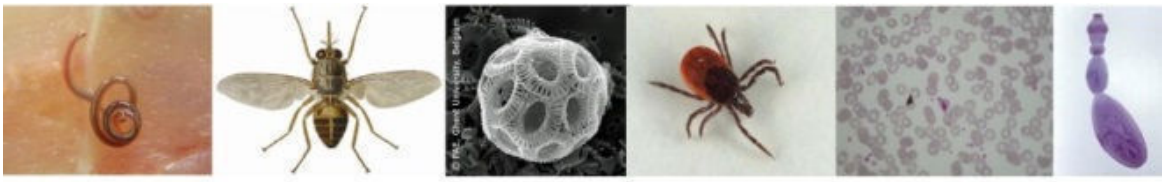

Belgian Society of
Parasitology & Protistology

Annual Meeting of the Belgian Society of Parasitology and Protistology

Vrije Universiteit Brussel
05 May 2017

Abstract Book

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PROGRAM

09:30	Registration	
09:45	BSPP President: Guy Caljon	Welcome address
Session 1 – Chair: Thomas Geurden		
10:00	**Keynote 1:** Henry-Michel Cauchie (Luxembourg Institute of Science and Technology)	PATHOGEN DISTRIBUTION HETEROGENEITY IN SPACE AND TIME: HOW TO DESIGN THE MONITORING SCHEME OF YOUR WATERBODY TO DEAL WITH IT?
10:45	Johnny Vlaminck (UGent)	THE FUTURE OF SEROLOGY AS A DIAGNOSTIC TOOL FOR ASCARIASIS
11:00	Piet Cools (UGent)	COMPARISON OF SINGLE KATO-KATZ, DUPLICATE KATO-KATZ, MINI-FLOTAC AND FECPAK^{G2} FOR THE ASSESSMENT OF ANTHELMINTHIC EFFICACY OF SINGLE DOSE ALBENDAZOLE
11:15	Tine Huyse (KUL & Africa Museum)	DON'T OVERLOOK THE SNAIL: BARCODING AND INFECTION DYNAMICS OF INTERMEDIATE SNAIL HOSTS OF HUMAN AND LIVESTOCK SCHISTOSOMES IN SENEGAL
11:30	**Coffee break**	
Session 2 – Chair: Edwin Claerebout		
12:00	Irina Matetovici (ITM)	ANALYSIS OF THIOESTER-CONTAINING PROTEINS SUGGESTS A ROLE IN THE INNATE IMMUNITY OF THE TSETSE FLY
12:15	Lieselotte Van Bockstal (UA)	ASSESSING THE EFFECTS OF THE ACQUISITION OF MILTEFOSINE RESISTANCE ON PARASITE VIRULENCE
12:30	Bart Cuypers (ITM)	SPLICED-LEADER SEQUENCING: A HIGH-THROUGHPUT, SELECTIVE METHOD FOR RNA-SEQ OF TRYPANOSOMATIDA
12:45	**Lunch**	
13:30	**BSPP General Meeting**	
Session 3 – Chair: Johannes Charlier		
14:15	**Keynote 2:** Edwin Claerebout (UGent)	THE GHENT LAB: BRIDGING TWO CENTURIES IN PARASITOLOGY
15:00	Christopher K. Kariuki (VUB)	APPLYING THE NANOBODY TECHNOLOGY® TO TARGET THE BLOODSTREAM TRYPANOSOMAL TRANSFERRIN RECEPTOR
15:15	Eline Eberhardt (UA)	IMPACT OF MILTEFOSINE RESISTANCE AND HOST IMMUNE SUPPRESSION ON PARASITE FITNESS
15:30	Magali Van den Kerkhof (UA)	MECHANISM OF ACTION OF THREE DISTINCT SERIES OF NOVEL ANTILEISHMANIAL COMPOUNDS
15:45	**Coffee break**	
Session 4 – Chair: Guy Caljon		
16:15	Frank Dumetz (ITM)	ANEUPLOIDY VARIATION DURING THE LIFE CYCLE OF *LEISHMANIA DONOVANI*
16:30	Gaston Amzati (UNamur)	POLYMORPHISM IN THE GENE ENCODING *THEILERIA PARVA* TP2 ANTIGEN RECOGNISED BY BOVINE CTL: A PROSPECTIVE FOR CATTLE IMMUNIZATION PROGRAM IN THE DEMOCRATIC REPUBLIC OF CONGO AND BURUNDI
16:45	Piet Cools (UGent)	MOLECULAR TYPING OF *TRICHOMONAS VAGINALIS* CLINICAL ISOLATES FROM KILIFI, KENYA BY ACTIN GENE SEQUENCING AND DETECTION OF *TRICHOMONAS VAGINALIS* VIRUSES
17:00	**Avia-GIS Best Presentation Award & Zoetis Travel Grant**	
17:30	**Reception**	

SESSION 1

INVITED SPEAKER

Henry-Michel Cauchie is heading the Research Unit (60 members) on "Water Security and Safety" at the Luxembourg Institute of Science and Technology (www.list.lu). Within the Research Unit, he is furthermore responsible for the Research Group on Bioindication and Microbial Biosensors which deals with the application of bioindicators under global change pressures, the understanding of waterborne pathogen dispersal and related risk assessment as well as the development of microbial sensors. Dr Cauchie also gives several courses (ecology, monitoring of environment quality) at the University of Liège (Belgium) and the University of Luxembourg.
The Water Safety and Security Research unit includes about 60 researchers with complementary backgrounds bringing innovative solution to the contemporary water issues such as floods and drought, the increasing need for drinking water in intense urbanization context, the protection of water resources,... The missions of the Water Security and Safety unit are (1) to understand how catchments store and release water, microbes and pollutants; (2) to monitor and understand how flowpaths and flow sources control streamwater and groundwater chemistry and microbiology; and (3) to design, craft, test and bring to the market new technological solutions for keeping our water resources safe. Dr Cauchie's missions encompass the responsibility for the overall management, coordination and performance of the RDI activities of a unit.

PATHOGEN DISTRIBUTION HETEROGENEITY IN SPACE AND TIME: HOW TO DESIGN THE MONITORING SCHEME OF YOUR WATERBODY TO DEAL WITH IT?

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Faecal contamination of water resources is a recurring problem in developing countries causing high mortality rates. Although less frequent in industrialised countries, they have important impacts due to the number of people that can be affected by a microbial contamination of drinking water networks. Establishing water safety plans (WSP) i becoming a standard procedure in many countries. To reduce the risk of microbial contamination of water resources, WSP recommends to characterise precisely the watershed, notably in terms of microbial contamination sources. Monitoring resources for the detection of microbial pathogens is however not an easy task, essentially because of the large spatial and temporal variability of microbial contaminations in water.
The objective of this presentation is to review the major problems to tackle when establishing a monitoring scheme for pathogens in water. The focus will be made on *Cryptosporidium* and *Giardia* and their dynamics in rivers, lakes and groundwater. The interrelations with the hydrological and meteorological conditions will be analysed in details, allowing to define adequate sampling strategies.

SELECTED ORAL PRESENTATIONS

THE FUTURE OF SEROLOGY AS A DIAGNOSTIC TOOL FOR ASCARIASIS.

Vlaminck Johnny[1], Daniel Dana[1,2], Lien Demeyer[1], Zeleke Mekonnen[2], Gary J. Weil[3], Peter Geldhof[1], Bruno Levecke[1].

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The nematode parasite *Ascaris lumbricoides* is estimated to infect over 800 million people and is considered to be an important neglected tropical disease pathogen. Ascariasis has a substantial impact on public health, but routine diagnosis still relies on the detection of eggs in stool. This technique has important limitations in terms of both application and interpretation. Unfortunately, the development of novel, more sensitive diagnostic tools has been rather limited. For the veterinary field, where they struggled with similar diagnostic challenges, a new serological test for the detection of *A. suum* exposure in fattening pigs was developed in our lab a few years back and has since been widely applied in a number of European countries. This test is based on the immune recognition of the *A. suum* haemoglobin antigen (AsHb) by infected pigs. Given the nearly identical genetic and antigenic constitution of pig and human *Ascaris*, we recently modified the test to detect human *Ascaris* infection. Evaluation of this test using a serum sample set from a clinical trial in an Indonesian community has shown that a serological tool could be a potential asset in certain stages of STH control programs.
The goal of this talk will be to provide an overview of the recent developments in serodiagnosis for *Ascaris* and to highlight the possible applications for serology. The general difficulties involved in evaluating and interpreting serodiagnostic tests for STH will also be discussed.

COMPARISON OF SINGLE KATO-KATZ, DUPLICATE KATO-KATZ, MINI-FLOTAC AND FECPAK^{G2} FOR THE ASSESSMENT OF ANTHELMINTHIC EFFICACY OF SINGLE DOSE ALBENDAZOLE

Cools P.[1], Vlaminck J.[1], Albonico M.[2], Ame S.[3], Ayana M.[4], Keiser J.[5], Mekonnen Z.[4], Montresor A. [2], Sayasone S.[6], Vercruysse J.[1], Levecke, B.[1]

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INTRODUCTION: The soil-transmitted helminths (STHs; *Ascaris*, *Trichuris*, hookworm) are responsible for the highest burden among all neglected tropical diseases.
Mass drug administration (MDA) programs, in which a single-oral dose of albendazole or mebendazole are periodically administered to (pre)school-aged children are the main strategy to control for STHs. This drug pressure makes MDA programs highly vulnerable to the development of anthelminthic resistance (AR), as has been described in veterinary medicine. This necessitates thoroughly designed surveillance systems that allow detection of any changes in anthelmintic drug efficacy that may arise through the evolution of AR. We aimed to assess the performance of new diagnostic tools to monitor the efficacy of anthelminthic drugs.
METHODOLOGY: In Tanzania, Ethiopia and Laos, stool specimens from school-aged children (5-14 years) were collected during a baseline visit, where children received a single dose of albendazole. Stool specimens were analyzed for the presence of *Ascaris lumbricoides*, *Trichuris trichiura* and hookworm using single Kato-Katz, duplicate Kato-Katz, Mini-FLOTAC and FECPAK^{G2}. A follow-up stool sample was collected within two to three weeks for each child positive at baseline for at least one STH with at least one technique, aiming at a predefined number of 110 completed cases of *Ascaris*

and *Trichuris*, and of 100 cases of hookworm, for each site. Egg-reduction rates (ERR) were calculated for the different STHs using the different techniques, and was the time needed to process and analyze the samples using each technique.
RESULTS: ERR for *Ascaris* and hookworm were comparable for each technique but differed markedly for *Trichuris* in a study site dependent manner. Single Kato-Katz was the fastest technique in all settings. Overall, FECPAK^{G2} had the lowest sensitivity.
CONCLUSIONS: The choice of diagnostic techniques to monitor drug efficacy will depend on both the species present in endemic regions and their intensity.

DON'T OVERLOOK THE SNAIL: BARCODING AND INFECTION DYNAMICS OF INTERMEDIATE SNAIL HOSTS OF HUMAN AND LIVESTOCK SCHISTOSOMES IN SENEGAL.

Tine Huyse[1,2], Nele Boon[1,3], Nathalie Smitz[2], Alessandro De Sciscio[2], Bruno Kanage[1], K. Polman[3], F. Volckaert[1], & Frederik Van den Broeck[3]

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The epidemiology of schistosomiasis in Northern Senegal is very dynamic. While *Schistosoma mansoni* was the dominant parasite at the onset of the epidemic in the nineties, the urinary species, *S. haematobium*, was mostly absent. Nowadays this pattern is almost completely reversed. In addition, recent molecular analyses revealed that Senegalese children were infected with hybrid crosses between *S. haematobium* and *S. bovis*, the latter being a livestock parasite. This species is very closely related to the former but uses a different freshwater snail host to complete its life cycle. However, nearly nothing is known about the distribution of this snail host. If this snail host is very abundant, this hybridization could explain the rise in urinary schistosomiasis. To test this hypothesis we sampled in 2012 the main snail intermediate host species in the lower and middle delta of the Senegal River Basin. We barcoded the bulinid snail species by sequencing a partial cytochrome oxidase 1 (*cox*1) fragment and tested each snail for schistosome infection using a diagnostic PCR. The most dominant snail species was *Bulinus truncatus*, the host of *S. bovis*, followed by *B. globosus,* the main host of *S. haematobium.* The former was exclusively infected by pure *S. bovis* parasites, while the latter was infected with *S. haematobium* and with hybrid parasites. The distribution of both species was heterogeneous along the river basin, as was the distribution of the hybrid parasites in children that was obtained during a previous study. A detailed comparison between host and parasite distribution will be made in order to better understand the driving factors behind schistosomiasis epidemiology.

SESSION 2

SELECTED ORAL PRESENTATIONS

ANALYSIS OF THIOESTER-CONTAINING PROTEINS SUGGESTS A ROLE IN THE INNATE IMMUNITY OF THE TSETSE FLY

Irina Matetovici [1] and Jan Van Den Abbeele [1]
[1] Unit of Veterinary Protozoology, Department of Biomedical Sciences, Institute of Tropical Medicine Antwerp (ITM), Antwerp, Belgium

Thioester-containing proteins (TEPs) are conserved proteins among insects, that are thought to play a role in innate immune response. In the current study, we functionally characterized the seven members of the TEPs family present in the genome of two tsetse fly species, *Glossina morsitans* and *G. palpalis*. Phylogenetic analysis of the family shows that tsetse species harbor specific TEPs, not found in any other dipteran. *G. morsitans* TEP genes display a tissue-specific pattern of expression with some that are markedly up-regulated in the presence of the trypanosome parasite. Of note, a different TEP response was observed to infection with *Trypanosoma brucei* compared to *T. congolense*, indicating that the tsetse TEP response is trypanosome-specific. Taken together our findings suggest the involvement of the TEP family in tsetse innate immunity with a possible role in the specific control of the trypanosome parasite in the fly.

ASSESSING THE EFFECTS OF THE ACQUISITION OF MILTEFOSINE RESISTANCE ON PARASITE VIRULENCE

Van Bockstal L.[1], Sadlova J.[2], Eberhardt E.[1], Hendrickx S.[1], Maes L.[1], Volf P.[2], Caljon G.[1]
[1]Laboratory of Microbiology, Parasitology and Hygiene, University of Antwerp, Antwerp, Belgium; [2]Department of Parasitology, Faculty of Science Charles University, Prague, Czech Republic; Corresponding author: Lieselotte.vanbockstal@uantwerpen.be

BACKGROUND: Oral miltefosine (MIL) is used to treat visceral leishmaniasis (VL) but is increasingly failing. Two isogenic bioluminescent lines of *Leishmania infantum* (MHOM/FR/96/LEM3323) were selected *in vitro* for differential susceptibility to MIL. This was followed by transfection to express red shifted luciferase (PpyRE9) which is a proficient tool for downstream *in vivo* bioluminescent imaging. Compared to the susceptible (MIL-S) line, tissue parasite burdens in mice revealed a severely hampered infectivity of the resistant (MIL-R) line in the main target organs.
METHODS: The *in vitro* macrophage infectivity and intracellular parasite expansion of the MIL-R and MIL-S matched lines was compared in relation to the observed *in vivo* infection deficit arising from the acquired MIL resistance. Following the observations made in Balb/c mice, infectivity was also compared in *Phlebotomus perniciosus* sand flies, a key vector of *L. infantum* in the Mediterranean area. Female sand flies were infected by feeding through a chick-skin membrane on heat-inactivated rabbit blood containing 10^6 promastigotes/mL. Flies were dissected at different time intervals post-bloodmeal in order to determine the abundance and location of the *L. infantum* infections with special emphasis to colonization of the stomodeal valve as prerequisite for successful parasite transmission.
RESULTS: Acquisition of MIL resistance, which was linked to a frameshift mutation in the MIL transporter, resulted in a reduced intracellular macrophage growth. This indicated a reduced *in vitro* fitness as compared to the MIL-S parental line and was also reflected by the severely compromised virulence in mice. Infection studies in sand flies suggest that the infectivity of the MIL-R strain is also hampered.

CONCLUSIONS: Evaluation of the *in vivo* fitness of the two *L. infantum* parasite lines with differential MIL resistance profile indicate a significant impact of a non-functional MIL transporter on virulence/infectivity in Balb/c mice and in the *P. perniciosus* sand fly vector.

SPLICED-LEADER SEQUENCING: A HIGH-THROUGHPUT, SELECTIVE METHOD FOR RNA-SEQ OF TRYPANOSOMATIDA

Bart Cuypers[1,2]*, Malgorzata A. Domagalska[1], Geraldine De Muylder[1], Pieter Meysman[2], Manu Vanaerschot[1$], Hideo Imamura[1], Thomas-Wolf Verdonckt[1], Peter J. Myler[3], Gowthaman Ramasamy[3], Kris Laukens[2#] and Jean-Claude Dujardin[1,4#]

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The Trypanosomatida family contains many human pathogenic species including Leishmania donovani (visceral leishmaniasis), Trypanosoma gambiense/rhodesiense (sleeping sickness) and Trypanosoma cruzi (chagas disease). Transcriptome studies of these parasites are essential for fundamental insights in parasite development, pathogenicity and drug resistance. However, in most tissue samples, host RNA is much more abundantly present than parasite RNA, imposing complicated and time consuming parasite isolation prior to sequencing. Interestingly, the mRNA of Trypanosomatida differs from the host's by starting with a fixed 39 nucleotide sequence or spliced-leader (SL). We exploited this feature and developed a protocol (SL-seq) to specifically amplify and sequence SL-containing parasite RNA out of a pool of host cell RNA and assessed its validity, specificity and sensitivity. We also provide a corresponding bioinformatics pipeline. Briefly, SL-Seq first converts SL-containing mRNA to cDNA using a SL-specific primer. Amplification is carried out with overhang-extension PCR, which adds additional motives and indexes, allowing multiplexing hundreds of samples on a single sequencing lane. We verified the validity of the SL-seq results by comparing them with those obtained with the Illumina Stranded mRNA kit (ILL-seq) and observed a strong correlation between the expression values obtained with both methods ($p<2e-16$ and $R^2=0.8$). We successfully sequenced Leishmania transcriptomes directly from infected THP-1 cells, without prior isolation of the parasites. With ILL-seq only 1.6% of the ILL-seq data was Leishmania mRNA, while this was 65.0% using the SL-seq protocol, indicating SL-seq resulted in a 42 fold enrichment of parasite mRNA. In conclusion, SL-seq completely abolishes the need for parasite isolation from the host tissue prior to RNA sequencing. SL-seq could also be useful for other SL-containing organisms including nematodes, trematodes and primitive chordates.

SESSION 3

INVITED SPEAKER

Edwin Claerebout graduated as a veterinarian at Ghent University in 1990. After a short stay in a rural practice in the UK, he started working at the Laboratory of Parasitology, Faculty of Veterinary Medicine, Ghent University, where he obtained his PhD in veterinary sciences (parasitology) in 1998. He became lecturer in parasitology at Ghent University in 2001 and full professor in 2010. Since 2003 he is a Diplomate of the European Veterinary Parasitology College (EVPC). He lectures on various topics in veterinary parasitology, but his main area of expertise is control of gastrointestinal parasites of cattle.

HELMINTH CONTROL IN CATTLE: BRIDGING TWO CENTURIES IN VETERINARY PARASITOLOGY RESEARCH AT GHENT UNIVERSITY

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Helminth infections of cattle affect productivity in all age classes and are amongst the most important production-limiting diseases of grazing ruminants. Current control of helminth infections is largely based on the (preventive) use of anthelmintics. However, indiscriminate use of anthelmintics is increasingly controversial, due to the public awareness for drug residues in the environment and in animal products and the development of resistant helminth strains. Consequently, there is a need for a more sustainable use of anthelmintics as well as alternative methods for the control of helminth infections.
Initial research at the Laboratory of Parasitology, Ghent University, focused on the evaluation of the (persistent) efficacy of anthelmintic drugs and the design of preventive treatment schedules to protect grazing stock against parasitic gastroenteritis. Attention was also given to potential side effects of intensive chemoprophylaxis, such as interference with the development of immunity and emerging anthelmintic resistance. Over the last decades, the focus of our research shifted to more sustainable use of anthelmintics and the development of vaccines as an alternative for chemoprophylaxis. Current vaccine research focuses on comparing immune responses against protective vs. non-protective experimental vaccines, combined with structural analyses of the vaccine antigens, in order to improve recombinant antigen production and vaccine delivery. In parallel, sustainable use of anthelmintics is based on improved diagnosis and integration of parasite control into the farm management. Diagnosis evaluated from merely detecting presence/absence of helminth infections towards detecting their impact on production, which allowed to integrate diagnostics in economic evaluation frameworks for improved decision making. Importantly, farmer attitudes and behaviour regarding helminth control are now also taken into account to design and implement sustainable parasite control programmes. Combining new parasite control tools and better economic impact assessments of helminth infections together with a deeper understanding of the (non-)economic factors that drive a farmer's animal health decisions should result in more effective control strategies and increased farmer satisfaction.

SELECTED ORAL PRESENTATIONS

APPLYING THE NANOBODY TECHNOLOGY® TO TARGET THE BLOODSTREAM TRYPANOSOMAL TRANSFERRIN RECEPTOR

Kariuki C.K[1], Mainye S.K[1], Stijlemans B.[1,2] & Magez S[1,3]
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INTRODUCTION: African trypanosomosis is a disease of medical and veterinary importance threatening both humans and animals in Sub-Saharan Africa. Currently, there are no anti-disease vaccines available and disease control relies heavily on the treatment with trypanocidal drugs. Therefore, targeting a trypanolytic/toxic molecule to a parasite surface molecule or receptor is ideal for increasing the drug targeting efficiency. In this study, we opted to use the trypanosomal transferrin receptor (TfR), a GPI-surface linked heterodimeric protein (ESAG6 and 7) bearing no similarity to the mammalian transferrin receptor involved in iron-uptake, as a target molecule. We propose to use a Nanobody technology® platform to generate and characterize Nanobodies® against the bloodstream trypanosomal transferrin receptor (ESAG6) as a tool to target trypanosomes.
METHODOLOGY: Following immunization of a llama with recombinant ESAG6 and selection against the ESAG6 via phage-display technology, three monovalent anti-ESAG6 Nanobodies® were selected. These were initially characterized *in silico* and after purification, via thermostability assays. The anti-ESAG6 Nanobodies® were subsequently tested in an Enzyme Linked Immunosorbent assay (ELISA) using whole cell lysate from different purified bloodstream-derived trypanosome species. In addition, Flow cytometry was performed with Alexa Fluor® 647 labelled anti-ESAG6 Nanobodies® on purified bloodstream-derived and blood-borne monomorphic trypanosomes.
RESULTS: The Nanobody® characterization assays revealed stable proteins with an expected molecular weight of 14 kDa and good thermal stability. ELISA assays indicated specific binding to *T. brucei* - derived lysate with little cross-reactivity to non - *T. brucei* related trypanosomal lysates. Flow cytometry analysis confirmed that one Alexa Fluor® 647 anti-ESAG6 Nanobody® could detect and bind to its antigen on intact monomorphic *T. b. brucei* Antat 1.1 parasites. In addition, a stronger signal was obtained on fixed and permeabilized parasites.
CONCLUSIONS: The available anti-ESAG6 Nanobodies® can bind their antigen in parasite lysate as well as on intact parasites. Moreover, binding of anti - ESAG6 Nanobodies® on fixed and permeabilized parasites indicate that the majority of TfR molecules are present intracellularly. Collectively, these results suggest that anti - TfR Nanobodies® might have diagnostic and therapeutic (targeting) potential.

IMPACT OF MILTEFOSINE RESISTANCE AND HOST IMMUNE SUPPRESSION ON PARASITE FITNESS

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INTRODUCTION: The increase in treatment failures and occurrence of drug resistance is a growing problem in the treatment of visceral leishmaniasis (VL). To study the impact of the acquisition of miltefosine (MIL) resistance on the parasite infection dynamics, bioluminescent *L. infantum* reporter lines were generated of a MIL-susceptible strain (MIL-S, MHOM/FR/96/LEM3323) and a syngeneic experimental MIL-resistant (MIL-R) strain that is hampered in MIL uptake due to a frameshift mutation in the transporter gene. The *in vivo* fitness and course of infection were compared in single and in co-infections in immunocompetent and immunocompromised hosts using bioluminescent imaging.
METHODOLOGY: BALB/c mice were infected intravenously with $1x10^8$ metacyclic promastigotes of the luminescent MIL-S or MIL-R lines, while only one of the 2 strains was luminescent in co-infections. To

assess the effect of immunosuppression, half of the (single) infected animals received 150 mg/kg cyclophosphamide weekly. *In vivo* imaging was performed at 1 day post-infection (dpi) and at 1, 2, 3, 4, 6, 8, 10 and 12 weeks post-infection (wpi) upon which they were sacrificed to determine parasite burdens by RT-qPCR and microscopy.
RESULTS: The MIL-S parental strain produced a distinct liver and spleen peak respectively at 3 wpi and 10-12 wpi. In contrast, MIL-R only showed a limited bioluminescent signal in the liver disappearing at about 3-4 wpi, despite fully comparable initial levels to the MIL-S line at 1dpi. In the co-infected mice, the MIL-S infection course was unaltered while the MIL-R line disappeared more rapidly, actually suggesting that MIL-S outcompetes MIL-R. Immune suppression did not rescue the observed fitness loss of MIL-R, but resulted in higher overall organ burdens for both strains.
CONCLUSIONS: Both in single and co-infections, the MIL-R strain fails to multiply and establish visceral infections *in vivo*, while the MIL-S strain induces a clear and typical VL infection course. This impaired fitness is associated to the resistant phenotype of the parasite and cannot be rescued by immunosuppression.

MECHANISM OF ACTION OF THREE DISTINCT SERIES OF NOVEL ANTILEISHMANIAL COMPOUNDS

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INTRODUCTION: Current antileishmanial treatment regimens show limitations, including toxicity, treatment failure and drug resistance, hence stressing the need for novel drugs. The Drugs for Neglected Diseases initiative (DNDi) is currently developing three novel 'lead' series (oxaboroles, aminopyrazoles and nitro-imidazoles). *In vitro* and *in vivo* experiments are being performed to better understand their mode-of-action.
METHODOLOGY: 1/ *In vitro* susceptibility assays on intracellular and extracellular stages and *in vivo* studies in Syrian Golden hamsters with reference strains of *Leishmania donovani* (MHOM/ET/67/L82) and *L. infantum* (MHOM/MA(BE)/67/ITMAP263); 2/ Cross-resistance *in vitro* against several resistant strains for paromomycin, Sb^{III} and miltefosine to exclude common mode-of-actions; 3/ Time-to-kill (TTK) assays of the laboratory strains to identify the minimal exposure time needed to eliminate all viable intracellular amastigotes and 4/ the involvement of specific efflux pumps in both parasite stages on the *in vitro* potency of the 'lead' compounds.
RESULTS: All three "lead' series show potent and selective activity against both *Leishmania* species, both *in vitro* and *in vivo*. No cross-resistance against the current antileishmanial drugs was noted indicating a different mode-of-action. All selected DNDi 'lead' compounds showed a similar or better TTK than the reference drugs, with the nitro-imidazoles being the better series. TTK were fully comparable for both *Leishmania* species, except for the aminopyrazoles which were not cidal within the 15 days of drug-exposure for *L. donovani*. The studies on the involvement of efflux pumps are still ongoing, however, preliminary results showed that the aminopyrazoles are not substrates of the intracellular parasite and macrophage pumps.
CONCLUSIONS: The new DNDi 'lead' compounds are highly promising as they have a higher potency, a shorter time-to-kill, and most likely have a mode-of-action that is different from the current antileishmania drugs.

SESSION 4

SELECTED ORAL PRESENTATIONS

ANEUPLOIDY VARIATION DURING THE LIFE CYCLE OF *LEISHMANIA DONOVANI*

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Many studies have demonstrated aneuploidy and its potential role as an adaptive mechanism in *Leishmania* promastigotes. We have shown that karyotype polymorphism in *L. donovani* clinical isolates from the Indian sub-continent significantly correlated with genetic diversity. In this study we investigated for the first time the genome and transcriptome dynamics (using high-throughput sequencing) of *L. donovani* throughout its complete life cycle, using *Phlebotomus argentipes* as vector and hamster as mammalian host. The study was initiated with *in vitro* promastigotes of the aneuploid (8/36 chromosomes) reference strain. After inoculation to hamster and 3 successive *in vivo* passages, amastigotes were purified and sequenced: 7 of the 8 aneuploid chromosomes were disomic (chr31 remaining tetrasomic), while chr8 became trisomic. In contrast, we found only a small reduction in aneuploidy after passage of the same *in vitro* promastigotes to sand flies. *P. argentipes* cyclically transmitted promastigotes were used to infect another set of hamsters: after the first passage we observed only a slight reduction of aneuploidy in amastigotes, suggesting that aneuploidy changes occur gradually during the course of infection. Somy variation was the only variable genome feature during the study, as no local CNVs or *de novo* SNPs were observed. In a next stage, we performed RNAseq analysis of all the samples and observed an overall correlation (excluding chr31) between chromosome copy number and transcript abundance. However, for the same aneuploidy profile, transcription changed significantly according to the life stage, revealing a complex interplay between genome dosage and regulation of gene expression.

POLYMORPHISM IN THE GENE ENCODING THEILERIA PARVA TP2 ANTIGEN RECOGNISED BY BOVINE CTL: A PERSPECTIVE FOR CATTLE IMMUNIZATION PROGRAM IN THE DEMOCRATIC REPUBLIC OF CONGO AND BURUNDI

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INTRODUCTION: The tick-borne disease East Coast fever (ECF), caused by the protozoan *Theileria parva*, is a lympho-proliferative disease of cattle which occurs in central, southern and eastern Africa. It represents a major challenge to livestock production causing important economic losses to farmers due to high mortality and related control cost. ECF kills over ~1 million cattle per year corresponding to an annual economic losses of ~USD300 million. Immunization of cattle with a cocktail of three *T. parva* isolates, called Muguga cocktail (Muguga, Serengeti-transformed and Kiambu-5), by infection and treatment method (ITM) induces long term immunity against homologous strains. It was discovered that exposure of vaccinated cattle to heterologous field strains could result in breakthrough infection. This shows that the immunity is strain specific. Introducing new parasite strains, through cattle displacement or vaccination with exotic strains can lead to recombination with local strains and may result in new more virulent parasite variants causing endemic instability. In the great lakes region of central Africa, transboundary movement of cattle, associated to ecological

variations, constitute a major risk factor of spreading infected ticks with various *T. parva* stocks. Hence, effective immunization of cattle requires good knowledge of parasites strains circulating in the region. A number of antigen targets of bovine major histocompatibility complex class I restricted cytotoxic T-lymphocytes (CTLs), induced by ITM vaccine have been identified. Those antigens are good markers to discriminate the antigenic variation between *T. parva* stocks.
METHODOLOGY: In order to access the genetic polymorphism in *T. parva* strains circulating in DR Congo and Burundi, we sequenced 123 isolates of *T. parva* from five ecological zones, using Nested PCR and Sanger sequencing of the gene encoding *T. parva* Tp2 antigen, which is major target for immune response.
RESULTS: Molecular analysis identified 181 variable positions (35.91% of the analyzed region of 504 bp length), giving 10 antigen variants. The overall nucleotide diversity was 12%. The lowest polymorphism was observed in the highlands region of RD Congo (π =0.1% and two variants) where all sequences are similar to *T. parva* Muguga strain. New epitope variants were identified in samples from lowlands and midlands of Congo and Burundi. Phylogenetic analysis showed two clusters. The major cluster comprised samples similar to Muguga cocktail stocks (63.52%). This result indicates that *T. parva* populations are distinct across regions, although genetic exchanges between lowlands of Congo and Burundi occurred. This could imply using different immunization stocks in the region. Additional molecular studies, such as microsatellite markers could be carried out to refine the genetic diversity of *T. parva* and provide more accurate information for the vaccination stock to be used in the field.

MOLECULAR TYPING OF *TRICHOMONAS VAGINALIS* CLINICAL ISOLATES FROM KILIFI, KENYA BY ACTIN GENE SEQUENCING AND DETECTION OF *TRICHOMONAS VAGINALIS* VIRUSES

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INTRODUCTION: The protozoan parasite *Trichomonas vaginalis* is the most common non-viral sexually transmitted pathogen. Even though *T. vaginalis* is highly prevalent among women in Kenya, little is known about the degree of strain variability. Several techniques have been utilized to perform typing of *T. vaginalis,* including detection of carriage of *Trichomonas vaginalis* viruses (TVVs) by some isolates.
METHODOLOGY: Using a previously described typing scheme we determined the genetic variability of *T. vaginalis* isolates from Kilifi, Kenya. Sequencing of the actin gene of *T. vaginalis,* coupled with polymerase chain reaction (PCR) to identify TVVs, was used to type 22 *T. vaginalis* isolates obtained after culture of vaginal specimens of pregnant women. *T. vaginalis* diversity was inferred by the neighbor-joining algorithm, while the *T. vaginalis* actin genotypes were determined *in silico* by simulation of cleavage of the *T. vaginalis* actin sequence by HindII, RsaI and MseI restriction enzymes.
RESULTS: Five actin genotypes were identified, 50.0% of the isolates were of actin genotype E, 27.3% of actin genotype N, 13.6% of actin genotype G and 4.5% of actin genotypes I and P each. Prevalence of TVVs was 43.5% (95% Confidence interval (CI): 23.2-65.5). TVV1 was the most prevalent, present in 39.1% of the strains. Most, i.e. 90%, of *T. vaginalis* isolates harboring TVVs had more than one type of TVV. None of the isolates of actin genotype E harbored TVVs.
CONCLUSION: This study demonstrates the diversity of *T. vaginalis* occurring among pregnant women in Kilifi, Kenya. The actin genotype E was the most the dominant genotype among pregnant women in Kilifi, Kenya and is associated with lack of TVVs.