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# BSP Autumn Symposium 2018 - Parasite Glycobiology

## Welcome

Among the most important human deaths are those caused by parasitic protozoans and helminths. More than a million people die each year from diseases like malaria and several neglected tropical diseases. Patients affected by these diseases also endure disabilities that cause lifelong suffering and that hamper productivity and development. Importantly, parasitic diseases also generate vast economic losses, since they also affect farming.

Glycobiology has become a well-established area of study in recent decades and is currently providing drug targets against several pathogens and diseases, including those caused by parasites. Research in parasite glycosylation provides new opportunities for the discovery of vaccine candidates and for the development of novel chemotherapy approaches and diagnostic tools. Thus, for instance, besides its effect modulating the host immune response against the infection, glycans from *Schistosoma mansoni* are currently being explored as targets for vaccination and/or serodiagnosis of human schistosomiasis and infections caused by kinetoplastid parasites.

Today's symposium will have presentations from experts in different aspect of parasite glycobiology to discuss cutting-edge aspects like evolution of glycosylation pathways, structure and biosynthesis parasite glycoconjugates, disease biomarkers, glycovaccine development and other translational applications. There is also a section for younger scientists on how to exploit social media for science communication.

I would like to express my gratitude to Mr Julian Fuller (BSP) and Mrs Mary Creegan (LSTM) for their invaluable logistic help, and Cambridge University Press, the British Society for Parasitology and the Liverpool School of Tropical Medicine for their support.

I hope you all enjoy the symposium!

Alvaro Acosta Serrano

## Contents

## Event Schedule

**Session A** - 10:15 to 12:10

Chair - Igor Almeida

10:15 (15 mins) - *Welcome & introduction* (Alvaro Acosta-Serrano)

10:30 (25 mins) - *ER-associated degradation and disposal of misfolded GPI-anchored proteins in Trypanosoma brucei* (James Bangs)

10:55 (25 mins) - *Comparative Invertebrate Glycomics* (Iain Wilson)

11:20 (25 mins) - *Diversification of UDP-glycosyltransferase genes in trypanosomatid genomes* (Andrew Jackson)

11:45 (25 mins) - *Protein C-mannosylation in Toxoplasma gondii* (Françoise Routier)

**Speed talks 1** - 12:10 to 12:25

Chair - Alvaro Acosta-Serrano

12:10 (5 mins)- *New approaches to studying the GPI biosynthesis pathway in T. brucei: uncovering the missing links.* (Zhe Ji)

12:15 (5 mins) - *New insights in the structure-function relation of trans-sialidase from T. congolense* (Mario Waespy)

**Session B** – 13:30 to 14:45

Chair - Alvaro Acosta-Serrano

13:30 (25 mins) - *Pinpointing key glycan antigens of schistosomes targeted by the mammalian host immune response* (Cornelis Hokke)

13:55 (25 mins) - *An $\alpha$-Gal-based glycovaccine protects both mice and nonhuman primates against Chagas disease* (Igor Almeida)

14:20 (25 mins) - *In search of $\alpha$-Gal glycans in Plasmodium falciparum* (Luis Izquierdo)

**Speed talks 2** - 14:45 to 15:00

Chair - Alvaro Acosta-Serrano

14:45 (5 mins) - *Existence of Male Genital Schistosomiasis (MGS) in fishermen along Southern shores of Lake Malawi* (Sekeleghe Kayuni)

14:50 (5 mins) - *Antigenic cross-reactivity between the tropical helminth parasite Schistosoma mansoni and the house dust mite Dermatophagoides farinae: a role for cross-reactive carbohydrate determinants (CCDs) and implications for the hygiene hypothesis.* (Fatou Gai)

**Session C** - 16:10 to 17:50

Chair - Luis Izquierdo

16:10 (25 mins) - *Exploiting carbohydrate binding agents as anti-trypanosomals* (Dolores González-Pacanowska)

16:35 (25 mins) - *Insertion of N-terminal hinge glycosylation enhances interactions of human IgG1-Fc monomers to glycan-dependent receptors including influenza virus haemagglutinin* (Richard Pleass)

17:00 (25 mins) - *Potential of Glycomics to treatment efficacy, disease progression and therapeutic approaches in American trypanosoamiasis.* (Pedro Bonay)

17:25 (25 mins) - *Leishmania glycoproteins as master manipulators of sand fly and mammalian hosts.* (Matthew Rogers)

### Closing remarks

17:50 (70 mins) - Alvaro Acosta-Serrano

## Orals

### Session A

Presenter: **Prof James Bangs**, *University of Buffalo*

**ER-associated degradation and disposal of misfolded GPI-anchored proteins in *Trypanosoma brucei* - *A16360***

**J Bangs**[1];
[1] Department of Microbiology & Immunology, University of Buffalo, United States

African trypanosomes survive in the bloodstream and tissues in the face of the humoral immune system of the mammalian host by a remarkable process called antigenic variation. The lynchpin of this process is the variant surface glycoprotein (VSG), which is 10% of total cell protein. There is a repertoire of ~1500 VSG genes, only one of which can be expressed at time. Switching occurs primarily by gene conversion whereby the active gene is over-written by another gene from the silent repertoire. Often this process is segmental leading to fusion of sequences from multiple VSG genes. This will ultimately lead to assembly of incompatible segments that are incapable of folding and subsequent forward trafficking from the ER - a catastrophic event unless the cell can cope with the accumulated cargo. Misfolded secretory proteins are generally retained by ER quality control (ERQC) and degraded in the proteasome by ER associated degradation (ERAD). However, in yeast and mammals misfolded glycosylphosphatidylinositol-anchored proteins (GPI-APs) are preferentially degraded in the vacuole/lysosome. To determine if ERAD can handle misfolded GPI-APs in trypanosomes we exploit a misfolded GPI-anchored subunit (HA:E6) of the trypanosome transferrin receptor. HA:E6 is N-glycosylated and GPI-anchored, and accumulates in the ER as aggregates. Treatment with MG132, a proteasome inhibitor, generates a smaller protected polypeptide (HA:E6*), consistent with turnover in the proteasome. HA:E6* partitions between membrane and cytosol fractions, and both pools are proteinase K-sensitive, indicating cytosolic disposition of membrane associated HA:E6*. HA:E6* is de-N-glycosylated and has a full GPI-glycan structure from which dimyristoylglycerol has been removed, indicating that complete GPI removal is not a prerequisite for proteasomal degradation. However, HA:E6* is apparently not ubiquitin modified. Like yeast and mammals, the trypanosome GPI anchor is a forward trafficking signal, thus the dynamic tension between ERQC and ER exit favors degradation by ERAD. These results differ markedly from the standard eukaryotic model systems, and may indicate an evolutionary advantage related to pathogenesis.

Presenter: **Prof Iain Wilson**, *University of Natural Resources and Life Sciences, Vienna*

## **Comparative Invertebrate Glycomics** - *A16361*

**I Wilson**[1]; K Paschinger[1]; A Hykollari[1]; B Eckmair[1]; S Yan[1]; Y Vanbeselaere[1];
[1] Department für Chemie, Universität für Bodenkultur Wien, Austria

Invertebrates are often either parasites themselves or are hosts/vectors for parasitic species, whereby glycans often play key roles in inter-species interactions. Recent data indicates that the diversity of N-glycan modifications in invertebrate species is extremely high. From our own studies on nematodes, cestodes, molluscs and insects, a wide range of new glycan modifications has been demonstrated using an off-line HPLC/MALDI-TOF-MS workflow. Particularly underrepresented in older studies are the ‘charged’ modifications of N-glycans from lower eukaryotes, such as glucuronic acid, sulphate, phosphoethanolamine, aminoethylphosphonate and phosphorylcholine. Not only are there surprises in terms of complexity and similarities of the N-glycan structures, but interactions of potential biological relevance with pentraxins or antibodies can be defined in array or blotting formats. For instance, lepidopteran and cestode species display phosphorylcholine-modified glycans not dissimilar to those in some nematodes and which are potential epitopes for human C-reactive protein; also royal jelly glycoproteins, which have been claimed to be nematicidal, antibiotic or anti-hypertensive, carry phosphoethanolamine residues which can explain their binding to human serum amyloid P protein.

Presenter: **Dr Andrew Jackson**, *University of Liverpool*

## **Diversification of UDP-glycosyltransferase genes in trypanosomatid genomes** - *A16362*

**A Jackson**[1];
[1] University of Liverpool, UK

Trypanosomatid parasites such as *Trypanosoma* spp. and *Leishmania* spp. are a major source of infectious disease in humans and domestic animals worldwide. Fundamental to the host-parasite interactions of these potent pathogens are their cell surfaces, which are highly decorated with glycosylated proteins and other macromolecules. Trypanosomatid genomes contain large multi-copy gene families encoding UDP-dependent glycosyltransferases (UGTs), the primary role of which is thought to be cell-surface decoration. I will present a phylogenetic analysis of UGTs from diverse trypanosomatid genomes, the aim of which was to understand the origin and evolution of their diversity. I have compared UGT repertoire, genomic context and sequence evolution across 19 trypanosomatids. This identified a UGT lineage present in

stercorarian trypanosomes and a free-living kinetoplastid *Bodo saltans* that likely represents the ancestral state of this gene family. The phylogeny of parasite-specific genes shows that UGTs repertoire in Leishmaniinae and salivarian trypanosomes has expanded independently and with distinct evolutionary dynamics. In the former, the ancestral UGT repertoire was organised in a tandem array from which sporadic transpositions to telomeric regions occurred, allowing expansion most likely through telomeric exchange. In the latter, the ancestral UGT repertoire was comprised of seven subtelomeric lineages, two of which have greatly expanded potentially by gene transposition between these dynamic regions of the genome. The phylogeny of UGTs confirms that they represent a substantial parasite-specific innovation, and although they have diversified independently in the major trypanosomatid lineages, developmental regulation has been a strong driver of this innovation in all trypanosomatid genomes.

Presenter: **Prof Françoise Routier**, *Hannover Medical School*

### Protein C-mannosylation in *Toxoplasma gondii* - *A16363*

**F Routier**[2]; A Albuquerque-Wendt[2]; C Hoppe[2]; D Jacot[3]; G Bandini[4]; C E Costello[1]; D Soldati-Favre[3]; H Bakker[2];
[1] Department of Biochemistry, Center for Biomedical Mass Spectrometry, Boston University School of Medicine, United States; [2] Department of Clinical Biochemistry, Hannover Medical School, Germany; [3] Department of Microbiology and Molecular Medicine, University of Geneva Medical School, Germany; [4] Department of Molecular and Cell Biology, Boston University, United States

Trypanosomatid parasites such as *Trypanosoma* spp. and Leishmania spp. are a major source of infectious disease in humans and domestic animals worldwide. Fundamental to the host-parasite interactions of these potent pathogens are their cell surfaces, which are highly decorated with glycosylated proteins and other macromolecules. Trypanosomatid genomes contain large multi-copy gene families encoding UDP-dependent glycosyltransferases (UGTs), the primary role of which is thought to be cell-surface decoration. I will present a phylogenetic analysis of UGTs from diverse trypanosomatid genomes, the aim of which was to understand the origin and evolution of their diversity. I have compared UGT repertoire, genomic context and sequence evolution across 19 trypanosomatids. This identified a UGT lineage present in stercorarian trypanosomes and a free-living kinetoplastid Bodo saltans that likely represents the ancestral state of this gene family. The phylogeny of parasite-specific genes shows that UGTs repertoire in Leishmaniinae and salivarian trypanosomes has expanded independently and with distinct evolutionary dynamics. In the former, the ancestral UGT repertoire was organised in a tandem array from which sporadic transpositions to telomeric regions occurred, allowing expansion most likely through telomeric exchange. In the latter, the ancestral UGT repertoire was comprised of seven subtelomeric lineages, two of which have greatly expanded potentially by gene transposition between these dynamic regions of the genome. The phylogeny of UGTs confirms that they represent a substantial parasite-specific innovation, and although they have diversified independently in the major trypanosomatid lineages, developmental regulation has been a strong driver of this innovation in all trypanosomatid genomes.

## Speed Talks 1

Presenter: **Miss Zhe Ji**, *Biochemistry PhD student, University of Dundee*

### New approaches to studying the GPI biosynthesis pathway in *T. brucei*: uncovering the missing links. - *A16392*

**Z Ji**[1]; M Tinti[1]; L S Guther[2]; M A Ferguson[2];
[1] Biological Chemistry and Drug Discovery, School of Life Sciences, University of Dundee, UK; [2] Wellcome Trust Centre for Anti-Infectives Research, School of Life Sciences, University of Dundee, UK

The bloodstream form of *Trypanosoma brucei* (*T. brucei*) is coated with glycosylphosphatidylinositol (GPI) anchored variant surface glycoprotein (VSG). These GPI anchored VSG homodimers act as the first line of defence for the parasites and undergoes antigenic variation leading to persistent infection. A lot is known about GPI anchor biosynthesis in *T. brucei*, indeed the first studies of GPI anchor structure and biosynthesis were performed on this organism and these methodologies were subsequently applied to mammalian cells, yeast and other organisms. Despite conservation in the core structure of almost all GPI anchors across eukaryotic evolution, notable differences occur between the *T. brucei* and mammalian GPI anchor biosynthetic pathways making this pathway possible a drug target. Here we present a quantitative proteomics approach to identify the proteins that catalyse key steps of GPI anchor biosynthesis in *T. brucei* but are still

unknown. Known components in this pathway were epitope tagged with Myc sequences and co-immunoprecipitation was conducted using agarose beads to the tag. SILAC proteomics were then performed to identify associated proteins that may induce missing links in the GPI pathway. TbGPI12 encoding de-N-acetylase that catalyses the second step in the pathway was chosen first to act as the bait protein to perform such a pull down. NativePAGE Western blot after pulling down TbGPI112-Myc has shown TbGPI12 has formed complexes and the SILAC proteomics has identified specifically co-immunoprecipitated proteins. These results show that we have successfully established a SILAC proteomics method for the co-immunoprecipitation of proteins with epitope tagged components of the GPI anchor biosynthesis pathway in *Trypanosoma brucei*. This method can be expanded to other known components in this pathway and find the missing links.

Presenter: **Dr. Mario Waespy**, *PostDoc, University of Bremen*

### New insights in the structure-function relation of trans-sialidase from *Trypanosoma congolense* - *A16389*

**M Waespy**[1];

[1] Centre for Bimolecular Interactions Bremen CBIB, University of Bremen, Germany

Trypanosomes are protozoan parasites causing trypanosomiasis in South America (Chagas' disease) and Sub-Saharan Africa (sleeping sickness in humans and Nagana in livestock). African Animal Trypanosomiasis (AAT also called Nagana), predominantly caused by *Trypanosoma congolense* is a devastating disease in domestic African animals causing an annual loss in agriculture productivity of about 4.5 billion USD due to the death of more then 3 million cattle, whereas 40 millions are estimated to be threatened. Trypanosomes express unusual enzymes termed trans-sialidases (TS). TS are surface-bound enzymes, which catalyse the transfer of sialic acids (Sia) from host cell glycoconjugates to terminal galactose residues of target acceptor substrates, such as those of the parasite's surface [1]. Several studies have shown that TS play important roles in the pathology of trypanosomiasis in mammalian host and are essential for survival of the parasite also in the insect vector, representing TS as a major virulence factor [1].

Our studies on *Trypanosoma congolese* TS (TconTS), revealed 14 active variants exhibiting significantly different enzymatic activities, although these cannot be sufficiently explained by amino acid variations at the catalytic centre [2,3]. Besides the catalytic domain (CD), all TS contain a lectin-like domain (LD), whose biological function still remains unknown. Recently we demonstrated the carbohydrate binding ability of TconTS-LD to mannose oligosaccharides and high-mannose type N-glycans of glycoproteins [4]. However, mannose oligosaccharides do not represent substrates for the catalytic Sia transfer but are commonly found as part of several glycoconjugates of parasite's, vector's and host's cell surface molecules [5]. Interestingly, in further studies focusing on the potential of LD's to modulate enzyme activities we demonstrated that loss of TconTS-LD carbohydrate binding ability significantly affects TS activities. Finally we demonstrated that N-glycosylation of TconTS enzymes itself plays a more pivotal role in enzyme activity. In this vein, it appears that many underlying factors regulate TconTS enzyme function as previously thought, which might represent one of the reasons for the absence of potent TS inhibitors. In conclusion, unravelling the different roles played by these factors will provide more perspectives in understanding the mechanisms of these interesting enzymes and consequently open new avenues towards the development of new strategies to control African trypanosomiasis.

**References**:

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## Session B

Presenter: **Prof Cornelis Hokke**, *Leiden University Medical Center*

### Pinpointing key glycan antigens of schistosomes targeted by the mammalian host immune response - *A16369*

**C Hokke**[1]; [2];

[1] Leiden University Medical Center, Netherlands; [2] Leiden University Medical Center, UK

Helminths express an abundance of proteins and lipids with complex glycosylation patterns. Glycomics studies are providing more and more insights into glycan and glycoconjugate expression by different helminth species, including major trematodes, nematodes and cestodes of clinical and veterinary importance such as *Schistosoma*, *H. contortus*, *F. hepatica* and *Echinococcus*. Antigenic glycans of different life stages of most helminths induce specific antibodies in the mammalian host, and helminth-associated glycan motifs trigger innate mechanisms that modulate inflammatory immune responses.
In particular schistosomes have been extensively studied with respect to structural and functional glycomics. To provide a map of schistosome glycosylation in support of functional studies of host-parasite glycobiology my research group applied mass spectrometric (MS) glycomics approaches to determine expression profiles of hundreds of protein- and lipid-linked glycans during the schistosome life cycle. In addition, we have generated a microarray of hundreds of N-, O-, and lipid-glycans covering the entire glycome of *S. mansoni*. The constructed glycan microarray was used to determine IgG and IgM to each glycan in human and animal infection cohorts.

Striking shifts and switches in the expression of putative functional glycan motifs during during worm and egg development were identified suggesting various roles of glycans and associated anti-glycan responses during infection. For instance, using clear examples, I will discuss how glycan motifs contribute to characteristic immunomodulatory properties of schistosome egg antigens such as omega-1. Also, based on cross-sectional and longitudinal studies of antibodies in sera from natural and experimental schistosome infections, I will discuss the potential of antibodies against specific glycan motifs as targets for schistosomiasis vaccines and diagnostics. The schistosome glycomics data will be discussed in relation to the glycome and glycogenome of other helminth species. It is concluded that schistosome glycans are attractive targets for novel therapeutics and control tools.

Presenter: **Prof Igor Almeida**, *The University of Texas at El Paso*

## An α-Gal-based glycovaccine protects both mice and nonhuman primates against Chagas disease - *A16370*

**I Almeida**[1];
[1] Department of Biological Sciences, The University of Texas at El Paso, United States

Chagas disease (ChD) caused by *Trypanosoma cruzi* affects 6-8 million people worldwide. Chemotherapy is partially effective and toxic, and there is no preventive or therapeutic vaccine. *T. cruzi* has a surface coated by highly immunogenic mucins, which contain the immunodominant glycotope αGal(1,3)Galβ(1,4)GlcNAc (Galα3LN) that induces abundant lytic anti-α-Gal antibodies. Here we evaluated the efficacy of the Galα3LN glycotope, covalently attached to the carrier protein human serum albumin (HSA), as potential vaccine in both murine and nonhuman primate models of ChD. First, the neoglycoprotein (NGP) Galα3LN-HSA was tested in the α1,3-galactosyltransferase-knockout (α1,3-GalT-KO) mice, which akin to humans and Old-World nonhuman primates do not express linear α-Gal glycotopes. α1,3-GalT-KO Mice vaccinated with Galα3LN-HSA were fully protected against lethal *T. cruzi* challenge by inducing a strong anti-α-Gal antibody-mediated humoral response and a balanced Th1/Th2-type cellular immunity. Furthermore, Galα3LN-HSA-vaccinated mice exhibited significant reduction (91.7-99.9%) in parasite load in all tissues analysed, cardiac inflammation, myocyte necrosis, and T-cell infiltration. Next, nonhuman primates (baboons) were immunized with NGP Galα3LN-HSA plus adjuvant (LMPLA), or LMPLA alone (control). Animals were subsequently challenged twice with *T. cruzi* metacyclic trypomastigotes (previously isolated from baboons) and humanely euthanized 14 weeks following the first parasite challenge. A strong anti-α-Gal Ab response, considerable reduction in cardiac parasite burden and inflammation, and protective Th1- and Th17-mediated response were observed in NGP24h+LMPLA-vaccinated baboons but not in control animals. Untargeted metabolomics identified overall cardiac correlates of vaccine protection and regiospecific alterations between cardiac ventricles. This is the first proof-of-concept study to demonstrate the efficacy of a prophylactic α-Gal-based glycovaccine for experimental acute and chronic Chagas disease, in both mice and non-human primates.

Presenter: **Dr Luis Izquierdo**, *Barcelona Institute for Global Health*

## In search of α-Gal glycans in *Plasmodium falciparum* - *A16354*

**L Izquierdo**[1];
[1] Instituto de Salud Global de Barcelona, Spain.

Annually, malaria causes more 200 million clinical cases and 450,000 deaths. Recent studies suggest the presence of unknown α-galactose (α-gal)-containing glycans on the surface of Plasmodium sporozoites, which invade the mosquito salivary glands and are delivered to the human host during blood meal. Due to gene inactivation of α-1,3-galactosyltransferase (α1,3GT) during evolution, human cells lack α-gal epitopes and produce anti-α-gal antibodies, which are the most abundant immunoglobulines in humans. Notably, individuals with higher anti-α-gal antibody levels are protected against malaria infection in endemic areas, and vaccination against α-gal confers sterile malaria protection in α1,3GT-deficient mice. Therefore, immunization with appropriate α-gal epitopes and the boost of anti-α-gal antibody levels could offer a unique opportunity to protect against malaria infection and significantly reduce its transmission. Remarkably, the malaria parasite produces UDP-galactose, the sugar nucleotide donor required by galactosyltransferases. Nevertheless, much remains unknown about the P. falciparum glycoconjugates containing α-gal epitopes, including their putative expression in other parasite stages. We have investigated the α-gal presence in the different stages of *P. falciparum* development with the goal to map glycoconjugates putatively carrying α-gal epitopes. Using anti-α-gal antibodies and lectins that recognize α-gal, we were unable to detect α-gal-containing glycans in the parasite sporozoite stages. However, we have observed α-gal-containing proteins in ring and trophozoite stages. Interestingly, α-gal is not present in schizonts, merozoites or gametocytes, suggesting a tightly regulation of α-gal expression through the different stages of intraerythrocytic sexual and asexual development. We are currently carrying out anti-α-gal pull downs for LC-MS/MS based protein identification to tackle the analysis of α-gal glycoconjugates. The identification and characterization of these α-gal-containing glycans may be exploited for the development of novel glycan-based protein conjugate vaccines against the parasite.

## Speed Talks 2

Presenter: **Dr Sekeleghe Kayuni**, *Commonwealth PhD scholar, Liverpool School of Tropical Medicine / UoL*

### Existence of Male Genital *Schistosomiasis* (MGS) in fishermen along Southern shores of Lake Malawi.

**S A Kayuni**[1]; J R Stothard[1]; E J Lacourse[1]; P Makaula[4]; F Lampiao[3]; L Juziwelo[2];
[1] Liverpool School of Tropical Medicine / UoL, UK; [2] National Schistosomiasis and STH Control Program, CHSU, Ministry of Health, Malawi; [3] Physiology department, College of Medicine, University of Malawi, Malawi; [4] Research in Health, Environment and Development (RHED), Malawi

**Introduction**

Male genital schistosomiasis (MGS) is a specific manifestation of schistosomiasis associated with snail-borne trematode *Schistosoma* eggs and related pathologies in genital tract of men inhabiting or visiting endemic areas especially of Sub-Saharan Africa. The first recognized MGS report was made by Madden in 1911, followed by several case reports and research studies in subsequent years. However, the epidemiology, diagnostic testing and case management of MGS are not well described owing to limited research and diminishing focus in endemic countries over several decades. There is less awareness on the clinicopathological consequences of MGS in seminal vesicles, vas deferens and prostate due to the emphasis on the urinary system pathology of urogenital schistosomiasis (UGS) by *Schistosoma haematobium*. Furthermore, the presence of immune cells and mediators indicates an associated genital pathology that may increase the susceptibility to Human immunodeficiency virus (HIV) infection and transmission among infected males and females in schistosomiasis-endemic regions.

Longitudinal cohort research studies were conducted in Malawi to investigate the current burden of MGS among fishermen living along Lake Malawi shores in Mangochi and assess the possible increased risk of HIV transmission through seminal viral shedding.

**Methods**

Fishermen aged 18+ years from fishing villages along the Lake Malawi shores were recruited after providing informed written consent. Individual questionnaires were administered to assess their knowledge, attitudes and practices on MGS and HIV. Thereafter, participants submitted urine in a container at the nearby health facility and semen into a clear, sealable plastic bag, following 2 days of coital abstinence. In addition, transabdominal ultrasonography of their genital organs were conducted and at the end, Praziquantel therapy was provided to all participants, together with the follow-up date, one, three and six months post-therapy.

**Results**

A total of 386 fishermen, 320 without HIV and 56 with HIV and on Antiretroviral treatment (ART), aged 18 to 70 years (mean 30.6 years), were recruited into the study and had questionnaire interviews. Preliminary results indicate that 31 participants (14.8%, n 209) had *S.*

*haematobium* eggs in urine at baseline, with mean egg count of 2.3 per 10ml and range from 0 to 137.8. Eight participants (3.8%) tested positive for circulating cathodic antigen (CCA) which is mainly indicative of hepato-intestinal S. mansoni infection. Eleven (12%, n 92) had MGS, thus S. haematobium eggs in their semen (mean 1 egg / ejaculate, range 0 - 14 eggs / ejaculate). Out of 125 participants with transabdominal ultrasonography at baseline, 25 (20%) had abnormalities in the genital organs, displaying pathological consequences of MGS.

At 1-month follow-up, only 4 participants still had UGS and 2 were new infections, with reduced mean egg count of 1.4 per 10ml urine, and range of 0 to 29.6. None of the participants had eggs in their semen, suggesting clearance of MGS infection after therapy. At 3-months follow-up, more participants had UGS (16.9%, n 65), and MGS (8.2%, n 61) supposedly due to reinfections. On 6 months follow-up, only four participants (6.5%, n 62; mean 0.03 eggs per 10ml) had UGS, while 3 participants had 1 S. haematobium egg in semen (5.6%, n 51).

**Conclusion**

Male genital schistosomiasis is as prevalent as UGS in fishermen living and working in Lake Malawi. Interestingly, the current treatment is capable of clearing MGS similarly to UGS, raising the need for availability and accessibility of praziquantel to all people in endemic areas, together with other disease control interventions.

Presenter: **Mrs Fatou Gai**, *PhD student, University of Nottingham*

### Antigenic cross-reactivity between the tropical helminth parasite *Schistosoma mansoni* and the house dust mite Dermatophagoides farinae: a role for cross-reactive carbohydrate determinants (CCDs) and implications for the hygiene hypothesis. - *A16395*

**F Gai**[1]; F Falcone[1]; M Faham[1]; M Doenhoff[1];
[1] University of Nottingham, UK

In recent decades, in countries with advanced health systems, there has been a marked increase in diseases attributed to immunological disorders such as asthma and allergies. However, people infected with parasitic helminths including schistosomes have been found to suffer less from allergy. This has led
to formulate a helminth parasite variant of the so-called 'hygiene' or 'old friends' hypotheses. Previous studies have found that rabbit IgG antibodies raised against *Schistosoma mansoni* egg antigens cross-react with allergens such as peanut, grass pollen and natural rubber latex. In this work, we describe how rabbit IgG antibodies raised against *Schistosoma mansoni* soluble egg antigens (SmSEA) are cross-reactive with molecules in house dust mite (HDM) *Dermatophagoides farinae* somatic extracts. A cross-reactive molecule from HDM with approximate mass of 98 kDa was identified by tandem mass spectrometric (TMS)analysis to be the allergen Der f 15. Rabbit anti-schistosome IgG antibodies eluted from the HDM molecule reacted with the three major *S. mansoni*< egg glycosylated antigens IPSE/alpha-1, omega-1 and kappa-5. Moreover, anti-*S. mansoni* egg antibodies that had been eluted from the HDM cross-reactive antigen also reacted with antigenic constituents of a variety of plants which are known to be allergenic in humans. This extensive cross-reactivity was ablated by sodium metaperiodate treatment of the film carrying the plant antigens, indicating it was due to cross-reactive carbohydrate determinant (CCDs). Amino acid sequence analysis of the allergen indicated it had potential N- and O-linked glycosylation sites. In this work, we have also used the humanized Rat Basophilic Leukemia RS-ATL8 reporter system which is used to detect allergen specific IgE. RS-ATL8 cells were sensitized overnight with high dilution of sera of the patient with allergy to HDM and stimulated with a wide range of HDM allergen concentrations the next day, luminescence was measured 4 hours after stimulation we found the range from 10 ng/ml to 100 pg/ml represent the optimal concentrations. These findings are novel, and provide a possible explanation for the hygiene hypothesis and a potential starting point for improved allergen-specific immunotherapy.

## Session C

Presenter: **Prof Dolores González-Pacanowska**, *CSIC – Granada*

### Exploiting carbohydrate binding agents as anti-trypanosomals - *A16375*

**D González-Pacanowska**[2]; V M Castillo-Acosta[2]; M Valente[2]; L M Ruiz-Pérez[2]; E J Van Damme[3]; Y Igarashi[1]; J Balzarini[4];

[1] Biotechnology Research Center, Toyama Prefectural University, Japan; [2] Instituto de Parasitología y Biomedicina "López-Neyra". CSIC., Spain; [3] Laboratory of Biochemistry and Glycobiology, Ghent University, Belgium; [4] Rega Institute for Medical Research, KU Leuven, Belgium
In *Trypanosoma brucei*, surface VSGs are modified by mannose rich or complex glycans. We have identified a series of carbohydrate-binding agents (CBAs) that bind to surface glycoproteins and exhibit a strong trypanocidal activity against the clinically relevant bloodstream form. Analysis of the mode of action showed a rapid internalization of glycoprotein-CBA complexes and accumulation in the lysosome leading to perturbation in endocytosis and in the progression of the cell cycle. Long-term exposure to peptidic agents such as Hippeastrum hybrid agglutinin yielded resistant parasites with reduced CBA binding and uptake. Resistant cell lines present modifications in the glycosylation profile of VSGs as a result of genetic rearrangements in the TbSTT3B oligosaccharyltransferase (OST) gene. The resistance phenotype was associated with the total loss of the expression of this OST, and a reduction of infectivity and virulence in mice. Carbohydrate-binding nonpeptidic compounds were also tested and exhibit pronounced antiparasitic activity. As an example, pradimicin and its derivatives are non-peptidic CBAs that adhere to the carbohydrate moiety of Trypanosoma brucei (etiological agent of sleeping sickness) surface glycoproteins inducing parasite lysis in vitro. Notably, pradimicin S enables cure of an acute form of sleeping sickness in mice. By inducing resistance in vitro we have established that the composition of the sugars attached to the variant surface glycoproteins are critical to the mode of action of pradimicins and play an important role in infectivity. Thus, specific glycosylation patterns are important for CBA cytotoxicity and parasite fitness in vivo and agents binding efficiently to surface glycoproteins may provide a unique and highly novel avenue for the development of treatments against parasitic diseases. We are currently exploring the potential of this class of compounds in the treatment of other parasitic diseases.

Presenter: **Prof Richard Pleass**, *Liverpool School of Tropical Medicine*

## Insertion of N-terminal hinge glycosylation enhances interactions of human IgG1-Fc monomers to glycan-dependent receptors including influenza virus haemagglutinin - *A16376*

**R Pleass**[1]; P A Blundell[1]; S Haslam[2]; A Dell[2]; D Lu[2];

[1] Department of Parasitology, Liverpool School of Tropical Medicine, UK; [2] Dept. Life Sciences, Imperial College, University of London, UK
In therapeutic applications where the Fc of IgG is critically important, the receptor binding and functional properties of the Fc are lost after deglycosylation, or removal of the unique Asn297 sequon. A population of Fc-bearing sialylated glycans has been identified as contributing to this functionality, and high levels of sialylation also lead to longer serum retention times which are advantageous for therapy. The efficacy of sialylated Fc has generated an incentive to modify the unique N-linked glycosylation site at Asn297, either through chemical and enzymatic methods, or by mutagenesis of the Fc that disrupts the protein Asn297-carbohydrate interface. Here we took an alternative approach, by inserting or deleting N-linked attachment sites into the body of the Fc, to generate a portfolio of mutants with tailored effector functions. For example, we describe mutants with enhanced binding to low-affinity inhibitory human Fc$\gamma$ and glycan receptors that may be usefully incorporated into existing antibody engineering approaches to treat or vaccinate against disease. The IgG1-Fc mutants containing complex sialylated glycans attached to the N-terminal Asn221 sequon bound hemagglutinin and may disrupt influenza A-mediated agglutination of erythrocytes and 293T cells

Presenter: **Prof Pedro Bonay**, *Universidad Autónoma de Madrid*

## Potential of Glycomics to treatment efficacy, disease progression and therapeutic approaches in American trypanosomiasis. - *A16377*

**P Bonay**[1]

[1] Centro de Biología Molecular "Severo Ochoa"- Universidad Autónoma de Madrid, Spain

Chagas' Disease or American trypanosomiasis is a chronic, debilitating illness endemic in central and south America. It is caused by *Trypanosoma cruzi*, affecting nearly 20 million persons. The transmission is mainly vectorial by hemiptera of the Reduviidae family, followed by blood transfusions and organs transplants, transplacentally from mother to child, and recently, orally by consumption of contaminated beverages, in urban zones where vectorial transmission is controlled. The oral outbreaks have been characterized as highly virulent. The disease characterizes by an acute phase in which parasitemia is evident among a broad unspecific symptoms that can last for about 4-6 weeks, followed by a long asymptomatic indeterminate phase that can last up to decades before just 20-30% of patients develop terminal

complications, mainly chronic cardiomyopathy and mega syndromes. New approaches are needed for blood bank screening, and for improved diagnosis and prognosis. Many studies have shown that changes in the glycosylation pattern are associated with specific diseases. Here, we present for the first time, an analysis of the serum N-glycome in Chagas' disease patients and controls. For this, blood samples were collected from Chagas' disease patients in the acute phase (oral and vectorial transmission from Venezuela) before and after treatment with Benznidazol, in the chronic phase (from Venezuela and Bolivia). After release and labeling, serum glycans were subjected to hydrophilic interaction high performance liquid chromatography, which resulted in 38 chromatographic glycan peaks (GP). The abundances of the GPs were compared between the study groups. We found a statistically significant difference between groups when considering fucosylated and disialylated glycans. Furthermore, performing a linear discriminant analysis, an excellent separation and prediction ability were observed. One of the most interesting results derived from the clear discrimination in the n-glycan profiles of the two subgroups of acute patients (oral and vectorial infection). In addition, we observed a "normalization" of the profile upon chemotherapy. These alterations in the glycosylation signature could provide new aspects to the pathophysiology of the disease and may serve as new potential markers in the future. Also, we present for the first time a study of the anti-glycan antibodies repertoire present in those patients. These alterations in the glycosylation signature could provide new aspects to the pathophysiology of the disease and may serve as new potential markers in the future.

Presenter: **Dr Matthew Rogers**, *London School of Hygiene and Tropical Medicine*

### ***Leishmania*** **glycoproteins as master manipulators of sand fly and mammalian hosts.** - *A16378*

**M Rogers**[1];

[1] London School of Hygiene and Tropical Medicine, UK

*Leishmania* rely on their surface and secreted glycans for survival in both sand fly and mammalian hosts. In the sand fly midgut multiplicative promastigotes secrete a gel-like plug formed from secreted proteophsophoglycans. The promastigote secretory gel (PSG) is intimately involved in *Leishmania* transmission and infection as it can influence sand fly bloodfeeding, resulting in the regurgitation of parasites and facilitate infection – leading to exacerbated disease. Our recent studies explore the complexity of these interactions to understand the biology of the transmission event. In the sand fly vector the PSG interacts with the incoming bloodmeal to enrich the infectious dose for metacyclic promastigotes. To do this, PSG co-opts the components of the host's sera to differentially allow metacyclic forms to migrate through the PSG for regurgitation into the skin. Shortly following deposition, we show that *Leishmania* use regurgitated PSG to take advantage of the wound created by the vector bite and the host's need to control excessive collateral tissue damage during the initial pro-inflammatory phase of wound healing. To do this, PSG exaggerates the inflammatory phase of the early wound response to induce IGF1-signalling and IGF1-dependent expression of Arginase 1 in macrophages. As a result, the PSG redirects macrophages to the site of parasite deposition and alters their activation and metabolism to a state which is highly beneficial for parasite survival and multiplication. In this talk I will discuss these results in the context of other recent key findings of *Leishmania* transmission.

## Posters

Presenter: **Ms Nethravathi Puttappa**, *Research Scholar, JSS College of Pharmacy*

### Poster 3 : **Artesunate-Quercetin loaded self-nanoemulsifying drug delivery system for oral malaria therapy: Pharmacokinetics and Pharmacodynamic evaluation studies**

**N Puttappa**[1];

[1] JSS College of Pharmacy, India

**Background:** Malaria is one of the major global health challenges with 300 million new cases annually. Currently, artemisinin combination therapy (ACT) has begun to emerge resistance in various parts of the world such as Cambodia and Greater Mekong Subregion. In order to counteract this, we hypothesize artesunate (ART) and quercetin (QRT) loaded self-nanoemulsifying drug delivery system (SNEDDS) which could provide an alternate drug combination to ACT induced P. falciparum resistance to treat malaria.

**Objective:** The main objective of the work is to overcome the solubility and bioavailability problems associated with Artesunate and adjuvant (Quercetin) combination therapy by formulating as lipid-based nanoemulsion, in an attempt to effectively treat the resistant forms of *falciparum* species of malaria.
**Method:** ART-QRT nanoemulsion was prepared using spontaneous nanoemulsification method and optimized by Box Behnken design. The optimized SNEEDS were evaluated for particle size, PDI, percentage transmittance, refractive index, drug content, viscosity and release rate. Further, these nanoemulsion are evaluated for pharmacokinetic and *in vivo* antimalarial efficacy in combination with QRT in animal model.
**Results & discussion:** Compatibility studies revealed that the selected drug ART and QRT are compatible with each other without any unwanted interactions. The LC-MS/MS method was successfully developed for the simultaneous analysis of ART, DHA and QRT in rat plasma for the pharmacokinetics studies. The optimized SNEDDS composed of Capryol 90, cremophore EL and PEG-400 The ART-QRT which could withstand the extensive dilution and did not show any phase separation or drug precipitation. The SNEDDS exhibited mean globule size <80 nm, with a percentage transmittance of 98. Release rate of the drug from the optimized batch was found to be quite significant ($P < 0.001$) as compared to the plain drug. The in vitro cytotoxicity studies confirmed that the nanoformulation is safe and nontoxic. *In vivo* oral bioavailability of the nanoemulsion formulation in wistar rats of either sex was found to be higher observed from pharmacokinetic studies. The antimalarial activity against *Plasmodium bergheii* infection in swiss albino mice showed improved parasite clearance and survival rate in combination with QRT. Hence, SNEEDS of ART in combination with QRT have yielded promising results, which might help to establish better therapeutic strategies for malaria treatment. Nevertheless, extensive investigation is essentially required in the near future for the same.
**Keywords:** Malaria, Self-nanoemulsifying drug delivery system, Artesunate, Quercetin, *Plasmodium bergheii*.

Presenter: **Mr Umar Anjum**, *PhD Student, De Montfort University*

## Poster 4 : **Studying the presence of *Cyclospora* and *Cystoisospora* in urban parks from Leicester, UK**

**U Anjum**[1]; F Izquierdo[2]; A Peña-Fernández[1];
[1] De Montfort University, UK; [2] Universidad San Pablo CEU, Spain

*Cyclospora cayetanensis* and *Cystoisospora belli* (formerly known as Isospora belli) are emerging coccidian parasites that can spread by ingesting contaminated food or water. Despite their presence is more common in tropical and subtropical regions, different studies have described domestic outbreaks due to these pathogens around the world. Zoonotic transmission of these pathogens is under discussion as they have been found in various animals and birds. We have performed a preliminary study to investigate their potential presence in an English urban environment. 132 animal faecal samples were collected between Summer 2017 and Spring 2018 from 7 different urban parks across Leicester (UK). A veterinarian confirmed animal species as: 78 avian (25 pigeon, 14 waterfowl, 12 songbird, 27 uncertain due to diarrhoea), 37 deer, 13 dogs and 4 cats. Smears were microscopically analysed by Kinyoun's acid-fast staining technique. Cyclospora spp. were observed in three faecal samples (2.3%), two from deer and one from avian (diarrheic sample); however, further analysis are required to determine if the oocysts observed are from *Cyclospora cayetanensis*. Contrarily, *Cystoisospora* spp. were not found in any of the screened stool samples. Despite our results should be considered as preliminary, the presence of *Cyclospora* spp. oocysts in 2.3% of the animal faecal samples collected across Leicester might represent a potential human risk that, although minor, should be throughly studied to protect the local community. Moreover, *Cyclospora* spp. have been found in different animal species, which may require different interventions to target those specific animals to protect the public health.

Presenter: **Dr Samuel Duncan**, *University of Dundee*

## Poster 3 : **Identifying highly divergent glycosyltransferases in the African trypanosome**

**S M Duncan**[1]; M Damerow[1]; M A Ferguson[1];
[1] Wellcome Trust Centre for Anti-Infectives Research, School of Life Sciences, University of Dundee, UK

*Trypansoma brucei* is a protozoan parasite that infects humans and cattle via a tsetse fly vector. Key to parasite survival during progression through this complex life cycle is the expression of cell surface and endocytic pathway glycoproteins, modified with glycosylphosphatidylinositol (GPI) membrane anchors and/or N-linked oligosaccharides. We estimate that protein glycosylation in this parasite requires at least 38 distinct glycosyltransferases (GTs), only a few of which can be predicted by bioinformatics. Interestingly, a family of 21 putative trypanosome GTs has been identified that share a single beta 1-3 transferase ancestor but catalyse a diverse array of glycosidic

linkages. Inhibition of such highly divergent GTs is therefore a promising therapeutic avenue, yet 17 of these putative TbGTs require characterisation. This project aims to identify their function by utilising reverse genetics and RIT-Seq approaches.

Presenter: **Dr Mohammad Feiz Haddad**, *Ahvaz Jundishapur University of Medical Sciences*

## Poster 4 : **Evidence for Toxoplasmosis associations in thyroid disorders in pregnant women, Southwest, Iran, 2017-2018**

**M H Feiz Haddad1**[3]; M Ansari[1]; R Haddad[1];
[1] Dezful University of Medical Sciences, Dezful, Iran, Iran; [2] Health Research Institute, Infectious and Tropical Diseases Research Center, Ahvaz

**Background:** *Toxoplasma gondii* has a worldwide prevalence and mankind get infected by this parasite as an interface host. The parasite reaches to different parts of body through blood and lymph after the entrance. It may enter the pituitary and thyroid glands and effect the production of thyroid hormones and TSH. Thyroid disorders are relatively common among pregnant women which may cause complications such as preeclampsia, abortion, preterm delivery. *Toxoplasma* parasite itself could cause abortion in pregnant women and they might also lead to cerebral, ocular damages in fetus. Therefore, this study was designed to investigate the relation between toxoplasmosis and thyroid disorders in pregnant women, Southwest, Iran, 2018.
**Material & Methods:** Serums of 630 pregnant women were examined for detection of *Toxoplasma gondii* IgG and IgM immunoglobulins and thyroid disorders through TSH test using AUTOBIO kits. The biometric and serological obtained results were analyzed by SPSS application and chi-square test.
**Results:** The *Toxoplasma gondii* IgG immunoglobulin was detected in 70.6% of the pregnant women with hyperthyroidism and there was a significant relation between above variations (*P*=0.001). Prevalence of *Toxoplasma gondii* IgG and IgM immunoglobulins were 31.9% and 1.1%, respectively. While, hypothyroidism and hyperthyroidism prevalence among pregnant women were 28.1% and 2.7%, respectively and there was a statistically significant correlation between IgG immunoglobulin and education (*P* =0.004), contact with cats (*P* =0.019), and contact with soil (*P*=0.046) variations.
**Conclusion:** This study proved that there is a statistically significant correlation between hyperthyroidism and *Toxoplasma gondii* IgG immunoglobulin in pregnant women (*P*=0.001) and being infected with *Toxoplasma gondii* parasite can possibly be one of the reasons of hyperthyroidism among pregnant women. The hyperthyroidism complications such as spontaneous abortion, preeclampsia, heart failure, premature and low birth weight in pregnant women could be prevented by taking into account health measures for the avoidance of toxoplasmosis.

Presenter: **Mrs Fatou Gai**, *PhD student, University of Nottingham*

## Poster 5 : **Antigenic cross-reactivity between the tropical helminth parasite *Schistosoma mansoni* and the house dust mite *Dermatophagoides farinae*: a role for cross-reactive carbohydrate determinants (CCDs) and implications for the hygiene hypothesis.**

**F Gai**[1]; F Falcone[1]; M Faham[1]; M Doenhoff[1];
[1] University of Nottingham, UK

**Key words**: Allergy, *Schistosoma mansoni,* house dust mite, cross-reactive carbohydrate determinants (CCDs), IgG blocking antibodies, hygiene hypothesis.

In recent decades, in countries with advanced health systems, there has been a marked increase in diseases attributed to immunological disorders such as asthma and allergies. However, people infected with parasitic helminths including schistosomes have been found to suffer less from allergy. This has led to formulate a helminth parasite variant of the so-called ‘hygiene’ or ‘old friends’ hypotheses. Previous studies have found that rabbit IgG antibodies raised against *Schistosoma mansoni* egg antigens cross-react with allergens such as peanut, grass pollen and natural rubber latex. In this work, we describe how rabbit IgG antibodies raised against *Schistosoma mansoni* soluble egg antigens (SmSEA) are cross-reactive with molecules in house dust mite (HDM) *Dermatophagoides farinae* somatic extracts. A cross-reactive molecule from HDM with approximate mass of 98 kDa was identified by tandem mass spectrometric (TMS)analysis to be the allergen Der f 15. Rabbit anti-schistosome IgG antibodies eluted from the HDM molecule reacted with the three major *S. mansoni* egg glycosylated antigens

IPSE/alpha-1, omega-1 and kappa-5. Moreover, anti-*S. mansoni* egg antibodies that had been eluted from the HDM cross-reactive antigen also reacted with antigenic constituents of a variety of plants which are known to be allergenic in humans. This extensive cross-reactivity was ablated by sodium metaperiodate treatment of the film carrying the plant antigens, indicating it was due to cross-reactive carbohydrate determinant (CCDs). Amino acid sequence analysis of the allergen indicated it had potential N- and O-linked glycosylation sites. In this work, we have also used the humanized Rat Basophilic Leukemia RS-ATL8 reporter system which is used to detect allergen specific IgE. RS-ATL8 cells were sensitized overnight with high dilution of sera of the patient with allergy to HDM and stimulated with a wide range of HDM allergen concentrations the next day, luminescence was measured 4 hours after stimulation we found the range from 10 ng/ml to 100 pg/ml represent the optimal concentrations. These findings are novel, and provide a possible explanation for the hygiene hypothesis and a potential starting point for improved allergen-specific immunotherapy.

Presenter: **Miss Zhe Ji**, *biochemistry PhD student, University of Dundee*

## Poster 6 : **New approaches to studying the GPI biosynthesis pathway in *T. brucei*: uncovering the missing links.**

**Z Ji**[1]; M Tinti[1]; L S Guther[2]; M A Ferguson[2];

[1] Biological Chemistry and Drug Discovery, School of Life Sciences, University of Dundee, UK; [2] Wellcome Trust Centre for Anti-Infectives Research, School of Life Sciences, University of Dundee, UK

The bloodstream form of *Trypanosoma brucei* (*T. brucei*) is coated with glycosylphosphatidylinositol (GPI) anchored variant surface glycoprotein (VSG). These GPI anchored VSG homodimers act as the first line of defence for the parasites and undergoes antigenic variation leading to persistent infection. A lot is known about GPI anchor biosynthesis in *T. brucei*, indeed the first studies of GPI anchor structure and biosynthesis were performed on this organism and these methodologies were subsequently applied to mammalian cells, yeast and other organisms. Despite conservation in the core structure of almost all GPI anchors across eukaryotic evolution, notable differences occur between the *T. brucei* and mammalian GPI anchor biosynthetic pathways making this pathway possible a drug target.

Here we present a quantitative proteomics approach to identify the proteins that catalyse key steps of GPI anchor biosynthesis in *T. brucei* but are still unknown. Known components in this pathway were epitope tagged with Myc sequences and co-immunoprecipitation was conducted using agarose beads to the tag. SILAC proteomics were then performed to identify associated proteins that may induce missing links in the GPI pathway.

TbGPI12 encoding de-N-acetylase that catalyses the second step in the pathway was chosen first to act as the bait protein to perform such a pull down. NativePAGE Western blot after pulling down TbGPI112-Myc has shown TbGPI12 has formed complexes and the SILAC proteomics has identified specifically co-immunoprecipitated proteins.

These results show that we have successfully established a SILAC proteomics method for the co-immunoprecipitation of proteins with epitope tagged components of the GPI anchor biosynthesis pathway in *Trypanosoma brucei*. This method can be expanded to other known components in this pathway and find the missing links.

Presenter: **Dr Victoria Adamu Pam**, *Senior Lecturer, Federal University Lafia*

## Poster 7 : **Evaluation of Parasitic Contamination of Local Drinking Water Source in Doma Local Government Area of Nasarawa State, Nigeria**

**V A Pam**[1]; A A Idris[1]; V A Adejoh[1]; A Ombugadu[1];

[1] Federal University Lafia,Nassarawa State,Nigeria, Nigeria

Water is essential for existence, and its importance for individual health and to the well-being of a nation cannot be underestimated. This study evaluates the parasitic contamination of drinking water sources in Doma Local Government Area of Nasarawa State, Nigeria. A total of 48 water samples were collected from different selected sources (boreholes, wells and streams) between the months of March to July 2017. The water samples were analyzed using the Calcium carbonate ($CaCO_3$) floatation method and the samples were examined microscopically for the presences of parasites. A total of 32 water samples were found with parasites. These include a Trematode (*Fasciola hepatica*), 2 protozoans (*Entamoeba histolytica, and Giardia lamblia*) and 3 nematodes (*Trichuris trichiuria, Ascaris lumbricoides and* Hookworm). The nematodes had the highest contamination rate 19(59.38%) followed by the protozoans 12(37.50%) while the trematode was least with 1(3.13%). The stream water sources has the most contamination in late dry and early wet season with 69.23% and 94.74% respectively. The wells had 30.77% and 5.26% in late dry and early wet season respectively. The boreholes had zero contamination for both seasons. Prevalence of parasites in

relation to sources of water showed a high significant differences ($c^2$=49.741, df =2, P=0.0000001), while there was no significant difference in relation to late dry and early wet seasons ($c^2$=2.3438, df=1, P=0.1258). Result indicated high rate of parasitic contamination of drinking water source in the study area. Hence there is need for advocacy and enlightenment on the importance of proper drinking water treatment.

Presenter: **Dr Clair Rose**, *Post Doc, Liverpool School of Tropical Medicine*

## Poster 8 : **Characterisation of a glycosylated glutathione transferase of *Onchocerca ochengi***

**C Rose**[1]; Z Stead[1]; G Praulins[1]; B Makepeace[3]; A Acosta-serrano[1]; E J Lacourse[2];
[1] Liverpool School of Tropical Medicine, UK; [2] Liverpool School of Tropical Medicine / UoL, UK; [3] University of Liverpool, UK

The cattle filarial nematode *Onchocerca ochengi* is a well-established model for the study of human onchocerciasis, the causative agent of which is O. volvulus. Despite the immune competence of their cattle or human hosts, both Onchocerca spp. typically survive for many years in close proximity to host-derived inflammatory and immune cells. One multi-functional protein superfamily which may aid worm survival through their potential for immunomodulatory product production and/or detoxification of drugs and host-derived immunochemical attack are the glutathione transferases (GSTs). O. volvulus GST1 (OvGST1) is a novel GST found in O. volvulus and is similar to GST (OoGST1) found in O. ochengi, both of which are novel in being the only GSTs shown to date as glycosylated. However, the structure and functions of the glycosylation are still largely unknown and yet to be elucidated through different biochemical techniques.
Here, *O. ochengi* GSTs were purified through a two-step affinity chromatography approach, prior to proteomic and enzymatic investigations to resolve, characterise and unravel the structure of native OoGST1 glycans modifications. Bioinformatics analysis revealed the mature OoGST1 protein is potentially *N*-glycosylated at five different sites. Phylogenetic analysis confirmed the OoGST1 protein is most closely related to *O. volvulus* GST1b protein and that all three known glycosylated sigma class GSTs from *O. volvulus* and *O. ochengi* are clustered. Mass spectrometry analysis revealed the OoGST1 glycans are mainly composed of paucimannose glycans and also contain a hybrid-type glycan. Lectin-affinity blot analysis confirmed these results by illustrating the presence of both mannosylated and fucosylated glycans in the purified GST sample. Finally, recombinant unglycosylated OoGST1 protein was successfully sub-cloned, expressed and purified using an *Escherichia coli* expression system for use in future comparative analysis.

Presenter: **Ms Judith Weber**, *PhD student, University of Bremen*

## Poster 9 : **Microbiomic sialidases as potential targets to prevent Trypanosoma congolense colonization in tsetse**

**J Weber**[5]; J Rosenau[5]; M Waespy[5]; T T Gbem[1]; S H Ngomtcho[6]; A Gupta[5]; S S Shaida[3]; D Achukwi[4]; A Dotzauer[5]; B Reinhold-Hurek[5]; K Badu[2]; J A Nok[1]; S Kelm[5];
[1] Ahmadou Bello University, Nigeria; [2] Kwame Nkrumah University Of Science and Technology, Ghana; [3] Nigerian Institute for Trypanosomiasis Research, Nigeria; [4] TOZARD Research Laboratory, Cameroon; [5] University of Bremen, Germany; [6] University of Ngaoundéré, P.O. Box 454, Ngaoundéré, Cameroon

African trypanosomes are protozoan parasites causing Human African Trypanosomiasis (HAT) and Animal African Trypanosomiasis (AAT, also called Nagana) in sub-Saharan Africa. During development, parasites circulate between insect vector and mammalian host. To ensure their survival in both, trypanosomes have evolved different strategies. One includes the expression of an unusual surface glycosyltransferase termed trans-sialidase (TS) [1]. TS catalyse the transfer of terminal sialic acids (Sia) from glycoconjugates to terminal galactosyl residues on target glycoproteins. Due to the lack of Sia synthesis de novo parasites utilise TS to scavenge Sia from host glycoconjugates and decorate its own surface molecules [2]. This generates a negatively charged glycocalyx, which protects the parasite in the fly gut. Our research focus is on the biochemical characterisation of TS from *Trypanosoma congolense* (TconTS). Enzymatic activities of different TconTS enzymes were determined and showed different ratios of Sia transfer over sialidase activity [3,4]. While major studies have focused on TS catalytic domain, we recently demonstrated the carbohydrate binding ability of the lectin-like domain (LD) [5]. We investigated the presence of bacterial expressed sialidases in the fly gut as potential competitors for TconTS activity to prevent colonization of trypanosomes. Sialidases catalyse the hydrolysis of terminal Sia and therefore deplete substrates for TconTS present in the bloodmeal. Along this line we have developed a sialidase activity assay as a broad screening method for application in the field. This assay has been used to identify sialidase activity in the fly gut. In a second step several bacteria were isolated, cultured and are currently tested for their potential sialidase expression. Besides bacterial sialidases, modified TconTS only exhibiting sialidase activity could be introduced into the fly gut via transgenic bacteria as a second approach. In conclusion, understanding the mechanism and function of TS will open new avenues towards the control of trypanosomiasis in Africa.

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Presenter: **Dr Jael Asabe Yohanna**, *Senior Lecturer, University of Jos Plateau State, Nigeria*

## Poster 10 : **Gastrointestinal Parasites among Pregnant Women Attending Antenatal in Parts of Jos, Plateau State Nigeria**

**J Yohanna**[4]; M A Ike[4]; S T Joseph[4]; S O Okodhi[1]; A Ombugadu[2]; F Omini[3];
[1] DEE Medical Center Buruku, Plateau State, Nigeria; [2] Federal University Lafia,Nassarawa State,Nigeria, Nigeria; [3] Hasiya Bayero Paediatric Hospital Kano, Nigeria, Nigeria; [4] University of Jos Plateau State, Nigeria, Nigeria

Gastrointestinal parasites are endemic in Nigeria. Cases of asymptomatic infections also exist which maintains transmission of these parasites through ways which include vertical transmission. A total of five hundred and ten (510) stool samples were obtained from volunteer pregnant women attending anti-natal is some hospitals in Jos. they were analyzed using iodine and normal saline wet preparations as direct smears while saturated flotation and formol ether concentration methods were used. Pearsons's Chi-square test was used to compare infections in relation to age, trimesters, PCV and occupational groups. One hundred and sixty seven (32.7%) had single parasite infection, 32 (6.3%) were infected with two parasites while 3 (0.6%) had three parasites. The age groups >45 years had 3 (42.9%) infection, 16 - 25 years age groups had 35.8% and least infection was in the 26 - 35 years age group. infection across age groups showed no significant difference ($c^2$ = 2.4078, df = 3, P > 0.05). Those in the third trimesters had the highest infection (34.7%) and least in the first trimesters. Infection across trimesters showed no significant difference ($c^2$ = 0.31645, df = 2, P> 0.05). There was however significant difference in relation to PCV groups ($c^2$ = 35.559, df = 2, P < 0.0001) and occupational groups ($c^2$ = 16.738, df = 2, P = 0.0001). The highest infection was with *Entamoeba histolytica*, followed by *Ascars lumbricoides* and least with *Strongiloides* spp. Of those infected, 18 (10.78%) were bloody, 14 (8.38%) were watery, 6 (3.59%) were mucoid and 2 (1.20%) had fatty stool. There was significant difference ($c^2$ = 54.29, df = 6, P<0.0001) between parasites species pooled infection rates recorded in individuals. The result shows the need for routine screening of all pregnant women for gastrointestinal infection during anti-natal visits for treatment. Sensitization campaigns should be given to avoid habits that predispose to infection and effects.

## Index