Annual Scientific Meeting of the Belgian Society of Parasitology and Protistology

From protists to metazoan parasites: gained insights for future research

Thursday 26 November 2015

Aula Janssens
Institute of Tropical Medicine

Antwerp

www.bspp.be

PROGRAMME

09:15	Registration	
09:45	BSPP President: Guy Caljon	Welcome address
Session 1 – Chair: Stefan Magez		
10:00	**Keynote 1:** MATTHEW HIGGINS (UNIVERSITY OF OXFORD, UK)	STRUCTURAL INSIGHTS INTO INNATE IMMUNITY IN TRYPANOSOMES AND ERYTHROCYTE INVASION BY THE MALARIA PARASITE
10:40	Fontaine Frédéric (ULB)	COUPLING OF LYSOSOMAL AND MITOCHONDRIAL MEMBRANE PERMEABILIZATION IN TRYPANOLYSIS BY APOL1
10:55	Prajapati Surendra (ITM)	ROLE OF COMPLEMENT RECEPTOR 1 IN *PLASMODIUM VIVAX* RETICULOCYTE INVASION
11:10	**Coffee break**	
Session 2 – Chair: Johannes Charlier		
11:35	Madinga Joule (UCL-ITM)	GEOSPATIAL AND AGE-RELATED PATTERNS OF *TAENIA SOLIUM* TAENIASIS IN THE RURAL HEALTH ZONE OF KIMPESE, DEMOCRATIC REPUBLIC OF CONGO
11:50	Vanhee Merijn (Vives)	*TOXOCARA* PREVALENCE IN SANDPITS OF PUBLIC PLAYGROUNDS AND KINDERGARDENS IN A BELGIAN CITY
12:05	Bui Tui Dung (ULg)	MOLECULAR AND MORPHOLOGICAL CHARACTERIZATION OF *FASCIOLA* SPP COLLECTED FROM CATTLE IN SLAUGHTERHOUSES AND THE SUSCEPTIBILITY OF LYMNAEID SNAILS TO *FASCIOLA* SPP.
12:20	Verschave Sien (UGhent)	GLOWORM-PARA: A FLEXIBLE MODEL FRAMEWORK FOR THE PARASITIC PHASE OF GASTROINTESTINAL NEMATODE PARASITES IN RUMINANTS.
12:35	Chemurot Moses (UGhent)	FACTORS INFLUENCING THE PREVALENCE AND INCIDENCE OF TWO HONEYBEE PARASITES IN TWO AGRO-ECOLOGICAL ZONES OF UGANDA
12:50	**Lunch**	
Session 3 – Chair: Peter Geldhof		
13:50	**Keynote 2:** RICK MAIZELS (UNIVERSITY OF EDINBURGH, UK)	GASTROINTESTINAL NEMATODES: SECRETED PRODUCTS IN MODULATION OF HOST IMMUNITY
14:30	González-Hernández Ana (UGhent)	ANALYSIS OF THE VACCINE-INDUCED IMMUNITY AGAINST GASTROINTESTINAL PARASITES SUGGESTS A CRUCIAL ROLE FOR MUCOSAL IgG1 AND MEMORY NK CELL RESPONSES
14:45	Bittencourt-Cunha Paula (ULB)	THE IMPORTANCE OF HAPTOGLOBIN-HEMOGLOBIN RECEPTOR DOWN REGULATION DURING THE CYCLING OF *TRYPANOSOMA BRUCEI* IN TSE TSE FLY
15:00	Kauffmann Florence (VUB)	REFINING THE ROLE OF T HELPER POLARIZATION-ASSOCIATED MOLECULES USING *LEISHMANIA MAJOR* AS A MODEL
15:15	**Coffee break**	
Session 4 – Chair: Louis Maes		
15:45	Mondelaers Annelies (UA)	GENOMIC AND MOLECULAR CHARACTERIZATION OF MILTEFOSINE RESISTANCE IN A *LEISHMANIA INFANTUM* STRAIN THAT ACQUIRED RESISTANCE THROUGH EXPERIMENTAL SELECTION AT INTRACELLULAR AMASTIGOTE LEVEL.
16:00	Schoonvaere Karel (UGhent)	TOWARDS CHARACTERIZATION OF THE PATHOSPHERE OF FOUR WILD BEE SPECIES IN BELGIUM
16:15	Lempereur Laetitia (ULg)	EQUINE INFECTIOUS ANAEMIA VIRUS AND ITS VECTORS: AN ASSESSMENT OF THEIR DISPERSION POTENTIAL.
16:30	Van den Broeck Frederik (ITM)	WHOLE GENOME ANALYSIS OF *LEISHMANIA BRAZILIENSIS* AND *L. PERUVIANA* CLINICAL ISOLATES
16: 50	**Dafra Pharma Best Presentation Award & Zoetis Travel Grant**	
17:00	**BSPP statutory meeting**	
17:30	**Reception**	

INVITED SPEAKERS

STRUCTURAL INSIGHTS INTO INNATE IMMUNITY IN TRYPANOSOMES AND ERYTHROCYTE INVASION BY THE MALARIA PARASITE

Matthew Higgins, University of Oxford, UK

The structural characterisation of host-parasite interactions can help us to understand how parasite surface molecules diversify in response to immune pressure, and to identify conserved features that can be targeted by vaccines. In this presentation I will discuss our studies of erythrocyte invasion by the malaria parasite, *Plasmodium falciparum*, the adhesion of *Plasmodium*-infected erythrocytes within the brain, and the uptake of lytic factors by trypanosomes.

GASTROINTESTINAL NEMATODES: SECRETED PRODUCTS IN MODULATION OF HOST RESPONSES

Maizels R.M., Johnston C.J.C, Harcus Y., Hewitson, J.P., Smith, K.A., Smyth, D.J., and McSorley, H.J.
Institute of Immunology and Infection Research, University of Edinburgh, EH9 3JT, Edinburgh UK. E-mail: r.maizels@ed.ac.uk

INTRODUCTION. Gastrointestinal nematode parasites are highly prevalent throughout the world today reflecting their ability to suppress host immunity, down-regulating both anti-parasite responses and inflammatory responses to bystander specificities such as autoantigens, alloantigens and allergens. To analyse immunological mechanisms underpinning these effects, we are studying a model intestinal nematode *Heligmosomoides polygyrus*, which is related to human hookworm parasites.

RESULTS. Infection of susceptible mouse strains expands immunosuppressive host cell populations including both natural and adaptive Foxp3$^+$ regulatory T cells (Tregs) and regulatory B cells, which inhibit allergic inflammation and autoimmune disease when transferred to uninfected animals. Promoting Tregs in resistant mice (with IL-2:anti-IL-2 complex) enhances infection, while Treg depletion in susceptible mice engenders worm expulsion. The immunoregulatory effects of *H. polygyrus* can be recapitulated in vivo with soluble products (termed HES) secreted by live parasites in vitro, which inhibit systemic autoimmunity, airway allergy and colitis in vivo. HES induces de novo Foxp3 expression and Treg function in peripheral T cells, acting through a parasite-encoded mimic of host TGF-β, termed TGM. HES also blocks the release of the alarmin IL-33 by epithelial cells, in a TGF-β-independent manner; through a novel molecule termed ARI (alarmin release inhibitor). The immunoregulatory properties of *H. polygyrus* can be neutralized by immunization of mice with HES in adjuvant; vaccination elicits 100% sterile immunity, accelerating myeloid cell extravasation and trapping tissue-phase larvae in the wall of the small intestine. Immunity requires activated myeloid cells driven by IL-4R-mediated signaling, together with stimulation by IL-25 and macrophage migration inhibitory factor (MIF). IL-25, together with IL-4, can also induce worm expulsion in RAG-deficient animals lacking B or T cells.

CONCLUSIONS. Nematodes can release potent immunomodulatory molecules, but targeting these for vaccination drives a novel innate effector pathway of defense that mediates immunity.

ORAL PRESENTATIONS

COUPLING OF LYSOSOMAL AND MITOCHONDRIAL MEMBRANE PERMEABILIZATION IN TRYPANOLYSIS BY APOL1

Vanwalleghem G.[1,*], Fontaine F.[1,*], Lecordier L.[1], Tebabi P.[1], Klewe K.[2], Nolan D.[3], Yamaryo-Botté Y[4], Botté C.[4], Kremer A.[5], Schumann Burkard G.[6], Rassow J.[2,] Roditi I[6], Pérez-Morga D.[1,7] and Pays E.[1]
[1]Laboratory of Molecular Parasitology, IBMM, ULB, ffontain@ulb.ac.be; [2]Institute of Biochemistry and Pathobiochemistry, Ruhr-Universität Bochum ; [3]Molecular Parasitology Group, Trinity College Dublin; [4]Apicolipid Group, CNRS; [5]IRC/VIB Bio Imaging Core, Gent; [6]Institute of Cell Biology, University of Bern; [7]Center for Microscopy and Molecular Imaging (CMMI), ULB; *Equal co-authorship

Humans resist infection by the African parasite *Trypanosoma brucei* owing to the trypanolytic activity of the serum apolipoprotein L1 (APOL1). Following uptake by endocytosis APOL1 forms pores in endolysosomal membranes and triggers lysosome swelling. We show that APOL1 induces both lysosomal and mitochondrial membrane permeabilization (LMP, MMP). However, trypanolysis only results from MMP that releases the mitochondrial *Tb*EndoG endonuclease into the nucleus. APOL1 is associated with the kinesin *Tb*KIFC1, of which both the motor and vesicular trafficking VHS domains are required for MMP, but not for LMP. The presence of APOL1 in the mitochondrion is accompanied by mitochondrial membrane fenestration, which can be mimicked by knock-down of a mitochondrial mitofusin-like protein (*Tb*MFNL). The BH3-like peptide of APOL1 is required for LMP, MMP and trypanolysis. Thus, trypanolysis by APOL1 results from apoptosis-like MMP occurring together with *Tb*KIFC1-mediated transport of APOL1 from endolysosomal membranes to the mitochondrion.

ROLE OF COMPLEMENT RECEPTOR 1 IN *PLASMODIUM VIVAX* RETICULOCYTE INVASION

Prajapati S.K.[1], Borlon C. [1], Rovira-Vallbona E. [1], Gamboa D.[2], Erhart A. [1], Nosten F. [3], Abbeele J.V.D. [4], d'Alessandro U. [5], Rosanas-Urgell A[1].
[1] Unit of Malariology, Department of Biomedical Sciences, Institute of Tropical Medicine, Antwerp, Belgium (arosanas@itg.be), [2] Departamento de Ciencias Celulares y Moleculares, Universidad Peruana Cayetano Heredia, Peru, [3] Shoklo Malaria Research Unit, Thailand, [4] Unit of Veterinary Protozoology, Department of Biomedical Sciences, Institute of Tropical Medicine, Antwerp, [5] Medical Research Council Unit, The Gambia.

Plasmodium vivax is the most widespread human malaria parasite outside sub-Saharan countries, causing huge morbidity to its human host. This parasite is characterized by invasion of reticulocytes through interaction with Duffy antigen on reticulocyte. However, inability of *P. vivax* to invade Duffy positive mature red cells and numerous emerging reports of *P. vivax* infections in Duffy negative populations suggest invasion mechanisms are incompletely understood. Maturation process of reticulocytes shows significant reduction of various membrane and cytoskeleton proteins including Complement receptor 1 (CR1), which is also a known invasion receptor for *P. falciparum*. Here, we investigated the role of CR1 in *P. vivax* reticulocyte invasion using *ex vivo* invasion assays and population genetics approaches. Trypsin treatment of reticulocytes (CR1 cleavage) showed reduction of invasion rate by 50% (p=0.027) whereas chymotrypsin treatment (cleavage of CR1 and Duffy receptor) showed invasion reduction over 95%. Invasion assays using low CR1-expressor reticulocytes, soluble CR1 competition and anti-CR1 monoclonal antibodies showed CR1-specific invasion inhibition by 40%, 44.9% and 39%, respectively. Overall, the reduction (but not prevention) of invasion after CR1 inhibition suggests CR1 may act as a co-receptor during the invasion process of reticulocytes. The interaction between CR1 and *P. vivax* (invasion mechanism) is strongly supported by population genetic analysis showing departure of CR1 low allele (L allele, associated with low CR1 expression) from Hardy-Weinberg equilibrium (p=<0.001) in malaria endemic countries where *P. vivax* is/was the major parasite (Italy and Asian countries) as opposed to *P. falciparum* (African countries). This suggests *P. vivax*-linked natural selection of CR1 L allele has a beneficial advantage to human host. This study establishes for the first time a role of CR1 in *P. vivax* invasion process and advances our current understanding on *P. vivax* invasion biology, despite the lack of *in vitro* culture system for this parasite.

GEOSPATIAL AND AGE-RELATED PATTERNS OF *TAENIA SOLIUM* TAENIASIS IN THE RURAL HEALTH ZONE OF KIMPESE, DEMOCRATIC REPUBLIC OF CONGO

Joule Madinga[1,2,3*], Kirezi Kanobana[2], Philippe Lukanu[4], Emmanuel Abatih[2], Sylvain Baloji[5], Sylvie Linsuke[3], Nicolas Praet[2], Serge Kapinga[3], Katja Polman[2], Pascal Lutumba[3,6], Niko Speybroeck[1], Pierre Dorny[2] , W. Harrison[7] and Sarah Gabriel[2]

[1] Institute of Health and Society, Université Catholique de Louvain, Brussels, Belgium 2. Department of Biomedical Sciences, Institute of Tropical Medicine, Antwerp, Belgium 3. Institut National de Recherche Biomédicale, Kinshasa, Democratic Republic of Congo 4. Zone de santé Rurale de Kimpese, Democratic Republic of Congo 5. Programme National de Lutte contre la Trypanosomiase Humaine Africaine, Kinshasa, Democratic Republic of Congo 6. Department of Tropical Medicine, University of Kinshasa, Democratic Republic of Congo 7. Department of Infectious Disease and Epidemiology, Imperial College, London, UK * corresponding author: jmadinga@yahoo.fr

BACKGROUND: Knowledge on patterns of *Taenia solium* infections in both human and pig is crucial to design effective control strategies. This study assessed the prevalence, risk factors and spatial distribution of taeniasis in a rural area of the Democratic Republic of Congo (DRC), in the prospect of upcoming control activities.

METHODS: A cross-sectional study was conducted in 24 villages of the health zone of Kimpese. Individual and household characteristics were recorded; stool samples were collected from willing participants and analyzed using the copro-antigen enzyme-linked immunosorbent assay for the detection of taeniasis. Blood samples were collected from pigs and analyzed using the B158/B60 monoclonal antibody-based antigen ELISA to detect porcine cysticercosis. Logistic regression and multilevel analysis were applied to identify risk factors. Global clustering and spatial correlation of taeniasis and porcine cysticercosis were assessed using K functions. Local clusters of both infections were identified using the Kulldorff's scan statistic.

RESULTS: Of 4,751 participants (age median: 23 years; IQR: 11-41) included, 1,112 were positive (23.4% (95%CI: 22.2-24.6). Taeniasis prevalence, ranged from 1 to 60% between villages, with a significant between-household variance of 2.43 (SE=0.29, $p<0.05$). Taeniasis was significantly associated with age ($p<0.05$) and the highest prevalence was found in the 5-10 years age group. The overall prevalence of porcine cysticercosis was 45.6% (95% CI: 40.2-51). K functions revealed a significant overall clustering of taeniasis and porcine cysticercosis but no spatial dependence between them. Two significant clusters of taeniasis ($p<0.001$; n=276 and n=9) and one cluster of porcine cysticercosis ($p<0.001$; n=24) were found.

CONCLUSION: This study confirms high endemicity and geographical dispersal of *T. solium* infections in the study area, evidenced the role of age in taeniasis patterns and the absence of spatial correlation between taeniasis and porcine cysticercosis as well as the need for control activities in this endemic area.

TOXOCARA PREVALENCE IN SANDPITS OF PUBLIC PLAYGROUNDS AND KINDERGARTENS IN A BELGIAN CITY

Vanhee M.[1], Depuydt L.[1], Dalemans A.C.[2] & Viaene J.[1]

[1]Department of Biotechnology, Vives University College, Roeselare, merijn.vanhee@vives.be; [2]Bayer Health Care, Animal Health Division, Diegem

INTRODUCTION: Roundworm infections (*T. canis* and *T. cati*) are common in dogs and cats, and eggs shed in the environment by defecation can cause toxocariasis in man. Accidental infection of humans with embryonated *Toxocara* eggs can end up with migrating larvae, resulting in a wealth of symptoms. Environmental contamination with worm eggs is considered the key transmission route, and mainly young children are at risk. Contamination of soil with *Toxocara* eggs has been demonstrated in several different countries worldwide, but data are lacking for Belgium.

METHODOLOGY: This study aimed to investigate faecal contamination and *Toxocara* prevalence in sandpits of public playgrounds (n=45) and kindergartens (n=27) in a large Belgian city. Every location was visited once, and 200g of sand was collected from all sandpits present, resulting in 104 samples. The presence of *Toxocara* eggs was investigated by enrichment on a series of sieves, followed by centrifugation and flotation. In case of faecal contamination, the origin (dog or cat) was determined by PCR and the faeces were investigated for the presence of *Toxocara* eggs.
RESULTS: Faeces, of which 85% originated from cats, were found in one fourth of the public playgrounds and one fifth of the kindergartens. *Toxocara* prevalence in faeces was 8,8%, and *Toxocara* eggs were found in 16,4% of sandpits in public playgrounds, and 2,3% in kindergartens. These data suggest that environmental contamination with *Toxocara* is widespread in urban areas in Belgium, with cats being the main source.

MOLECULAR AND MORPHOLOGICAL CHARACTERIZATION OF *FASCIOLA* SPP COLLECTED FROM CATTLE IN SLAUGHTERHOUSES AND THE SUSCEPTIBILITY OF LYMNAEID SNAILS TO *FASCIOLA* SPP.

Bui T.D.[1,2], Saegerman C.[2], Losson B.[2]
[1] Institute of Ecology and Biological Resources, Vietnam Academy of Science and Technology, Hanoi, Vietnam, PhD student at University of Liège, Belgium, dung.buithi@student.ulg.ac.be; [2] Center for Fundamental and Applied Research for Animals and Health, Faculty of Veterinary Medicine, University of Liège, Belgium, Claude.Saegerman@ulg.ac.be; blosson@ulg.ac.be

INTRODUCTION: In Vietnam, *Fasciola gigantica* is a major species causing both human and animal fasciolosis. Recently hybrids forms were reported in some areas. Additionally, the identity of Vietnamese lymnaeid snails acting as intermediate hosts of *Fasciola* spp is questionable. The present study was conducted to determine the prevalence of *Fasciola* spp infection in cattle, to identify Fasciola species and to assess the receptivity of 3 lymnaeid snail species (*Austropeplea viridis, Radix auricularia, R.* rubiginosa) to *Fasciola* spp.
METHODOLOGY: A total of 360 gall bladders from cows and water buffaloes were examined. Fasciola specimens were measured. Genetic markers (ITS-2 and COX1) were obtained and the representative amplicons were sequenced. Three lymnaeid snails were collected from different locations and bred in the laboratory. Experimental infection of snails to *F. gigantica* (Vietnamese isolate) and *F. hepatica* (Belgian isolate) were carried out. Snail infections were monitored by crushing method and multiplex PCR.
RESULTS: A significantly higher prevalence of *Fasciola* spp infection was observed in cattle (63.53%; 216/340) in comparison with buffaloes (30%; 6/20) (Chi2=8.98; P-value=0.003). Four different phenotypes were observed. Type 4 (medium-narrow; 25.43±2.08-11.7±1.4) presented a phenotype compatible with *F. hepatica* while the others were compatible with *F. gigantica*. All Fasciola COX1 sequences fell into *F. gigantica* cluster. ITS-2 sequences of Fasciola type 1, 2, 3 fell into *F. gigantica* cluster while Fasciola type 4 and Belgian isolate fell into *F. hepatica* cluster. Fasciola type 4 sequence showed two peaks at 207, 327 nucleotide positions and 7 different nucleotide positions between Fasciola type 4 and other types were observed. By morphometric and molecular analysis, Fasciola type 4 as being a hybrid form of *F. gigantica* and *F. hepatica.* Under experimental conditions *A. viridis* only is receptive to *F. gigantica*. All experimental infections of Vietnamese lymnaeid snails with a Belgian isolate of *F. hepatica* failed. The multiplex PCR was found to be a sensitive technique allowing the detection of Fasciola DNA as early as 2 days post exposure.

GLOWORM-PARA:
A FLEXIBLE MODEL FRAMEWORK FOR THE PARASITIC PHASE OF GASTROINTESTINAL NEMATODE PARASITES IN RUMINANTS

Verschave S.H.[1], Claerebout E.[1], Morgan E.R.[2], Charlier J.[3], Vercruysse J.[1], Rose H.[2]
[1] Department of Virology, Parasitology and Immunology, Faculty of Veterinary Medicine, Ghent University, Belgium, Sien.Verschave@ugent.be; [2] School of Biological Sciences, University of Bristol, United Kingdom, Hannah.Rose@bristol.ac.uk; [3] Avia-GIS, Zoersel, Belgium, jcharlier@avia-gis.com

Gastro-intestinal nematodes remain an important threat to livestock farming. Parasite transmission models could be useful decision-support tools to develop or to validate sustainable worm control strategies. Recently, a general model framework for the free-living phase of gastro-intestinal nematodes in ruminants was created (GLOWORM-FL). However, to explore the effect of different worm control approaches, a complementary model for the parasitic phase is needed.
GLOWORM-PARA provides a model framework for the parasitic phase that can be applied to a range of nematode species. The framework was parameterised and validated for first season grazing cattle infected by two species that are of major importance in cattle, i.e. *Ostertagia ostertagi* and *Cooperia oncophora*. Two different approaches were compared for the parameterisation of the immune response, i.e. estimation of the immune response rate from tracer calves vs. fitting the immune response rate to field observations. Host grazing behaviour in relation to the vertical distribution of infective larvae on herbage was incorporated. The model performed well in predicting faecal egg counts of first season grazing cattle throughout the grazing season. The estimation of the immune response rate from field observations was preferred over fitting the immune response rate as the former gave more meaningful predictions of acquired immunity. Incorporating host grazing behaviour improved model performance and is therefore likely to be important in the transmission of gastro-intestinal nematodes. GLOWORM-PARA can be integrated with models of the free-living stages to understand gastro-intestinal nematode epidemiology under changing climate and management scenarios, facilitating adaptation to and mitigation of climate change impacts.

FACTORS INFLUENCING THE PREVALENCE AND INCIDENCE OF TWO HONEYBEE PARASITES IN TWO AGRO-ECOLOGICAL ZONES OF UGANDA

Moses Chemurot[1, 2] Lina de Smet[1] and Dirk C. de Graaf[1]
[1]Laboratory of Molecular Entomology and Bee Pathology, Ghent University, Krijgslaan 281 B-9000 Ghent, Belgium; [2]Department of Biological Sciences, School of Bio-sciences, College of Natural Sciences, Makerere University, P.O. Box 7062 Kampala, Uganda. mchemurot@cns.mak.ac.ug

The honeybee ectoparasitic mite *Varroa destructor* was first noticed in Uganda in 2011 while the microsporidian gut parasite *Nosema* spp has not been investigated. Due to limited studies in Uganda, scarce information is available on honeybee parasite infestation levels and factors that influence them. In this study, we investigated the prevalence and incidence of varroa mites and *Nosema* spp in two highland agro-ecological zones of Uganda and explored potential factors that influence them. Our findings show mean mite prevalences in apiaries of 52.7% and 39.5% for the Eastern and Western zones respectively during the dry season. Over the wet season, mean mite prevalence increased considerably in the Western (58.6%) compared to the Eastern (50.6%) zone. Incidence of Varroa mites in colonies in the Eastern zone was significantly higher than that in the Western during the dry season (p=0.022). Factors that were associated with Varroa mite incidence include altitude, nature of apiary slope and apiary management practices. Colony productivity and strength were not correlated to varroa mite incidence. Preliminary results on nosemosis indicate the presence of probably a new species of *Nosema* in Uganda because its molecular signature was not closely related to neither *Nosema apis* nor *Nosema ceranae*. *Nosema* spp prevalence rates were 55.5% and 86.1% in the Eastern and Western agro-ecological zones respectively during the dry season. Increased incidence of *Nosema* spore counts were associated with apiaries located in *Eucalyptus* plantations

and low beehive height from ground. These results point the need to survey unstudied regions which could be habouring pathogens that may be spread in this era of globalization.

ANALYSIS OF THE VACCINE-INDUCED IMMUNITY AGAINST GASTROINTESTINAL PARASITES SUGGESTS A CRUCIAL ROLE FOR MUCOSAL IgG1 AND MEMORY NK CELL RESPONSES

González-Hernández A.[1], Van Coppernolle S.[1], Peelaers I[1]. , Claerebout E.[1], Geldhof P.[1]
[1]Laboratory of Parasitology, Faculty of Veterinary Medicine, University of Ghent
Salisburylaan 133, 9820 Merelbeke, Belgium

Introduction: The aim of our study was to unravel the immune mechanisms underlying the protective vaccine-induced immune responses against the gastrointestinal parasites *Ostertagia ostertagi* and *Cooperia oncophora* in cattle.
Methods: Cattle and mice were immunized with native experimental vaccines, and the antigen-specific antibody and cellular responses were analyzed and compared with the responses induced by non-protective recombinantly produced versions of these vaccines.
Results: *In vitro* re-stimulation of lymphocytes from calves vaccinated with the protective experimental vaccines resulted in a marked proliferation of NK and $CD3^-CD335^-CD21^-$ cells. In the case of the *C. oncophora* vaccine, antigen-specific proliferation of CD4 and CD8 T cells was also observed. In addition, a strong mucosal IgG1 response was observed in vaccine-protected calves following challenge infection. Injection of mice with the native vaccines similarly resulted in an antigen-specific NK and $CD3^-CD335^-CD21^-$ cell proliferation together with a strong IgG1 response, indicating that this antigen-specific immune response is conserved among species. In contrast to the native vaccines, the recombinant versions failed to both induce a strong cellular memory response in cattle and mice, and to trigger a secondary mucosal IgG1 response in cattle following challenge.
Conclusion: The outcome of this research suggests an important role for NK cells, $CD3^-CD335^-CD21^-$ cells and IgG1 antibodies in the protection induced by both native anti-parasite vaccines. The data also indicates that mice could potentially be used as a model to test recombinant anti-parasite vaccines for their immunogenicity.

THE IMPORTANCE OF HAPTOGLOBIN-HEMOGLOBIN RECEPTOR DOWN REGULATION DURING THE CYCLING OF *TRYPANOSOMA BRUCEI* IN TSE TSE FLY

Bittencourt-Cunha P. [1], Lecordier L.[1] , Van Den Abbeele J.[2] , Vanhollebeke B[1]
[1]Laboratory of Molecular Parasitology, IBMM, Université Libre de Bruxelles (ULB), 12 rue des Prof Jeener et Brachet, B-6041 Gosselies, Belgium.; [2] Unit of Veterinary Protozoology, Department of Biomedical Sciences, Institute of Tropical Medicine Antwerp, Antwerp, Belgium

The parasite *Trypanosoma brucei* has a complex life cycle that alternates between mammalian and insect hosts. This cycling requires a complete change of gene expression pattern, in particular for the surface proteins. . Previous studies have described a surface glycoprotein receptor able to bind with high affinity and to uptake the haptoglobin-hemoglobin complex (HpHbR). The endocytosis of hemoglobin is a major source of haem, an important molecule for the growth of the parasite. During the mammalian infection, the parasites differentiate from the slender, multiplicative form, to the stumpy, quiescent form, which is pre-adapted to the transmission to Tse tse fly. During this differentiation in stumpy forms, the HpHbR gene is down regulated. To evaluate the importance of the receptor down regulation for the transmission, different pleiomorphic cell lines were generated in which the HpHbR gene regulation has been altered to maintain its expression at the stumpy stage. The different mutants were able to differentiate both *in vitro* and *in vivo*, but only one was able to uptake the HpHb complex at the stumpy stage. The following step will be to infect the flies with those parasites capable of HpHb uptake and observe their behaviour during the Tse tse fly infection.

REFINING THE ROLE OF T HELPER POLARIZATION-ASSOCIATED MOLECULES USING LEISHMANIA MAJOR AS A MODEL

Kauffmann F.[1], Muraille E.[2], De Jonge K.[1], Meert E.[1], Cnops J.[1], Magez S.[1] and De Trez C.[1]
[1]Laboratory of Structural and Functional Immunoparasitology, VUB, Pleinlaan 2, 1050-Brussels, Belgium florence.kauffmann@vub.ac.be; [2] Laboratory of Parasitology, ULB, Route de Lennik 808, 1070-Brussels, Belgium.

Leishmania major causes cutaneous leishmaniasis (CL) and has been extensively studied for years. A particular feature of CL is that it exhibits a typical T helper 1 (Th1) resistant/ T helper 2 (Th2) susceptible dichotomy. In this project, we aim to elucidate the impact of impairing simultaneously the Th1- and Th2-differentiation process on the outcome of CL caused by *L. major* IR75 intradermal footpad infection. Therefore, we used IL-12p40 (a Th1-associated cytokine), STAT6 (a Th2-associated transcription factor) and IL-12p40/STAT6 double KO (DKO) mice on a susceptible BALB/c background. Upon infection three phenotypes were observed: BALB/c and IL-12p40$^{-/-}$ mice developed large necrotic lesions, STAT6$^{-/-}$ mice developed small lesions and DKO mice developed smaller footpad lesions than IL-12p40$^{-/-}$ mice, with less necrotic lesions. However, the number of *in situ* parasites in DKO mice was similar to those observed in IL-12p40$^{-/-}$ mice, and much higher than in STAT6$^{-/-}$ mice. Flow cytometry indicated that the footpads of infected DKO mice had low percentages of inflammatory monocytes, which are important for parasite control, as observed in IL-12p40$^{-/-}$ mice. This correlates with the high number of parasites in the footpads of DKO mice. Neutrophils are associated with pathology during *L. major* infections. We observed high percentages of neutrophils in DKO mice, to the same extent as in IL-12p40$^{-/-}$ mice, and low percentages in STAT6$^{-/-}$ mice. Interestingly, neutrophils present in the footpads of DKO mice expressed lower levels of Ly6C and CD11b than in IL-12p40$^{-/-}$ mice. This might indicate that the neutrophils present in DKO mice are phenotypically different and possibly have a lower activation status due to the absence of both Th1 and Th2, than neutrophils found in IL-12p40$^{-/-}$ mice. This potential lack of neutrophil activation in the DKO mice might explain the lower pathology observed in these mice upon *L. major* IR75 infection.

GENOMIC AND MOLECULAR CHARACTERIZATION OF MILTEFOSINE RESISTANCE IN A *LEISHMANIA. INFANTUM* STRAIN THAT ACQUIRED RESISTANCE THROUGH EXPERIMENTAL SELECTION AT INTRACELLULAR AMASTIGOTE LEVEL.

Mondelaers A.[1], Sanchez-Canete M.[2], Hendrickx S.[1], Eberhardt E.[1], Hernandez R.G.[2], Kauffmann F.[3], Lachaud L.[4], Cuypers B.[5], Hideo I.[5], Dujardin J.C.[1,5], Delputte P.[1], Cos P.[1], Gamarro F.[2], Castanys S.[2], Maes L.[1]*
[1]University of Antwerp, Universiteitsplein 1, 2610 Wilrijk (* Louis.Maes@uantwerpen.be); [2]Instituto de Parasitologia y Biomedicina "Lopez-Neyra", Avda. Conocimiento S/N, 18100 Armilla – Granada; [3]VUB/VIB, Laboratory for Cellular and Molecular Immunology, Pleinlaan 2, Brussels, Belgium; [4]Centre Hospitalier Universitaire et Université de Montpellier, France; [5]Institute of Tropical Medicine, Nationalestraat 155, B-2000 Antwerp, Belgium

Miltefosine (MIL) is used as first-line treatment for visceral leishmaniasis in endemic areas with antimonial resistance, however, a decline in clinical effectiveness is now being reported. Phenotypic MIL-resistant *L. donovani* isolates have not yet been identified while two MIL-resistant *L. infantum* strains from HIV co-infected patients have been documented. A clear understanding of the factors contributing to increased MIL-treatment failure is necessary. Given the paucity of MIL-resistant strains, our research group succeeded in experimental selection of MIL-resistance in a *L. infantum* isolate at intracellular amastigote level. A naturally MIL-resistant clinical isolate was included to correlate both datasets. In-depth exploration of the MIL-resistant phenotype was performed by coupling genomic with phenotypic data to gain insight into gene function and mutant phenotype.
This study provides evidence that the *in vitro* amastigote resistance selection model may be a good proxy for the *in vivo* field situation since both strains showed mutations in the same inward transporter system that is responsible for the acquired MIL-resistant phenotype. In line with literature, our data suggest a defect in the inward transporter through inactivation of the LiMT/LiROS3 protein as a main mechanism for MIL-resistance. Phenotypically, resistance was based

on intracellular amastigote susceptibility *in vitro* and MIL-uptake. Gene sequencing analysis revealed the presence of a 2 base pair deletion in the LiMT gene of the experimentally induced strain, leading to an early stopcodon and truncation of the LiMT protein. Interestingly, the MIL-resistant clinical isolate revealed mutations in both genes LiMT/LiROS3. To verify that these mutations were indeed accountable for the observed acquired resistance, transfection experiments were performed to re-establish MIL-susceptibility. Susceptibility of the *in vitro* resistant strain was restored by transfection with a LiMT plasmid, whereas the resistant clinical isolate was made susceptible again after transfection with the pX-ROS3 vector. Western blot experiments using anti-ROS3 and anti-LiMT antibody and [^{14}C]MIL accumulation assays supported the restoration of MIL-susceptibility.

TOWARDS CHARACTERIZATION OF THE PATHOSPHERE OF FOUR WILD BEE SPECIES IN BELGIUM

Schoonvaere K.[1], Meeus I.[2], Smagghe G.[3], de Graaf DC[4]
[1,4]Laboratory for Molecular Entomology and Bee Pathology, Department of Biochemistry and Microbiology, Ghent University, Ghent, Belgium. Karel.schoonvaere@UGent.be[1], Dirk.degraaf@UGent.be[4]; [2,3]Laboratory of Agrozoology, Department of Crop Protection, Faculty of Bioscience Engineering, Ghent University, 9000 Ghent, Belgium. Ivan.meeus@UGent.be[2], Guy.smagghe@UGent.be[3]

Hymenopteran insects are widely affected by parasitic protists, fungi, nematodes and mites. In contrast to the domesticated bee *Apis mellifera*, little is known about parasites and pathogens of wild bees including solitary bees. Here, we applied a metagenomic strategy to explore the diversity of eukaryotic parasites in three common wild bee genera (*Bombus, Osmia* and *Andrena*). Two nematode species were found: the well-established parasite *Sphaerularia bombi* (Nematoda: Tylenchida: Sphaerulariidae) was carried by *Bombus* sp. queens and a close relative of *Koerneria hylobii* (Nematoda: Neodiplogasteridae) was found in *Andrena vaga*. Further, two parasitic protists were found in *Bombus terrestris* queens: *Apicystis bombi* (Apicomplexa: Neogregarinorida) and *Crithidia bombi* (Kinetoplastida: Trypanosomatidae). Four mites species could be detected including the parasitic mite *Chaetodactylus krombeini* in *Osmia* and the storage mite *Thyrophagus longior* in *Bombus.* No conclusions could be drawn from metagenomics data on the presence of pathogenic fungi, although the presence of *Nosema* sp. (Microsporidia) in all three bee genera was demonstrated by conventional PCR. Next to eukaryotic parasites, we found preliminary evidence for 10 as yet unknown viruses with half of them showing similarity to known viral entomopathogens. In summary, we identified a diverse set of eukaryotic parasites in three wild bee genera of which remarkably more eukaryotic parasites are associated with social bumblebees compared to solitary bees.

EQUINE INFECTIOUS ANAEMIA VIRUS AND ITS VECTORS: AN ASSESSMENT OF THEIR DISPERSION POTENTIAL.

Lempereur L[1], Sohier C[2], Smeets F[3], Madder M[2], Francis F[3], Losson B[1]
[1] Laboratory of Parasitology and Parasitic Diseases, Farah, ULg; [2] Veterinary Entomology Unit, Institute of Tropical Medicine, Antwerp, Belgium; [3] Functional and evolutive entomology unit, Agro-Bio Tech, ULg, Gembloux, Belgium. Corresponding author: llempereur@hotmail.com

The recent outbreaks of equine infectious anaemia (EIA) in Belgium have dramatically increased the concern about this viral disease and its transmission in our country. Mechanical transmission seems to be the major route of spread of EIA and Tabanidae and Muscidae are considered as the main arthropod vectors.
The potential dispersion of unfed or partially fed Tabanidae and Muscidae was assessed using challenging and innovative techniques such as *in vitro* feeding and mark-recapture experiments.

3600 biting flies (*Stomoxys calcitrans, the stable fly)* and 2900 tabanids of the genus *Haematopota* were collected in farm and on horses respectively. These flies and tabanids were colored using fluorescent dyes and half of them were partially fed with heparinized blood using an *in vitro* technique.
They were released at increasing distances from bait horses surrounded by traps in order to assess the distance that unfed and partially fed vectors could fly and consequently the potential dispersion of the virus. This experiment was repeated several times without other animals in the vicinity and under appropriate weather conditions.
These experiments demonstrated that the present control measures based on a 200 meters buffer zone applied in case of introduction of the virus in Belgium are insufficient in order to prevent virus transmission.

SPECIATION, DIVERGENCE AND GENOME STRUCTURE IN NEW WORLD *LEISHMANIA* POPULATIONS ALONG THE ANDES

Van den Broeck F[1], Imamura H[1], Van der Auwera G[1], Vanaerschot M[2], Arevalo J [3], Adaui V[3], Jara M[1,3], Garcia L[4], Berriman M[5], Cotton J[5], Dujardin JC[1]
[1]Institute of Tropical Medicine, Antwerp, Belgium, fvandenbroeck@itg.be, jcdujardin@itg.be; [2]Columbia University College of Physicians and Surgeons, New York, USA; [3]Instituto de Medicina Tropical A. von Humboldt, Lima, Peru; [4]CUMETROP, Cochabamba, Bolivia; [5]Wellcome Trust Sanger Institute, Hinxton, United Kingdom

New World leishmaniasis is caused by *Leishmania* parasites that show an extensive phenotypic diversity from an evolutionary, ecological and clinical point of view. The two main species of *Leishmania* encountered in Peru and Bolivia illustrate this diversity. *Leishmania braziliensis* is endemic in the Amazonian lowlands and causes severe cutaneous lesions. *Leishmania peruviana* is mainly found in the western slopes of the Andes and in some inter-Andean valleys; it is a disease of altitude and is characterized by benign cutaneous lesions. Early molecular karyotype work suggested that both species significantly rearrange and adjust their genome, with *L. peruviana* showing lower gene copy number and even total gene loss compared to *L. braziliensis*. In the present study we hypothesize that differences in evolutionary history and/or genome structure may explain the observed phenotypic diversity between both species. We therefore characterized the genomes of 116 *L. braziliensis* and *L. peruviana* clinical isolates that were collected in Peru and Bolivia from 1991 to 2003. All isolates were sequenced on Illumina HiSeq2000 platforms, and sequence data were mapped against the *L. braziliensis* M2904 draft genome. A consensus calling scheme based on five calling methods revealed a total of 319,261 SNPs, which is significantly higher than the 2,418 SNPs that were described within previously studied Indo-Nepalese *L. donovani* populations. *L. peruviana* was characterized by a low amount of heterozygosity compared to *L. braziliensis*, suggesting a founder effect in the former, and supporting the hypothesis that *L. peruviana* diverged from *L. braziliensis* by colonization of the Andean region though the Porculla pass. A large variation of chromosome and local copy number was observed among all *Leishmania* promastigotes, with a significant number of small partial duplications (200-500bp) and deletions (100-800bp), and expansion/contraction of genes within tandem arrays. Further work is ongoing to describe the structural variations in both species.

OUR SPONSORS

Zoetis is a global animal health company dedicated to supporting customers and their businesses in ever better ways. Building on 60 years of experience, we deliver quality medicines and vaccines, complemented by diagnostic product and genetics tests and supported by a range of services. We are working every day to better understand and address the real-world challenges faced by those who raise and care for animals in ways they find truly relevant.
The name, Zoetis (zō-EH-tis), has its root in zo, familiar in words such as zoo and zoology and derived from zoetic, meaning "pertaining to life." It signals our company's dedication to supporting the veterinarians and livestock producers everywhere who raise and care for the farm and companion animals on which we all depend.

Dafra Pharma International NV is a European Pharmaceutical company, founded in 1997, with focus on export to Sub-Sahara Africa. Our head-office is based in Turnhout, Belgium and export to Sub-Sahara Africa is centralized in Boningen, Switzerland.
We are a dynamic, innovative pharmaceutical company that cares about the health and wellbeing of people in Africa.
Recognized by our customers as a reliable health solution provider we deliver a wide range of high quality pharmaceutical products which are at the reach of every patient.
We are present in 30 countries with a growing product portfolio that counts over 25 brands. We focus strongly on teamwork and aim for valued and trustworthy partnerships. Dafra Pharma R&D is the research branch of Dafra Pharma International and focuses on drug development in the field of Global Health. The branch specifically targets unmet medical needs and is currently developing a new oral drug against leishmaniasis. This drug is currently in clinical Phase 1 and Phase 2 trials are in preparation.

HOSTED BY