

# KINETOPLASTID DISEASES 2006

*Hôtel de l'Indépendance, Dakar, Senegal*

*Saturday, March 11<sup>th</sup> to Monday, March 13<sup>th</sup>, 2006*

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### **Also Participating at the ITC, Kololi, The Gambia on Thursday March 16th**

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### **Running Order of Oral Presentations**

1st – Jeremy Sternberg (1)	7th – Oumar Gaye	13th – Bruno Bucheton
2nd – M. Sohail Sajid	8th – Annette MacLeod	14th – Jeremy Sternberg (2)
3rd – Harry de Koning (1)	9th – Chris Peacock (1)	15th – Chris Peacock (2)
4th – Oumar Diall	10th – Abdul Jabbar	16th – Harry de Koning (2)
5th – Alicia Couto	11th – Henry Tabel (1)	17th – Henry Tabel (2)
6th – Reinaldo Bestetti	12th – Liam Morrison	

## **Titles/Abstracts of Presentations / Summaries of Participants**

Listed Alphabetically by Family Name [ Number and/or Type of Presentation(s) in Parentheses ]

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**Reinaldo Bestetti [ Oral 6 ]**

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### **PREDICTORS OF MORTALITY AND TREATMENT OF PATIENTS WITH CHAGAS' CARDIOMYOPATHY (AMERICAN TRYPANOSOMIASIS).**

Reinaldo B. Bestetti, MD, PhD; Augusto Cardinali-Neto, MD, MSc; Tatiana A. D. Theodoropoulos, MD. Faculty of Medicine of São José do Rio Preto and Faculty of Medicine of Centro Universitário Barão de Mauá, Ribeirão Preto, Brazil.

This paper summarizes our experience in predicting prognosis and treating patients with Chagas' cardiomyopathy. Chagas' disease (American Trypanosomiasis) is caused by the protozoan *Trypanosoma cruzi*, which is transmitted to humans via the feces of a bug (*Triatoma infestans* in Brazil). Chronic cardiomyopathy is the most frequent clinical manifestation of Chagas' disease, affecting about 30% of infected patients. Recognizing patients with Chagas' cardiomyopathy at risk of dying is a major goal for physicians working in areas where the disease is endemic. Mode of death in Chagas' cardiomyopathy are usually sudden cardiac death, death from intractable chronic heart failure and death from thromboembolic phenomena. In the vast majority of cases, sudden cardiac death is caused by malignant arrhythmia (Sustained Ventricular Tachycardia or Ventricular Fibrillation). Independent predictors of sudden cardiac death are left ventricular diastolic dimension and left ventricular apical aneurysm, a hallmark morphological sign of the disease. Thus, in contrast to what has been observed in non-Chagas' disease patients, malignant arrhythmia can be seen in Chagas' disease patients with no left ventricular systolic dysfunction. Amiodarone is able to suppress malignant arrhythmia, but no study has proved or disproved its efficacy to abort sudden cardiac death. In contrast, Automatic Implantable Defibrillators have successfully been used to treat Chagas' disease patients with malignant arrhythmia. However, the impact of this therapy on long-term prognosis of patients with this condition is uncertain. In Chagas' disease patients with chronic heart failure, left ventricular ejection fraction, atrial fibrillation, right ventricular pacemaker implantation, maximal oxygen consumption rate, and no tolerability to carvedilol are independent predictors of mortality. The treatment of chronic heart failure in Chagas' cardiomyopathy patients are diuretics, digoxin, B-blocking agents (carvedilol or metoprolol) and Angiotensin Converting Enzyme Inhibitors. In the overwhelming majority of cases, however, Chagas' disease patients are unable to tolerate ACEI and B-blockers, mostly if they are given simultaneously. This fact can account, at least in part, for the poorer prognosis of Chagas' cardiomyopathy patients in comparison to that of non-Chagas' disease patients. Heart transplantation is a valid option for the treatment of end-stage Chagas' cardiomyopathy. Cardioembolic stroke is seldomly observed in milder forms of Chagas' cardiomyopathy. In advanced cases of the disease, however, cardioembolic stroke can independently be predicted by the presence of chronic heart failure, apical aneurysm, arrhythmia on the 12-lead ECG and female gender. Therefore, anticoagulation therapy is indicated for this subset of Chagas' disease patients. Thus, predictors of prognosis should be known by physicians working where Chagas' disease is endemic in order that an adequate treatment can be given to patients with Chagas' cardiomyopathy.

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**Caroline Boda [ Poster ]**

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*Title is Awaiting Confirmation by the Author*

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**Bruno Bucheton [ Oral 13 ]**

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### **IDENTIFICATION OF NEW CANDIDATE GENES FOR HUMAN VISCERAL LEISHMANIASIS SUSCEPTIBILITY BY A GENOME-WIDE LINKAGE APPROACH.**

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A striking observation in visceral leishmaniasis (VL) endemic areas is that most subjects, although exposed to *Leishmania* infection do not develop any clinical sign of the disease. Whereas socio-economic and parasite virulence factors have been shown to influence host susceptibility to VL, growing evidence now indicate that the host genetic make-up also plays an important role in determining the infection outcome. Genetic studies in the mice experimental models have identified several genes and/or genetic loci accounting for the differences of susceptibility between different inbred strains of mice thus opening the way to a "mouse to man" strategy to identify disease susceptibility genes on the human genome. During a longitudinal epidemiological survey carried out in a village of Eastern Sudan heavily affected by an outbreak of VL (1996-2000), we identified environmental and host risk factors for VL. Although environmental risk factors (presence of Neems and cows) probably influenced exposure to sandfly bites in the first phase of the outbreak, we showed that VL was more frequent in certain families and ethnic groups thereby suggesting that host genetic factors played an important role in the development of VL. A genomewide linkage study was performed on 63 families selected from the most affected ethnic group and including 169 children that developed kala azar during the outbreak. Highly significant linkage (LOD score 3.5 [ $P=3 \times 10^{-5}$ ] in all patients; LOD score 3.9 [ $P=10^{-5}$ ] in patients who were affected early in the outbreak) was obtained with markers on chromosome 22q12. Conditional analysis was also suggestive of a second locus controlling disease susceptibility on chromosome 2q23 (LOD score 2.29 [ $P=0.0006$ ]). Noteworthy, candidate genetic loci identified in mice (e.g. NRAMP1, 5q31, HLA, ...) only yield weak linkage results in our study population. Thus other genes than those identified in mice may play a more important role in human genetic susceptibility to *Leishmania* infection. Analysis of new candidate genes in 22q12 and 2q23 is underway and preliminary results suggest a role for the IL2 receptor B chain gene.

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**Alicia Couto [ Oral 5 & Poster ]**

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**STUDIES ON PARASITE GLYCOBIOLOGY**

*Vilma G. Duschak*<sup>1</sup> and *Alicia S. Couto*<sup>2</sup>.

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Biochemical and molecular characterization of glycoproteins and glycolipids present in haemoparasites is required for a detailed functional analysis: their role as surface receptor molecules, their contribution to the membrane physical properties, their function as signaling molecules affecting the host's response to parasite infection. By this reason, we focused our work on the structural characterization of glycoconjugates, present in *Trypanosoma cruzi* and *Plasmodium falciparum*.

In the last years, we have studied the carbohydrate post-traslational modifications of cruzipain, the major cystein proteinase of *T. cruzi*. Using HPAEC-PAD combined with UV-MALDI-TOF mass spectrometry we have characterized the O- and N-glycosidic chains of this enzyme. Interestingly, sulfated N-glycans have been detected for the first time. In addition, preliminary results showed that sulfate-bearing glycoproteins in Trypanosomatids are antigenic for humoral immune responses, which might contribute to clarify whether these structures play a role in the control of *T. cruzi* infection or in pathogenesis of Chagas heart disease.

On the other hand, the glycosphingolipid (GSLs) pathway has also been attracting our attention as targets for new antiparasitic drugs. The physiological functions of the GSLs have only been documented in mammalian cells whereas very little information of their roles in other systems, is available. The core structure of the majority of GSLs, glucosylceramide, GlcCer, is synthesized by the action of a UDP-glucose:ceramide glucosyltransferase (GCS, glucosylceramide synthase EC 2.4.1.80) which was originally found in animal tissues. GlcCer is modified by a series of Golgi glycosyltransferases to produce higher order GSL structures. Since over 400 different glycolipids are derived from GlcCer, GCS is an extremely important glycosylating enzyme. In *Plasmodium falciparum*, we have described for the first time, the presence of an active GCS in the intraerythrocytic stages of the parasite. Two different assays, using UDP-[<sup>14</sup>C]glucose as donor with ceramides as acceptors, or UDP-glucose as donor and fluorescent ceramides as acceptors, were performed. In both cases, we found that the parasitic enzyme was able to glycosylate only dihydroceramide. The enzyme activity could be inhibited *in vitro* with low concentrations of D,L-threo-phenyl-2-palmitoylamino-3-morpholino-1-propanol (PPMP). In addition, *de novo* biosynthesis of glycosphingolipids was shown in the three intraerythrocytic stages of the parasite and the structure of some hexosylceramides was analyzed by UV-MALDI-TOF mass spectrometry. When PPMP was added to parasite cultures, a correlation between arrest of parasite growth and inhibition of glycosphingolipid biosynthesis was observed. The particular substrate specificity of the malarial GCS must be added to the already known unique and amazing features of *P. falciparum* lipid metabolism; therefore this enzyme might represent a new attractive target for malarial chemotherapy.

In addition, we have developed similar studies on the GCS of *Trypanosoma cruzi*. In contrast with the plasmodial enzyme, *T. cruzi* enzyme glycosylates the unsaturated ceramide. The use of different drugs affecting the GSLs pathway has been tried. Interestingly, 10  $\mu$ M PPMP inhibited glucosylceramide synthesis and showed 79 % of blood trypomastigote lysis. In contrast, 10  $\mu$ M tamoxifen, a known anticancer drug, although increased all

glycosphingolipid synthesis, produced 90% of blood trypomastigote lysis. Purification of both parasitic enzymes has been achieved and studies are in progress to clarify the metabolic glycosphingolipidic pathway regulation.

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## Oral 5

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**Title: Glucosylceramide Synthase as Target for New Antiparasitic Drugs**

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## Poster

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**Involvement of Sulfated Oligosaccharides in the Antigenicity of the Major Cysteine Proteinase of *Trypanosoma cruzi*.**

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## Harry de Koning [ Oral 3 & 16 ]

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## Oral 3

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### Drug resistance in African trypanosomiasis Harry P. de Koning

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In the past, African trypanosomiasis control has been extremely successful, leading to the near-eradication of the disease in many parts of Africa by the 1960's. Many of the control measures that were so successful then are not available now:

- Vector control through massive application of insecticide and clearing of habitat.
- Culling of wild reservoir animals.
- Many mobile teams of doctors screening entire populations for human African trypanosomiasis (HAT).
- Mass-prophylaxis with pentamidine.

A vaccine, even for a single *Trypanosoma* species, is still a very distant prospect, and control is now further hampered by the onset of drug resistance to some of the first-line medications. In the veterinary field, resistance to all current drugs has been reported for numerous locations, particularly where high transmission rates have necessitated intensive treatment with diamidines (Diminazene Aceturate (Berenil<sup>®</sup>)), phenanthridines (Isometamidium (Samorin<sup>®</sup>) and Ethidium (Homidium<sup>®</sup>)) or Quinapyramine (Antrycide<sup>®</sup>). For HAT, the main problem is with the main treatment for late stage disease, melarsoprol, where in some treatment centres failure rates of well over 30% have been reported. For West African sleeping sickness, difluoromethylornithine (DFMO or Eflornithine<sup>®</sup>) is an alternative, but the involves numerous intravenous infusions of the drug, and thus requires proper hospital conditions that are not always available. Trials with the Chagas' disease drug nifurtimox (Lampit<sup>®</sup>) in combination with melarsoprol or eflornithine are currently underway, but new treatments are necessary.

To have any impact, the new treatments must not be cross-resistant with existing drugs and it thus becomes vital to study the mechanisms by which trypanosomes have become resistant to the various chemotherapeutic agents. The current drugs, with the exception of Eflornithine, have been in heavy use for half a century or more. Of the HAT treatments, the diamidine compound pentamidine has seen the most intensive use, as it was used prophylactically on a very large scale for almost two decades. Yet, few if any well-documented cases of human infective and pentamidine resistant sleeping sickness exist. The marked contrast in the resistance situation for the veterinary diamidine Berenil and its human equivalent, pentamidine, can be explained by the way they gain entry to the parasite's cytosol. While Berenil uses a single *T. brucei* adenosine transporter, called P2, to enter the trypanosome, pentamidine can enter through at least two additional routes, probably because this molecule is much more flexible than diminazene. We have identified and characterised the two transporters for this drug, and are currently trying to clone them and

establish their role in the trypanosome's physiology. Preliminary results show that pentamidine resistant laboratory strains are much less infective, though they have the same growth rate as parental strains in vitro. The High Affinity Pentamidine transporter also appear to be involved in high-level arsenical resistance. These laboratory findings need urgently be tested with drug resistant isolates from the field.

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## Oral 16

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Title: The ENT Family of Purine Transporters in Protozoa

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### Oumar Diall [ Oral 4 ]

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Shortened protocols for field detection and surveillance of trypanocide resistance in the cotton zone of West Africa

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The use of trypanocidal drugs is the most popular strategy in the control of bovine trypanosomosis in West Africa. In this region, trypanocidal drug resistance has become the major threat for the continuation of this strategy. The development of a better strategy for trypanosomosis control in a given area requires an evaluation of resistance levels to trypanocidal drugs currently in use, namely Isometamidium (preventive) and Diminazène (curative). It is therefore important to provide relevant national institutions with a rapid and an easy-to-use tool for the diagnosis of chemoresistance in the field. Previous methods used for such diagnosis were based on long protocols of 70- 90 days, and targeted only Isometamidium. In the present work we first evaluated a shortened protocol of 56 days in 5 villages identified as high prevalence sites in Sikasso area, south of Mali, through a cross-sectional parasitological survey of 25 villages. The analysis of data suggested that even a 28- day observation could be sufficient to get significant results in high risk areas. Thus a 28-day protocol was developed and tested in villages in the same area and results were encouraging.

This last protocol comprises only two parasitological controls on days 14 and 28 after preventive treatment with Isometamidium (1mg/kg) of 40-50 cattle, compared with a control group. During the follow up, all infections detected in both groups were treated with Diminazène (3.5mg/kg)

Resistance to Isometamidium was assessed by comparing cumulative incidences (CI) in both treatment and control groups using the test Relative Risk Reduction (RRR) and deducting the rate of treatment failure. Resistance to Diminazène was assessed through the rate of treatment failures observed at 14 days post treatment in the control group alone. For both drugs, the threshold of 25% treatment failure was adopted as criteria of presence of chemoresistance. These shortened protocols have revealed different levels of resistance to both Isometamidium and Diminazène in the surveyed villages, and allowed economies in time and resources.

**Key words:** trypanosomosis, chemoresistance, cotton zone of West Africa

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### Abdul Jabbar [ Oral 10 ]

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**Trypanosomiasis in Camels: 1. Prevalence 2. Comparative efficacy of Suranol-T and Cymelarsan**

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*Trypanosoma evansi* infection adversely affects the health status and working of camels all over the world. The disease is transmitted by the biting of Tabanids and causes huge economic losses. The present study was carried out to determine the prevalence of trypanosomiasis in camels using the parasitological and serological methods. A total of 150 dromedary camels were selected randomly. Out of total selected animals 5(3.3%) and 6(4%) camels were positive by the parasitological and serological examination, respectively. The animals detected positive by the tests were treated with Suranol-T (a locally claimed effective homeopathic preparation) and Cymelarasan. Following the treatment the blood and serological examination was carried out on day 7, 14 and 21. It was observed that Suranol-T was 25% effective at day 14 and 50% at day 21, while the camels treated with Cymelarasan exhibited no parasitemia on all days observed post-treatment and it was found to be 100% effective.

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**Helen-Lydia Kutima [ Poster ]**

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**Transformation Studies in *Glossina Morsitans Morsitans* and Some Haematophagous Insects (*Phlebotomus Duboscqi*, *Aedes Aegypti* and *Stomoxys Calcitrans*) in Relation to *Trypanosoma Brucei Brucei***

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The transformation of parasites in the guts of *Glossina morsitans morsitans* (W.), *Phlebotomus duboscqi* (L.), *Aedes aegypti* (L.) and *Stomoxys calcitrans* (L.) both *in vitro* and *in vivo* was studied. Fifty percent of bloodstream forms *T. b. brucei* (Bruce) had transformed into procyclics (midgut forms) in *G. m. morsitans* by 6-h post-feeding. On the contrary, no parasites had transformed in, *P. duboscqi*, *A. aegypti* and *S. calcitrans* even after 24-h post-feeding. Transformation in *G. m. morsitans* was inhibited by the addition of D-glucosamine to the bloodmeals. The parasite count *in vivo* reduced with increasing time interval post feeding. Parasites were observed up to 96 hours post feeding in *G. m. morsitans* and *S. calcitrans* in fixed midgut. Fewer parasites were found in the fixed midguts of *P. duboscqi*, *A. aegypti* form 24 hours post feeding and some were agglutinated and lysed clumps of parasite remain. All the four insect species were able to transmit *T. b. brucei* mechanically.

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**Annette MacLeod [ Oral 8 ]**

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**THE GENETIC MAP OF *TRYPANOSOMA BRUCEI* AND ITS USE IN LOCALIZING GENES THAT DETERMINE DRUG RESISTANCE AND HUMAN INFECTIVITY.**

**Annette MacLeod**, Sarah McLellan, Alison Tweedie, Anneli Cooper, Lindsay Sweeney, Liam Morrison, C. Michael R. Turner and Andy Tait

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The genetic system in *T. brucei* has recently been proven to be a conventional diploid Mendelian system with allele segregation and the independent segregation of alleles at unlinked loci. This opens up the possibility of generating a genetic map of the parasite and using forward genetics and positional cloning to identify genes that are involved in a number of phenotypic traits, such as, drug resistance, host specificity, pathogenesis and virulence. To this end, we have used the available genome sequence to identify mini- and microsatellite markers distributed throughout the genome and have constructed the first genetic map of *T. brucei* (TREU 927). Meiotic crossover activity in the genome is high (approximately 15 kilobases per centiMorgan) with regions of higher recombination frequency (hot spots) and regions where recombination is rare (cold spots), which include putative centromeres. This genetic map has been used in linkage analysis to identify a novel single locus responsible for arsenical drug resistance.

Similarly, by the analysis of a cross between *T.b. gambiense* and *T.b. brucei*, a partial genetic map of *T.b. gambiense* has also been constructed. Analysis of the inheritance of human infectivity in this cross indicates that this trait segregates in a Mendelian fashion. We have used quantitative trait analysis to identify a major locus responsible for the majority of the phenotype in the West African subspecies, *T.b. gambiense*.

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### **Jacques Mauël**

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Jacques Mauël, Professor Emeritus, Department of Biochemistry, Faculty of Biology and Medicine, University of Lausanne, Switzerland

Graduated in biochemistry, then Ph.D. thesis (1967) at the Swiss Institute for Experimental Cancer Research, studying the lytic mechanisms of killer lymphocytes towards target cells. To this end, I started and developed the use of chromium-labelling of target cells (using <sup>51</sup>Cr) for measuring the cytotoxic potential of lymphocytes, a technique now widely used. Post-doctoral studies at the Wistar Institute, Philadelphia, USA, where my interests shifted towards the study of macrophages and I developed a permanent line (IC-21) of SV40-transformed murine macrophages which have retained most of the physiological properties of *bona fide* macrophages. On returning to Switzerland, started to work at the WHO Laboratories within the Department of Biochemistry of the Lausanne Medical School, working on the interaction of macrophages with the intracellular parasite *Leishmania*. From 1977 to 1984, served as member, then chairman of the Steering Committee on Immunology of Leishmaniases, WHO TDR Programme, Geneva. Together with my colleague Dr. Reza Behin, we demonstrated that the most likely mechanism of recovery from the infection by this obligate parasite was through *macrophage activation*, a phenomenon whereby macrophages respond to certain lymphokines by producing molecules that are toxic for microorganisms. Similar to other groups, we then found that *nitric oxide* (NO) was a major toxic product of activated macrophages. Recent interests include : (1) the role of gp63, or leishmanolysin, a surface protease of *Leishmania* that appears to cleave macrophage MARCKS-related protein (MRP), potentially leading to profound changes in the physiology of macrophages, (2) vaccinating against *Leishmania* infections using defined parasite molecules, and (3), especially, trying to develop an "anti-smoking" vaccine using immunogens made of nicotine coupled to protein carriers.

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### **Liam Morrison [ Oral 12 ]**

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#### **A GENETIC APPROACH TO THE STUDY OF VIRULENCE CAUSED BY *TRYPANOSOMA BRUCEI***

**L.J. Morrison**, S. MacLellan, A. MacLeod, A.Tait and C.M.R. Turner.

Wellcome Centre for Molecular Parasitology and Division of Infection and Immunity, University of Glasgow, Biomedical Research Centre, 120 University Place, Glasgow G12 8TA.

*Trypanosoma brucei* is a protozoan parasite that causes serious human and animal disease in sub-Saharan Africa. The disease is characterised by anaemia, spleno- and hepatomegaly, and eventually neurological symptoms and death. The pathogenesis of trypanosomiasis is relatively poorly understood. We have examined the progression of several disease indicators (red blood cell count, hepatomegaly, splenomegaly, nitric oxide levels), erythropoiesis (reticulocyte count, erythropoietin expression) and cytokine expression (IL4, IL10, IL12, IFN $\gamma$ , TNF $\alpha$ ), in parallel infections of two *T. brucei* strains, TREU 927 and STIB 247. Statistically significant differences were observed in nine of these parameters between the two strains of trypanosomes, showing a clear cut difference in virulence, with strain TREU 927 causing acute anaemia, low red blood cell production and splenomegaly, whereas infections with strain STIB 247 showed little anaemia, less splenomegaly, but a marked transient reticulocytosis.

The genetic basis of these strain differences has been analysed by examining the different parameters in infections with 35 F1 hybrids from a cross between the two trypanosome strains. Several of the phenotypes exhibit segregation in the progeny, and open up the possibility of identifying the loci involved, using the recently developed genetic map of the parasite. The identification of the trypanosome gene(s) responsible will provide insight into how pathology is induced by the parasite, and potentially provide a target for disease intervention.

This work is funded by the Wellcome Trust.

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**Theresa O'Brien [ Poster ]**

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### **Targeting Cathepsins for New Therapy Against African Sleeping Sickness**

T. O'Brien, Z.B. Mackey, C. Caffrey, and J. McKerrow

In the last 80 years, only four drugs have been discovered to treat African Sleeping Sickness. Patients undergoing chemotherapy with any of these drugs may suffer severe side effects. Cathepsins are lysosomal cysteine proteases that are critical to the life cycle and pathogenicity of protozoan parasites. Previous work has shown that chemical inhibitors of cysteine proteases are effective at killing *T. brucei* in culture and in experimentally infected mice. Two cathepsins, rhodesain, a cathepsin L-like protease, and TbcAtB, a newly identified cathepsin B-like protease, are expressed in *T. brucei*. We have shown using RNA interference (RNAi) that TbcAtB, but not rhodesain, is required for parasite survival in culture. However, in a mouse model of infection, knockdown of rhodesain enhances host survival suggesting that this protease may function in immune evasion. When TbcAtB mRNA expression is silenced, the trypanosomes become dismorphic and die after 72 hours. We hypothesize that TbcAtB may represent an exploitable target for future drug chemotherapy. Current studies include the expression and biochemical characterization of recombinant TbcAtB, localization of TbcAtB in bloodstream parasites, and deciphering the role that TbcAtB plays in host protein degradation. We have evidence to suggest that the rhodesain zymogen is a potential substrate for TbcAtB, and that TbcAtB is required for degradation of host transferrin.

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**Chris Peacock [ Oral 9 & 15 ]**

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### **Comparative Genome Sequencing of *Leishmania* and *Trypanosomes*: searching for answers to disease diversity**

**C. Peacock**, C Hertz-Fowler, M. Berriman

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

The Wellcome Trust Sanger Institute has had a long-term investment and commitment to sequencing *Leishmania* spp. and African trypanosomes. The recent publication of the complete genome sequences for *Leishmania major*, *Trypanosoma brucei brucei* and *Trypanosoma cruzi* have shown the usefulness of comparative genomic analysis across related organisms. *Leishmania* spp. cause a broad range of disease phenotypes in humans that can be broadly categorised as localised cutaneous, mucocutaneous and visceral leishmaniasis. Although host genetics influence the course of infection, disease type is determined by the infecting species of *Leishmania*. Pathogenic strains of *Leishmania major*, *Leishmania braziliensis* and *Leishmania infantum* have been selected for sequencing as representative members of each of the three disease phenotypes. All three species have now been completely sequenced and manually annotated. Extensive comparative genome analysis has revealed that there are relatively few species-specific genes that may account for the disease differences. Further analysis has identified genes that are

significantly diverged from the respective orthologues in the other species. Most of these have no putative function but are likely to be involved in host-parasite interaction.

Apart from the recently published *Trypanosoma brucei brucei*, the Wellcome Trust Sanger Institute is sequencing a further three species of African trypanosomes, including the human pathogen *Trypanosoma gambiense* and the two cattle pathogens *Trypanosoma congolense* and *Trypanosoma vivax*. Comparative analysis of these human and non-human species will shed light on those factors that determine pathogenicity in the respective hosts.

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### Oral 9

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**Title: The TriTryp Genome Projects**

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### Oral 15

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**Title: Comparative Genomics of Leishmania**

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## M. Sohail Sajid [ Oral 2 ]

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### **A PRELIMINARY REPORT ON *TRYPANOSOMA EVANSI* INFECTION IN CHARMING HIMALIYAN BLACK BEARS**

G. MUHAMMAD, M. SAQIB, M. S. SAJID<sup>\*</sup> and A. NAUREEN

**Department of Clinical Medicine & Surgery, Faculty of Veterinary Science, <sup>\*</sup>Department of Veterinary Parasitology, Faculty of Veterinary Science, University of Agriculture, Faisalabad, 38040-Pakistan.**

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Asiatic or Himalayan black bear (*Selenarctos thibetanus*) is an endangered wild animal species. In South-Asian countries, captive tamed Himalayan bears are commonly used by roving bear-charmers to entertain the people in rural and urban areas. In unnatural habitats, this species confronts several psychophysical traumas and communicable diseases, which are prevalent in other domestic species. The present report describes 4 cases of *Trypanosoma evansi* infection in live Himalayan charming bears, which originated from Faisalabad and Jhang districts of Pakistan. The condition was characterized by fever (38.3-40.7°C), accelerated pulse (74-104 beats/min) and respiration (39-46 breaths/min) rates, depression, anemic mucous membranes, recumbency (n = 1), ataxia (n = 3) and crackles on auscultation of lungs (n = 1). Microscopic examination of peripheral wet blood films revealed moderate (n = 2) to very large (n = 2) number of *Trypanosoma evansi*. All 4 bears treated twice at 3 days interval with suramin sodium (Naganol, Bayer; Germany) using almost two fold the dose recommended for common domestic species (10 mg/kg b.wt.) were found aparasitemic on repeat blood testing on days 2, 3 and 7 of institution of therapy. No untoward effect was noticed and all 4 cases recovered in 3-7 days after completion of second round of treatment. One bear died 8 day after completion of treatment. This is the first report of *T. evansi* in live bears.

Key words: Himalayan black bear, *Selenarctos thibetanus*, Suramine sodium, *Trypanosoma evansi*, Naganol Pakistan

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## Jeremy Sternberg [ Oral 1 & 14 ]

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### Oral 1

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#### **Virulence variation in Human African Trypanosomiasis**

Jeremy M Sternberg and Lorna MacLean, University of Aberdeen, School of Biological Sciences, Aberdeen AB24 2TZ UK. email: j.sternberg@abdn.ac.uk

We recently described two foci of *T.b.rhodesiense* sleeping sickness with dramatically different virulence profiles. In Malawi, disease was chronic and progression to late stage rare, while in Uganda the disease was acute and rapidly progressed to late stage (MacLean et al., 2004). I will review these data and present new findings on localised virulence variation in Ugandan cases of sleeping sickness. These will be discussed in the context of host-immune responses, with particular reference to cytokines regulating inflammatory responses.

MacLean, L., Chisi, J.E., Odiit, M., Gibson, W.C., Ferris, V., Picozzi, K., Sternberg, J.M., 2004. Severity of Human African Trypanosomiasis in East Africa is associated with geographic location, parasite genotype and host-inflammatory cytokine response profile. *Infect Immun* 72, 7040-7044.

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## Oral 14

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### Does the inflammatory/counterinflammatory cytokine balance determine the onset of late stage human African Trypanosomiasis

Jeremy M Sternberg, Lorna Maclean, Jean Rodgers\*, Barbara Bradley\* and Peter G.E. Kennedy\* (School of Biological Sciences, University of Aberdeen, Aberdeen AB24 2TZ, UK; \*Dept of Veterinary Clinical Studies and Dept. of Neurology, University of Glasgow, UK) email: j.Sternberg@abdn.ac.uk

The late or meningoencephalitic stage of African trypanosomiasis follows the penetration of the CNS by trypanosomes and is characterised by increasingly severe neurological disturbance. Previous studies in experimental mouse models have implicated parasite-triggered microglial and astrocyte activation and the production of pro-inflammatory cytokines in this process. However recent data from trypanosomiasis patients in Uganda and Malawi suggest that both the onset of and progression of the late stage of disease is controlled by the dynamic balance of inflammatory and counter-inflammatory cytokines expressed in the CNS (Maclean et al., 2006). We describe a mouse model which supports this hypothesis (Sternberg et al., 2005).

Maclean, L., Odiit, M., Sternberg, J.M., 2006. Intrathecal cytokine responses in *Trypanosoma brucei rhodesiense* sleeping sickness patients. *Trans R Soc Trop Med Hyg* 100, 270-275.

Sternberg, J.M., Rodgers, J., Bradley, B., Maclean, L., Murray, M., Kennedy, P.G., 2005. Meningoencephalitic African trypanosomiasis: Brain IL-10 and IL-6 are associated with protection from neuro-inflammatory pathology. *J Neuroimmunol* 167, 81-89.

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## Ryan Swenerton [ Poster ]

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### Gene Targeting and Biochemical Characterization of Clan SB and SC Serine Proteases in *Leishmania donovani*

R.K. Swenerton, B.L. Kelly, M. Sajid, and J.H. McKerrow

Serine proteases have been implicated in key stages of the infectious lifecycle of kinetoplastid parasites. Treatment of *Leishmania donovani* with the broad serine protease inhibitor, Pefabloc, arrested replication *in vitro*. Over twenty distinct serine protease genes can be identified in the published *L. major* genome. Our initial biochemical and proteomics studies of *L. donovani* extracts confirmed the presence of an active clan SC protease, oligopeptidase B (OpdB). Previous work in other trypanosomatids has shown that this subfamily of enzymes plays a role in host-cell invasion and contributes to the pathogenesis of disease. We have begun functional characterization of this protease and recently deleted it by gene targeting. Phenotypic analysis of these knockouts is underway. We have also cloned the OpdB gene and added reporter- and epitope-tags for localization and overexpression in *L. donovani*. Additionally we have identified a *L. donovani* clan SB subtilisin. This enzyme has been deleted by gene targeting and knockout parasites are currently under phenotypic analysis. Initial investigations suggest that these latter mutant parasites have impaired promastigote to amastigote differentiation axenically. The *L. donovani* subtilisin gene has also been cloned and is currently being recombinantly overexpressed for biochemical analysis.

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## Henry Tabel [ Oral 11 & 17 ]

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## Oral 11

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### Immune responses in experimental African trypanosomiasis

Henry Tabel, Dept. of Veterinary Microbiology, University of Saskatchewan, Saskatoon, Canada

Protective immune responses comprise the production of antibodies to the variant surface glycoproteins (VSG) and to common antigens. We have recently analysed the interactions of IgM anti-VSG antibodies to *Trypanosoma congolense*. We studied the effect on phagocytosis of trypanosomes *in vitro* (1) and *in vivo* (2, 3). Macrophages play a predominant role in clearance of the parasite but also in pathogenesis. I intend to discuss the complexity of antibody responses and their effects.

1. Pan, W., Wei, G., Shi, M., and Tabel, H. 2006. CR3 (CD11b/CD18) is the major macrophage receptor for IgM antibody-mediated phagocytosis of African trypanosomes: diverse effect on subsequent synthesis of TNF- $\alpha$  and nitric oxide. *Microbes Infect.*, in press.

2. Shi, M., Wei, G., Pan, W., and Tabel, H. 2004. *Trypanosoma congolense* infections: antibody-mediated phagocytosis by Kupffer cells. *J. Leukocyte Biol.* 76: 399-405.

3. Shi, M., Wei, G., Pan, W., and Tabel, H. 2005. Impaired Kupffer cells in Highly susceptible mice infected with *Trypanosoma congolense*. *Infect Immun.* 73: 8393-8396.

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## Oral 17

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### **Immunopathogenesis in experimental African trypanosomiasis**

Henry Tabel, Dept. of Veterinary Microbiology, University of Saskatchewan, Saskatoon, Canada

African trypanosomes are protozoan parasites that infect both humans and livestock. BALB/c mice are highly susceptible and C57BL/6 mice are relatively resistant to infections by *Trypanosoma congolense* or *T. brucei*. Rapid death observed in BALB/c mice infected with virulent strains of *T. congolense* or *T. brucei* is due to a systemic inflammatory response syndrome (SIRS) (1). A subset of pathogenic, MHC class II-restricted CD4<sup>+</sup> T cells (Tp cells), activated during the course of *T. congolense* infection, mediates early mortality in infected BALB/c mice via excessive synthesis of IFN- $\gamma$  (2). In the infected mice, IFN- $\gamma$  is produced predominantly by MHC class II-restricted, CD3<sup>+</sup>Thy1.2<sup>+</sup>TCR $\beta$ <sup>+</sup>CD4<sup>+</sup> T cells. Since these pathogenic T cells are matrix-adherent, they could be distinguished from conventional Th1 cells. Our working hypothesis is that this subset of CD4<sup>+</sup> Tp cells are not simply hyperactive Th1 cells but might be a novel subset with unique surface markers.

1. Shi, M., Pan, W., and Tabel, H. 2003. Experimental African trypanosomiasis: IFN- $\gamma$  mediates early mortality. *Eur. J. Immunol.* 33: 108-118.
2. Shi, M., Wei, G., Pan, W., and Tabel, H. 2006. Experimental African trypanosomiasis: a subset of pathogenic, IFN- $\gamma$ -producing, MHC class II-restricted CD4<sup>+</sup> T cells mediates mortality in highly susceptible mice. *J. Immunol.* 176: 1724-1732.