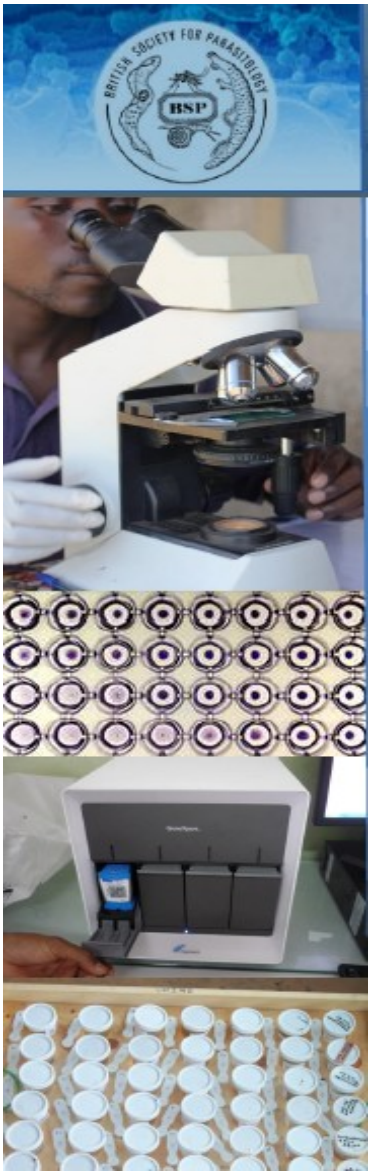
2013 BSP Autumn Symposium

"Advances in diagnostics for infectious diseases"

25th – 26th September

Ness Gardens, UoL



Outline timetable

&

abstract booklet

organised by

Russell Stothard & Emily Adams

.

We hope that you will enjoy this scientific meeting at Ness Gardens, and have the opportunity to make new friendships or solidify existing ones.

Most of all, we hope you will be inspired by the presentations and posters. Collectively these nurture the growing importance of applied diagnostics, with applications from the bedside-clinic to adventures out in-the-field.

All of the presentations, and several of the posters, will form manuscripts within a special issue of *Parasitology.* This volume will have an expected publication date of Summer 2014, so is something we all look forward to.

Thank you for attending,

Russell and *Emily*

TIMETABLE BOOKLET:

Advances in diagnostics for infectious diseases

Wednesday 25th September

08.30 -10.15 **Business breakfast: local enterprise and LSTM/UoL** in the Hulme Reception Room

08.45 - **Meeting registration** and set up of posters on the walls in the Foyer/Hulme Reception Room

09.30 - **Morning coffee & tea** in the Foyer

10.50 - **Opening**: welcome and meeting orientation in the lecture theatre

11.00 - 12.00 **Session 1: Evaluation and implementation of diagnostics**. Chair: Russ Stothard
KEYNOTE. Diagnostic tests: from bench to bedside - **Rosanna Peeling**
12.00 - 13.00 Modelling diagnostics and health systems - **Bertie Squire** & **Ivor Langley**
Evaluation of diagnostics - **Mariska Leeflang**

13.00 - 14.00 **Lunch** in the Hulme Reception Room

14.00 - 15.30 **Session 2: Diagnostics at home and abroad**. Chair: Henk Schallig
Diagnostic metagenomics - **Mark Pallen**
Malaria diagnostics, now and the future - **Peter Chiodini**
Non-falciparum diagnostics - **Martha Betson**

15.30 - 16.00 **Afternoon tea** in the Foyer

16.00 - 17.30 **Session 3: Veterinary parasitology**. Chair: David Rollinson
Veterinary helminthology - **Jozef Vercruysse**
Fasciolosis in livestock - **Diana Williams** & **Jane Hodgkinson**
Fascioliasis in people - **Santiago Mas-Coma** & **Delores Bargues**

17.45 - 18.45 **Poster Session** in the Foyer and Hulme Reception Room, with poster walk at 18.15

19.00 - 21.45 **Conference Dinner** in the Hulme Reception Room, exit by 22.00

Thursday 26th September

09.00 - 11.00 **Session 4: Mapping and NTDs**. Chair: Peter Chiodini
Diagnosis and NTD maps - **Laura Rinaldi** & **Giuseppe Cringoli**
Schistosomiasis - **Russell Stothard** & **David Rollinson**
Leishmaniasis & trypanosomiasis - **Michael Miles**
Lymphatic filariasis - **Moses Bockarie**

11.00 - 11.30 **Morning coffee & tea** in the Foyer

11.30 - 13.00 **Session 5: Novel diagnostics and their implementation**. Chair: Emily Adams
Clinical use of qPCR - **Jaco Verweij**
Novel diagnostics for insecticide resistance - **Martin Donnelly**
New diagnostics for LMIC and 'blue sky' thinking - **Mark Perkins**

13.05 - **Closure of meeting**, thanks and highlighting the special issue of *Parasitology*

13.00 - 14.00 **Lunch** in the Hulme Reception Room

14.00 - **Exit**, but don't forget to have a walk around Ness Gardens before you leave!

ABSTRACT BOOKLET PRESENTATIONS:

All lectures will be given in the main theatre, a short presentation from Partec and also ICOPA 2014 will take place at the end of each lunchtime session from 13.45 on each day, respectively.

Wednesday 25th September 2013

Keynote - 11.00am:

The future of diagnostic technologies: bench to bedside

Professor Rosanna Peeling

London School of Hygiene and Tropical Medicine, UK

Rapid advances in diagnostic technology platforms and data capture have provided tools to transform the detection of infectious diseases to improve global health and health security. However, the bench to bedside pathway is lengthy, costly and fragmented, and the uptake of innovation has been slow. Public-private collaboration to accelerate the development, evaluation and adoption of affordable quality-assured diagnostics for global health is an urgent priority.

Session 1 - 12.00pm:

Modelling diagnostics within health systems

Professor Bertie Squire & Ivor Langley

Liverpool School of Tropical Medicine, UK

New diagnostic tools for infectious diseases have the potential to deliver substantial reductions in the burden of disease. However, the scale of these impacts will vary by the epidemiology and programmatic conditions of the local context. The health system and resource constraints of the setting will influence the implementation policies and diagnostic algorithms that are affordable, sustainable and cost effective. We will present an innovative, linked operational and transmission model which we have developed for tuberculosis. The approach is comprehensive and models patient, health system and transmission impacts of new TB diagnostics including Xpert MTB/RIF. We intend to explore ways in which the approach could also be employed for other infectious diseases.

Session 1 – 12.30pm:

Evaluation of diagnostics: for whom?

Dr Mariska Leeflang

Academic Medical Center, Holland

Evaluating diagnostics is more than just running them over some samples with confirmed disease and some samples from healthy controls and calculating the sensitivity and specificity. The perfect test in the lab can be useless in the field, for several reasons: pathophysiological variation, non-optimal lab circumstances, or because subsequent treatment choices are being based on other things than test results. We therefore need more practically relevant evaluations, focused around user-driven questions.

Session 2 – 14.00pm:

Diagnostic metagenomics

Professor Mark Pallen

University of Warwick, UK

The term "metagenomics" is used to describe the extraction and shotgun sequencing of DNA from complex environmental or clinical samples without culture or target-specific amplification or capture. With the easy accessibility of high-throughput sequencing, metagenomics brings the promise of a simple one-size-fits-all diagnostic approach that could potentially replace the onerous and complex laboratory workflows based on microscopy and culture. Here I will review recent progress in the applications of diagnostic metagenomics, including pathogen discovery, detection of an outbreak strain of *E. coli* in faecal samples and the recovery of sequences from viral, bacterial and protozoal pathogens from frozen or mummified tissues.

Session 2 – 14.30pm:

Malaria diagnostics, now and in the future

Professor Peter Chiodini

Hospital for Tropical Diseases, UK

Malaria is the most common problem seen in travellers returning home with parasitic infection, typically presenting with fever at the Hospital for Tropical Diseases, London for later diagnostic triage. Over the last two decades we have helped to pioneer the use of a battery of rapid diagnostic tests (pLDH and HRPII) alongside conventional blood film microscopy. This has also provided a fascinating insight into the changing epidemiology imported malaria. With the advent of PCR-based assays that allow very sensitive and precise quantification and characterisation of *Plasmodium* spp., I highlight how these tools have been included into our current patient management algorithm.

Session 2 – 15.00pm:

Diagnostics of non-falciparum malaria

Dr Martha Betson

Royal Veterinary College, UK

The advent of molecular methods for diagnosis of *Plasmodium* infections has revealed that non-falciparum malaria infections, in particular *P. malariae* and *P. ovale*, are much more prevalent than originally thought, especially as co-infections with *P. falciparum*. In addition, molecular studies have demonstrated that *P. ovale* actually consists of two distinct sub-species, which have been named *P. ovale curtisi* and *P. ovale wallikeri*. During community-based surveys in Uganda, we found surprisingly high prevalences of co-infection of *P. falciparum* with *P. malariae* and/or *P. ovale* sspp. in young children. Using novel PCR-based assays we demonstrated that both species of *P. ovale* circulate in the same communities. Although a number of rapid diagnostic tests (RDTs) showed excellent diagnostic performance for detection of *P. falciparum* in this setting, none of the RDTs used were able to detect *P. malariae* or *P. ovale* infections. The implications of our findings for community-based malaria control will be discussed.

Session 3 – 16.00pm:

Diagnosis of helminthiasis in animal health: chasing the helminth or its economic impact?

Professor Jozef Vercruysse

University of Ghent, Belguim

Even though helminth infections typically don't cause clinical symptoms in animals, they are responsible for important production losses because of their impact on e.g. weight gain, feed conversion, milk yield and fertility. Several cost-efficient sampling methods and diagnostic tests have been developed to assess production losses in ruminants and pigs e.g. measurement of antibody levels against *Ostertagia, Fasciola* and *Ascaris* in milk, meat juice and serum.

Session 3 – 16.30pm:

Why do we need to know if an animal is infected with *Fasciola hepatica*?

Professor Diana Williams & Dr Jane Hodgkinson

University of Liverpool, UK

We will review the significance of *Fasciola hepatica* infection in sheep and cattle in the UK, evaluate current methods used to detect infection, discuss the impact of *F. hepatica* on diagnosis of other pathogens. To close we will consider why we need tests to detect resistance to flukicides.

Session 3 – 17.00pm:

Diagnosis of human fascioliasis by stool and blood techniques: update for the present scenario

Professor Santiago Mas-Coma & Dr Delores Bargues

Universidad de Valencia, Spain

Diagnostic tools available for the different epidemiological situations of human fascioliasis hyperendemic, mesoendemic and hypoendemic areas are reviewed. We highlight several methods for the diagnosis of individual patients in hospitals and health centres, as well as, for monitoring of individual post-reatment/mass control objectives. We assess the usefulness and advantages/disadvantages of each technique within each situation and purpose of analysis.

Thursday 26th September 2013

Session 4 – 09.00am:

Diagnosis and NTD maps

Dr Laura Rinaldi & Professor Giuseppe Cringoli

University of Naples, Italy

Advances in epidemiological sampling, diagnostic tools, maps and geospatial methodologies are needed for the surveillance and control of NTDs. We emphasize the need for integrating sound epidemiological designs with innovative diagnostic tools and strategies (e.g. Mini-FLOTAC for detection of parasitic elements) and high-resolution geospatial tools for mapping. Recognizing these challenges, standardization of quality procedures, and innovating, validating and applying new tools and strategies will foster and sustain long-term control and eventual elimination of NTDs.

Session 4 - 09.30am:

Diagnostics in schistosomiasis: from people to snails and back again, with praziquantel in between

Professor Russell Stothard & Professor David Rollinson

Liverpool School of Tropical Medicine & The Natural History Museum, UK

For diagnosis and detection of schistosomiasis in clinical management of disease there is no single test that covers all aspects of what is needed. This is also true in public health settings for operational diagnostics is a pragmatic compromise with available in-field resources and constraints. Furthermore the repertoire of point-of-care markers of morbidity is rather sparse. For environmental epidemiological studies, detection of schistosomes in their aquatic habitats is also important. In this latter setting whilst qPCR methods are available with future cross-over into clinical applications, their scalability is a major bottleneck. In this presentation we review the state-of-the-art for present needs of control and for future elimination settings.

Session 4 – 10.00am:

South American trypanosomiasis and visceral leishmaniasis: towards higher resolution diagnostic and prognostic indicators

Professor Michael Miles

London School of Hygiene and Tropical Medicine, UK

Given the severity of disease and risks associated with treatment, accurate diagnosis of South American trypanosomiasis (Chagas disease) and visceral leishmaniasis (VL) is important. Diagnostics of these parasites can be challenging when set in context with the extensive diversity of these kinetoplastids, and I highlight our ongoing efforts to develop lineage specific diagnostic markers for *Trypanosoma cruzi* using oligochromatographic dipsticks and for VL, present our work revealing the substantive molecular diversity of kinesin genes within *Leishmania donovani*. This latter diversity likely accounts for the poorer performance of the rK39 immunochromatographic rapid diagnostic test (RDT) in East Africa.

Session 4 – 10.30am:

Diagnostics in lymphatic filariasis and vector competence

Professor Moses Bockarie

Liverpool School of Tropical Medicine, UK

With ongoing mass drug administration (MDA), the diagnostic landscape for monitoring lymphatic filariasis (LF) in people has moved away from inspection of 'classic' night-drawn blood films towards rapid diagnostic tools. This is especially true when endpoints of MDA are approached. In these settings, more sensitive and field-appropriate techniques for transmission surveillance are required and The Global Program to Eliminate Lymphatic Filariasis (GPELF) guidelines call for using filarial antigen testing. With the introduction of a new rapid diagnostic test, the Alere Filariasis Test Strip, this appears to have much promise over the current BinaxNOW Filariasis card. As programmes move more towards post-MDA transmission surveillance, DNA-based methods of parasite detection will become more important.

Session 5 – 11.30am:

Parasitic PCRs in clinical diagnostics

Jaco Verweij

St. Elisabeth Hospital Tilburg, Holland

For many years DNA-based methods are available in most clinical microbiology laboratories, however, until recently these tools were not routinely exploited for the diagnosis of parasitic infections. Laboratories were reluctant to implement PCR not knowing how to incorporate such an approach in the algorithm of tools available for the most accurate diagnosis of a large variety of parasites. Especially in the diagnosis of intestinal parasitic infections the diversity of parasites that one can expect in most settings is far less than the parasitological textbooks make you to believe. Therefore, using the classical algorithm based on population, patient groups, use of immuno- suppressive drugs, travel history etc. is also applicable to decide to perform additional techniques when a multiplex PCR panel is used as a first line diagnostic.

Session 5 – 12.00pm:

Detecting insecticide resistance within mosquitoes with new genetic tools

Professor Martin Donnelly

Liverpool School of Tropical Medicine, UK

Given the limited number of insecticides in the public health armamentarium, management of insecticide resistance is a crucial to sustainable malaria and filarial vector control campaigns. Insecticide resistance commonly results from alterations to target sites or copy number variation, over expression or allelic variation in detoxification enzymes. In this presentation I will describe efforts to identify resistance associated variants using genomic and transcriptomic approaches and the challenges we have faced in integrating the resultant DNA diagnostics into disease surveillance programmes.

Session 5 – 12.30pm:

Novel diagnostics for LMIC and 'blue sky' thinking

Professor Mark Perkins

FIND, Switzerland

What are the future diagnostic needs of low and middle income countries (LMIC)? At FIND we have developed a research portfolio, and international network of collaborators, to address some of the most pressing diagnostic needs. Foremost is tracking and responding to the threat of multi-drug resistant strains of *Mycobacterium tuberculosis* and forming a communication network to empower LMIC health services in making best choices with available technologies. For malaria, FIND has been active in developing new methods, e.g. loop isothermal DNA amplification (LAMP), as well as, ensuring quality control and best use of existing rapid diagnostic tests (RDTs), highlighting the needs for better case management and reporting of non-malarial febrile illness (NMFI). Other neglected diseases receive attention, foremost with human African Trypanosomiasis (HAT) with the introduction of a new RDT, the SD BIOLINE HAT test.

ABSTRACT BOOKLET POSTERS:

Posters will be in numerical order along the walls, with any additional posters at the end of this series.

POSTER 1

Proteomic selection of immunodiagnostic antigens for *Trypanosoma congolense* and *Trypanosoma vivax* with the generation of a proof-of-concept lateral flow immunodiagnostic device for the latter

Jennifer R Fleming, Lalitha Sastry, Thomas W M Crozier, Steven J Wall, Lauren Sullivan, Michael A J Ferguson
College of Life Sciences, University of Dundee, Dundee, UK; BBInternational, Alchemy House, Dundee, UK.

African Animal Trypanosomosis (AAT) presents a severe problem for agriculture development in sub-Saharan Africa with large economic losses. The two main parasites responsible are *Trypanosoma congolense* and *T. vivax*. Current diagnostic methods are either symptom-based or too costly and technologically demanding for use the endemic regions. With this in mind, we set out to develop a new diagnostic for *T. congolense* and *T. vivax*. The aims are to take advantage of recombinant protein production technology to produce a lateral flow test, which will allow for a standardised, large-scale, low cost solution to create an easy to use pen-side diagnostic. In order to identify antigens for recombinant production and test development, we utilised a non-biased proteomic approach. We report the successful identification, production and evaluation of new potential biomarkers for AAT caused by *T. congolense* and *T. vivax* infection, two previously unidentified ISGs. A proof-of-concept lateral flow test for *T. vivax* was produced and underwent preliminary evaluation.

POSTER 2

Paralogous sequences in nuclear ribosomal DNA of triatominae vectors of Chagas disease: a new field of applied research

MD Bargues, MA Zuriga, S Mas-Coma
Universidad de Valèncía, Spain.

The internal transcribed spacer region (ITS1, 5.8S rDNA, ITS2) represents the most widely applied nuclear marker in eukaryotic phylogenetics. Although this region has been assumed to evolve in concert, the number of investigations revealing high degrees of intra-individual polymorphism connected with the presence of pseudogenes has risen. The 5.8S rDNA is the most important diagnostic marker for functionality of the ITS region. In Triatominae, intra-individual 5.8S and ITS-2 rDNA polymorphisms have been found in 13 taxa of the genus *Meccus* and *Triatoma* covering North America, Central America and northern South America. In the total of the triatomines analysed, functional and pseudogenic variants of the 5.8S+ITS-2 were present in 75.60% of the specimens, whereas the pseudogenic variant was not detected in only nine species (21.95%) of the genera *Triatoma, Dipetalogaster, Panstrongylus* and *Rhodnius*. The discovery of a pseudogene in many phylogenetically related species is unique in animals and allowed for an estimation of its palaeobiogeographical origin based on molecular clock data, inheritance pathways, evolutionary rate and pattern, and geographical spread. Additional to the technical risk to be considered henceforth, this relict pseudogene, designated as "ps(5.8S+ITS-2)", proves to be a valuable marker for specimen classification, phylogenetic analyses, and systematic/taxonomic studies. It opens a new research field, Chagas disease epidemiology and control included, given its potential relationships with triatomine fitness, behaviour and adaptability.

POSTER 3

Molecular diagnosis for specific differentiation of *Fasciola hepatica* and *Fasciola gigantica*

MD Bargues, P Artigas, S Mas-Coma
Universidad de Valencia, Spain.

Human fascioliasis presents a marked heterogeneity including different epidemiological situations and transmission patterns in the different endemic areas. Different situations appear related, among others, to the presence of only one (*F. hepatica* or *F. gigantica*), the two species (endemic area presenting total, partial or no overlapping of two fasciolid species), or the two species plus hybrids. For genetic characterisation, two aspects should be considered: (i) *F. hepatica* and *F. gigantica* are very close one another and have diverged evolutionarily only recently, and (ii) their present geographical distribution is the consequence of spreading events that have taken place recently, but mostly in prehistorical times. Intraspecific and interspecific variabilities of “pure” *F. hepatica* and “pure” *F. gigantica* were analysed by means of complete sequences of rDNA ITS-2 and ITS-1 spacers and mtDNA *cox*1 and *nad*1 complete genes from areas with only one fasciolid species. Problems posed by fasciolid crossbreeding, introgression and hybridisation in

overlap areas are analysed. Nuclear rDNA appears to correlate with phenotype (adult fluke characteristics and fasciolid/lymnaeid specificity), whereas mtDNA does not. However, flukes sometimes appear so intermediate that they cannot be ascribed to either *F. hepatica*-like or *F. gigantica*-like forms. The sequence analysis of four molecular markers allow to define the nucleotide positions providing molecular diagnosis for specific differentiation between both species.

POSTER 4

Diagnostic accuracy under field conditions of HAT Sero K-SeT, a rapid diagnostic test for sleeping sickness

Philippe Büscher, Pascal Mertens, Thierry Leclipteux, Quentin Gilleman, Diane Jacquet, Dieudonné Mumba-Ngoyi, Patient Pati Pyana, Joris Menten, Veerle Lejon

Institute of Tropical Medicine, Department of Biomedical Sciences, Nationalestraat 155, B-2000 Antwerp, Belgium; Coris BioConcept, Crealys Park, rue Jean Sonet 4a, B-5032 Gembloux, Belgium; Institute of Tropical Medicine, Applied Technology and Production Unit, Nationalestraat 155, B-2000 Antwerp, Belgium; Institut National de Recherche Biomédicale, Avenue de la Démocratie, BP 1197, Kinshasa Gombe, Democratic Republic of the Congo; Institute of Tropical Medicine, Department of Clinical Sciences, Nationalestraat 155, B-2000 Antwerp, Belgium.

With the steadily decreasing prevalence of *Trypanosoma brucei* (*T.b.*) *gambiense* human African trypanosomiasis (HAT), the cost-effectiveness of large-scale screening of populations at risk with CATT/*T.b.gambiense* becomes low. Limited specificity causes low positive predictive value of CATT when the prevalence is <1%. Within the NIDIAG network supported by the EC FP7 we developed a new generation rapid diagnostic test for detection of *gambiense*-specific antibodies to fulfil the needs of HAT control programmes in low endemic foci. The HAT-Sero-K-SeT successfully passed phase I evaluation on plasma and reconstituted blood and underwent phase II evaluation on 134 patients and 359 endemic controls in Bandundu Province, Democratic Republic of the Congo. HAT patients were confirmed by the presence of parasites in lymph or blood (with mini-anion exchange centrifugation technique) or cerebrospinal fluid (with modified single centrifugation). Sensitivity was 98.5% (95% Confidence Interval (CI): 95.9–100%) and specificity was 98.6% (95% CI: 97.1–100%). Phase III evaluation is on-going in Democratic Republic of the Congo and in Côte d'Ivoire.

POSTER 5

Point-of-care device for malaria diagnosis and drug resistance status

Cathy Moore, Sanjeev Krishna, Henry Staines, Pedro Gil, Peter Kremsner, Elaine Warburton, Sam Whitehouse, Jonathan O'Halloran

St George's University of London, UK; Karolinska Institutet, Sweden; University of Tübingen, Germany; QuantuMDx, UK.

Prompt diagnosis of malaria allows appropriate treatment and reduces both morbidity and mortality. However, present diagnostics at point-of-care do not provide information regarding the resistance status of the plasmodial parasite underlying the infection. The NANOMAL consortium aims to use innovative new technologies to confirm malaria diagnosis and detect drug resistance in malaria parasites by analysis of resistance-associated mutations (both point mutations and copy number variations), using nanowire technology. This will result in the development of a simple, rapid (sample to result time of < 15 min) and affordable point-of-care handheld diagnostic device. The device will be useful at many levels in malarial control including optimising individual treatments for patients, assessing the epidemiology of drug resistance in malaria endemic areas and assessing population impacts of antimalarial interventions. Here we present the latest advances in this ground breaking project.

POSTER 6

Epidemiology and molecular genotyping of echinostome metacercariae in *Filopaludina* snails in Lamphun Province, Northern Thailand

Waraporn Noikong

Applied Parasitology Research Laboratory Department of Biology, Chiang Mai University, Thailand.

To analyse the prevalence of echinostome metacercariae in *Filopaludina dorliaris* and *F. martensi martensi* and genotype variation of echinostome metacercariae by using random amplified polymorphic DNA (RAPD) analysis. *Filopaludina* sp. snails were collected from eight localities of Lamphun Province, Northern Thailand and examined for echinostome metacercariae. RAPD-PCR was used to for analyze genotype variation of echinostome metacercariae. A total of 3226 *Filopaludina dorliaris* and *F. martensi martensi* snails were collected from eight localities from Lamphun Province, Northern Thailand. The overall prevalence of echinostome metacercariae in *F. dorliaris* and *F. martensi martensi* was 40.89% and 36.27% infected, while the intensity of infection was 20.37 and 12.04 respectively. The dendrogram constructed base on RAPD profiles, 4 well supported domains were generated; (i) group of metacercariae

from Ban Hong, Mae Ta, Meaung, Pa Sang, Toong Hua Chang, and Weang Nong that were clustered in the group of E. revolutum, (ii) Ban Thi, (iii) Lee, and (iv) 3 adults of an out group.

POSTER 7

Immunoblotting with human antigen detects early changes in cyst viability in a longitudinal study of hepatic cystic echinococcosis

M Mariconti, F Tamarozzi, V Meroni, F Genco, E Brunetti.
IRCCS Policlinico San Matteo Hospital Foundation, Italy; University of Pavia, Italy; WHO Collaborative Centre for Clinical Management of Cystic Echinococcosis, Via Taramelli, 27100 Pavia, Italy.

The diagnosis of hepatic cystic echinococcosis is based on ultrasonography and confirmed by serology, but the latter is not standardized and its relationship with cyst stages has rarely been addressed. However, no biological marker of cyst viability is currently available implying years-long patient follow-up, hardly feasible in endemic areas. We characterized the performances of an immunoblotting test based on human hydatid cyst fluid as an antigen and tested it in a longitudinal study on a cohort of patients with single liver cysts that shifted from active to transitional to inactive, as the result of albendazole treatment, in a short time (1-2 years). Sera from healthy volunteers, and from patients with other helminthiases were included as a control group. Antigens of 8, 16, 24, 34-50 kDa were identified, corresponding with AgB (8, 16 and 24 kDa) and Ag5 (34-50 kDa). Sera from 55 CE patients with different cyst activity recognized different fractions of the native antigen. The rapid change in cyst viability observed by US in 5 patients was paralleled by a clear change in band recognition pattern on the experimental IB, while routine serology remained positive. These results strongly support the hypothesis that different antigens are expressed by different cyst stages of the larval phase of *E. granulosus*, as suggested by functional studies, and not just by different phases of the parasite life cycle. These differences might be useful in clinical practice as a biological marker of cyst viability and to shorten patients' follow-up.

POSTER 8

Detection of leishmanial antigens in patient urine by quantitative ELISA and its potential as a test-of-cure

Audrey Albertini, Marcel Hommel, Sally Ellis, Rashidul Haque, Abu Toha, Rezuanul Haque Bhuiyan, Ridwanur Rahman, M Abul Faizf, Dinesh Mondal, Michael Childerstoneg, Neil Purcellg, Joseph Ndung'u, Mark D. Perkins
FIND, 16 avenue de Budé, 1202 Geneva, Switzerland; Institut Pasteur, Paris, France; DNDi, 15 Chemin Louis-Dunant, 1202 Geneva, Switzerland; ICCDDR, GPO Box 128, Dhaka 1000, Bangladesh; Shaheed Suhrawardy Medical College and Hospital, Sher-e-Ban, Dhaka 1207, Bangladesh; Malaria Research Group, Chittagong 4000, Bangladesh; Kalon Biological Limited, Merrow Lane, Guilford, UK.

Early identification of treatment failure in visceral leishmaniasis (VL) patients is critical to the successful control and elimination of the disease. An assay that could do this would also have potential as a pharmacodynamic marker. Both test indications would require a sensitive and quantitative assay. We report on the development of an antigen affinity purified (AAP) polyclonal double-antibody sandwich ELISA assay for *Leishmania* antigens in urine, and its testing on patient and endemic control samples from South-East Asia. The AAP ELISA uses the same antigen as the KAtex latex agglutination test but is quantitative and does not require boiling of urine samples before testing. Urine samples were collected by DNDi in Bangladesh, to determine the feasibility of implementing new treatment regimens for VL recommended by WHO in a primary healthcare setting. The samples were obtained from parasitologically confirmed VL patients (collected at pre-treatment, and on days 7, 15, 45 and 180 after initiation of treatment) and from healthy endemic controls. When a cut-off of 1 UAU/ml (arbitrary units of urinary antigen per ml) was used, the AAP ELISA prototype was positive in 95.3% (102/107) of *Leishmania* patient samples before treatment (baseline), while all the endemic controls (48/48) were negative. During the first 45 days after initiation of treatment, the level of *Leishmania* antigen in urine decreased by 95% in majority of the patients. While this assay has good potential as a test of cure, the appropriate time for testing needs to be defined, in line with patient follow-up visits.

POSTER 9

A molecular diagnostic assay for enzootic abortion of ewes: validation of a swab sampling method

Erin Williams, Rita Dempsey, **Mary Sekiya**
School of Veterinary Medicine, UCD Dublin, Belfield, Dublin, Republic of Ireland.

A multiplex real-time PCR assay for the diagnosis of enzootic abortion of ewes (EAE) in tissue samples was previously developed at UCD Dublin in cooperation with the Department of Agriculture. The two primary causative organisms, *Chlamydia abortus* and *Toxoplasma gondii*, are specifically detected. The assay has the potential to be a valuable tool for surveillance of EAE, therefore we were interested in determining whether a swab sample of aborted foetuses and

placenta would be sufficiently sensitive for detection. We assayed samples submitted by the Regional Veterinary laboratories and also by the sheep farming community. During the spring lambing season of 2011, swabs were collected from 160 individual animals. Eight of these were healthy lambs from the Lyons Estate farm, which tested negative for both organisms. Of the remaining 152 animals, we determined that 26% were positive for *C. abortus*, 10% were positive for *T. gondii* and <1% (one animal) was positive for both. This result is similar to the findings from a previous validation study on farm samples. A subset of the test samples was submitted by the Regional Veterinary labs and comparisons of the results from the real-time PCR assay with other clinical findings will be presented.

POSTER 10

Development of rapid diagnostic tests for surra and sleeping sickness based on recombinant variant surface glycoproteins

Rogé Stijn, M Magali, Y Guisez, Q Gilleman, T Simon, P Büscher
Institute of Tropical Medicine, Department of Biomedical Sciences, Nationalestraat 155, 2000 Antwerp, Belgium; University of Antwerp, Laboratory for Molecular Plant Physiology and Biotechnology, Department of Biology, Groenenborgerlaan 171, 2020 Antwerp, Belgium; Coris BioConcept, Science Park Crealys, Rue Jean Sonet 4A, 5032 Gembloux, Belgium.

Serodiagnosis of surra and sleeping sickness (HAT) is still based on native antigens purified from bloodstream form trypanosomes grown in rodents. To avoid the use of laboratory rodents in antigen preparation we expressed the N-terminal part of the variant surface glycoproteins (VSGs) used in these tests, RoTat 1.2, LiTat 1.3 and LiTat 1.5, recombinantly in *Pichia pastoris*. The secreted recombinants are affinity purified with yields of up to 20 mg per liter cell culture. The diagnostic potential of the recombinant proteins was confirmed through ELISA on 185 sera of naturally infected and control dromedary camels (rRoTat 1.2) or 162 human sera (rLiTat 1.3 and rLiTat 1.5). Coris BioConcept developed a lateral flow singular test format based on the recombinant RoTat 1.2 (Surra Sero K-SeT) with a specificity of 97.3 % and a sensitivity of 100 % compared to trypanolysis on 146 camel sera. The results suggest that the recombinant RoTat 1.2 can replace the native RoTat 1.2 VSG for serodiagnosis of surra. Currently, a similar rapid diagnostic test for HAT (HAT Sero K-SeT) is developed based on native LiTat 1.3 and LiTat 1.5 antigens. As for the surra test, we aim to replace the native antigens by recombinants to eliminate the infection risk and the use of laboratory animals for antigen production.

POSTER 11

Anti-alpha-galactosyl antibodies as a novel diagnostic tool for the detection of cutaneous leishmaniasis in Saudi Arabia

Waleed Saleh Al Salem, Daniela Ferreira, Naomi Dyer, Emily Adams, Salah M Balghonaim, Ahmed Y Al Muhanaa, Mohamed Alzahrani, Ziad Memish, Igor C. Almeida and Alvaro Acosta- Serrano
Liverpool School of Tropical Medicine, Liverpool, UK; Saudi Ministry of Health, Riyadh, Saudi Arabia; The Border Biomedical Research Center, Department of Biological Sciences, University of Texas, El Paso, Texas USA.

Cutaneous leishmaniasis (CL) is the major VBD throughout the Kingdom of Saudi Arabia (KSA). Despite the positive impact of programmes carefully designed to control and curtail CL cases in KSA, the disease remains a serious problem due to uncontrollable urbanization, socioeconomic factors. Currently, in KSA CL is only clinically diagnosed and no diagnostic method is available to further confirm *Leishmania* infection. In this study, we report that patients with either active CL lesions or clinically cured present high levels of anti-galactosyl (herein referred as CL-Gal) antibodies compared to healthy individuals from several endemic regions of KSA. We also compared the CL-Gal levels in CL patients to current diagnostic methods like wound aspiration and microscopy. Serum, wound aspirate and skin biopsy from 400 individuals clinically diagnosed with CL and serum samples from healthy individuals, were collected from five CL endemic regions of KSA. Parasite species were identified using PCR-RFLP of the ribosomal internal transcribed spacer 1 region (ITS1). Serum levels of IgG CL-Gal antibodies were assessed using a novel chemiluminescent ELISA that uses BSA conjugated with the trisaccharide Gal-1-3Gal-1-4GlcNAc as antigen. We found high levels of IgG CL-Gal antibodies in individuals infected with either *L. major* (98%) or *L. tropica* (97%) compared to the results obtained by PCR-RFLP (92% and 93% for *L. tropica* and *L. major*, respectively) and microscopic smears (68% and 45% for *L. major* and *L. tropica*, respectively). Interestingly, the IgG CL-Gal levels were also highly elevated (~3-fold) in cured individuals even 1 year post treatment.

POSTER 12

Understanding differential regional sensitivity of rapid diagnosis for visceral leishmaniasis: antigenic diversity of East African *Leishmania donovani*, and comparatively higher IgG levels among Indian patients

Tapan Bhattacharyya, Duncan E. Bowes, Sayda El-Safi, Shyam Sundar, Andrew K Falconar, Om Prakash Singh, Rajiv Kumar, Osman Osman, Marleen Boelaert, Michael A. Miles.

Faculty of Infectious and Tropical Diseases, London School of Hygiene and Tropical Medicine, Keppel Street, UK; Faculty of Medicine, National University of Khartoum, Sudan; Institute of Medical Sciences, Banaras Hindu University, Varanasi, India; Departamento de Medicina, Universidad del Norte, Baranquilla, Colombia; Department of Public Health, Institute of Tropical Medicine, Antwerp, Belgium

Visceral leishmaniasis (VL) remains an important public health problem in the endemic regions of South Asia, East Africa, and Brazil. A point-of-care rapid diagnostic test (RDT) for anti-*Leishmania donvani* antibodies, based on the rK39 kinesin antigen, has given high sensitivity in South Asia but is less effective in East Africa. This may be explained by regional diversity of the *L. donovani* kinesin antigen or by differential levels of IgG production among Indian and East African VL patients. To investigate antigenic diversity, we amplified and sequenced the section of the kinesin gene corresponding to the rK39 diagnostic antigen from a panel of East African *L. donovani* and compared the sequences to those of South Asian *L. donovani*. We observed regional specific polymorphisms, with substantial non-conservative changes in the N terminal half of each antigen repeat, and small stretches of conserved residues towards the C terminus. We also compared anti-*L. donovani* IgG titres among Indian and Sudanese VL patients by ELISA. We showed a clear difference between the two groups, namely that Indian patients had a significantly higher level of anti-*Leishmania* antibodies compared to those from Sudan. These observations remained consistent when further analysed by age, gender or geographical source of the lysate antigen used in the ELISA. Thus both diversity of the rK39 diagnostic antigen and the distinct anti-*Leishmania* IgG response levels between Indian and Sudanese VL patients are likely to contribute to the differential regional efficacy of the rK39 RDT.

POSTER 13

Estimating prevalence of urogenital schistosomiasis in pre-school and primary-school aged children using antibody responses

Welcome Mkululi Wami, Norman Nausch, Nicholas Midzi, Takafira Mduluza, Mark Woolhouse, Francisca Mutapi

Institute for Immunology and Infection Research, Center for Infection, Immunity and Evolution, Ashworth Laboratories, University of Edinburgh, West Mains Road, Edinburgh EH9 3JT, UK; Research Council of Zimbabwe, Mount Pleasant Business Park, Harare, Zimbabwe; Department of Biochemistry, University of Zimbabwe, Harare, Zimbabwe.

Urogenital schistosomiasis is caused by *Schistosoma haematobium* and in infected young children, egg excretion is low and this may result in underestimated infection prevalence. Egg count in urine is the gold standard for quantifying *S. haematobium* infection levels in the population. Frequency of treatment as outlined by the WHO depends on the prevalence and thus more sensitive methods of estimating prevalence are essential for the correct implementation of control strategies. To compare prevalence of infection in children determined using antibodies to the prevalence based on egg counts. The study was conducted on children aged 1-10 years residing in *S. haematobium* endemic area in Zimbabwe. Prevalence and force of infection were determined using parasitological data and IgM antibodies directed against eggs. Bimodal normal mixture and generalized regression models were applied to estimate overall prevalence and age-dependent prevalence respectively. Estimated prevalence based on parasitology was 41% (37-46%), and 82% (70-94%) using serology and it increased with age. Infection intensity and antibody levels were both significantly associated with age ($p<0.0001$). IgM antibodies are indicative of exposure to schistosome antigens. Levels of egg-specific antibodies increased with age, which may be due to an increase in exposure to infective water. Prevalence based on parasitological data was lower compared to that based on serology. The findings highlight the potential of underestimation of prevalence based only on parasitology in young children.

POSTER 14

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

Tapan Bhattacharyya, Andrew Falconar, Alejandro Luquetti, Michael A. Miles

Faculty of Infectious and Tropical Diseases, London School of Hygiene & Tropical Medicine, Keppel Street, London, UK; Departamento de Medicina, Universidad del Norte, Baranquilla, Colombia; Hospital das Clinicas, Universidade Federal de Goiás, Goiânia, Brazil.

Chagas disease, caused by the protozoan *Trypanosoma cruzi*, remains an important parasitic disease in the Americas. It can be fatal in the acute phase, but life-long chronic infection may be asymptomatic, or lead to debilitation and death

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

POSTER 15

The novel use of TPPA on dried blood spots to test for yaws in Vanuatu

Stephanie Migchelsen, RA Dabbs, W Donald, CJ Drakeley, A Hightower, J Kool, G Taleo, DCW Mabey, LS Vestergaard, AW Solomon

London School of Hygiene and Tropical Medicine, UK; Ministry of Health, Vanuatu US Centres for Disease Control, USA; WHO, Vanuatu

Yaws is a re-emerging endemic treponemal infection, with some of the Pacific Island countries a major focus of infection. WHO is aiming to eradicate yaws by 2020 with a strategy that rely on epidemiological mapping of cases and use of single-dose azithromycin treatment. To map the burden of yaws in Vanuatu, a population-based prevalence survey of yaws was carried out as part of a standard malaria indicator household survey. Clinical signs of yaws were recorded and blood samples collected as dried blood spots from children <15 years. A standardised questionnaire was used to collect clinical history and information on previous yaws treatment received. The samples were tested using the Serodia TPPA. The protocol was modified as per Smit et al, 2013 for DBS, with the samples first being eluted, then tested at 1:8 concentration using the TPPA kit. Results were read after the two hour incubation period, and interpreted according to the settling patterns of the sensitised and unsensitised particles. Here we present preliminary data on the prevalence of treponemal/yaws infection in Vanuatu. Our initial testing shows approximately 12% prevalence nationally, though this varies greatly from island to island; for example, samples from Tanna show nearly 20% prevalence, while samples from Pentecost show zero prevalence. This work contributes significantly to our understanding of the epidemiology of yaws in Vanuatu and offers a proof of principle for the use of dried blood spots and TPPA kits as a convenient and easy-to-use method of sample collection and testing for treponemal infection.

POSTER 16

Evaluation of loop-mediated isothermal amplification (LAMP) as diagnostic tool for *Paragonimus westermanni* in The Philippines

Charlotte Gower, Asuncion C, Naig JR, Webster JP, Belizario VY

Department of Infectious Disease Epidemiology, Imperial College London, Norfolk Place, London W2 1PG, UK; National Institute of Health, University of Philippines, Manila,Philippines

Current diagnosis of paragonomiasis, via detection of eggs by microscopy in sputum or faeces, has limited sensitivity. Inappropriate diagnosis can results in confusion with TB and recent studies in the Philippines suggest that 25-50% of suspected TB patients in some endemic areas, were suffering instead from *Paragonimus* infection. Preliminary experiments were conducted to optimise a robust LAMP reaction for detection of *Paragonimus* DNA based on a published assay. This assay was also demonstrated to detect other *Paragonimus* species, including *P. africanus* and *P. uterobilateralis*. Experiments were conducted to compare DNA extraction methods to detect *Paragonimus* in clinical samples (sputum, urine and stool), and extraction using a commercial Qiagen extraction technique was found to be the most reliable method. Field trials were carried out in Zamboanga del Norte, The Philippines. 278 local residents aged 3-86 who reported persistent cough were examined and provided clinical samples. LAMP using sputum, urine and faecal samples was compared to detection of eggs in sputum or faeces and the use of a questionnaire regarding history of crab consumption (crabs are the route of infection for this disease) and clinical symptoms. Preliminary analyses reported prevalence by microscopy of 6.7% and by LAMP of 13%. Stool microscopy showed low sensitivity compared to detection of eggs in sputum, but similar sensitivity in stool and sputum was reported by LAMP detection. Clinical history (cough, hemolysis and history of eating raw crabs or shrimps) appeared relatively non-specific on initial analyses.

POSTER 17

Evaluation of Loop-mediated isothermal amplification (LAMP) for *Schistosoma mansoni* detection in post-treatment situations in Uganda

Charlotte Gower, LG Prager, ET Muheki, RHL Lamberton
Department of Infectious Disease Epidemiology, Imperial College London, Norfolk Place, London W2 1PG, UK; Vector Control Division, Ministry of Health, 15 Bombo Road, Kampala, Uganda

Published LAMP assays for *S. mansoni* detection were adapted for human clinical samples. Initial optimisations determined that stool was the most sensitive sample for testing, and storage and DNA extraction techniques suitable for LAMP testing were developed. The specificity and sensitivity of Kato-Katz (KK) microscopy, CCA tests and LAMP for diagnosis of *S. mansoni* was compared in 100 children from each of three schools in the highly endemic Mayuge district pre-, 1 week post- and 3 weeks post-treatment with praziquantel. At baseline LAMP showed a lower than expected sensitivity with prevalences of 90.6%, 72.4%, 90.6% and 61.1% recorded by 3 days KK, 1 day KK, CCA and LAMP respectively. At one week post treatment, LAMP was more sensitive at 69.2% but still lacked sensitivity compared to the gold standard of 3 days KK of 78.6%. LAMP showed similar sensitivity to one day KK and CCA, which had estimated costs £1.66 and £1.80 per test, in comparison to £6.38 per test for the LAMP reaction. At 3 weeks post-infection there was a strong discordance between KK (3 day and 1 day) and CCA results, with prevalences of 5.1%, 1.5% and 64.2% recorded. Detection of *S. mansoni* by LAMP in 51.5% of infections suggested that this resulted from a lack of sensitivity by Kato-Katz rather than a lack of specificity by CCA. This suggests very rapid re-infection in this region, as well as limited use of microscopy in determining drug efficacy.

POSTER 18

The development of a novel serodiagnostic test for the detection of ascariosis in fattening pigs

Johnny Vlaminck, J Vercruysse, P Geldhof
Laboratory of Parasitology, Ghent University, Belgium.

Every day, pigs all over the world get infected with the intestinal parasite *Ascaris suum*. As a consequence, many farmers see their profits decreased by the negative impact these roundworms have on the growth and general health of their pigs. However, due to the subclinical nature of the disease together with the lack of proper diagnostic tests, ascariosis often remains undiagnosed. The development of new and improved tools for the detection of roundworm infections in pigs is therefore necessary. Here we describe the development of a novel immuno-diagnostic ELISA using the purified *A. suum* haemoglobin (AsHb) molecule as an antigen. Initial validation of the test using sera of 190 piglets after 7 and 14 weeks of experimental infection with *A. suum* demonstrated that the AsHb ELISA is able to detect long-term exposure to *A. suum* with high sensitivity and specificity (99.5% and 100.0% respectively). This serological test proved to be more sensitive than faecal examination on week 7 and 14 of the experiment (99.5% and 100% compared to 59.5% and 68.4% respectively). Additionally, analysis of sera from 14 Flemish fattening farms showed significant correlations between the mean ODr results and the average daily gain and the days to market (ρ=-0.761;P<0.05 and ρ=0.732;P< 0.01 respectively). ELISA tests performed on sera from fattening farms show highest numbers of A. suum positive farms in France (64%) followed by Belgium (55%) and The Netherlands (45%). This new ELISA could offer farmers and veterinarians an easy tool to assess the Ascaris-status of their herd.

POSTER 19

Evaluation and application of a next generation droplet digital PCR assay for the detection of ocular *Chlamydia trachomatis* infection in a trachoma-hyperendemic population in the Bijagas Archipelago of Guinea Bissau, West Africa

Anna Last, C Roberts, E Cassama, M Nabicassa, S Burr, D Mabey, R Bailey, M Holland
Clinical Research Department, London School of Hygiene and Tropical Medicine, UK; Programa Nacional de Saúde de Visão, Ministerio de Saude Publica, Guinea Bissau; Disease Elimination Theme, MRC Unit The Gambia.

Trachoma is caused by ocular *Chlamydia trachomatis* (Ct) infection. The WHO advocates mass drug administration (MDA) with antibiotics for trachoma control. MDA impact is evaluated by measuring TF (follicular trachoma) prevalence in 1-9 year olds. Testing for infection may be useful in measuring MDA impact, where TF persists but infection has been reduced/eliminated. We evaluated and applied a novel droplet digital PCR (ddPCR) assay for Ct detection and quantitation in conjunctival swabs against the Roche Amplicor® CT/NG test in trachoma-hyperendemic treatment-naïve communities. PCR by Amplicor and ddPCR was undertaken on extracted DNA from 1507 samples with corresponding clinical phenotype at baseline. The cohort was followed up one year post-MDA with repeat examination, sampling and ddPCR. ddPCR was precise and accurate (R2~0.995). Sensitivity against Amplicor was 73.3% (67.9-78.7%) and specificity

99.1% (98.6-99.6%). Negative and positive predictive values were 94.6% (93.4-95.8%) and 94.5% (91.3-97.7%). TF in 1-9 year olds at baseline was 21% (136/660) and Ct infection 26%. Prevalence of Ct infection overall was 16%. Median Ct load was 2038 copies/swab (15-274,835). Most infections had low loads (<1000 copies/swab). At follow-up, overall infection prevalence was 3.5%, very low load infections were absent and the prevalence of TF (7.5% (29/384) (t=5.74,p<0.0001)) and Ct infection (6.8% (t=7.64,p<0.00001) in 1-9 year olds were significantly reduced. There may be a threshold of Ct infection prevalence and intensity below which infection spontaneously disappears from a population ('Allee effect'). Quantitative tests such as this ddPCR assay may be valuable to determine thresholds and in decisions to discontinue MDA.

POSTER 20

Screen my genome to reveal my secrets - multiplex ligation-dependent probe amplification (MLPA) as a tool to identify drug resistance in *Plasmodium falciparum* and *Mycobacterium tuberculosis* isolates

Petra Mens, Henk Schallig, Sarah Sengstake, Indra Bergval, Anja Schuitema, Laura de Bes, Kiki Tuin, **Richard Anthony**
KIT Biomedical Research, Royal Tropical Institute, Amsterdam, The Netherlands; MRC Holland, Amsterdam, The Netherlands.

MLPA is a tool for targeting genetic markers such as single nucleotide polymorphisms (SNP), in a (patho)genome of choice. The technique amplifies sequence-specific MLPA probes rather than target DNA. Standard MLPA amplicons are length coded and readout is by fragment analysis. At the Royal Tropical Institute we have developed a *Mycobacterium tuberculosis* (MTB)-specific MLPA (Bergval et al. 2008 JCM) with zipcode readout. With this assay we screened the DNA of cultured isolates for the presence of genotypic markers and mutations conferring drug resistance. Comparison of the MLPA results with the the gold standard methods for drug resistance determination and strain identification was performed. Readout is on a liquid bead array (Luminex MagPlex-TAG) allowing high throughput and multiplexing of up to 50 targets per isolate (Bergval, Sengstake et. al. 2012 PLOS One). Assay implementation in central hospitals as well as in district level laboratories of high burden middle income countries isongoing. Furthermore, MLPA was developed to detect, in the genome of the malaria parasite *Plasmodium falciparum*, SNPs causing anti-malaria drug resistance. For example, mutations in the dhps and dhfr genes associated with sulphadoxine–pyrimethamine (SP) resistance. MLPA was developed for the known mutations conferring SP resistance, dhfr codons 51, 59, 108 and dhps codons 437, 540 and 613. Assay readout used gel analysis or capillary electrophoresis. .The assay was validated on P. falciparum reference strains and clinical samples. Further applications for monitoring resistance within control programmes are being explored.

POSTER 21

Isolation and characterisation of novel candidate *Leishmania donovani* antigens from urine of visceral leishmaniasis patients

Tegwen Marlais, Andrew Falconar, Tapan Bhattacharyya, Shyam Sundar, Sayda El-Safi, Osman Osman, Om Prakash Singh, Carolyn Hyde, Marleen Boelaert, Michael Miles
Faculty of Infectious and Tropical Diseases, London School of Hygiene and Tropical Medicine, Keppel Street, UK; Departamento de Medicina, Universidad del Norte, Baranquilla, Colombia; Institute of Medical Sciences, Banaras Hindu University, Varanasi, India; Faculty of Medicine, National University of Khartoum, Sudan; Wolfson Institute for Biomedical Research, University College London, UK; Department of Public Health, Institute of Tropical Medicine, Antwerp, Belgium.

Visceral Leishmaniasis (VL) is caused by the protozoan parasites *Leishmania donovani* and *L. infantum*. Symptomatic VL is fatal if untreated and affects about 500,000 people/year worldwide, with 90% of cases in the Indian subcontinent and Sudan. Diagnosis for VL currently relies on demonstration of parasites in spleen or bone marrow aspirates, and serology. Serological tests cannot confirm cure or detect relapsed infection because patients remain seropositive for several years after cure. Rapid serological tests, such as the rK39 lateral flow test, are most convenient but have variable sensitivity in different regions. The only existing urine antigen-detecting test, KATex, suffers from low sensitivity. A sensitive antigen detection test using urine would overcome these limitations. We aim to identify suitable alternative *Leishmania* antigens from the urine of Sudanese and Indian VL patients. Rabbit IgG anti-L*eishmania* was used to probe western blots of VL and control urine and to affinity purify *Leishmania* antigen from Indian VL urine. Human VL serum successfully detected affinity-purified 16-24 kDa antigen on western blot. Bands of interest were excised from the gels and submitted to MALDI-TOF mass spectrometry to identify the proteins. Strongly antigenic proteins of 55 and 33 kDa were recognised in VL urine by rabbit IgG anti-*Leishmania*. So far one 40 kDa *L. donovani* protein is a possible match for peptides identified by mass spectrometry. Further work is also in progress to affinity-purify *Leishmania* proteins from Sudanese urine and to characterise the relevant antigens.

POSTER 22

Host cytokine response to rapidly confirm the effect of tuberculosis treatment

RM Anthony, AL Den Hertog, MM Montero, JB Sherchand, LE Cuevas
KIT Biomedical Research, Royal Tropical Institute, The Netherlands; Liverpool School of Tropical Medicine, UK; Tribhuvan University Institute of Medicine, Nepal.

Many patients with symptoms of TB are treated empirically without confirmation. The lack of simple diagnostic methods for TB, especially for smear-negative patients and those who have drug resistant strains led us to investigate a novel Treat-to-Test strategy to confirm a diagnosis of TB. We have previously shown in mice that serum levels of IFNy, IP-10, MIG and MCP-1 decreased within 1 week of treatment among TB infected subjects compared to placebo. We therefore hypothesized that the killing of large numbers of mycobacteria after initiation if therapy in vivo would elicit similar kinetic patterns among selected biomarkers in patients with TB. Measuring these biomarkers at pre-established time points could confirm a clinical diagnosis, long before cultures become available and identify patients that require additional testing for drug resistance. Here we present serum samples from 24 patients with an without TB in Nepal at days 0, 1, 3, 5, 7 and 14 of antituberculosis therapy in which cytokines IFNy, IP-10, MIG, MCP-1, TNFa and IL-6 were measured. IFNy, IL-6, IP-10 and MIG serum levels decreased significantly within the first week of treatment in culture positive patients (n=16). Serum levels of culture-negative patients (n=8) remained unchanged. IFNy, IL-6 and IP-10 had a strong decrease with time in 3 out of 4 smear-negative culture positive patients. Rapid immunological changes occurring soon after starting therapy have the potential to confirm the diagnosis in patients with smear-negative TB. Future work will include a larger number of patients and drug resistant pathogens.

POSTER 23

A novel assay to test the immunogenicity of *Leishmania* DNA vaccines

Khdija Suleman Ali, Robert C Rees, Christopher Terrell-Nield, A Ali Selman
Interdisciplinary Biomedical Research Centre, School of Science and Technology Nottingham Trent University, Clifton lane, Nottingham, NG11 8NS, UK; The John van Geest Cancer Research Centre School of Science and Technology Nottingham Trent University Clifton Lane Nottingham, NG11 8NS, UK.

A novel assay was developed to investigate and compare the immunogenicity of *Leishmania* DNA constructs as potential vaccines. The principle of the test is based on the failure of the amastigotes generation in macrophages derived from immune mice to transform into promastigotes. In this assay Balb/c mice were immunized three times (day 0, 14, and 32), using gene gun with pcRT7/CT-Topo, VR1012, and pcDNA3.1 encoding *L. mexicana* gp63, *L. donovani* centrin1, and *L. donovani* centrin3, empty plasmids were used as controls. Two weeks after the last immunization, bone marrow derived macrophages, (BM-DMs) were generated from hind limbs of naïve and immunized mice. BM-DMs were infected with late log phase virulent *L. mexicana* promastigotes, the non-attached promastigotes were removed 4 hours later by washing, and infected cells were further incubated for 48 hours at 37°C to produce the amastigotes stage. The results show the inability of amastigotes isolated from immunised macrophages to transform to the promastigotes stage when incubated at 25°C,

POSTER 24

Schistosomiasis diagnosis at the single worm level: approaching the ultimate sensitivity by UCP-CAA lateral flow

Govert J van Dam, Claudia J de Dood, Dieuwke Kornelis, Paul LAM Corstjens
Department of Parasitology (Parasite Diagnostics), & Department of Molecular Cell Biology, Leiden University Medical Center, Leiden, The Netherlands.

The well-studied schistosome antigen detection ELISA's (CCA- and CAA-ELISA) have been transformed into a Point-of-Care rapid test (urine POC-CCA) and an ultra-sensitive UCP lateral flow based assay (serum/urine UCP-CAA). The rapid POC-CCA test may replace the Kato-Katz testing for prevalence mapping of community-level *S. mansoni* infections using a single drop of urine. The UCP-CAA assay, applicable to serum and urine samples, detects antigen at sub-pg levels, a sensitivity allowing detection of single worm infections. In experimentally infected baboons and in humans with very low egg counts, CAA is easily detected and correlates with worm and egg counts. The effect of praziquantel treatment can be clearly shown within days after treatment. The dry reagent field applicable test is currently used in several African countries. The UCP-CAA assay therefore is a highly valuable sensitive diagnostic tool, for screening and case finding in very low prevalence areas, including pre-elimination settings.

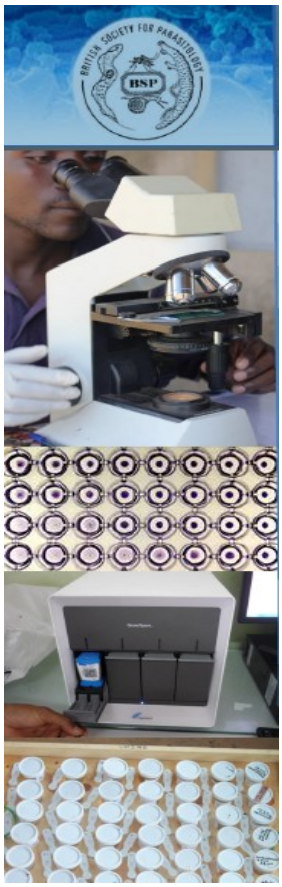
Acknowledgments

Immediate thanks to the speakers for their efforts in attending this meeting, to those delegates who are to present a poster, as well as, those in the audience registered to attend.

Special thanks are due to Ness Gardens, UoL and to Ms Jen Lemon for hosting us at this lovely venue on the Wirral and to RW Bespoke Events for catering throughout the meeting.

Thanks are also due to Mary Creegan for helping in production of this abstract booklet and other helpful tasks.

The meeting gratefully received support from the following organisations in terms of sponsorship:



Boehringer-Ingelheim

Cambridge University Press

Epistem

MAST group

Partec

Royal Society Publishing

The BSP looks forward to seeing you again at

The 2014 Spring Meeting in Cambridge

***see* www.bsp.uk.net**