). The frequency of the mutations in (*Pfmdr1* and *Pfcrt)* was 69%. The frequency of the mutations among the CQ *in vitro* sensitive isolates was 55.6%, and the frequency of the mutations among those resistant to CQ by *in vitro* micro-test was 4.4%. Also it was observed that 25 /45 sensitive by *in vitro* test have mutation genes *Pfcrt, Pfmdr1* or both and 2 of 45 of the resistant by *in vitro* test have two mutation genes (Pfcrt and Pfmdr1), 14 /45 have no mutant genes and 13/14 were *in vitro* CQ sensitive and 1/14 was *in vitro* CQ resistant.
On the basis of these findings, it appears that the CQ *in vitro* sensitivity is increasing, and molecular markers are not consistently predictive of CQ resistance in the study area although might be effective.

P40 Investigating Anthelmintic Resistance through Protein-Protein Interactions

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Parasitic nematodes are a major constraint on livestock production world wide with an estimated loss 10^6 tonnes of animal protein annually. Nematode resistance to Benzimidazole (BZ) anthelmintics is a global livestock problem. However, BZ resistance is a model for studying the mechanism of anthelmintic resistance to other more recently released anthelmintics. BZ anthelmintics are known to target □-tubulin with resistance correlated to mutation. Preventing the spread of resistance has become compromised as no reversion, in the absence of anthelmintics, to wild type □-tubulin has been seen. □-tubulin itself is predicted to interact with many protein partners. It is among these partners, that 'modifier' proteins, compensating for any loss of fitness in resistant strains, may be found.
Investigating protein-protein interactions in parasitic nematodes will therefore further our understanding of anthelmintic resistance and potentially identify novel anthelmintic targets. We are currently using the model nematode *Caenorhabditis elegans* to investigate protein-protein interactions of □-tubulin associated with BZ resistance. To study these interactions, tandem affinity purification for *in situ* interactions and traditional 'pull down' proteomic methods are being used to systematically characterize the protein complexes concerned, how they respond to the presence of anthelmintics and how they are altered in resistant strains.

P41 Flow cytometry in anthelmintic resistance research.

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The time demands inherent in microscopy are one of the most important limitations which, through their effects on the number of samples that can be examined, slow the rate of progress in anthelmintic resistance research. Large particle flow cytometry is one system which has the potential to allow greater numbers of samples to be examined, which could be used to improve the sensitivity and throughput of current diagnostic techniques. Comparision between results gained through conventional microscopy and a Cytosense benchtop flow cytometer (1mm aperture) for the Egg Hatch Assay (EHA), Larval Feeding Inhibition Assay (LFIA) and peanut agglutinin staining of eggs of *Haemonchus contortus* has shown that the two methods are equally repeatable and sensitive but that the latter has a higher sample throughput. Since the Cytosense system also enables fluorescent samples to be sorted it has the capacity to enable the isolation of individual parasites on the basis of their phenotypic response in bioassays. These characterised populations provide an invaluable resource for studies seeking to identify genetic markers of anthelmintic resistance which are related to a particular parasite phenotype.

P42 Investigating macrocyclic lactone resistance in cyathostomin populations using the larval migration assay

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Cyathostomins are highly pathogenic horse nematodes with resistance recorded to all three classes of broad-spectrum anthelmintic. Of particular concern, is the emergence of resistance against the most effective class, the macrocyclic lactones (ML). These are the only anthelmintics effective against pathogenic mucosal stages. Due to high levels of nematode genetic diversity and large population size, the rapid spread of resistance through populations is inevitable and so early methods of detection are required. In-vivo methods (i.e. faecal egg count reduction test) lack sensitivity for early resistance detection and so an in-vitro larval migration assay (LMA) was developed and optimised for cyathostomins. This assay utilises the paralysing effects of MLs (e.g. ivermectin, IVM, and moxidectin) to gauge the effect on motility of infective L3 over a range of concentrations. Dose-response curves and LMI50 values are then produced for comparative analysis. Significant differences in LMI50 values were observed between populations of cyathostomins shown to have different sensitivities to ML in vivo. ML-resistant populations exhibited LMI50 values of 2.41 µg/ml and 2.86 µg/ml in an IVM-LMA, compared to 0.26 µg/ml observed in a ML-naïve population and 0.57 µg/ml in a ML-sensitive population. These findings indicate that the LMA shows potential for early resistance detection.

P43 A novel model to study of CNS infection *in vivo* due to *Acanthamoeba* spp. (T4 genotype).

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It is shown that the African migratory locust can be used as a model to study *Acanthamoeba* pathogenesis. Mature adult locusts are injected intra-abdominally with 10µl of a suspension of 10^6 *Acanthamoeba* (a clinical isolate of the T4 genotype) in culture medium, or with the same volume of sterile culture medium. Infected locusts show significant weight loss, and reduced production of faeces compared with control locusts. At room temperature, all infected locusts die within 17 days, although the speed of kill is temperature and dose-dependent. When faecal pellets or various tissues of infected locusts are cultured on non-nutrient agar plates containing bacterial lawns, live amoebae are recovered from haemolymph, flight muscle, and fat body samples, but not faeces. When brains dissected from locusts are incubated with an anti-amoebic drug (100µM chlorhexidine) to kill extracellular amoebae, washed, homogenized, and cultured on bacterial-seeded non-nutrient agar plates, only lysates from amoebae-infected locusts are positive for *Acanthamoeba*. This suggests strongly that amoebae invade the locust brain and, indeed, trophozoites of *Acanthamoeba* can be identified within the brain in histological sections of brains from infected locusts, but not from non-infected locusts.

P44 Occurrence of the swimbladder nematode *Anguillicola crassus* in European Eels (*Anguilla anguilla*) within the United Kingdom

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Anguillicola crassus is a parasitic nematode that originates from the Indo-Pacific region, where it naturally invades the swimbladder of the Pacific eel, *Anguilla japonica,* causing little, if any, pathology to the host. Following accidental introduction into European waters, *A. crassus* has rapidly spread through populations of the European eel, *Anguilla anguilla*, causing severe pathology to the host swimbladder. The histopathological and physiological effects of the parasite upon the eel swimbladder have led to proposals that *A. crassus* infection is a contributory factor to the current decline of European eel populations.
A limited number of eel surveys have been carried out in UK river systems to assess the geographic extent and prevalence of *A. crassus*. In order to assist development of eel management plans, this study aims to assess the extent of prevalence of *A. crassus* infection within eel populations indigenous to UK waters. The results of this ongoing study will be presented at the meeting.

P45 Isolation and characterisation of protease genes from the parasitic nematode *Anisakis simplex*

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Nematode proteases have been shown to be involved in numerous biological processes and are attractive drug targets in parasites. *A. simplex* is an ascaridian nematode of marine mammals that is responsible for anisakiasis in humans. With a view to further understanding the roles of peptidases in the biology of *A. simplex*, this study has focussed on isolating and characterising three protease genes; 1), a cytosolic non-specific dipeptidase (CNDP), 2), a matrix metalloprotease and 3), the puromycin-sensitive aminopeptidase (pam-1). Briefly, a PCR strategy was used to isolate the full length CNDP and MMP genes and an almost full length pam-1 gene from cDNA prepared from both larval and adult stages of the parasite. A differential expression pattern was acquired for CNDP and the MMP, showing that the larval proteases are expressed at higher levels than in adults. EXPression analysis of pam-1 has been carried out in the model nematode *Ceanorhabitis elegans* using transgenic gfp expressing lines and also a PAM-1 specific antibody. Further translational studies of the CNDP and the MMP are planned in *C.elegans* to assist understanding of the biological roles of these proteases in nematodes.

P46 Liveweight gain as a marker for targeted selective treatments in lambs

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The development of anthelmintic resistance in ovine gastrointestinal nematodes now threatens the sustainability of small ruminant production systems. One approach that may prolong the efficacy of current anthelmintics is the maintenance of a parasite population in refugia through the use of targeted selective treatments (TSTs). In this approach treatment is only given to those animals that are most likely to benefit.
Replicated field trials, conducted in lambs over 3 years at Moredun, have compared a TST approach with 3 other commonly used anthelmintic treatment regimes; i.e. treatments given neo-suppressively (NST), prophylactically (SPT) or metaphylactically/therapeutically (MT). A decision support system, utilising production efficiency, has been developed and used to identify individuals requiring treatment. The TST, NST and SPT groups performed similarly but the MT group had daily liveweight gains of around 20 % lower than these groups. For each treatment given to the TST lambs, the NST, SPT and MT lambs received 1.9, 1.1 and 0.9 treatments respectively. Faecal egg count reduction tests show that efficacy was maintained in all groups apart from the NST group where efficacy declined each year to reach a low of 43% during the final season.
These studies suggest that the use of a TST approach may usefully prolong the efficacy of current anthelmintics.

P47 Host-Parasite Interactions in the Large Arionids: Is Parasitology Linked to Species Invasions?

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Using a combination of morphological and molecular techniques this study tests the hypothesis that parasitology is a significant factor driving the invasive nature of the large Arionids. Preliminary results, based on 116 specimens, suggest a strong species specific relationship with differences in parasite diversity and abundance. Morphological results may also show evidence that carotenoid pigments could be involved in a resistance response. Furthermore, the use of morphological analysis alone to identify species of large Arionid was shown to be accurate within “pure” individuals but of limited use within areas of species introgression.

P48 Molecular epidemiology of ascariasis

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It is estimated that more than 1 billion people are infected with the giant intestinal roundworm, *Ascaris lumbricoides*. Although the greatest numbers of infected individuals are found in Asia and sub-Saharan Africa, ascariasis shows a cosmopolitan distribution and is found in many deprived communities in temperate and tropical areas.
We are using molecular epidemiology techniques to study the population structure of *A. lumbricoides*. Worms were obtained from human hosts on Zanzibar and in Uganda, Bangladesh, Guatemala and Nepal. Additionally, Ascarid worms were collected from pigs in Uganda, Tanzania, Denmark, Guatemala and the Philippines. Genomic DNA was extracted from all worms. A 450 base pair region of the mitochondrial cytochrome c oxidase 1 gene (CO1) was sequenced for each worm. A 300 base pair region of the internally transcribed spacer 1 of nuclear rDNA was also sequenced for some samples. Sequences were aligned to identify non-synonymous substitutions, and phylogenetic analysis and assessment of genetic diversity was undertaken. In addition, we are starting microsatellite analysis of the *Ascaris* DNA.
So far 15 different CO1 haplotypes have been detected in *A. lumbricoides* from Zanzibar and 3 different haplotypes in Uganda, 2 of which match Zanzibari haplotypes. Further analysis should provide valuable insights into the population structure and transmission dynamics of *A. lumbricoides* and help to address whether *A. lumbricoides* of humans represents a different species to *A. suum* of pigs.

P49 Parasites of Bats in the United Kingdom

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Bats (*Chiroptera*) are one of the most successful and diverse of mammalian orders, with an estimated 1100 species worldwide. They are host to a range of infectious agents including rabies, SARS and ebola viruses, and also harbour a plethora of parasites.
Studies of bat parasites are relatively limited when compared with that of other mammalian orders, for example Rodentia. Within the UK, invasive studies on bats are difficult to perform, particularly given their protected species status. As such, there are many fundamental questions concerning parasites of British bats that remain unanswered.
In order to address some of these questions, one hundred pipistrelle bat (*Pipistrellus pipistrellus/ P. pygmaeus*) corpses were obtained between 2006 and 2008 via the South Lancashire Bat Group. Sample dissection, microscope analyses and PCR-based screening techniques have been undertaken to detect both protozoan and helminth parasites in these specimens.
Confirmation of the presence of *Eimeria* spp. in British bats is presented, as is the first comprehensive analysis of helminth communities in British bats. Prevalence of *Trypanosoma* spp., *Babesia vesperuginis*, *Bartonella* spp., *Eimeria* spp., and five trematode species has been used to analyse the parasite communities of bats in relation to a range of factors including host genotype.

P50 Hsp90 and the biology of parasitic nematodes

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Heat shock protein 90 (Hsp90) represents an ubiquitous group of proteins that function both as molecular chaperones and as stress proteins. The molecular architecture and sequence of Hsp90 is well conserved in most eukaryotes, and consists of a N-terminal domain which contains a well-characterised ATP binding site, a highly charged region and the C-terminus containing the dimerization domain. Previous studies on nematode Hsp90s had demonstrated functional differences in Hsp90 from *Caenorhabditis elegans* and the parasitic species, *Brugia pahangi*. *C. elegans* Hsp90 failed to bind to Geldanamycin (GA), a specific inhibitor of Hsp90, in solid phase pull down assays, while *B. pahangi* Hsp90 binds to GA. In this study, we examined the GA binding of a range of nematode species from different clades with a view to determining whether *C. elegans* GA-resistant phenotype is shared with other nematodes. Our results demonstrate that Hsp90 from both free-living and parasitic species belonging to Clade V are non-binding while Hsp90 from *T. spiralis* (Clade I) and from Clade III nematodes, including other filarial worms and ascarids, bind to GA. Thus the life history of the species may determine whether or not Hsp90 binds to GA; species that have a free-living larval stage in the soil do not bind GA, while those species which are obligate parasites (*Trichinella* and the filarial worms), or which are enclosed within a protective egg shell while in the environment (Ascarids), possess an Hsp90 that binds GA. Current efforts are focused on understanding the molecular basis of GA sensitivity.

P51 Identification and description of *Bucephalus minimus* (Digenea: Bucephalidae) life cycle in Portugal: morphological, histopathological and molecular data

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The cercaria of *Bucephalus minimus* infects the digestive gland and gonads of its first intermediate host, the edible cockle, *Cerastoderma edule*. Light and scanning electron microscopy of the cercaria showed a tail formed by a central stem with 2 long contractile arms presenting distinct morphological surfaces. The encysted metacercaria naturally infected the flathead grey mullet, *Mugil cephalus.* The cysts found in the heart, liver, and spleen were shown to be identical by the ITS1 sequence and morphological features and were associated with encapsulation , recruitment of cell infiltrates, and presence of melanomacrophages and adipose tissue. To establish the life cycle, we compared the ITS1 sequence in an adult from the known definitive host, *Dicentrarchus labrax,* encysted metacercariae from the liver, heart, and spleen of *M. cephalus*, and a cercaria from *C. edule*. It was determined that they had a 100% similarity. Therefore, the ITS1 sequence data clearly indicate that these 3 parasitic stages belong to the same species, i.e., *B. minimus*.

P52 Morphological and molecular studies on life cycle stages of *Diphtherostomum brusinae* (Digenea: Zoogonidae) from northern Portugal

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Diphtherostomum brusinae was first recorded by the present study in the North of Portugal. Sporocysts, containing cercariae and encysted metacercariae, were observed in the gonads and digestive gland of the gastropod *Nassarius reticulatus*. Metacercariae were also found infecting the foot, mantle border and gills of the cockle *Cerastoderma edule*. The adult form was lodged in the rectum of the definitive host *Diplodus sargus*. The morphology of the three parasitic stages was studied by light and scanning electron microscopy (SEM). Despite the close similarity between cercaria and metacercaria, SEM data provided information that allowed its differentiation. The adult showed characteristics of *D. brusinae* species, in particular the presence of acetabular lips, compact vitellaria and large elliptical eggs. Sequenced ITS1 data clearly demonstrated that the cercariae and metacercarial cysts from *N. reticulatus*, the cysts from *C. edule* and the adult isolated from *D. sargus* were life cycle stages that belonged to the same species, i. e., *D. brusinae*. Two transmission strategies in the life cycle of this species were observed: 1) Cercariae encyst within the sporocysts of *N. reticulatus* and await ingestion by the definitive host; 2) *N. reticulatus* naturally emits cercariae; they encyst in *C. edule* or the environment and are ingested by the definitive host.

P53 Expression of parasitic nematode enzymes using *Caenorhabditis elegans*

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Haemonchus contortus is a blood-feeding gut parasite of sheep which causes severe anaemia and can be fatal. Anthelmintic drugs currently available to control infection are losing efficacy due to the emergence of drug resistant nematodes and alternative controls are needed. Previous studies have identified a number of protective proteins and protein complexes extracted from the parasite that can induce significant protective immunity. However, recombinant forms of these proteins expressed in bacteria, yeast and insect cells are far less effective. We are using the free-living nematode *Caenorhabditis elegans* to express identified control targets of *H. contortus* with the aim of producing these in a form with similar conformation and glycosylation to the native proteins. We have already express *H. contortus* cathepsin L cysteine protease in *C. elegans* and shown functional rescue of a *C. elegans* cathepsin L (*cpl-1*) mutant, demonstrating active enzyme. We are now testing expression of the previously identified protective proteins, members of the cathepsin B protease family and gut aminopeptidase.

P54 The possible impact of climate change on the composition of parasite communities

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A long term investigation into the parasites of wild rabbits (*Orcytolagus cuniculus*) at a study site in eastern Scotland has shown that the intensity of the infections of the stomach nematode *Graphidium strigosum* increased markedly since 1977. At a near by meteorological station elevated air and soil temperatures were recorded over the same period. Increased temperatures may have had a direct effect on the survival of the free-living stages of *G. strigosum* possibly increasing its ability to over-winter. No such increase in intensity was observed in another parasitic nematode species of the rabbit, *Trichostrongylus retortaeformis,* suggesting the free living stages of this species was not markedly influenced by raised temperatures. However an interaction between these two nematode parasites within the rabbit implicating differential immune responses has been reported and this could complicate future epidemiological patterns seen in these rabbit parasites. Where parasitic communities contain more parasitic species than the simple parasitic community found in rabbits then interactions between changing external abiotic factors and host /parasite and parasite/parasite interactions may produce complicated epidemiological patterns which are difficult to predict and unravel.

P55 Analysis of Trends in the Performance of Water Laboratories Participating in the Inter-Laboratory Cryptosporidium Proficiency Testing Scheme (CRYPTS) 1. Microscope Slides.

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All UK and some overseas water laboratories that carry out Cryptosporidium analysis participate in the monthly inter-laboratory Cryptosporidium proficiency scheme (CRYPTS). Primary aim of CRYPTS is to promote quality in the measurement of Cryptosporidium oocysts in treated water supplies to assess Participating Laboratory performance for the following three analytes a) microscope slides, b) filters and c) suspensions containing differing numbers of Cryptosporidium spp. oocysts.
The Assigned Value (AV) for each slide is determined from the two enumerations performed at SPDL prior to dispatch (Initial Count) and re-enumeration when returned to SPDL (Count Back). Only oocysts fulfilling defined and published morphological and morphometric criteria are enumerated.
The data presented from 105 rounds of CRYPTS show a high percentage of slides deviating from the AV in the first 24 rounds (44% to 81%). As participants become more familiar, the percentage number of slides deviating lowers in R24 to R58 (21% to 63%). However, the introduction of increased numbers of oocysts, different organisms and Cryptosporidium species used in R59 to R80, show an increase in deviation (65% to 92%), lowering from R81 to R105 (30% to 71%).

P56 Analysis of Trends in the Performance of Water Laboratories Participating in the Inter-Laboratory Cryptosporidium Proficiency Testing Scheme (CRYPTS). 2. Filters.

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All UK and some overseas water laboratories that carry out Cryptosporidium analysis participate in the monthly inter-laboratory Cryptosporidium proficiency scheme (CRYPTS). Primary aim of CRYPTS is to promote quality in the measurement of Cryptosporidium oocysts in treated water supplies. It assesses participant performance for three analytes a) microscope slides, b) filters and c) suspensions all containing differing numbers of Cryptosporidium oocysts. Satisfactory recovery is considered to be 30% for filter analysis (elution and concentration, immunomagnetic separation and microscopy), indicating laboratory performance. Oocyst suspensions (Assigned Value; n = 10% prepared) using flow cytometry are used to seed Filters prior to dispatch.
The data presented from 105 monthly rounds of CRYPTS filters indicate that 11 of 24 participating laboratories have a high proportion of satisfactory performances (53% to 78.8%); eleven have a high number of less than satisfactory performances (52.4% to 82.1%); two participants have an equal proportion of performances that are satisfactory and less than satisfactory, and three laboratories had less than satisfactory performances in all distributions. In the first 4 years of the scheme, the average percentage recovery (15.4% to 28.9%) was consistently low for each round (≤29.9%), but a steady increase in recovery occurred thereafter (30.0% to 51.9%), probably due to familiarity and optimisation of methods.

P57 Analysis of Trends in the Performance of Water Laboratories Participating in the Inter-Laboratory Cryptosporidium Proficiency Testing Scheme (CRYPTS) 3. Suspensions.

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All UK and some overseas water laboratories that carry out Cryptosporidium analysis participate in the monthly inter-laboratory Cryptosporidium proficiency scheme (CRYPTS). Primary aim of CRYPTS is to promote quality in the measurement of Cryptosporidium oocysts in treated water supplies to assess Participating Laboratory performance for the following three analytes a) microscope slides, b) filters and c) suspensions containing differing numbers of Cryptosporidium oocysts.
The Assigned Value (AV; n = 10% prepared) is determined using the average number of oocysts recovered from membranes following Flow Cytometry. Suspensions are dispatched to participants and oocysts enumerated following Immuno-Magnetic Separation in order to monitor participant performance, a satisfactory recovery is considered to be ≥60%.

The data presented from 105 rounds of CRYPTS show 21 of the 24 participants have a high proportion of satisfactory performance (63.9% to 88.6%); three participants have a higher proportion of unsatisfactory performance (53.8% to 100%); five participants performing better than average (93.3% to 96.2%). The average percentage recovery of oocysts was taken for each round ranges between 63.6% and 95.6% depending on number of oocysts present. Greatest increases in recovery are seen in eight rounds with recoveries ≥100% due to the presence of participant contamination.

P58 A study of serological markers for *Cryptosporidium* exposure and variation over time associated with changes in drinking water treatment.

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Drinking water contaminated with *Cryptosporidium* is an internationally recognised risk for human illness. Contamination can arise from a variety of sources, ranging from raw water to distribution systems, with oocysts from infected humans, livestock and feral animals present in a catchment. Inadequate drinking water treatment permits oocyst transmission to susceptible consumers of that water. Continuous low-level exposure via unfiltered water can result in higher background immunity to *Cryptosporidium* among consumers. Paradoxically, low-level exposure may stimulate a protective effect among people subsequently exposed to *Cryptosporidium* from other sources e.g. zoonotic. The unintended consequence of filtration in Loch Katrine might therefore reduce levels of 'herd immunity' to *Cryptosporidium* in the relevant population. In collaboration with Scottish National Blood Transfusion Service, blood was collected from healthy blood donors residing in Dundee and Glasgow. Western blot analysis was used to facilitate the understanding of the sero-epidemiology of *Cryptosporidium*. Initial statistical analyses and risk factors reveal that Dundee donors were more likely to drink unboiled tap water than their Glasgow counterparts. However, Glasgow donors who had higher antibody responses compared with Dundee donors drank more cups of unboiled water. Preliminary data suggest identification in changes of water treatment as being associated with potential exposures of *Cryptosporidium* within a population.

P59 Identification and characterisation of a potential immunodiagnostic marker for larval cyathostominosis

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Parasitic nematodes of the group *Cyathostominae* are an important cause of equine disease. This group consists of approximately 50 species which all have a similar life cycle, which involves the encystment of parasitic larval stages in the large intestinal wall. Large numbers of developing larvae can emerge synchronously, damaging the intestinal mucosa and result in a pathogenic condition known as larval cyathostominosis. A diagnostic tool which estimates larval burden would allow targeted treatment and may delay drug resistance; however such a test has yet to be developed. We report the identification of a larval protein that shows promise for utility as a diagnostic antigen for larval cyathostomin burdens. This antigen was identified via immunoscreening of a cyathostomin mixed-larval stage cDNA library. Recombinant antigen was expressed in *E. coli* and was shown to be immunogenic, displaying low cross-reactivity to proteins present in other equine helminths. Transcription of the antigen is restricted to mucosal larval stages, and the protein was localised to the nematode gut. The equivalent gene encoding this antigen was identified in ten separate cyathostomin species, indicating its ubiquity. This study indicates the promise of this antigen as a diagnostic marker for larval cyathostominosis.

P60 Response of the domestic fowl following exposure to *Dermanyssus gallinae*

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Two groups of laying hens had mite chambers secured to their backs and were exposed to either 200 *D. gallinae* for up to 48 hours or acted as controls. Birds were euthanased either on the day prior to infestation or 1, 2 and 5 days after infestation. The expression of GAPDH, IFN-γ and IL-10, IL-13 and IL-18 was measured in spleen and skin samples from the feeding site by semi-quantitative PCR. Cytokine expression was standardized against GAPDH expression in each sample.

No significant differences in cytokine expression in the skin or spleen were detected between treatments at any time point. There were no numerical differences in gene expression between treatments in skin samples. In spleen samples, infested birds had numerically higher IFN-γ expression than controls on Days 1 and 5, whilst IL-18 was higher in infested birds on Days 1, 2 and 5. IL-13 expression was detected in infested birds on Day 5 but not in controls, whilst IL-10 was detected in a single bird from both groups on Day 1.
This study confirmed both that mite feeding chambers could be used successfully to allow *D. gallinae* to feed on birds and feeding mites did stimulate numerically detectable differences in Th1 cytokine expression. It is probable that higher numbers of *D. gallinae* are required to stimulate a more pronounced immune response.

P61 Phylogenetic linkage between poultry red mite populations in Europe

Marianna Marangi[1], Carlos de Luna[2], Mariassunta Cafiero[3], Antonio Camarda[4], Sophie Le Bouquin[5], Didier Huonnic[5], Annunziata Giangaspero[1] and Olivier Sparagano[2]
[1]Dipartimento PrIME, Università di Foggia, Italy; [2]School of Agriculture, Food and Rural Development, Newcastle University, Newcastle upon Tyne, NE1 7RU, UK; [3]Istituto Zooprofilattico Sperimentale della Puglia e Basilicata, Italy; [4]Dipartimento di Sanità pubblica e Zootecnia, Università di Bari, Italy; [5]Unité EBEAC, AFSSA - BP 53 -F 22 440 Ploufragan, Francexphylogenetic analysis of *Dermanyssus gallinae* mites (also known as the poultry red mite) originating from UK, France and Italy was performed using partial amplification of the mitochondrial COI gene. Results showed that UK samples reveal the greatest variation and diversity and are linked to one of the French populations highlighting North-South genetic transitions in European red mite populations. Intra-farm variations between mite samples highlighted the diversity between national populations and possibly its origin from the different chemical strategies used in each country.

P62 Echinococcosis in Wild and Domestic Canids in Eastern Tibet

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A survey was conducted within a Tibetan County of Sichuan Province, China to estimate the infection rate of *Echinococcus* spp. in domestic dogs and Tibetan foxes (*Vulpes ferrilata)* from a region where both human cystic echinococcosis (CE) (*Echinococcus granulosus)* and alveolar echinococcosis (AE) (*E. multilocularis)* are co-endemic[a]. A third recently discovered species on the Tibetan plateau *Echinococcus shiquicus* shows significant infection rates in Tibetan foxes and Pika (*Ochotona* spp.). In order to investigate the role of canids in transmission more than 600 faecal samples from Tibetan foxes and owned domestic dogs have been analysed by *Echinococcus* genus specific coproantigen- ELISA and a species specific multiplex PCR. In addition, a cohort of 300 dogs was followed up for two years after a single treatment with praziquantel for a re-infection study. Likelihood of peri-domestic transmission for *E.multilocularis* in dogs as well as their capability to act as hosts for *E.shiquicus* is considered.
[a]Li et al (2005) Emerging Infectious Diseases. 11(12) 1866- 1873

P63 Transformation of bovine monocytes by *Theileria annulata* renders them susceptible to infection with *Ehrlichia ruminantium*

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The tick-borne protozoan parasite *Theileria annulata* infects and transforms bovine cells of the macrophage/monocyte lineage. Such infection results in alterations in host cell gene expression and function, including down-regulation of surface expression of CD11b and CD14 and abrogation of the ability to produce IFN-α. We have shown that the obligate intracellular tick-borne bacterial pathogen *Ehrlichia ruminantium*, which causes heartwater disease in ruminants, invades and replicates within *T. annulata*-transformed cells. Treatment of the cells with cycloheximide to inhibit host cell division facilitated up to 26% *E. ruminantium* infection rates over a 3-4 day period. Ultrastructural examination revealed intracellular bacterial colonies resembling those reported in early-stage endothelial cell infections. In contrast, although *E. ruminantium* was taken up by freshly harvested bovine blood monocytes, the organisms failed to replicate within these cells. Since *T. annulata* transformed cells are known to be efficient antigen-presenting cells, we plan to use parasitised cells superinfected with *E. ruminantium* to analyse T cell responses of calves immunised with *E. ruminantium*. In addition, this system provides a useful model to study how infection with *T. annulata* modulates host cell activation pathways involved in defence against infection with intracellular pathogens.

P64 Single nucleotide polymorphism analysis of population structure and virulence in *Entamoeba histolytica*

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Virulent amoebiasis is a relatively rare outcome of *Entamoeba histolytica* infection. Virulence may be at least partly genetically determined, and identification of virulent strains would be valuable in targeting treatment. The population structure of *E. histolytica* is poorly understood, nor is it known if the parasite undergoes a sexual cycle, which would both affect the spread of virulence determinants. We are using ABI SOLiD sequencing and 454 pyrosequencing technologies to determine entire genomic sequences of 5 parasite strains of differing virulence and from different geographic locations. We will use these data to define SNPs and genotype a large number of parasites from several disease foci to address three questions: does sexual recombination occur in parasite populations? What is the extent of gene flow between populations in different geographical regions? Is there a genetic basis for virulence and did it evolve once or multiple times? Clonality will be assessed from linkage disequilibrium between SNPs. If clonal, parasites will be analysed using phylogenetic methods to assess inter-population divergence and the putative genetic basis for virulence. If sexual, they will be analysed using Bayesian clustering methods to assess population structure, and LD mapping and population genetic tests of neutrality will be used to identify candidate virulence loci.

P65 Glutathione Transferases of *Fasciola gigantica*: a Proteomic Approach to Identification.

Neil Eccleston[1], John McGarry[1], Samirah Perally[2], Peter Brophy[2], Diana Williams[1] and James LaCourse[1].
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Fasciolosis is an important foodborne, zoonotic disease of domestic animals and humans, with global annual health and economic losses estimated at several billion US$. Digenean trematodes of the Genus *Fasciola* are the causative agents of fasciolosis; *Fasciola hepatica* are the major species in temperate regions whilst *F. gigantica* dominates in the tropics.In the absence of commercially available vaccines to control fasciolosis, increasing reports of resistance to current chemotherapeutic strategies and the spread of fasciolosis into new areas, much research is underway to identify potential new drug targets and vaccine candidates.The glutathione transferase (GST) superfamily of enzymes has received much attention as both drug detoxification targets and as vaccine candidates. In this study we report the identification, via a sub-proteomic approach, of a previously undiscovered GST in the tropical liver fluke *F. gigantica*. This new GST shares a greater identity with the *Schistosoma* GST component of the human schistosomiasis vaccine currently at Phase II clinical trials, than previously discovered *F. gigantica* GSTs. As a putatively more closely related protein to the human schistosomiasis GST vaccine, this new *F. gigantica* GST is a potential component of a vaccine for tropical fasciolosis.

P66 The use of RNAi to understand the role of fatty acid binding protein in the metabolism and resistance to triclabendazole in *Fasciola hepatica*

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In the absence of vaccines effective control of *Fasciola hepatica* infections of livestock depends largely on the chemical Triclabendazole (TCBZ) due to its activity on both immature and adult parasite stages. Dependence on this single drug and poor parasite management in climate change are probably responsible for the increasing fluke resistance to TCBZ. The mode(s) of action and biological target(s) of TCBZ are unknown, limiting the ability to detect and monitor TCBZ resistance. Previous proteomic analyses of TCBZ tolerant and TCBZ susceptible isolates of *F. hepatica* with and without in vitro exposure along with interaction studies of recombinant forms has already strongly suggested the involvement of a fatty acid binding protein (Fh15). RNA-mediated interference is now currently in progress to firstly determine a role for Fh15 in TCBZ metabolism and resistance and secondly to investigate its potential for use in chemotherapy in conjunction with TCBZ against Fascioliasis.

P67 Tools for monitoring drug resistant *Fasciola hepatica* in cattle and sheep

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Fasciolosis has a significant global impact on livestock health and welfare, with productivity loses of ~$US3 billion per annum. In the absence of vaccines *Fasciolosis* is controlled by anthelmintics. Triclabendazole (TCBZ) is the most important *Fasciolicide*, and an Achilles heel of chemo-control as it is the only drug with significant efficacy against both adult worms and juveniles. TCBZ resistance is well established. The mode of action of TCBZ at the molecular level has yet to be resolved and therefore early resistance detection via target mutation is not possible. Proteomic approaches have identified that a fatty acid binding protein (FABP) is up-regulated in a TCBZ resistant liver fluke isolate (Sligo) compared to a TCBZ sensitive isolate (Cullompton). We report cloning and sequence analysis of FABP in liver fluke populations and our progress to detect liver fluke FABP in faecal samples.

P68 *Fascioloides magna* in deer population in Serbia

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Giant liver fluke (*Fascioloides magna*) infection is an important health problem of cervids in southeast Europe. The present study confirmed the presence of *F. magna* infections in Serbia and tested a therapeutic control protocol. From November 2007 to February 2008, faecal samples and livers of fallow deer (*Dama dama*) were used to determine the prevalence and intensity of infection in a selected fenced area in northwest of Serbia. Coprological examination was performed on 30 faecal samples, and three livers of hunted deer were dissected to confirm *F. magna* infection. Sixteen (53.33%) of the 30 examined fecal samples contained eggs of giant fluke. All dissected livers contained adult or juvenile flukes and had pathologic lesions. We then applied flukicide-triclabendazole in medicated feed. Medicated corn bait was given to 124 fallow deer: 11mg/kg body weight per deer, per day for seven days. Medicated bait was offered for one week in early February 2007 and additionally 14 days later. Four weeks after treatment <15% of faecal samples contained *F. magna* eggs. One dissected liver contained live flukes, a second liver contained only dead parasites, a final liver contained no parasites. This study confirms *F. magna* infection in Western Vojvodina. Baiting corn feed with triclabendazole satisfied expectations and should be included in *F. magna* control programs in the Serbian deer population.

P69 Sexual selection and parasitism in amphipods

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Parasites affect host reproduction in a variety of ways, including manipulation of mating behaviour and sperm allocation strategies. Sperm competition theory predicts that males should prudently allocate limited sperm reserves, based upon the perceived return on investment. It has previously been shown that males of the amphipod *Gammarus duebeni* become sperm-limited after 3 consecutive matings. We therefore predict that *Gammarus* males will be selected to tailor their ejaculate size in response to female quality to achieve maximum reproductive success. This is supported by evidence that male *G. duebeni* donate less sperm to females infected by the microsporidian sex ratio distorter *Nosema granulosis* than to more fecund unparasitised females. However, *Gammarus* species are infected by a wide range of other parasites, including acanthocephalan and trematode species, and it is not yet known how these might affect sperm allocation. My PhD investigates the influence of several different parasites on gammarid mating strategies. Here we present data on the impact of infection on male and female fecundity, and aim to determine how males might allocate sperm differentially between infected and uninfected females.

P70 Effects of antioxidants on midgut infection rates in *Glossina palpalis palpalis*

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Glossina spp. vector African trypanosomes the causative agents of sleeping sickness and nagana throughout sub-Saharan Africa. Despite high prevalence in the animal populations infection rates in tsetse are usually quite low. Although susceptibility to infection in the fly is maternally inherited the actual mechanism remains a mystery although several theories have been put forward. We recently published the effects of antioxidants on infection rates of *T. b. brucei* in *G. m. morsitans*, showing that glutathione (GSH), N-acetyl-cysteine (NAC), uric acid and ascorbic acid all increased infection rates. Here we present the same experiments carried out in *G. p. palpalis*. Although addition of GSH and NAC to the infective bloodmeal could raise midgut infection rates to >95%, higher concentrations were required than that needed for *G. m. morsitans*. However, when uric acid or ascorbic acid were added to the infective bloodmeals, no significant difference was found when compared to control flies. The increased refractory of riverine tsetse seen in these experiments is similar to previously published work and suggests that the *G. p. palpalis* gut is a more hostile environment to trypanosomes than the *G. m. morsitans* gut.

P71 Comparative analysis of gene regulation in nematodes

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Haemonchus contortus is one of the most pathogenic nematodes of ruminants. It is a blood sucking abomasal parasite that can cause anaemia, oedema and subsequently death.
Nematode resistance to all three classes of anthelmintic drugs currently available is becoming an increasing threat to the livestock industry thus alternative control approaches are urgently needed. The increasing understanding of parasite gene organisation, function and regulation can be exploited to develop new ways to interfere with parasite development and survival.
The aim of this project is to compare gene regulation in the parasitic nematode *H. contortus* with the free-living nematode *Caenorhabdatis elegans.* The *H. contortus* genome is currently being sequenced at the Sanger Institute with approximately 9x coverage complete. We will focus on mechanisms of nematode gene regulation, looking at upstream regulatory regions and potential regulatory motifs. These motifs will then be tested in *C. elegans,* looking at expression both spatially and temporally. It has been suggested that in *C. elegans* gut expressed genes are controlled by a master regulator, the ELT-2 GATA transcription factor; using RNAi to target this regulator has shown a significantly reduced gut function. Our work will initially focus on regulation of *H. contortus* gut expressed enzymes.

P72 Screening of plant compounds for anthelmintic activity against ovine gastro intestinal nematodes.

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The increasing prevalence of anthelmintic resistance has led to research examining phytotherapeutics as a means of controlling gastro-intestinal nematodes.
Preliminary screening for 513 European plant extracts has been conducted as part of an EU Framework Six project (www.replace-eu.com) in an attempt to identify plants with anthelmintic activity.
A variety of *in vitro* tests can be used for detecting anthelmintic activity but the larval feeding inhibition test has proved to be the most sensitive where feeding activity can be disrupted through direct effects on the neuromusculature and/or other target sites for extract products. The larval feeding inhibition test (LFIT) has been adapted for use with aqueous plant extracts and was used as a primary screen. Polyethylene glycol (PEG) and polyvinylpyrrolidone (PVPP) were used as inhibitors to identify principal active plant secondary metabolites (PSM). Of the initial 513 plants samples, 119 were active i.e had an LFI_{50} estimate <1.25mg/ml. Twenty three of the most active plants were subsequently analysed further on the basis of these primary tests. The egg hatch test (EHT), larval exsheathment test (LET) and adult motility test (AMT) were used as a secondary screen. Five plants exhibiting anthelmintic properties from the primary and secondary screens were selected for tertiary *in vivo* screening. Worm-burden efficacies for the five plant compounds ranged between 0-42% and 21-37% against *Haemonchus contortus* and *Trichostrongylus colubriformis* respectively.

P73 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 differential profiles of resistant and susceptible nematodes, with subsequent application in biomarker and novel drug-target discovery, elucidation of drug action and resistance mechanisms. However, proteomic profiling of parasitic nematodes is limited by genomic sequence availability. This study combines expressed sequence tag (EST) data, bioinformatics and proteomic technologies to investigate parasitic nematodes of significant veterinary importance.

P74 Effect of genetic growth potential and nutrition on immune response to *Heligmosomoides bakeri* infection

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Using “Roslin” mice, divergently selected for high (ROH) and low (ROL) body weight, we have previously shown that narrow selection for high growth can reduce host resistance (increased parasitism) and resilience (reduced growth following infection). We have also shown that protein scarcity augments this penalty on resilience but that nutritional sensitivity of resistance is less pronounced. Here, we test the hypothesis that these effects concur with reduced underlying immune responses in ROH compared to ROL mice and that protein scarcity impairs immune function more pronouncedly in ROH than in ROL mice. A 2x2x2 factorial design was used with two levels of *Heligmosomoides bakeri* infection (0 and 200 L_3) and two levels of crude protein nutrition (40 (LP) and 230 (HP) g per kg) for both ROH and ROL mice. Worm burdens were higher in ROH than in ROL mice on LP only. Other interactions between mice line and protein nutrition were not observed. However, results showed that infection induced a Th2 biased (IL-4, IL-5) immune response, that ROL prioritised “inflammatory” cytokines (IL-6, TNFα, IFNγ) over antibody production (IgG1, IgG2a) compared to ROH and that a LP diet impaired both Th2 response and inflammatory cytokines compared to feeding HP.

P75 ES antigens of *Heligmosomoides bakeri* modulate TLR-Stimulated Dendritic cell activation

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Heligmosomoides bakeri (H. bakeri) is capable *of* surviving in the small intestine for extended periods of time in immunocompetent mice. *H. bakeri* is able to modulate the immune response through the release of excretory / secretory (ES) molecules. Dendritic cells (DC) play a vital role in shaping and driving the adaptive immune response and DC maturation begins with the binding of pathogen associated molecular patterns (PAMP’s). The effects of *H. bakeri* ES on DC activation of PAMP’s such as toll like receptor (TLR) ligands was investigated *in vitro*. *H. bakeri* ES alone failed to induce DC activation. However *H. bakeri* ES modulates TLR-initiated dendritic cell activation, both in terms of reduced cell surface expression of maturation markers such as CD40, CD86 and MHC II and in the production of inflammatory and regulatory cytokines. *H. bakeri* ES is therefore capable of reducing the responsiveness of DC to wide range of TLR ligands. It appears that the ES molecules responsible for this down modulation are unlikely to be proteins as heat inactivated *H. bakeri* ES also modulates TLR-initiated DC activation. Data illustrating the varying effects of a *H. bakeri* ES on a panel of TLR ligands will be presented with results indicating that this nematode is indeed capable of actively instructing DCs *in vitro.*

P76 Heterogeneity of antimonial-resistant *Leishmania donovani* in natural populations

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Antimonial (SbV) resistance in *L. donovani* has been repeatedly reported in the Indian subcontinent. The action mechanism of SbV is complex and includes stimulation of host macrophages to impose lethal oxidative stress on the intracellular parasites and reduction of SbV to SbIII, which has been shown to interfere with the parasite's redox system. In this study, we analysed Nepalese *L. donovani* populations that have adapted to SbV through clinical exposure. Clinical isolates with variable SbV susceptibility from two genetic subgroups were comparatively characterised through (i) transcriptomic/proteomic profiling of genes/proteins with a central role in parasite oxidative stress defence; (ii) quantitation of parasite thiols and (iii) assessment of parasite susceptibility to direct oxidative stress challenges. SbV-resistant isolates were found to have heterogeneous profiles with respect to their biochemical characteristics and oxidative stress susceptibility. The results suggest that natural adaptations emerging under antimonial treatment pressure are pleomorphic and depend on the genetic background of the parasite. The significance of these findings will be discussed in the context of epidemiological surveillance of drug resistance.

P77 Leishmania oligopeptidase B2

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Oligopeptidase B (OPB) is is a member of the prolyl oligopeptidase family of serine peptidases (Clan SC, Family S9). OPB has a restricted taxonomic range that includes plants, fungi, bacteria and trypanosomatids. In *Trypanosoma* OPB has been determined to be an important virulence factor. We have identified a second OPB-like gene in *Leishmania* (OPB2). 3D models of *L. amazonensis* OPB and OPB2 were obtained by homology modelling based on the structure of mammalian prolyl oligopeptidases using the Modeller program. *L. amazonensis* OPB and OPB2 differ in their protein surface, with a positive and neutral charge in OPB2, compared to a negative charge in OPB. However, there is conservation in the interface between the B-propeller and the catalytic domain with a mainly negative charge, mostly in S1 site. The putative S2 subsite of OPB2 has a Serine and Methionine instead of dibasic amino acids, indicating that OPB2 has a difference substrate preference. To characterize a substrate specificity of OPB2, recombinant protein has been expressed and purified from *E. coli*. Antibody raised against OPB2 in rabbit has shown expression of OPB2 in promastigotes. Molecular genetic analysis of OPB2 is underway, which will lead to a greater understanding of the role and necessity of OPB2 in *Leishmania*.

P78 DNA immunisation using different methods of immunisation results in different models of protection in *Leishmania mexicana*

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Leishmaniasis is a worldwide disease prevalent in 88 tropical and sub tropical countries. Many attempts have been made and different strategies have been approached to develop a potent vaccine against *Leishmania*. It has been shown that immunity to *Leishmania* depends upon the activation of Th1 immune responses but the role of cytotoxic T cells is not yet established. DNA immunisation with a bias to Th1 immune response is capable to employ CD4+ and CD8+ T cells to generate a high immunity to *Leishmania*. It has been shown that the alteration in the method of administration of DNA can alter the immunity induced by the administered gene. In the present study, *L. mexicana* gp63, an immunogenic surface glycoprotein, cDNA was administered I.M. and by using the gene gun and the immunogenicity induced by two methods of immunisation was compared. In addition, the CTL activity induced by *L. mexicana* gp63 was investigated. The results showed that gene gun immunisation induced higher immunity to *L. mexicana* infection compare to I.M. injection of the DNA inducing a sharp rise of Th1 immune response determined by detection of a high level of IgG2a antibody in the serum soon after the immunisation, and establishing a long-term CTL activity during the course of the infection.

P79 Intranasal vaccination with extracellular serine proteases of *Leishmania amazonenis* protects mice against homologous infection

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Leishmania amazonensis is the main agent of anergic diffuse cutaneous leishmaniasis. Our previous studies demonstrated that intramuscular (i.m.) immunization with whole *L. amazonensis* antigens (LaAg) enhances mouse susceptibility to cutaneous leishmaniasis, however, intranasal (i.n.) vaccination with LaAg confers protection. Serine proteases are known to play a crucial role in the host-parasite interaction regarding different protozoans. Using a single-step aprotinin-agarose chromatography, serine proteases were partially purified from aqueous cellular and extracellular extracts of *L. amazonensis* promastigotes (Sol-SP and Extr-SP, respectively). To try their effectiveness in i.m. and i.n. BALB/c mice were twice vaccinated by the i.m. or i.n route with 25ug of serine proteases fractions, prior to footpad infection with *L. amazonensis*. We found that i.m. immunization with either Sol-SP or Extr-SP promoted increased susceptibility to subsequent infection, similar to found previously with LaAg, irrespective of the presence of saponin as adjuvant. The same finding was observed with i.n. Sol-SP. However, i.n. Extr-SP induced a strong protective immunity, as seen by significantly smaller lesion sizes and parasite burden. Protection was accompanied by increased IFN-gamma and decreased production of TGF-beta and TNF-alpha in the infected footpads, as compared with non-vaccinated mice, whereas IL-10 remained unchanged. This study shows that Extr-SP is a good and more defined vaccine candidate for adjuvant-free mucosal vaccination against cutaneous leishmaniasis.

P80 Irradiated *Leishmania mexicana* metacyclics as a potential vaccine candidate

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Studies carried out using irradiated promastigotes as vaccine candidates against leishmaniasis have previously given varying degrees of protection in mice. EXPeriments carried out demonstrated that when metacyclics were gamma irradiated with 63 krad they remained viable but were unable to infect BALB/c mice. Furthermore vaccination of BALB/c mice with irradiated metacyclics was able to provide significant protection upon challenge with viable and infective parasites four weeks after immunisation. The significance of this protection was explored by utilisation of the different components of the infective sandfly challenge inoculums, including saliva and promastigote secretory gel.
Results show that irradiated *L. mexicana* metacyclics are also able to offer protection over a longer period of time; but eventually cause lesions at the site of vaccine inoculation. In-vitro studies were carried out to ascertain how this unexpected result has arisen. EXPeriments focussed on the ability of irradiated parasites to transform into different life cycle stages and assessment of DNA damage via utilisation of a “comet assay”. Results showed that both irradiated *L. mexicana* amastigotes and metacyclic promastigotes were able to transform post irradiation *in-vitro* but that metacyclics are unable to repair their DNA damage *in-vitro* post irradiation over a 14 day period. Longer term experiments reflecting the time taken for lesions at the site of inoculation to develop are currently being carried out.

P81 The Development of Rapid Diagnostics for Leishmaniasis

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Leishmaniasis is classed as a neglected tropical disease and is caused by the protozoan parasites found in the *Leishmania Ross*, 1903 genus. Transmission in mammals occurs during the bite from the female sandfly *Phlebotomus spp.*, in the old world and *Lutzomyia spp.* in the new world. Humans, wild and dosmesticated animals serve as the reservoir for future infections. The development of rapid, specific and sensitive assays remains a high priority due the variation in disease manifestation. The sequencing of different *Leishmania spp.* has opened avenues for new targets of diagnostic importance.
The various forms of disease manifested in humans include visceral/kala-azar [VL] (*L. donovani complex)*, post-dermal kala-azar [PDKL], *mucutaneous [ML]* (L. *viannia spp.*), cutaneous [CL] and diffuse cutaneous leishmaniasis [DCL]. The ability to distinguish *Leishmania spp.* in the *L. donovani complex* and in the *L. viannia* subgenus is necessary to select the appropriate treatment regime. Diagnostic work at LSHTM has resulted in the development of a real-time PCR (qPCR) assay for ML through targeting *L. viannia spp*. This assay has been implemented in a clinical setting at the Hospital of Tropical Diseases in London. The development of a one step PCR assay identifying organisms able to cause ML and VL is the ultimate aim. Ongoing work to identify new targets for qPCR diagnosis will be presented.

P82 Present status of Cutaneous Leishmaniasis spread from Afghan refugees to the local Pakistani population in some areas of North West Pakistan

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Leishmaniasis is a major health problem worldwide. Pakistan has a burden of Cutaneous Leishmaniasis and has been reported from all the provinces. The most common type is called anthroponotic Leishmaniasis. Prevalence appears to be much higher in Persian, Pashton, Tajak, Uzbek and Turkmen tribes migrated from Afghanistan and Iran .It is endemic in NWFP, Baluchistan, Sindh, and Punjab.
The present study characterized the epidemiology of cutaneous Leishmaniasis in Afghan refugees and local population in various areas of NWFP.
A total of 515 suspected people were examined of which 281 were found positive for dermal Leishmaniasis (Males 63.12%; Females 38.06%). Prevalence of Leishmaniasis was higher in local population (65.62%) than Afghan refugees (36.41%). The burden of infection was highest among children of 0-9 years (66%). Lesions were more common on face (61.53%).
Average duration of lesions was 1-9 months. Prevalence of dry lesions (62.32%) was higher than wet type (37.68%). Patients previously treated with sodium stibo-gluconate had lower incidence (28.98%) than untreated patients (63.92%). Isolates of *Leishmania species* were identified after culturing in NNN medium. Susceptibility to infection with *Leishmania* promastigotes was examined in 30 BALB/c mice divided in 19 groups in three different experiments. Majority of mice developed skin nodules with punch-out ulcers 5-8 weeks post-infection from 0.1-1.2 cm in diameter.

P83 Developmental adaptation to the mammalian immune response by a filarial nematode

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Parasites are known to suppress, subvert or otherwise evade the immune responses of their hosts. Reports even suggest that elements of the immune system can enhance the development of parasitic helminths. We have previously shown that the filarial nematode *Litomosoides sigmodontis* is able to modify its larval development in response to type 2 immunity. We and others have also shown that elements of this response, more specifically polynuclear eosinophils, are predictive of parasite killing at two distinct phases of the filariae's life cycle: at their delivery to the host and months later as adults. Eosinophils are also pivotal in the establishment of vaccine-induced immunity. Here we show that *L. sigmodontis* has evolved to accelerate its larval development in response to IL-5-driven eosinophilia at the point of infection, in the skin. We also show that this results in increased fertility and transmission potential of the worms, suggesting a fitness advantage of maintaining the immune-dependant developmental plasticity that we have described. Taken together, our data provides grounds for a better understanding of host-specificity and the evolution of parasitism, and, more urgently, calls for caution in the design of anti-filarial vaccines.

P84 Helminth parasites of three sub-species of lizards (genus Uromastyx)

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Lizards of the genus *Uromastyx* have become popular as pets during the last few years; the huge genus species *U. acanthinura* is edible in Saudi Arabia and probably other Arab countries.
A parasitological survey was carried out on three sub-species *U. ocellata ocellata, U. ocellata ornate*, and *U.acanthinura*.
Results revealed infection of most specimens with an oxyuroid gut, *U. ocellata ocellata* showed a prevalence rate of 85%, *U. ocellata ornate* 100%, and *U. acanthinura* 85.7%. This nematode is placed temporarily into genus *Thelandos*, as it exhibits certain features that should be further studied.
A cestode which belongs to genus Oochoristica (Luhue, 1898) was detected only in gut of *U. acanthinura* with a prevalence rate of 7.1%.
These animals were purchased from a herpetoculturist and were kept in captivity for a while. This may explain the scarcity of cestode infection as cestodes develop first in an intermediate host, an insect in this case. High prevalence of the gut nematode may be accounted for the direct life cycle.
Infection with trematode is NOT expected, because this needs living in environment rich in water and vegetation.
This work represents the first survey on parasites of *Uromastyx* lizard in the Sudan.

P85 The utility of the microturbellarian, *Macrostomum lignano* as a model for flatworm parasites

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Parasitic flatworms represent a growing economic burden as disease-causing agents in humans and animals that is exacerbated by the shortcomings in the efficacies of the drugs that are currently used to treat them. The flatworm nervous system is important in controlling a range of behaviours that are critical to worm survival and therefore represents a potential source of novel drug targets. Due to the practical difficulties associated with maintaining and manipulating laboratory populations of flatworm parasites, there has been little progress in drug target elucidation and validation. Potential flatworm models have recently been uncovered as a result of increasing genomic and transcriptomic datasets for flatworms and their receptiveness to key reverse genetics techniques including RNA interference (RNAi) and suitability to diverse phenotypic assays; these include the triclad planarians *Schmidtea mediterranea* and *Dugesia ryukyuensis*, and the microturbellarian *Macrostomum lignano*. This study aims to establish *Macrostomum lignano* as a laboratory model with which to probe the structure and function of the flatworm nervous system, and assess its utility as a representative for parasite species. Preliminary results highlight the susceptibility of a *Macrostomum actin*-encoding gene to RNAi as assessed by RT-PCR, immunoflouresence and phenotype analysis. Additional neuronal targets with homologues in parasite species are under investigation.

P86 Novel Control Strategies for the Root-Knot Nematode *Meloidogyne minor*

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The phasing out of popular chemical nematicides has lead to increasing difficulties in plant parasitic nematode management, in particular the newly emerged root-knot nematode *Meloidogyne minor.* Infestation by this nematode results in stunted development of turf-grass and consequently the appearance of yellow-patch disease. Although *M. minor* has been found in potato crops, its appearance is steadily increasing in sports amenity sites. Investigations in the UK and Ireland during 2006-2007 indicated that 70% of soccer pitches (n=76), and 76% (n=443) of golf greens were infected with *M .minor*. Sports turfs are exposed to an unnatural level of stress, and it is believed that a highly stressed plant will show severe symptoms of nematode infection, even at a moderate to low nematode level. Biostimulants are naturally derived compounds which are believed to increase a plants ability to tolerate stress. Biostimulants we have tested (Algaegreen, Nemago, and Eaglegreen) have shown significant increases in root development of turf-grass (29% increase in root mass, $p<0.01$, 8% increase in root length, $p< 0.01$), and we believe this increase to plant health may ease the appearance of disease symptoms. This study aims to show how environmental stress can affect infection level and appearance of symptoms, and to investigate if biostimulants can ease the effects of environmental stress.

P87 Molecular characterization of *Neospora caninum* from Axis deer (*Axis axis*) in Argentina

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Neospora caninun infection was diagnosed in Axis deer at the zoo of La Plata, Argentina by serological, histopathological and molecular techniques. Gerbils (*Meriones unguiculatus*) were inoculated with brain tissues of one fawn, which presented dilatation of the anal sphincter and incontinence at birth, developed weakness and ataxia, and died at 14 days of age. The fawn had an IFAT titer of 6400 for *N. caninum* and thick-walled protozoan cysts were observed in brain samples. Four neonates that died in the same enclosure between May and September 2007 and 12/13 asymptomatic adult axis deer had reciprocal IFAT titers for *N. caninum* between 25 and ≥ 6400. The *N. caninum* infection in gerbils was confirmed by serology (reciprocal IFAT titer ≥ 800) and PCR techniques. Two of five inoculated gerbils developed ataxia and bent head at 22 and 26 days post inoculation, respectively. *N. caninum* DNA was detected in brain samples from the fawn and from one neonate. By multilocus microsatellite analysis based on the *N. caninum* microsatellites MS1B, 2, 3, 5, 6A, 6B, 10, 12 and 21 both DNA samples showed the same microsatellite pattern. This pattern was different from all other isolates from Argentina and other countries.

P88 Presence of *Neospora caninum* in the genital tract of infected bulls

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The presence of *Neospora caninum* DNA in the genital tract tissues of 3 naturally and 9 experimentally infected bulls by means of a nested-PCR was performed. In addition, histology was carried out in genital tract tissues of some experimentally infected bulls. Neospora DNA was consistently found in the epididymus (100%) and testicles (76%). Other sporadic locations were accessory glands (33%). In naturally infected bulls, parasite was also detected in ductus deferens, and cremaster muscle and penis. In all experimentally infected bulls investigated, the histopathological findings were focal or multifocal aggregates of lymphocytes and plasma cells, mainly located in epididymus (68%), accessory glands (23%), testicles (5%), and penis (5%). Disseminated lymphocytes and plasma cells infiltrating were observed in 20% of the samples and were located mainly in epididymus and penis. The comparison between the presence of lymphoplasmacytic aggregates with PCR positive results showed low concordance (Đº-value = 0.104). No specific immunostaining was detected in any of the positive nested PCR samples. Acknowledgements: Funding for this work was provided by research grants from the Spanish government (AGL2000-0112-P4-03 Pr95-0780.OP and RTA2006-00086-C02).

P89 Epidemiology and Diagnostics of *Neospora caninum* infection in cattle

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Neospora caninum is an apicomplexan parasite, closely related to *Toxoplasma gondii*. The parasite has a world-wide distribution and was first described as a pathogen of dogs, the definitive host, in the early 1980s. Subsequently, it was shown to be a major cause of bovine abortion. The parasite may be transmitted horizontally by the definitive host or vertically transmitted from infected dam to foetus. Epidemic transmissions of the parasite can result in abortion storms which are usually followed by endemic transmission within the herd. Diagnostic methods for the parasite traditionally include pathology and serology. We have improved DNA based detection of Neospora in aborted foetuses. In 380 brain samples from aborted foetuses, 85 were found to be positive by PCR for the presence of Neospora. This confirmed, in conjunction with veterinary diagnosis of the same samples, that the parasite is the most frequently detected pathogen in bovine abortions. Although Neospora is a major cause of bovine abortion, relatively little is known about it's transmission dynamics. To discover more about Neospora epidemiology and the importance of the different transmission routes we have set up longitudinal studies within dairy herds in the Southwest of Scotland. Initial whole herd sero-prevalences from these farms were recorded as 8.5%, 11.8%, 32.6% and 41.5%.

P90 *Neospora caninum* in dairy herds in Schleswig-Holstein, Germany

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The aim of a cross-sectional survey performed between November 2005 and April 2006 was to obtain actual information on the occurrence of N. caninum infection in dairy cows of Schleswig-Holstein. It was planned to examine tank-milk of about 35% of the approximately 6,000 dairy herds in Schleswig-Holstein for *N. caninum*-specific antibodies using a p38-tank-milk ELISA. This test is able to detect a within-herd *N. caninum*-seroprevalence of >10%. Only 20 (1.0%) out of 1,950 tank-milk samples from Schleswig-Holstein reacted *N. caninum*-positive. A significantly higher proportion of positive herds came from districts with a human population density >125 per km^2 (P = 0.022, Fisher exact). These districts were located close to the city of Hamburg. A prior survey in Rhineland-Palatinate performed with the same ELISA had revealed a proportion of 7.9% of tank-milk-positive herds. The results of the present survey therefore confirm prior assumptions that *N. caninum* infections are variably distributed in different German federal states. Similar to the results obtained in Rhineland-Palatinate, the observation of positive herds in the present study was associated with an elevated population density.

P91 Comparative infection dynamics of newly isolated and laboratory passaged *Nippostrongylus brasiliensis*

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Anecdotal reports suggest that repeated passage of *Nippostrongylus brasiliensis* leads to abbreviated infection in laboratory rats. The recent isolation of *N. brasiliensis* from a wild rat in Japan led us to compare infection dynamics with a multiply-passaged laboratory strain. Male SD rats were infected sub-cutaneously with 2000 infective larvae of each strain and parameters of infection compared. The number of adult worms establishing in the jejunum was identical, indicating no difference in infectivity. Differential features of the Japanese isolate were a) greater persistence in the gut, with the majority of worms surviving 3 days longer than the laboratory strain, and b) higher fecundity and cumulative egg output. The immune response to each strain was very similar, with comparable levels and dynamics of type 2 cytokine production, goblet cell hyperplasia and mast cell protease release. Comparison of secreted proteins by 2D SDS-PAGE suggested a more complex profile for both L3 and adult worms of the Japanese strain. These data suggest that repeated high dose passage leads to a reduced ability to persist in the intestinal tract, but it is as yet unclear whether this is attributable to specific factors or a generalised loss of fitness.

P92 Investigating RNA interference in Nippostrongylus brasiliensis

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Since the discovery of RNA interference (RNAi) in the free living nematode *Caenorhabditis elegans,* its potential for the investigation of gene function has been exploited in many species. RNAi has been investigated in several species of parasitic nematodes with mixed results. Whereas in some systems, notably plant parasitic nematodes, the technology appears to give high levels of specific transcript knockdown, in most animal parasitic species the results are equivocal and difficult to reproduce. Such is the case in the rat parasite *Nippostrongylus brasiliensis*, in which the first report of RNAi in animal parasitic nematodes was made.
Two hypothesis for the inconsistency of RNAi in nematodes have been proposed; that some species may lack necessary genes for a functional RNAi pathway, or due to the difficulty of delivering sufficient RNA into the body and cells of the worms.
To address the latter hypothesis we have attempted RNAi by the standard method of soaking worms in double stranded (ds)RNA for several nematode genes which have been successfully silenced in previous studies. In addition we have tested other methods of delivery using dsRNA and fluorescent reagents in order to increase the efficiency of delivery. Data will be presented on the utilisation of electroporation, several different transfection reagents and biolistics for RNA delivery in *N. brasiliensis.*

P93 Screening of natural African plant extracts for anthelmintic effects in helminths

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Helminth infections are a major cause of worldwide economic loss in livestock farming through reduced animal productivity and death. The increasing reports of multi-drug resistant parasites of livestock and the absence of alternative chemotherapeutics, threatens the long-term sustainability of the livestock farming sector. Alternative control strategies need to be identified and explored. Natural products, including numerous plant species are commonly used for ethnoveterinary purposes in Africa to combat a wide variety of diseases including parasitism. This study examines the anthelmintic potential of a range of African plant species for which there is anecdotal evidence of utility as anti-parasite therapies. Specifically, crude methanolic extracts of nine African plant species were screened for their effects *in vitro* on (i) the motility of the free-living nematodes, *Panagrellus redivivus* and *Caenorhabditis elegans* and the microturbellarian, *Macrostomum lignano* and (ii) the contractile activity of the gastrointestinal parasitic pig nematode *Ascaris suum*. The promising biological activity displayed by a number of the extracts examined warrants further investigation and active compound identification. This study indicates that the ethnoveterinary use of plants may have potential to provide new lead compounds for the control of parasites of livestock.

P94 A novel Latex-agglutination test for the detection of histidine-rich protein II in malaria infected individuals.

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We have developed a latex agglutination test based on the specific detection of *Plasmodium falciparum* histidine-rich protein II (HRPII). Most rapid diagnostic tests for the detection of malaria on the market are lateral flow tests. An agglutination test has the benefits that, in general, production costs are lower and that they have increased stability compared to lateral flow and dipstick tests.
Polystyrene-carboxylated beads were covalently bound with two mouse monoclonal antibodies, an anti-HRPII IgG and IgM. The beads were spotted on agglutination cards. The agglutination test was evaluated with sera of patients with microscopy-confirmed *P. falciparum* malaria and with sera from patients suspected with the febrile illness leptospirosis for which they tested negative and with other control sera. The same samples were tested with a lateral flow test that detects HRPII, Paracheck Pf, in parallel. For the agglutination test a sensitivity of 92.1%±6.3% and specificity of 72.7%±10.4% compared to microscopy were obtained and for the Paracheck Pf 89.5%±7.1% and 69.7%±10.7%, respectively. The positive predictive value for the agglutination assay was 80% and 77% for the Paracheck Pf; the negative predictive values were 89% and 85%, respectively.
This project is part of a collaborative effort for the improvement of malaria diagnosis of The Foundation for Innovative New Diagnostics, Geneva, Switzerland

P95 Phenotype and genotype studies of two transfected lines of *Plasmodium berghei*.

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The genome of *Plasmodium* encode a number of large multipass-transmembrane (TM) domain proteins of unknown function. Several of these are phylogenetically-conserved and contain features that would indicate a role as integral membrane receptors or channels. We have identified two putative membrane receptors that are largely conserved in the *Apicomplexa*; a conserved membrane protein with largely unknown function, that is a homologue of a human protein empirically shown to activate MAPK signalling pathways, and a predicted 7-TM protein found in a family of eukaryotic 'lung 7-TM receptors' that are annotated as G-Protein Coupled Receptors. To investigate the function of these proteins we have carried out gene deletion in the rodent malaria model, *P.berghei*. We will describe the bioinformatic features of these proteins and the phenotype of gene-knockout parasites throughout the life-cycle in mouse and mosquito.

P96 Calcium-activated potassium channels as targets for novel antiparasitics.

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The world wide spread of diseases associated with parasitic worms is a huge economic and social burden on society. Anthelmintic drugs are effective tools to control nematode related infections. However, the overuse of currently available drugs has led to the increasing resistance of parasitic nematodes to these compounds. This resistance requires the design of new compounds with novel modes of action.
Emodepside belongs to the class of cyclooctadepsipeptides and is effective against a wide range of gastrointestinal nematodes resistant to other anthelmintics. In our lab this drugs was shown to paralyse body wall muscle and pharyngeal muscle in a model organism *Caenorhabditis elegans*. Emodepside also inhibits egg-laying by possibly exhibiting its paralytic effects on the egg-laying muscles.
A possible mode of action of emodepside is via latrophilin receptors by an unknown mechanism. Recent work in our lab has provided further insight into the mechanism of action of this drug. Mutagenesis screening for the worms resistant to the paralytic actions of emodepside revealed nine none complementing lines of *C.elegans* with mutations in gene V that refer to the calcium activated voltage gated potassium channel SLO-1 (also known as BK, Maxi K, KCNMA1). The possible mode of action of emodepside is inhibition of a neurotransmitter release in neuromuscular junction of nematodes through the pathway involving SLO-1 channels.

P97 Infestation of sheep with Psoroptes ovis, the sheep scab mite, results in recruitment of Foxp3$^+$ T cells into the dermis

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Regulatory T cells (Treg) play a central role in the termination of immune responses. Defects or dysfunction of these cells may lead to aggravated pathogen-induced inflammation. Infestation of sheep with *Psoroptes ovis* results in a pronounced cutaneous inflammatory response which appears to be crucial for mite survival. We hypothesise that (i) Treg are involved in sheep scab lesions and (ii) Treg responses may crucially affect lesion development and subsequent mite survival. FoxpP3 is a key transcription factor required for generation and maintenance of Treg in rodents and humans, and is the most widely used marker for Treg in these species. In this study we sequence ovine Foxp3 and show that it exhibits a high degree of homology with Foxp3 from other species. Using a validated immunohistochemical staining technique we demonstrate that infestation of sheep with *P. ovis* results in an influx of FoxpP3$^+$ T cells into the dermis. Future work will investigate the regulatory function of ovine Foxp3$^+$ T cells and determine whether *P. ovis* is capable of directly or indirectly modulating ovine Treg responses.

P98 Variation within the Internal Transcribed Spacer (ITS) of ribosomal DNA genes of *Biomphalaria pfeifferi*

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Schistosomiasis is a wide spread parasitic disease that affects man and animals. It is caused by a unisexual blood trematode of the genus *Schistosoma* and transmitted by fresh water snails. *Biomphalaria pfeifferi* serve as intermediate hosts for *Schistosoma mansoni* which cause intestinal human schistosomiasis.
The present study was carried out to investigate the intraspecific variation among *Biomphalaria pfeifferi*, intermediate host snails of intestinal schistosomiasis in Sudan. Snail were collected from field sites and were identified morphologically.
Intraspecific variation in *Biomphalaria pfeifferi* was assayed by Polymerase Chain Reaction (PCR) amplification of the ribosomal Internal Transcribed Spacer (ITS) region followed by Restriction Fragment Length Polymorphism (RFLP) analysis of this product with three restriction enzymes (*RsaI*, *AluI* and *MspI*). RFLP analysis of Internal Transcribed Spacer (ITS) with *RsaI*, *AluI* and *MspI* showed intraspecific variation in *Biomphalaria pfeifferi*. Each restriction enzyme yielded two profiles types in *Biomphalaria pfeifferi*.

P99 Metabonomic investigations of the intermediate snail hosts for *Schistosoma haematobium*: species differentiation and impact of infection

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Metabonomics has been used to describe metabolic changes associated with parasite infections, for example the mouse – *Schistosoma mansoni model.* In this study the approach has been extended to investigate biochemical factors in the intermediate snail host that might predispose or protect against schistosome infection. High – resolution nuclear magnetic resonance (NMR) has been employed to investigate differences between aqueous extracts of three species of *Bulinus,* two of which act as the intermediate host for *Schistosoma haematobium*. Multivariate analysis, such as principal component analysis (PCA), can differentiate species based on metabolic profiles. Results from preliminary investigations using ^{1}H – NMR spectroscopy on snails exposed to three miracidia of *S. haematobium* (at 72 hours post exposure) and unexposed snails differentiated between infection status. Metabolic profiling at different stages of the infection in the intermediate snail host should provide more information on biochemical changes associated with the host-parasite interaction.

P100 Modulation of human basophils function and characteristics by *Schistosoma mansoni* infection

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Helminth infections are associated with elevated levels of parasite-specific and of total circulating IgE. Basophils are the cell type with the highest surface expression of high affinity IgE receptors, FcεRI, which bind circulating IgE, therefore permanently arming this short-lived cell. IgE cross-linking of basophils' surface IgE results in the degranulation of the cell and the release of mediators such as histamine as well as helminth-associated type 2 cytokines IL-4 and IL-13. There is good evidence that schistosome infection may influence these activities. In the current study, in an area of Kenya endemic for *S.mansoni*, blood samples were donated by adults before and 7 weeks after anti-helmintic treatment. By measuring parasite specific IgE and using histamine-release as a measure of basophil function and flow-cytometry to phenotypically characterize these cells, we have investigated the influence of *S. mansoni* infection on basophil biology. We report the differences between infected and uninfected individuals and describe the changes that occur following chemotherapy.

P101 Human schistosomiasis: IgE responses to parasite allergen-like antigens in an infected population following drug treatment

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Human *Schistosoma mansoni* infections are characterised by a typical T-helper 2 response including eosinophilia, elevated IL-4, IL-5, IL-13 and IgE. Immunity to reinfection after praziquantel treatment increases with age, and is associated with high levels of parasite-specific IgE, which also increase in an age-dependent manner. Conversely, IgG4 has been linked with susceptibility to reinfection and may act to block protective responses. Exact target antigens and resulting effector mechanisms remain unknown; however, some members of a family of parasite-derived tegumental allergen-like (TAL) proteins have been identified as strong potential targets for this form of acquired immunity. A study cohort of 447 males aged 7-76 years were selected from Musoli, Uganda and blood samples were collected along with parasitological data prior to and 9-weeks post-treatment. Levels of IgG1, IgG4 and IgE specific for a series of TAL antigens were measured, with IgE measured in the presence and absence of IgG. Marked differences were found between adults and children with respect to their TAL-protein specific antibody responses, suggesting interesting changing patterns of competition between antibody isotypes, which may have a resulting effect on effector cell function.

P102 GeneDB: a curated resource for the analysis of pathogen genomes

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The challenge of providing access to and integrating sequence data with biological knowledge is immense. GeneDB (http://www.genedb.org/) provides a single point of entry for sequence and annotation of 17 parasitic organisms, with a particular emphasis on *Kinetoplastida*, *Apicomplexa* and the parasitic helminth *Schistosoma mansoni*. The resource combines data from finished and ongoing sequencing projects with computational predictions and manual curation, aiming to provide up-to-date, high quality and consistent datasets The poster will focus on how:
1) GeneDB displays primary annotation (e.g. predicted peptide properties and protein domains, gene ontology annotations, similarity information, database cross-references) and integrates these with expression, phenotype and orthologue data.
2) Coding sequences can be viewed in the genomic context via the genome browsers Artemis and Gbrowse.
3) Sequence updates and curation are handled within GeneDB. The majority of available pathogen genomes are still actively being finished. Frequent sequence updates are coupled with ongoing curation, including the re-annotation of *Plasmodium falciparum* and the curation of trypanosomatid genomes.
4) How GeneDB and EuPathDB interact to provide integrated and concurrent resources, supporting user choice of interface and data display yet simultaneously providing the ability to query the same data types.

P103 Sensitivity and specificity of Kato-Katz and CCA tests for Schistosoma mansoni after mass drug administration

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Due to logistical and financial constraints, the andlsquo;gold standard' for schistosome diagnosis of three to five double Kato-Katz (KK) smears, is often not possible. As mass drug administration (MDA) continues and infection intensities and prevalences reduce, the compromise of only two KK may no longer be acceptable. In three Ugandan primary schools we tested the sensitivity and specificity of one to three days of double KK and a circulating cathodic antigen (CCA) test pre-, one week post- and four weeks post-praziquantel treatment, with three double KK as the andlsquo;gold standard'. The sensitivity of two KK smears on one day was 77.1% pre-treatment but only 60% four weeks post-praziquantel with sensitivity positively associated with the number of smears pre- (r=0.947, p=0.004), one week post- (r=0.922, p=0.009) and four weeks post-treatment (r=0.926, p=0.008). Two KK readings had a specificity of only 55.6% pre-treatment. CCA sensitivity was 90.4% at baseline, but 66.7% one week post-PZQ-treatment. Although intra-specimen and inter-day variation has previously been demonstrated, we have shown here the importance of increasing smear numbers as infection intensities and prevalences decrease. As MDA continues more KK's are required so that treatment successes are not overestimated by inaccurate diagnoses particularly in low intensity regions, and that non-cleared infections and potential drug-resistant parasites are identified.

P104 Genomic Insights into the Evolution of Schistosoma Sex Chromosomes

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Schistosomes have evolved genetically predetermined sex and have defined sex chromosomes with females being the heterogametic sex. Using the *Schistosoma mansoni* genome data over 70 genes have been identified, of which 15 are also found on the sex chromosomes of other heterogametic organisms. These genes are found in both the non-recombining and psuedoautosomal regions of the sex chromosomes allowing an understanding of divergence levels between the Z and W to be estimated and indicate that parts of the sex chromosomes diverged at different intervals during the course of their evolution. Phylogenetic analysis of these sex-linked genes in the genus *Schistosoma* produces trees similar to those of mitochondrial genes, suggesting similar evolutionary processes and providing further support for the "Out of Asia" hypothesis.

P105 Effector and regulatory responses to schistosomiasis vary with host infection intensity and age

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Schistosoma haematobium induces a complex immunological response in humans, resulting in a distinct epidemiological pattern of immunity. Adult parasites, the life stage which is most long-lived in the human host are systemic and in constant contact with the host\'s blood. This study was designed to assay the antibodies and cytokines in the plasma to indicate the immunological milieu which the adult parasites exist in. Levels of parasite-specific antibodies (IgA, IgE, IgG1, IgG2, IgG3, IgG4 and IgM) directed against three different *S. haematobium* life stages cercariae, eggs and adult worms and plasma levels cytokines (IFNγ, IL-2, IL-4, IL-5, IL-10, IL-13, IL-17, IL-21, IL-23) were measured by ELISA and related to infection intensity and host age. Correlation and factor analyses showed a relationship between Th-2 and -regulatory responses against *S. haematobium*. While IL-2, 21 and 23 were strongly correlated with each other. IL-17 was not correlated to any other cytokines. Both the antibodies and cytokines showed distinct patterns with age which differed between egg positive and egg negative people. For example levels of IL-4, 5, 17, 21 and 23 were highest in the oldest egg negative people indicating that these responses were associated with resistance to infection/re-infection.

P106 Transgenesis of schistosomes: approaches with HIV-1 lentivirus

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Retroviral transduction of *Schistosoma mansoni* offers the potential for germ line transgenesis and thereby a tool for elucidating gene function and expression. Constructs were prepared using Invitrogen's promoterless lentivirus system. We engineered four lentivirus constructs, two which included *S. mansoni* promoters for the actin gene and two with the spliced leader RNA gene. Each promoter was cloned upstream of either the genes encoding enhanced green fluorescent protein or luciferase (act-GFP, act-Luc, SL-GFP and SL-Luc). We investigated these lentivirus virions pseudotyped with vesicular stomatitis virus glycoprotein (VSVG) for the ability to transduce *Schistosoma mansoni* eggs, sporocysts or schistosomules. We delivered the virus into the schistosome either by soaking the flukes in the presence of the virus or by square wave electroporation. Integration of lentiviral reporter transgene into the schistosome genome was investigated by PCR analysis; we identified numerous integration sites of the lentiviral transgene in schistosome chromosomes. These findings definitively demonstrated that lentiviral mediated somatic transgenesis of schistosome had taken place, and moreover they suggest a tractable route forward towards heritable transgenesis of schistosomes.

P107 New sequencing technologies applied to the transcriptome of *Schistosoma mansoni*

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Schistosoma spp. are helminth parasites responsible for schistosomiasis. *S. mansoni*, one of the most clinically relevant species, is endemic in regions of Africa and South America where it causes severe morbidity and economic losses. Praziquantel, active against the adult schistosome, is the front line drug for treatment of schistosomiasis. Although effective, it does not protect against re-infection and the possibility of the emergence of resistant strains is a constant threat. Therefore, the search for new drug targets for intervention and vaccine candidates is vital.
In this context, a combined effort has led to a draft genome sequence of S. mansoni. EST libraries and microarray data from different life stages have been successfully used in the identification of gene structures and the study of differentially expressed genes throughout the parasite's life cycle. However, due to the complex nature of the genome and its fragmentary draft sequence, even these data are insufficient to accurately resolve exon-intron boundaries or splice variants. Our work describes the use of ultra-high throughput sequencing technologies to provide accurate gene predictions based on transcriptome sequencing. With this approach we aim to gain better understanding of gene structures, further improve the annotation of the *S. mansoni* genome and provide a platform for more precise automated annotation. The prospects of using these data for quantitative analysis of gene expression are discussed.

P108 Protein kinase signalling in *Schistosoma mansoni*: a possible role for p38 MAPK in miracidial swimming

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Mechanisms of kinase signalling and the biological significance of such signalling in *Schistosoma mansoni* are not yet fully elucidated. This study focused on p38 mitogen-activated protein kinase (p38 MAPK) activity in different *S. mansoni* life stages and the functional role of p38 MAPK in miracidia. Western blotting with anti-phospho p38 MAPK monoclonal antibodies detected a 43 KDa protein in protein extracts of male and female adult worms, miracidia, post-miracidia, and mother sporocysts. Furthermore, enzymatic activity of the p38 MAPK-like protein was confirmed by immunoprecipitation and kinase assay. Osmotic stress induction with sorbitol (400mM) or treatment with the p38 MAPK activator anisomycin (2μM) increased p38 MAPK phosphorylation in miracidia after 15 min and, surprisingly, attenuated considerably miracidial swimming speed. Fluorescence confocal microscopy demonstrated that phosphorylated p38 MAPK is predominantly associated with the ciliated plates of intact miracidia, and that phosphorylation in this region is greater on miracidia that are stationary within the egg. Together these findings support a role for p38 MAPK in the regulation of swimming behaviour in *S. mansoni* miracidia.

P109 *Schistosoma mansoni* haemozoin affects macrophage phenotype in a changing cytokine milieu

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Adult worms of the genus *Schistosoma*, resident in the host circulation, are haematophagous; the digestion of haemoglobin releasing the pro-oxidant free heme moiety. Schistosomes, in common with *Plasmodium*, solve this problem via the crystallisation of heme into the less reactive pigment haemozoin. Heamozoin is regurgitated by the worm into the host circulation and accumulates in the liver, the primary site of immune driven pathology, where it is phagocytosed by macrophages. In murine experimental infections survival and pathology limitation have been shown to be highly dependent upon a subtly balanced and carefully regulated immune response, in which the activation status of macrophages may be key. Macrophages in the liver are exposed to a changing cytokine milieu during infection ranging from early Th1 type stimuli dominated by IFNγ, to later Th2 type stimuli dominated by IL-4 and IL-13. Using natural Hz extracted from *Schistosoma mansoni* worms and exhaustively purified, we demonstrate that this substance has no innate stimulatory effect on macrophages but that it does have notable effects on their activation in response to cytokine stimulation *in vitro.* This is especially marked during polarisation switching, from a classically to an alternatively activated phenotype in response to a shift from IFNγ to IL-4 stimulation. Under these circumstances, when haemozoin is present, macrophages develop a mixed activation phenotype indicated by both iNOS and arginase activity.

P110 Liver cellular responses to *Schistosoma mansoni* eggs

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Of the 200 million individuals with Schistosomiasis, 5-10% display chronic symptoms including liver fibrosis associated with deposition of parasite eggs in the sinusoids. Hepatic stellate cells (HSC) are thought to drive fibrosis and investigating their interactions with schistosoma eggs could lead to improved understanding of fibrogenesis in Schistosome infection.
HSC responses to either viable or dead *Schistosoma mansoni* eggs were determined in human cells (LX-2) by analysis of key fibrogenic markers using quantitative analysis of gene expression (qPCR) and morphological imaging techniques.
HSC display an active myofibroblastic phenotype with high expression of fibrotic markers including α- smooth muscle actin (α-SMA), type I collagen and connective tissue growth factor (CTGF) when cultured alone. As we reported previously, in the presence of viable *S.mansoni* eggs HSC adopted a quiescent phenotype. In this study, non-viable eggs also induced decreases in expression of several fibrotic markers and parallel increases in markers of quiescence, even in the presence of transforming growth factor-β (TGF-β), a potent activator of fibrosis, although differences in the magnitude of effects was noted compared to viable eggs. In particular, an increase in relative expression of CTGF by qPCR in non-viable-treated cells was observed. In summary, the data indicate HSC exhibit differential responses to viable and non-viable eggs but with a common trend towards quiescence of HSC. The mechanism by which eggs exert these anti-fibrotic effects requires further investigation.

P111 A study of the molluscicidal properties of some local plants on *Bulinus globosus*, intermediate host of *Schistosoma haematobium*

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A study was carried out to comparatively evaluate the molluscicidal properties of five Nigerian plants (*Tetrapleura tetraptera, Alternanthera sessilis, Duranta repens, Opuntia dillenii and Dialium guineense*) on the snail intermediate hosts of urinary schistosomiasis, *Bulinus globosus*, from some rivers in Southwest Nigeria. Hot water extracts of the local plants were prepared and applied to the snails at concentrations of 20, 50, 100, 200 and 500 mg/l for a period of 24 hours. Fruit extract of the plant *Tetrapleura tetraptera* was used as a benchmark for toxicity and two replicates were carried out on all the experiments. Toxicity tests showed *Tetrapleura tetraptera* recording 100% mortality in all concentrations used except the lowest concentration. Plant extracts of *Alternanthera sessilis* and *Dialium guineense* revealed medium and minimal toxicity respectively. Snails from Ere stream showed no response to all the plant extracts. Phytochemical analysis of the plant extracts used revealed the presence of saponins in all of them except *Opuntia dillenii* and absence of cardiac glycosides in all except *Tetrapleura tetraptera*. In addition, flavonoids and tannins were present in some of the plants. This study confirmed the powerful molluscicidal properties of *Tetrapleura tetraptera* and the need to study other plants.

P112 Circulating cytokines, their soluble receptors and human responses to praziquantel treatment of schistosomiasis

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Schistosomes live for years in the human bloodstream, effectively evading and regulating host immune responses. Praziquantel is an effective treatment, but reinfection is rapid in endemic areas. Older individuals develop a partial immunity to reinfection, associated with eosinophilia and anti-worm Th2-like responses: IL-4, IL-5, IL-13, IgE. Treatment itself boosts these responses, probably due worm antigens released into the bloodstream, although treatment does not induce anaphylaxis. This led us to examine immediate post-treatment responses and their regulation in Ugandan *Schistosomiasis mansoni* populations. We developed multiplex bead-based assays measuring numerous analytes simultaneously in just 5µl sample. This, alongside finger-prick blood-sampling, permits sampling and analysis of larger cohorts at multiple time-points. A Ugandan fishing community cohort (n=450) was treated and finger-prick samples taken pre-treatment and 24hours post-treatment. The samples were measured for IL-4, IL-5, IL-6, IL-10, IL-13 and their soluble receptors and these data are being analysed in the context of reinfection data and clinical, behavioural, demographic, immunological and geographical information. Substantial changes are observed in circulating cytokines post-treatment and some of these responses, particularly a large increase in plasma IL-5, appear to associate with immunity-related responses.

P113 Development of the 'dose pole' for the administration of praziquantel in preschool-aged children (≤6 year olds)

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Preschool-aged children (≤6 years) have long been overlooked by preventative chemotherapy campaigns aiming to control schistosomiasis. To eliminate the present health inequity, and to facilitate the administration of the drug of choice, praziquantel, the current 'dose pole' must be improved to accommodate this younger age-class. Data on height from 1144 preschool-aged children living along the coast of Lake Albert (Uganda) were used to predict bodyweight and dosage of praziquantel. This provisional 'dose pole' was then hypothetically tested on 470 children from Zanzibar, Tanzania, to validate the pole's applicability in another endemic population. Height was found to be a good predictor of weight, where >95% of individuals would have received a proven efficacious and safe praziquantel dose (30-60 mg/kg) in both Uganda and Zanzibar using two new thresholds for praziquantel administration: 60-84cm corresponding to half tablet and 84-94cm to ¾ tablets. Given the current evidence of infection risk in younger children, the proven pharmacological safety of praziquantel, and now the improvement of the 'dose pole', we strongly advocate the inclusion of preschool-aged children in current and future schistosomiasis control campaigns.

P114 Intestinal schistosomiasis around Lake Victoria: Building a cross-country dataset integrating schistosome and snail information

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Intestinal schistosomiasis, caused by infection with *Schistosoma mansoni*, is endemic along the shoreline of Lake Victoria, and is a large public health burden. From a perspective of national disease control programmes, up-to-date prevalence and distribution data of both the parasite and its host snail are needed in order to treat at-risk populations efficiently and effectively. To this end, extensive surveys around Lake Victoria have been undertaken, sampling both human populations for signs of infection and snail habitats for potential host species. The aim is to build an inclusive database, which, together with environmental variables, will be used to develop an infection risk map for the region. So far, 54 sites in Uganda and 40 sites in Tanzania have been visited, with a further expedition to Kenya completed in January 2009. Snails shedding *S. mansoni* were found at approximately 7.5% of sites in both cases. 27 schools in Uganda were also surveyed for infection prevalence, which varied widely across the lakeshore (range was from 0% to 100%, and average prevalence was 41.57%). In addition, molecular data from both snails and schistosomes has been collected to investigate in greater detail heterogeneities in the biology of disease transmission.

P115 Species identification of nematode eggs using PCR based methods.

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Anthelmintic resistance is now a key feature in UK sheep flock health plans, where monitoring is used to develop/optimise treatment strategies. The sensitivity of many resistance bioassays can be markedly increased through knowledge of the species implicated. Classical morphological identification techniques use third stage larvae (L_3) but have the drawback of being subjective, require an experienced/skilled operative and incorporate a lengthy (7-10 days) culture period. The studies reported here have compared two approaches to egg identification; using species-specific PCR (SSPCR) primers on pooled egg samples versus Pyrosequencing on individual eggs. In 2008, eggs were extracted from faecal samples with a moderate to high count which were obtained from Scottish VI Centres. Pooled samples provided between 55-500 ethanol fixed eggs for the SSPCR technique and 40 individual eggs for the Pyrosequencing assay. Preparation of clean eggs samples was found to be very important as the carry over of excessive amounts of faecal debris was shown to have the potential to inhibit downstream PCR reactions. The preliminary findings from the study confirmed Pyrosequencing to be a quantitative means of obtaining data on species prevalence but suffered from being relatively expensive. Although SSPCR only provided qualitative data it was nonetheless a cost-effective, rapid means for the determination of nematode species that were present in the samples.

P116 Parasitism-central genes in *Strongyloides ratti*

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Strongyloides spp. are major gastrointestinal parasites of humans and of other animals. Two species infect humans, *S. stercoralis* and *S. fuelleborni*: *c.*200 million people are infected worldwide. *S. ratti* is a natural parasite of rats. Recent EST and microarray analyses have identified genes whose expression is abundant and specific- or biased- to the parasitic stage. These expression data were used to define a set of otherwise uncharacterised genes that we hypothesise may play a central role in the parasitic life of this species, such that if the function of these genes were compromised then so would be the parasitic phase of this life-cycle. Detailed RT-PCR analyses of the expression of these genes throughout the *S. ratti* life-cycle have largely confirmed the parasite-specificity of these genes. RACE and further EST sequencing has provided the complete gene sequences. Bioinformatic analysis has identified putative homologues for these genes in other species. We are now expressing these gene products *in vitro*, to investigate the role and function of these gene products in the parasitic life of *S. ratti*.

P117 Gene exp**ression in third-stage larvae of the ovine abomasal nematode *Teladorsagia circumcincta* in response to changing environmental cues.**

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Parasitic gastroenteritis, caused by trichostrongylid nematodes, is the most commonly diagnosed systemic disease of sheep in the UK. The principal causative nematode (worm) is the abomasal parasite *Teladorsagia circumcincta*. Control, dependent on the use of anthelmintics, is failing due to the rapid emergence of drug resistance in the target nematodes. Vaccination is a feasible alternative but development is hampered by a lack of knowledge of the host-parasite interaction to incoming larvae, a prime effector of immunity in sheep. We are seeking to define the molecular interactions between the host site of infection (the true stomach or abomasum) and the incoming larvae - how do larvae respond to changing environmental cues? We are using subtractive suppressive hybridisation to compare gene expression in 3rd stage larvae at exsheathment as they encounter naïve or immune abomasal environments. Here, we describe a preliminary comparison between quiescent sheathed larvae and larvae exsheathed in a high CO2 environment primed for infection. Semi-quantitive PCR has shown up-regulation of several genes in the exsheathed population compared to the sheathed larvae. These include sequences with significant homology to activation-associated secreted proteins from *T. circumcincta* and *O. ostertagi*, which are similar to pathogenesis related ancyclostoma-secreted proteins from hookworms, and also sequences with homology to ESTs previously detected in L4 stage specific cDNA libraries.

P118 Both MHC genotype and T cell receptor (TCR) Vβ gene usage influence the parasite strain specificity of CD8 T cell responses induced by *Theileria parva*

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The recent identification of parasite antigens and epitopes recognised by CD8 T cells from cattle immunised with *Theileria parva* has provided the opportunity to examine the fine antigenic specificity of the CD8 T cell response. Analyses of memory CD8 T cells from animals homozygous for the A10 and A18 MHC class I haplotypes demonstrated that they had detectable reactivity with only one of 5 T. parva antigens tested. Over 60% of the responding T cells from the A10+ and A18+ animals were found to recognise dominant epitopes in the Tp2 and Tp1 antigens respectively. Sequencing of the Tp1 and Tp2 genes from different parasite strains revealed polymorphism, which resulted in coding changes within the epitopes. Analysis of TCR Vβ genes expressed by responding T cells demonstrated that the responses were polyclonal, but dominated by a limited number of abundant clonotypes. By testing recognition of peptides containing single amino acid substitutions in a dominant Tp2 epitope, it was shown that the residues that are critical for epitope recognition differ for T cell clones expressing different Vβ genes. Moreover, such T cell clones showed different patterns of reactivity with allelic variants of this Tp2 epitope.

P119 Investigation of *Theileria annulata* as an activator and modulator of the bovine transcription factor NF-*κ*B

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Theileria annulata is an apicomplexan tick borne haemoparasite that causes a debilitating lymphoproliferative disorder known as tropical theileriosis. The parasite induces transformation of the infected host leukocyte allowing establishment of immortalised cell lines in culture.
Host cell transformation by the parasite is associated with a number of signalling pathways that generate constitutive activation of bovine transcription factors, such as NF-*k*B and AP-1. Activation of these factors has been shown to confer protection against apoptosis and influence the metastatic potential of the infected cell, respectively. However, transcription factors have the potential to be detrimental as well as beneficial to the infected cell and experimental evidence suggest that the parasite may modulate the expression of genes targeted by the transcription factors it activates. I have commenced studies to investigate this possibility with emphasis on comparison of the mRNA expression profiles of uninfected and infected cells following NF-*k*B activation and drug treatment against the parasite. Preliminary results support the possibility that the parasite may modulate the outcome of the activation event.
Despite experimental evidence demonstrating that the parasite recruits the IKK signalosome to its surface, the responsible parasite molecule(s) is unidentified. In my project I have investigated the potential of a number of selected candidate parasite genes for their ability to activate NF-kB.

P120 Identification of parasite antigens recognised by CD8 T cells from cattle immunised with *Theileria annulata*: Evidence of polymorphism in a dominant antigen

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To investigate the role of parasite-specific CD8 T cell responses in immunity to *T. annulata*, we undertook a series of studies to identify and characterise the target antigens. Screening of a series of parasite genes, using CD8 T cell lines derived from immune cattle of different class I MHC genotypes, identified 3 antigens, each of which was recognised only by animals of one or two MHC genotypes. Further analysis of responses to one of these antigens, Tp9, demonstrated that it is consistently recognised by animals expressing the A10 class I MHC haplotype and that the reactive T cells recognise a single dominant epitope. Although the proportion of the response restricted by the A10 haplotype varied between animals (6-90%), virtually all of the A10-restricted T cells were specific for this Tp9 epitope. Nucleotide sequencing of the Tp9 gene from a series of *T. annulata* isolates revealed extensive polymorphism. The epitope was found to vary between strains and was differentially recognised by CD8 T cell clones. These results provide clear evidence of parasite strain restriction of the CD8 T cell response to *T. annulata*, which may account for previous observations of incomplete immune cross-protection between parasite strains.

P121 Does *Toxoplasma gondii* have any direct effect on animal behaviour through dopamine metabolism?

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T. gondii alters the behaviour of its host that will enhance its transmission, but the mechanism is unknown (Webster, JP. Schizophr Bull. 2007, 33(3):752-6; Vyas, A. PNAS 2007, 10:104(15):6442-7). It has been proposed that dopamine may play a role as antipsychotics that block the dopaminergic system interfere with the host manipulation. Previous work from our lab has shown the existence of two novel aromatic amino hydroxylase genes in the *T. gondii* genome that are bifunctional acting as phenylalanine and tyrosine hydroxylases. Tyrosine hydroxylase is the key enzyme in dopamine synthesis that converts tyrosine to L-dopa, the precursor of dopamine. Changes in dopamine levels can lead to serious diseases, like Parkinson's disease and schizophrenia. Whether *T. gondii*, which forms cysts in the brain, has any effect on dopamine levels is a crucial question. We have found that expression of the parasite hydroxylase is induced during differentiation to bradyzoites, the cyst stage, and enzyme is secreted into the parasitophorous vacuole. We are now addressing the question of whether this results in increased dopamine levels in the host brain. We are measuring levels of dopamine and other neurotransmitters in the brains of infected mice to confirm early studies that dopamine levels are increased in chronically infected mice (Stibbs, HH. Ann Trop Med Parasitol.1985;79(2):153-7). Furthermore we hope to detect any co-localisation of the parasite cyst and dopamine in vivo. These experiments will lead to development of knockout mutants for definitive studies of the impact of *T. gondii* tyrosine hydroxylase on dopamine levels and animal behaviour.

P122 Seroprevalence of toxoplasmosis among patients referred for hospital-based serological tests in Doha, Qatar

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The city of Doha in Qatar has a high density of feral cats and there is a high risk of toxoplasmosis for the resident population. We analysed the serological response to *Toxoplasma gondii* of 1625 subject referred for routine hospital based serological tests. Prevalence of current infection was assessed through presence of specific anti-*T. gondii* IgM antibodies, and previous history of infection through IgG. Overall prevalence of IgG responses was 29.8% but there was a marked age effect. Among children less than 1 year old prevalence was 22.9%, but then dropped to <4% in the 1 year old group, indicating that these antibodies were most likely acquired *in utero* from immune mothers. Prevalence then rose steadily to peak at 41.2% among the oldest age class (>45 years). There was also a significant difference between subjects from different geographical regions. No IgM antibodies were detected in any subjects younger than 19 years, but prevalence rose to plateau at 7 - 9% in subjects aged over 20 years. Although these data are based on a selected subset of the population, they nevertheless provide the first evidence that toxoplasmosis is endemic in Qatar, and most likely sustained by the high density of feral cats.

P123 Isolation of *Toxoplasma gondii* from the brain of a fourteen day old dog and its biological and molecular characterisation

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Toxoplasma gondii was isolated from the brain of a two week old pup. The identity of the parasite was confirmed by PCR, Western blotting, electron microscopy and cat bioassay. Genotyping of the isolate (TgDgAu1) performed using 10 PCR-RFLP markers showed it to be a Type II strain. Serology showed the bitch was probably infected during pregnancy and the *T. gondii* was transmitted to the pups *in utero*.

P124 Characterisation of a novel serine palmitoyltransferase from *Toxoplasma gondii*

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Sphingolipids are multi-functional amphipathic lipids which include components of the plasma membrane and play central roles in cell recognition, apoptosis, proliferation and stress responses. We have found that sphingolipid scavenging plays a significant yet limited role in *Toxoplasma gondii* proliferation within the host cell. It is therefore expected that *de novo* synthesis is important for parasite survival and proliferation *in vivo*. The first, step in sphingolipid biosynthesis is catalyzed by a serine palmitoyltransferase (SPT). Here we report the characterisation of the novel apicomplexan SPT from *T. gondii* (TgSPT1). The TgSPT1 protein sequence contains conserved active site residues and predicted phosphate binding pocket characteristic of this enzyme family. TgSPT1 is able to functionally complement a yeast SPT mutant. Surprisingly however, TgSPT1 is functional as a homomeric enzyme in contrast to the heterodimeric SPTs found in all other eukayotic species analysed to date. These data, together with phylogenetic analyses, identify the apicomplexan SPT as a new class of this biosynthetic enzyme. TgSPT1 possesses a type I signal-anchor which localises the protein to the endoplasmic reticulum and ongoing experiments will further characterise this domain. Additionally, construction of an SPT knock-out line in *T. gondii* is underway to determine the role of TgSPT1 in host cell invasion and pathogen proliferation.

P125 The effects of two parasite antigens (*Toxoplasma gondii* and *Toxocara canis*) on WEHI-164 fibrosarcoma growth in mouse model

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Background: Cancer is the main cause of death in developed countries. However in underdeveloped countries infections and parasitic disease are the main causes of death. There are raising scientific evidences indicating that parasitic infection induces antitumor activity against certain types of cancers. In this study the effect of *Toxoploma gondii* and *Toxocara canis* egg antigens in comparison with BCG (known to have anticancer distinctives)on WEHI-164 fibosarcoma transplanted to Balb/c mice was investigated.
Methods: Groups of 6 male Balb/c mice injected with *Toxoplasma gonidii* antigen, BCG or Toxocara canis egg antigen as case groups and alum alone as control groups. All mice were then challenged with WEHI -164 fibrosarcoma cells. The mice were examined for growth of solid tumor and the tumor sizes were measured every two days up to 4 weeks.
Results: The mean area of tumor in *Toxoplasma gondii*, BCG or alum alone injected mice in 4 different days of measurement were 25mm2, 23mm2 and 186mm2 respectively. Also the mean of tumor area in *Toxocara canis* injected mice in 4 different days was 25.5mm2 compared to control group (alum treated) which was 155mm2.
Conclusion: The mechanism by which, parasite antigens interfere with tumor growth is not clearly understood, it is possible that immune responses provoked by parasite antigens nonspecifically affect the tumor growth.

P126 An investigation into vertical transmission of *Toxoplasma gondii* within an inbred population of urban mice (*Mus domesticus*) using multilocus genotype data.

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Toxoplasma gondii is a protozoan parasite belonging to the Phylum Apicomplexa, it is an unusual member of the group in that it can sustain itself purely by asexual reproduction and infects an extremely broad range of intermediate hosts including man. The aims of our study are to investigate *T. gondii* infection in an inbred population of mice (*Mus domesticus*) to see if the parasite is being passed on vertically through successive generations. Parasite detection was carried out using SAG1 and SAG3 PCR and mice were genotyped at seven microsatellite loci. We have found a high prevalence of infection in these mice at 89% and only one strain of the parasite is present (Type I). This is strong evidence for serial vertical transmission in *T. gondii.*

P127 Seroprevalence of *Toxoplasma gondii,* Cytomegalovirus, Rubella virus and *Chlamydia trachomatis* among infertile women attending In vitro fertilization center, Gaza Strip, Palestine

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The aim of the present study was to determine the seroprevalence of Toxoplasma, rubella, CMV and *C. trachomatis* in Palestinian women through antenatal screening.
Methods**:** The study included 1954 Palestinian women records which were reviewed and analyzed statistically from 2000-2005. Those women attended In vitro fertilization centre in Gaza complaining from infertility and abortion. Anti-Toxoplasma, anti-rubella, anti-CMV and anti-Chlamydia IgM antibodies were assayed using an enzyme linked immunosorbent assay methods.
Results: The results showed that (7.9%) of women were positive for anti-*Toxoplasma gondii* IgM, (6.0%) were positive for ant-CMV IgM, (7%) were positive for anti-Rubella IgM and (12.8%) were positive for ant-Chlamydia antibodies. High significant infection rate was observed in year 2003 (P=0.001) for *T. gondii.* A clear variation with statistical significance was observed in the seroprevalence for all the studied pathogens due to years and regarding age of the women.
Conclusion: The study indicated that *T. gondii*, rubella, cytomegalovirus (CMV) and *Chlamydia trachomatis* are still constitute a public health problem among pregnant women and considered one of abortion factors. So, health authorities should care for these.

P128 An investigation into the prevalence of *Toxoplasma gondii* infection in *Apodemus sylvaticus* in North Yorkshire

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The coccidian parasite *Toxoplasma gondii* is ubiquitous and affects many mammals including humans. Felids both wild and domestic are the definitive host however *Toxoplasma* can infect all warm-blooded animals. Toxoplasmosis is a significant cause of abortion, stillbirth and disease in both humans and sheep. The strains of *Toxoplasma* can be subdivided into three clonal groups (Type I, II and III). The wood mouse *(Apodemus sylvaticus*) was sampled from North Yorkshire as part of a continuous study of parasites in the area. DNA was extracted from brain tissue using standard procedures and the presence of *Toxoplasma* was detected using nested PCR amplification of the SAG-1 gene. With 75/180 mice positive for *Toxoplasma gondii*, the mean prevalence over the last 8 years in the area is 42% (± 2.5%). This high level of prevalence is puzzling since this rural area of North Yorkshire is virtually free of cats. This suggests that other mechanisms of parasite transmission may be important in this population of *Apodemus sylvaticus.* Future work will be aimed at genotyping positive samples using the SAG-3 marker.

P129 Detection of *Neospora caninum* and *Toxoplasma gondii* in wild carnivores.

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Neospora caninum and *Toxoplasma gondii* are commonly diagnosed causes of reproductive losses in farm ruminants. Recent studies have demonstrated that the domestic dog (*Canis lupus familiaris*) is a definitive host of *N. caninum*, whilst the domestic cat (*Felis catus*) has long been known as the definitive host of *T. gondii*. Our study set out to determine whether wild British carnivores could be acting as reservoir hosts for these parasites, potentially helping to disseminate the infections. Brain samples were collected from ferret (*Mustela putorius furo),* European polecat (*Mustela putorius*), Eurasian badger (*Meles meles*), red fox (*Vulpes vulpes*), American mink (*Neovison vison*) and stoat (*Mustela erminea*). DNA from these samples was tested by species specific PCR for *N. caninum* and *T. gondii*. The results showed that ferrets, polecats, badgers and red foxes were positive for the presence of *N. caninum* in the brain, while mink and stoat samples were *N. caninum* negative. All species tested positive for *T. gondii*. A small number of ferrets, polecats and red foxes tested positive for both *N. caninum* and *T. gondii*. The presence of parasite DNA in these animals shows that they are persistently infected and are acting as reservoir hosts. The question still remains if any of these carnivorous animals could also be acting as definitive hosts as well?

P130 O-glycosylation in *Trichinella* muscle larvae. A lectin affino-blot and lectin histochemical study

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The aim of this work was to analyze oligosaccharide composition with the focus on the presence of O-linked glycoproteins in the total extract obtained from different *Trichinella* species muscle larvae by means of lectin affinity-blot, with lectins selected for their sugar specificities. The anatomical localization of the oligosaccharides of interest was also demonstrated in striated muscle tissue sections of *Trichinella spiralis* muscle larvae.
The absence of reactivity with *Tritrichomonas mobilensis* lectin and *Maackia amurensis* agglutinin indicated that species of the Trichinella genus do not synthesize sialic acid. Reactvity with *Helix pomatia* lectin (HPA), *Vicia villosa* lectin-B4 (VVL-B4), peanut aggltinin (PNA) and *Ulex europaeus* lectin-I (UEA-I) identified the presence of O-linked glycans analogous to carcinoma-associated Tn-antigen and T-antigen and also structures analogous to B- and H-blood group antigens. The existence of core 5 and core 7 structures is also possible, which was illustrated by the different pattern of labelling with HPA and VVL-B4. The products reactive to HPA and VVL-B4 were demonstrated in the hypodermis and on the larval cuticle. The main set of oligosaccharides reactive to PNA and UEA-I was localized in the stichosome.

P131 Complete absence of the GPI biosynthetic pathway in *Trichomonas vaginalis*

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

P132 The effect of vitamin A metabolites on inflammation in *Trichuris muris* infected mice

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Trichuris trichiura, or human whipworm, is a nematode tissue parasite that inhabits the colonic epithelium. It causes significant morbidity in approximately 1049 million people, primarily in areas of poor sanitation. The mouse analogue of this parasite, *Trichuris muris*, reflects the intestinal inflammation seen in the human infection and has been described as a good natural model of inflammatory bowel disease (IBD). In this model, the generation of a T helper type 2 (Th2) immune response confers resistance to the parasite and it is expelled. In contrast, mounting a Th1 response causes susceptibility and a chronic infection. Vitamin A metabolites, such as *all-trans*- and 9-cis-retinoic acid, act on the nuclear hormone receptors: retinoic acid receptor (RAR), retinoic X receptor (RXR) and peroxisome proliferator-activated receptor (PPAR). They have been shown to have a polarising effect toward the generation of a Th2 response, and both PPAR-γ and RXR agonists had anti-inflammatory actions in a mouse model of colitis. Therefore, we postulate that vitamin A metabolites and actions on their receptors will give protection from immunopathology in the context of *T. muris* infection, and we aim to deduce the cytokines and cells involved in the retinoic acid-regulated immune response. This work will produce a novel insight into the role of vitamin A metabolites in *T. muris*-mediated intestinal inflammation.

P133 Real time PCR to estimate infection load of *Glossina* species with *Trypanosoma congolense savannah*

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Trypanosoma congolense savannah is transmitted to the vertebrate host via tsetse flies (*Glossina* species) causing Nagana disease in cattle. Quantification of the midgut load with might aid in determining the vectorial capacity of tsetse flies. The major advantages of real-time PCR are its wide detection range due to the determination of the C_T (cycle threshold) values at any stage of the reaction, no requirement of gel electrophoresis, identification and quantification of DNA in a single step. In the current work, a quantitative PCR reaction has been developed and optimised for the quantification of *T. c. savannah* load in the midgut of experimentally infected *Glossina morsitans morsitans*. The PCR was conducted using satellite DNA sequence specific for *T. c. savannah*, the amplification was monitored using Sybr Green. The efficiency of the reaction was calculated to be 93%, moreover the reaction was tested against other trypanosome species and the results reviled the specificity of the reaction to the tested species.

P134 The uptake of trypanosome lytic factor in different strains and sub-species of *Trypanosoma brucei*

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Trypanosoma brucei is a major parasite of livestock and humans in sub-Saharan Africa. The sub-species *Tb.brucei* cannot infect humans due to sensitivity to trypanosome lytic factor (TLF) found in normal human serum. Two sub-species are able to resist lysis. *Tb.rhodesiesnse* achieves this via expression of Serum Resistance Associated gene (SRA) that inhibits a TLF component. The most prevalent human infective sub-species *Tb.gambiense* does not possess SRA. Furthermore, genetic and pathological studies suggest that there are two distinct types of *Tb.gambiense* and each may possess a different resistance strategy. There has been little investigation of the mechanism of resistance in *Tb.gambiense* due to the difficulty of working with the organism in a laboratory setting.
In this study, purified human TLF (gifted by J.Raper, NYU) was tagged with an AlexaFluor® dye. Representative members of each *T.brucei* sub-species, including both types of *Tb.gambiense*, were exposed to physiological levels of tagged TLF and imaged using a Deltavision system. The spatial distribution of TLF within the body of each trypanosome was measured for multiple parasites in each strain at various times. A time course with the degree of uptake and relative TLF position was created for each isolate to create a quantitative data set for comparison between lines. This revealed differences in the temporal and spatial distribution of TLF between strains.

P135 Role of Natural Inhibitor of Cysteine Peptidase in *Trypanosoma brucei*

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Inhibitor of cysteine peptidase (ICP) is a chagasin-family from clan IX, natural tight binding inhibitor of Clan CA, family C1 cysteine peptidases (CP), such as brucipain and cathepsin B. Endogenous mammalian and plant CP inhibitors from the cystatin class share no homology with ICP, which has been only identified in protozooa and bacteria. Analysis of phenotype of ICP null mutant in monomorphic bloodstream form *Trypanosoma brucei* 427 suggests that it regulates endogenous CP activity, and consequently plays a part in modulation of surface coat exchange during differentiation, intracellular proteolysis and parasite infectivity in mice. Previous studies using an in vitro model of the blood brain barrier (BBB) composed of the brain microvascular endothelial cells (BMECs) suggest that brucipain is important to the transendothelial migration of *T. b. rhodesiense* IL1852. To study the role of ICP in this process, we have generated two different pleiomorphic strains lacking ICP, *T. b. rhodesiense* IL1852 and *T. b. brucei* 247. In vitro, IL1852 ICP null mutants show increased BBB transmigration and human neutrophil adhesion in BMECs compared to WT, which correlates with increased CP activity in these mutants.

P136 Microsatellite analysis of a Gambian *Trypanosoma vivax* population

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Trypanosoma vivax, the causative agent of animal trypanosomiais, has a major impact on animal health in sub-Saharan Africa and as such is of intense economic importance. The parasite infects a host range including domestic breeds of cattle, horses and donkeys and is transmitted by tsetse flies or other biting flies and can be found over much of South America as well as Africa. While the related trypanosomes, *T. brucei*, *T. congolense* and *T. cruzi*, have all been shown to undergo some form of genetic exchange, this has not been examined in *T. vivax*. To address this question we have investigated a sympatric field population from The Gambia, using six *T. vivax* specific microsatellite markers developed specifically for the study. Our analysis of samples collected from donkeys, horses and cattle indicate that the *T. vivax* population is clonal, with no evidence of genetic exchange through mating. This finding has implications for the spread of important traits through populations and for the development of control strategies to combat this debilitating and life-threatening disease.

P137 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 10 microsatellite markers, 36 of these samples have been genotyped and the results compared to those from a previously published study (Maclean et al 2007). 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 phenotypes.

P138 Evolution of the amastin surface glycoprotein family in trypanosomatid parasites

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Amastins are transmembrane glycoproteins expressed during the vertebrate stages of trypanosomatid parasites, which cause various neglected tropical diseases in humans. Amastins comprise a large, structurally diverse and immunogenic gene family, which could have importance in pathology and vaccine development. Amastin complement is known to vary significantly between *Trypanosoma* spp. and *Leishmania* spp. parasites and so amastin loci are described from two non-human pathogens (*Crithidia deanei* and *Leptomonas seymouri*) that bridge the taxonomic gap between these genera. A molecular phylogenetic method is used to address both the microevolutionary processes affecting amastin loci and their macroevolutionary origins, to provide a systematic characterisation of total diversity among trypanosomatids.
Four distinct clades emerge from the phylogeny, which are reclassified as α–, β–, γ– and $\tilde{\delta}$-amastin respectively. While α– and $\tilde{\beta}$–amastin are structurally conserved across the trypanosomatids, substantial diversification of δ-amastin accounts for the expanded repertoire in *Leishmania* spp., but not in *C. deanei* or *L. seymouri*, indicating that this evolutionary change occurred after the origin of the vertebrate pathogen. Frequent transposition and tandem duplication of $\tilde{\delta}$–amastin has created genomic variation in repertoire between *Leishmania* species, while structural derivation of the extracellular protein domains and recombination of δ-amastin duplicates have affected genetic variation within the gene family. The identification of *Leishmania*-specific amastin gene copies strongly suggests that these genes contribute to particular features of the leishmanial life cycle or disease.

P139 Oral Melarsoprol cures CNS stage *Trypanosoma brucei brucei* infection in a murine model of human African trypanosomiasis

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Human African trypanosomiasis is a parasitic disease caused by the protozoan parasites *Trypanosoma brucei rhodesiense* and *T.b.gambiense.* Chemotherapy currently depends on a handful of drugs, many of which are difficult to administer and have undesirable side effects. Without appropriate chemotherapy the disease is invariably fatal. The development of novel or re-formulated trypanocides is therefore crucial.
Cyclodextrins are naturally occurring oligosaccharides widely utilised by the pharmaceutical industry to increase the solubility of drugs. Two melarsoprol cyclodextrin complexes: mel/hydroxypropyl-ß-cyclodextrin (HPCD) and mel/randomly-methylated-cyclodextrin (RAMCD) were synthesised and their *in-vivo* and *in-vitro* activity assessed.
CD-1 mice were infected with *T.b.brucei.* On day 21 post-infection, Mel/HPCD or Mel/RAMCD were administered at doses of: 0.0125, 0.025, 0.05, 0.1 and 0.2 mmol/kg while Melarsoprol, Trimelarsen or Cymelarsen were administered at 0.05mmol/kg. Each drug was given by oral gavage for seven consecutive days.
Mel/HPCD and mel/RAMCD successfully cured CNS stage trypanosome infection when administered at 0.05mmol/kg. Melarsoprol, Trimelarsen or Cymelarsen oral treatment was unsuccessful when administered at an equivalent dose. Mel/HPCD, mel/RAMCD and melarsoprol showed similar IC_{50} values as measured by Alamar Blue assay.
The authors acknowledge Stéphan Gibaud for preparing the cyclodextrin-melarsoprol complexes.

P140 Visualisation of the *Trypanosoma cruzi* life cycle using fluorescent proteins, including co-infection of individual host cells and hybrid organisms

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The reproductive mode of *Trypanosoma cruzi* is predominantly clonal, yet inter-lineage recombination has given rise to geographically widespread hybrid groups (TcIId and TcIIe), and intra-lineage hybridisation has been achieved in vitro. To facilitate the study of interactions between genetically distinct *T. cruzi* strains, including mating experiments, we generated a number of transgenic *T. cruzi* cell lines that constitutively expressed either red or green fluorescent protein (RFP or GFP) in combination with a selectable drug resistance marker (hygromycin B or neomycin). We were able to visualise complete life cycles by observing fluorescent epimastigotes, metacyclic trypomastigotes, intracellular amastigotes and bloodstream form trypomastigotes. After infection of Vero cell cultures with different combinations of RFP- and GFP-expressing strains, co-infection of individual Vero cells was observed at frequencies of up to 16.9%. For one pair of strains (TcIIc x TcIIc) a small number of Vero cells were found to contain individual parasites with co-localising red and green fluorescent signals suggesting that hybridisation had taken place. Double drug resistant organisms could not be recovered from this cross however, potentially reflecting low hybrid viability in this case. We have generated 19 transgenic fluorescent strains of *T. cruzi* covering 5 of the 6 genetic lineages, thereby enhancing the experimental options for ongoing studies of hybridisation in *T. cruzi*.

P141 Crystallography of *Trypanosoma brucei* phosphofructokinase highlights major differences from bacterial and mammalian enzymes

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Determination of the crystal structures of *T. brucei* phosphofructokinase (PFK) in both the apoenzyme and ATP-bound forms has enabled detailed comparisons with the structures of bacterial ATP-dependent and PPi-dependent PFKs, and with a model of human (ATP-dependent) PFK. The active site of *T. brucei* PFK is a chimaera of ATP-dependent and PPi-dependent PFKs, and possesses amino acid residues and structural features characteristic of both classes of enzyme. Although *T. brucei* PFK is strictly ATP-dependent, it belongs to the PPi-dependent family by sequence similarities. ATP binding causes conformational changes that include the opening of the active site to accommodate the two substrates, MgATP and fructose 6-phosphate, and a dramatic ordering of the C-terminal helices which serve to hold the tetramer together. These conformational transitions

are fundamentally different from those of other ATP-dependent PFKs such as observed for *E. coli* PFK. The major differences in structure and mechanism of *T. brucei* PFK from bacterial and mammalian PFKs give optimism for the discovery and development of parasite-specific drugs.

P142 *Trypanosoma brucei* CTP synthase - an essential gene and possible drug target.
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Cytidine triphosphate (CTP) is a central metabolite not only in nucleic acid synthesis but also for all glycolipid and phospholipid *de novo* biosynthesis. In *T. brucei*, the intracellular CTP concentration is unusually low (Hofer *et al*., 1998; Hofer *et al*., 2001), this is thought to be in part due to an absence of a salvage pathway for either cytosine or cytidine. Thus, *T. brucei* depend totally upon *de novo* synthesis of CTP, yet the CTP synthase (CTPS) enzyme has low catalytic activity (Hofer *et al*., 2001).
The CTPS has an aminotransferase domain and a glutaminase domain. The aminotransferase domain catalyses initial activation of UTP by phosphorylation, using ATP as a phosphate donor. An amino group (released by the glutaminase domain) is then substituted for the phosphate. We have investigated various inhibitors of CTPS, including three glutamine antagonists, in an *in vitro* assay, and chemically validated all three against *T. brucei*. We have also genetically validated CTPS in bloodstream form *T. brucei*.
An overview of the extensive biochemical phenotyping of the genetic and chemical validation will be presented.
(1998). JBC, *273*, 34098-34104; Hofer, A. *et. al*., (2001). PNAS, *98*, 6412-6416. This work is funded by The Wellcome Trust

P143 RNA editing as a drug target in trypanosomes: identification of inhibitors of the essential enzyme REL1 by virtual screening.
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Posttranscriptional RNA editing by uridine insertion and removal is essential for mitochondrial gene expression in trypanosomatids but absent from the host and therefore a potentially powerful novel drug target. The editing process is catalyzed by a multiprotein complex, the editosome, and involves a series of enzymatic steps. A key catalyst is RNA editing ligase 1 (REL1), an essential enzyme in bloodstream and insect stage *Trypanosoma brucei.* To identify potential REL1 inhibitors that could serve as hits in a drug discovery effort, we incorporated molecular dynamics simulations of the REL1 structure into a virtual screening strategy of the National Cancer Institute (NCI) diversity set. This was followed by a hierarchical similarity search with the most active compound over the full NCI database. Top compounds were tested for their effects on REL1 activity in biochemical assays. Three "drug-like" compounds were confirmed to inhibit REL1 with IC50 values around 1 µM. Tests of these compounds against the most closely related bacteriophage T4 RNA ligase 2, as well as against human DNA ligase IIIbeta indicated a considerable degree of selectivity for RNA ligases. These studies as well as preliminary tests of anti-parasite activity will be presented.

P144 Studies into the trypanocidal activities of NO-releasing agents
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Nitric oxide (NO) is an important cytotoxic and cytostatic agent that also possesses anti-parasitic activity. Various NO-donors have been shown to kill *Plasmodium falciparum*, *Leishmania major* and *Trypanosoma cruzi* in cell culture. There is increasing evidence that the parasiticidal effect of NO is through inhibition of cysteine proteinases which are validated targets for the rational design of new anti-parasitic chemotherapies. Recently, it has been shown that the NO-donor GSNO, NOR-3, SIN-1 and SNAP inhibit the catalytic activity of cysteine proteinases of *P. falciparum*, *T. cruzi* and *L. major*. In this study we investigated the effect the four NO-donors on *Trypanosoma brucei*, the causative agent of African sleeping sickness. With the exception of SIN-1, all NO-donors displayed trypanocidal activities against *T. brucei* with 50% growth inhibition (GI_{50}) values of around 30 µM. However, the NO-donors were ineffective in inhibiting significantly the major lysosomal cysteine proteinase (brucipain) activity within the parasites. This finding was confirmed by the ineffectiveness of the NO-donors to induce substantial accumulation of FITC-labelled transferrin in the lysosome of the parasite. Flow cytometric analysis revealed that only 3% of FITC-labelled transferrin accumulated in the lysosome of parasites treated with

NO-donors compared to parasites treated with the cysteine proteinase inhibitor Z-Phe-Ala-CHN_2. In conclusion, NO-releasing agents display only moderate trypanocidal activity which is not due to inhibition of the major lysosomal cysteine proteinase in *T. brucei*.

P145 Uptake of Diminazene Aceturate in *Trypanosoma brucei* clones lacking the TbAT1/P2 transporter.
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We have previously shown that diminazene aceturate (DA, Berenil) is taken up predominantly by the P2 adenosine transporter encoded by TbAT1 [1]. We have further shown that as a result, a Tbat1-/- line was approximately 18-fold resistant to DA [2]. However, this still leaves the parasites sensitive to as little as 1 microM DA, strongly suggesting the presence of a secondary uptake mechanism for this drug, as its target is intracellular and this cation has a negligible diffusion rate. We thus re-investigated the uptake of [3H]-DA in a number of DA-resistant lines. Uptake rates, measured over 5 - 10 minutes, were highest in wild-type *T. b. brucei*, followed by Tbat1-/- and was barely detectable in cell lines adapted from Tbat1-/- to higher DA or pentamidine concentrations in vitro. DA uptake in the absence of P2 activity displayed the characteristics of the previously identified High Affinity Pentamidine transporter HAPT1 [2,3]. The highly resistant line ABR had lost both P2 and HAPT transporter activities. However, the activity of the Low Affinity Pentamidine Transporter LAPT [2,3] was not affected.
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[2] Matovu et al. (2003) Eukaryot. Cell 2, 1003-1008.
[3] De Koning, H.P. (2001) Mol. Pharmacol. 59, 586-592.

P146 Human sleeping sickness: progress towards field friendly molecular diagnosis
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Trypanosoma brucei s.l. comprises three morphologically indistinguishable parasites including the two agents of human sleeping sickness, *T. b. gambiense* and *T. b rhodesiense*, as well as the non human pathogenic *T. b. brucei*. Accurate parasite identification is absolutely critical for the control of human sleeping sickness. *T. b. rhodesiense* must be distinguished from *T. b. brucei*, in domestic livestock, and *T. b. gambiense*, in humans, in order to identify cattle reservoirs of human disease and determine the best patient treatment strategy, respectively. At present field diagnosis differs for *T. b. gambiense* and *T. b. rhodesiense*, and high technology, laboratory based PCR approaches alone offer specific identification. To this end LAMP (loop-mediated isothermal amplification) assays have been developed for the identification of both human sleeping sickness agents. LAMP is a novel DNA amplification technique advocated as a field friendly diagnostic tool. Here we have looked to validate both published and novel LAMP reactions for *T. b. rhodesiense* and *T. b. gambiense*. We suggest that novel LAMP primers may form the basis for a common technology to assist the differential diagnosis of the two disease forms.

P147 An essential neutral sphingomyelinase in *Trypanosoma brucei*
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Phosphatidylcholine (GPCho) and sphingomyelin (SM) are the major phospholipid species in the protozoan parasite *Trypanosoma brucei* and thus are presumably essential structural components and metabolites in their membranes. 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, may be vulnerable to the inhibition of key enzymes in GPCho metabolism.A candidate membrane-bound neutral sphingomyelinase is being investigated for its potential role in maintaining lipid homeostasis by releasing choline phosphate from SM which can re-enter the Kennedy pathway, leading to the formation of GPCho.Recent findings confirm this neutral sphingomyelinase is essential to bloodstream form *T. brucei* and reveal a complex gene knockout phenotype with multiple defects in variant surface glycoprotein trafficking, endocytosis and cytokinesis. As such, it presents a promising therapeutic drug target for African sleeping sickness.

P148 New insights into the phenomenon of site-specific protein N-glycosylation in the protozoan parasite *Trypanosoma brucei*

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Asparagine-linked glycosylation is catalyzed by oligosaccharyltransferase (OTase). In the parasitic protozoan *Trypanosoma brucei* OTase activity is catalyzed by single-subunit enzymes encoded by three paralogous genes: TbSTT3A, TbSTT3B and TbSTT3C. It has been suggested that these genes encode enzymes with different oligosaccharide donor and/or peptide acceptor specificities. TbSTT3A and TbSTT3B, but not TbSTT3C, are transcribed in two lifecycle stages of *T. brucei*. Selective knockdown and analysis of parasite protein N-glycosylation showed that TbSTT3A selectively transfers biantennary $Man_5GlcNAc_2$ to specific glycosylation sites while TbSTT3B selectively transfers triantennary $Man_9GlcNAc_2$ to others. Mass spectrometric analysis of endogenous *T. brucei* glycosylation site occupancy showed that TbSTT3A and TbSTT3B prefer glycosylation sites in acidic and basic regions of polypeptide, respectively. This embodiment of distinct oligosaccharide donor and peptide acceptor specificities in single-subunit OTase's has not been previously described. In addition, TbSTT3A and TbSTT3B could be knocked down individually, but not collectively, *in vitro* without abolishing parasite growth. However, both were independently essential for parasite growth *in vivo*.

P149 Genome wide evidence of non-neutrality in mammalian protist microsatellites and their application to the epidemiology of *Trypanosoma cruzi*

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So-called 'junk' DNA is partly composed of microsatellites, which are short tandem repeats of 1 – 6 (mono – hexa-) nucleotide units. Until recently, it was thought that junk DNA was not transcribed and was non-functional. It has therefore been assumed that the accumulation of mutations in such DNA would not be subject to selection pressures and would reflect genetic drift. This assumption has led to the widespread application of microsatellites to the population genetics analysis of eukaryotes. However, several studies have identified 'non-neutral' behaviour of microsatellites in humans and fungi.
Firstly, we now reveal, using whole genome scan analyses of *Trypanosoma spp., Leishmania spp*., *Plasmodium spp.*, *Cryptosporidium spp.* and *Theileria spp*. that microsatellite sequences can not be regarded as neutral for many species of protists. We supplemented the comparative analysis with ciliate, amoeboid, fungal and insect genomes. The microsatellites for each genome (or where annotated each chromosome) were assessed using independent genetic traits and each trait distinguished the evolutionary groups of mammalian parasitic protists, resulting in trypanosomatid, *Plasmodium*, *Cryptosporidium* and *Theileria* specific groups as well as associations between related species. The systematic correlation of "neutral" genetic markers across millions of years of evolutionary divergence at the whole genome level, lead us to conclude that the complex mutational behaviour of microsatellites in parasitic protists may be explained by Darwinian selection, either acting directly, e.g. on gene regulation or through inherited bias in the mutation/ repair machinery.
Secondly, we demonstrate two aspects of non-neutral behaviour in a genome-scale microsatellite analysis for the population genetics of *T. cruzi*, by defining the sylvatic population structure of *T. cruzi* (genetic lineages TCI and TCIIc) using a panel of genome-wide microsatellite loci among ~200 *T. cruzi* isolates from over nine different countries and in the comparative context of domestic *T. cruzi* populations from Venezuela and Bolivia (TCI).

P150 Unravelling the epidemiology of *Trypanosoma cruzi* I using genome-scale microsatellites.

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Trypanosoma cruzi, the most important parasitic agent in Latin America, is highly genetically diverse, with at least six discrete typing units (DTUs) reported: TC I, IIa-e. However, the current six genotype classification is probably a poor reflection of the total genetic diversity present. To gather epidemiologically important information at the sub-DTU level, we developed a 48-marker panel of microsatellites to investigate population structure

across the geographic distribution of TCI. This DTU is the major cause of disease in northern South America but also occurs in silvatic transmission cycles throughout the continent. We demonstrate that silvatic TCI populations are extraordinarily genetically diverse, show spatial structuring at a continental scale and have undergone recent biogeographic expansion into the Southern USA. Conversely, the majority of human strains sampled are characterised by a considerable reduction in genetic diversity with respect to isolates from silvatic sources. In Venezuela, most human isolates showed little identity with local silvatic strains, despite frequent invasion of the domestic setting by infected adult vectors. Linkage indices indicate predominantly clonal parasite propagation among all populations. Excess homozygosity among silvatic strains and raised heterozygosity among domestic populations, however, suggest that some level of genetic recombination cannot be ruled out. The epidemiological significance of these findings is discussed.

P151 The function of the mitochondrion in bloodstream form trypanosomes

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The bloodstream form trypanosome does not use mitochondrial oxidative or substrate phosphorylation for energy generation, instead relying exclusively upon glycolysis for ATP production. Nonetheless, the mitochondrion and mitochondrial gene expression are essential in these forms. Recent work (Schnaufer *et al.,*. 2005) has shown that ATP synthase is essential for the maintenance of mitochondrial membrane potential and that dyskinetoplastid (dk) trypanosomes that lack mitochondrial DNA appear to maintain their mitochondrial membrane potential through the action of a mutated ATP synthase. Work in our laboratory now uses transgenic trypanosome cell lines and next-generation sequencing techniques to determine the exact role of ATP synthase mutations in the dk phenomenon and to identify other potential mechanisms that allow dk trypanosomes to survive without mitochondrial gene expression. Preliminary results from these studies will be presented.

P152 The trypanosomes of pigs in Arusha, Tanzania.

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In Uganda domestic cows are thought to be the main reservoirs of human infective *T. b. rhodesiense*, however, the roles of other domestic animals cannot be discounted. In this study we look at the levels of trypanosome infection in domestic pigs in Tanzania as well as the role these animals may play as potential reservoirs of cattle and human infective trypanosomes.
To ascertain if pigs have the potential to act as reservoirs for human and/or animal trypanosomiasis, this study investigated the prevalence of trypanosomes in domestic pigs from the Arusha region of Northern Tanzania. Blood samples collected on Whatman FTA cards underwent PCR based methods for the detection of parasite DNA.
Of the 168 domestic pigs tested, 28 (16.7%) were found to be infected with one or more species of trypanosome. These included 5 *T. vivax* infections, 3 *T. simiae*, 9 *T. b. brucei*, 8 *T. b rhodesiense*, and 4 suspected *T. godfreyi* infections.
These results show that domestic pigs have the potential to act as reservoirs for *T. vivax* and *T. brucei* s.l. infection in cattle, and more importantly as potential reservoirs for *T. b. rhodesiense,* the causative agent of human sleeping sickness.

P153 Marine fish trypanosomes from South Africa: morphological and molecular aspects of their development in fishes and a leech

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Life cycles in fish trypanosomes, particularly those of marine species, are largely unknown and their taxonomy and phylogenetic relationships are uncertain. However, all known fish trypanosome life cycles to date involve a blood sucking leech vector. Here, a variety of trypanosome stages found in both types of host (fishes and a leech), captured in rock pools from collection sites on the west and south coasts of South Africa in 2003 and 2008, are described. Attempts to elucidate the genetic relationships between the trypanosomes from some

fishes and leeches by means of polymerase chain reaction (PCR) and partial sequencing of the 18S ssu rRNA gene derived from Giemsa-stained fish blood films and leech squash preparations indicated that epimastigotes in leeches were those of a marine fish trypanosome. Knowledge of trypanosomes infecting fishes in South Africa is limited and, as far as is known, no previous reports exist of fish trypanosomes in marine teleosts from the collection sites in the current study. Moreover, trypanosome developmental stages have not been recorded previously from marine leeches in any part of Africa.

P154 LED fluorescence microscopy for diagnosis of human African trypanosomiasis

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Current diagnosis of human African trypanosomiasis (HAT) in the field relies on bright field microscopy, both to confirm infection and to stage the disease. Unfortunately this procedure is insensitive, and can be laborious when combined with concentration techniques. This leads to misdiagnosis, especially for the Gambian form of HAT where patients may present with very low parasitaemia.
Fluorescence microscopy could significantly improve the efficacy of parasitological tests, allowing immediate detection of parasites within a complex sample, such as blood or cerebrospinal fluid. Its use in the field today is possible thanks to new LED fluorescent microscopes, which are relatively inexpensive, easy to use, have a long life time and need low maintenance.
In order to develop a new diagnostic tool that exploits LED technology we need to identify fluorescent probes which are specific for trypanosomes, cheap and reliable. Our aim is to screen a wide number of new and commercially available compounds for this purpose.

P155 Cattle activity analysis using motion sensors in rural Zambia.

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IceTag pedometers are activity motion sensors that use electronic accelerometers to record the lying, standing and walking behaviour of animals. They were used to monitor Angoni cattle movement activity in the Petauke District of Zambia during the rainy season. Trypanosomiasis and tick-borne diseases are endemic in this region and low haemoglobin values are often associated with these and other parasitic infections. The study was designed to assess whether IceTag pedometers can be used in sub-Saharan Africa and to provide a baseline for future research on traditional cattle movement activity. Haemoglobin values were measured in 432 cattle in two villages. In each village ten pairs of co-grazing animals were selected on the basis of one high and one low haemoglobin value in each pair. The co-grazing pairs were age (as close as possible) and sex matched. Each animal had a pedometer placed on its hind leg, to continuously measure and record its activity for two weeks. Pedometer data were analysed using principal components analysis. Results indicated that cattle with high haemoglobin levels had higher step counts than low haemoglobin cattle. Unexpected differences were observed between the villages in terms of night-time cattle activity. Overall, pedometers successfully monitored and recorded cattle movement activity during adverse weather and animal husbandry conditions in Zambia.

P156 Religious violence, natural resource conflict and the epidemiology of African animal trypanosomiasis

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Over the past twenty years animal trypanosomiasis has emerged on the Jos Plateau, one of the main areas of intensive animal production in Nigeria. Riots in recent years have claimed thousands of lives and displaced over hundreds of thousands of people from the area. Although generally reported as religious conflicts, they are infact due to underlying conflict for valuable resources: political office in urban areas and land and water in rural areas.

There is intense competition for resources between farmers and cow herders on the plateau which has led to the latter being displaced from the area for over six months of the year. They spend this time grazing their cattle in trypanosomiasis endemic areas and this increased exposure to the disease has had the expected negative impact on the health and economic potential of cattle on the Plateau.
In 2008, a longitudinal prevalence survey and participatory rural assessment were carried out and results indicate that this land use pattern has created a significant difference between expected local transmission, farmer perception and actual prevalence of trypanosomiasis on the Jos Plateau.

P157 Tick cell lines in parasitology research

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Ticks are undoubtedly the most economically important ectoparasites of domestic animals and wildlife, because of the damage they cause during prolonged bloodfeeding and the wide range of protozoal, bacterial and viral pathogens they transmit. In addition, ticks are increasingly recognised as significant parasites of man and vectors of serious human disease. *In vitro* culture systems involving tick tissues and cells have been use for many years for propagation and study of arthropod-borne pathogens, and continuous cell lines are now available from a wide range of ixodid and argasid ticks of medical and veterinary importance. The development of new molecular technologies including stable cell transfection and RNA interference, combined with the availability of complete or partial genome sequences for several tick species, has opened up extensive possibilities for use of tick cell cultures as model systems for study and manipulation of biological processes in ticks and tick-pathogen interactions at the cellular and molecular level. The present status of tick cell culture at Edinburgh University will be presented, and the wide range of applications of tick cell lines in parasitology research will be reviewed.

P158 Do parasite fatty acid and retinol binding proteins modulate the immune responses?

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Nematodes secrete class-specific fatty acid and retinol (FAR) binding proteins into the tissues they occupy. These FAR proteins are characterized by a strong binding affinity for retinol (vitamin A). There have recently been a number of studies highlighting the immunomodulatory properties of the metabolic derivate of vitamin A, retinoic acid (RA). RAs are known to influence gene regulation by binding to nuclear receptors.
In particular, Vitamin A has been demonstrated to be important in the development of immune responses in the gut. It has been shown to affect the phenotypic development of T cells in this environment. Dendritic cells (DCs) in the intestine produce RA and both induce differentiation of T regulatory cells and generate a gut-homing phenotype on T and B cells. We therefore use the excellent model organism, the murine nematode *Heligmosomoides bakeri* (former *H. polygyrus*), which inhabits the intestine, to investigate the immunomodulatory properties of it's FAR protein and to evaluate if these could be due to its capacity to interact with retinol.
We will present data on the effects of *H. bakeri* and/ or *Onchocerca volvulus* recombinant FAR proteins on the maturation and activation of murine DCs.

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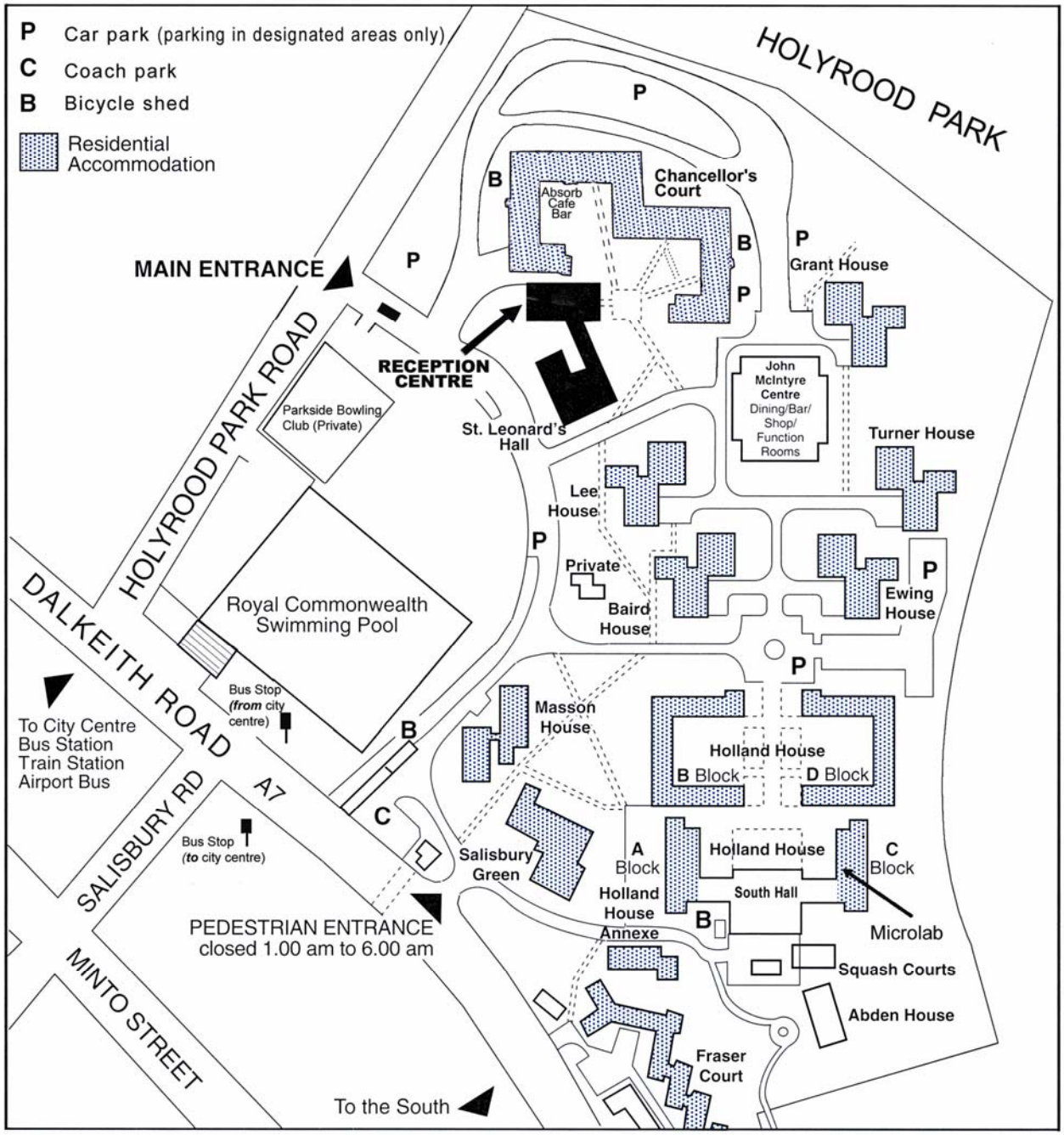

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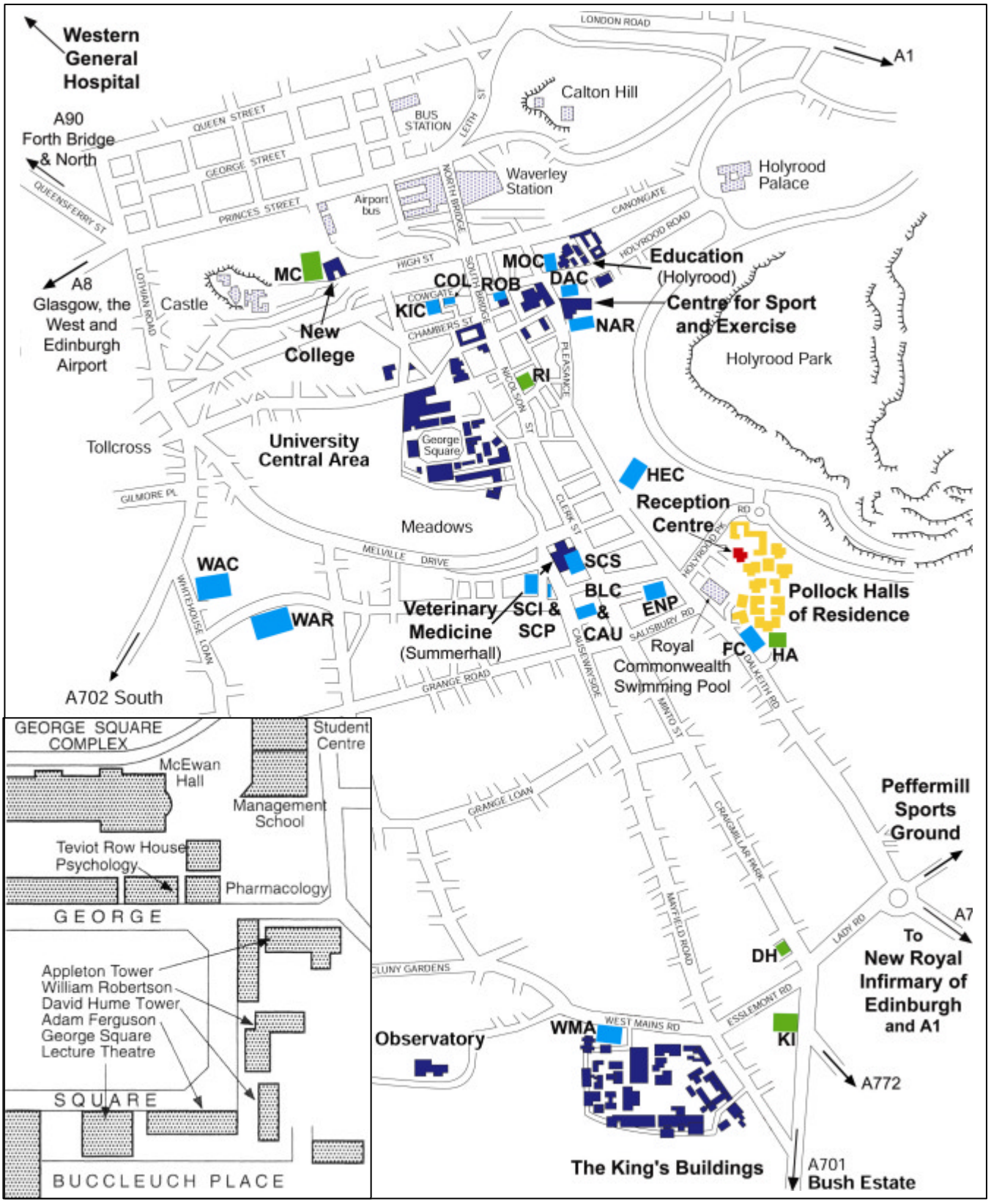

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RI	Richmond Place	**ENP**	East Newington Place	**MOC**	Morgan Court
WAR	Warrender Park Road	**HEC**	Hermit's Croft		
WAC	Warrender Park Crescent	**WMA**	West Mains Road		
SCI	Sciennes	**DAC**	Darroch Court		
SCP	Sciennes Place	**NAR**	New Arthur Place		