



Vectors & parasites

Joint spring meeting of the Belgian Society of Parasitology & Protistology (BSPP) and the Netherlands Society of Parasitology (NVP)
Friday 20th May 2016, Erasmus MC, Rotterdam

Abstract Book



PROGRAMME

09.00 Annual business meeting NVP

09.45 Registration for joint spring meeting + putting up posters
Coffee/tea

Session 1: Insect-borne parasites

10.30 **Teun Bousema** (Radboudumc): ***Where are we with malaria transmission control? (p. 5)***

11.05 Epke Le Rutte (Erasmus MC): *Feasibility of eliminating Visceral Leishmaniasis from the Indian Subcontinent (p. 5)*

11.20 Eline Eberhardt (University of Antwerp): *Molecular detection of infection homogeneity and impact of miltefosine treatment in a Syrian Golden hamster model of Leishmania donovani and L. infantum visceral leishmaniasis (p. 6)*

11.35 Eunice Betouke Ongwe (LUMC / Centre de Recherches Médicales de Lambaréné): *Effect of Plasmodium falciparum exposure on urinary metabolomics profiling (p. 6)*

11.50 Poster session

12.15 Lunch (poster session continued)

Session 2: Tick-borne parasites

13.30 **Michalis Kotsyfakis** (Czech Institute of Parasitology): ***High-Throughput Disease Vector Biology: the example of the tick Ixodes ricinus (p. 9)***

14.05 Theo Schetters (Protactivity): *Successful vaccination against Babesia parasites and their tick vectors using recombinant antigens (p. 10)*

14.35 Amzati Sefu Gaston (University of Namur / Université Evangélique en Afrique): *Relationship between Theileria parva entomological inoculation rate and prevalence in three agroecological zones of the Democratic Republic of Congo (p. 10)*

14.50 Coffee/tea

Session 3: Open session

15.20 Wilma Stolk (Erasmus MC): *Progress towards onchocerciasis elimination in the participating countries of the African Programme for Onchocerciasis Control: epidemiological evaluation results (p. 13)*

15.35 Dicky Tahapary (LUMC / Universitas Indonesia): *Effect of antihelminthic treatment on adiposity : a cluster-randomized placebo controlled trial in Nangapanda, Flores, Indonesia (abstract not provided)*

15.50 Marion Rolot (University of Liège): *Helminth-driven type 2 inflammation enhances CD8+ T cell-mediated control of acute gammaherpesvirus infection (p. 14)*

16.05 Oonagh Paerewijck (Ghent University): *Unraveling the role of IL-17A in the intestinal immune response against the protozoan parasite Giardia muris by an RNA sequencing approach (p. 14)*

16.20 Leonard Pelgrom (Leiden University Medical Centre): *Distinct metabolic requirements for T helper 1 and 2 polarization by dendritic cells (p. 15)*

16.35 BSPP Avia-Gis best presentation award ceremony

16.45 BSPP Zoetis travel grant award

16.55 Merial Award ceremony + presentation

17.30 Drinks

18.00 Buffet dinner (optional)

POSTERS

1. Caron Yannick *et al.* Swimmer's itch in Belgium: first recorded outbreaks, identification of the parasite species and intermediate hosts (p. 17)
2. de Jong Asscher Sanne E. (Vera *et al.*) CD4 T cell and $\gamma\delta$ T cell responses during malaria in Indonesian children (p. 17)
3. de Ruiter Karin *et al.* Randomized, placebo-controlled trial of three-monthly albendazole treatment on Th2 responses: Different effects seen on IgE and IL-5 (p. 18)
4. Frempong Kwadwo K. *et al.* Progress towards lymphatic filariasis elimination in Ghana: a model-based analysis of trends in infection prevalence during 15 years of mass drug administration (p. 18)
5. Haanstra Jurgen R. (Lindijer Dimitri V. *et al.*) Kinetic modelling of the trypanothione synthesis pathway in *Trypanosoma brucei* to identify the most promising drug targets. (p. 19)
6. Habteab Aklilu Ghebretinsae *et al.* Guidance in designing surveys for monitoring the progress of school-based deworming programmes to control soil-transmitted helminthiasis (p. 19)
7. Hendrickx Sarah *et al.* Evidence of a drug-specific impact of experimentally selected paromomycine and miltefosine resistance on parasite fitness in *Leishmania infantum* (p. 20)
8. Kaiser Maria M. M. *et al.* *Schistosoma* egg antigens prime dendritic cells for Th2 polarization via a prostaglandin E2-dependent mechanism (p. 21)
9. Labuda Łucja *et al.* Transcriptional and immune profiling of *Schistosoma haematobium*-infected Gabonese schoolchildren. (p. 21)
10. Malrait Karen *et al.* Prevalence of gastrointestinal nematodes in organic dairy goats in Flanders (p. 22)
11. Mebius Mirjam M. *et al.* Haemostatic changes occur in vivo already in the early non-hepatosplenic phase of schistosomiasis (p. 22)
12. Roth Johanna M. *et al.* Molecular malaria diagnostics for field conditions: development and validation of a multiplex direct on blood PCR-nucleic acid lateral flow immunoassay for the detection and differentiation of *Plasmodium* species. (p. 23)
13. Ruizendaal Esmée *et al.* Prevalence of mutations associated with sulphadoxine-pyrimethamine (SP) resistance in *Plasmodium falciparum* samples from the general population and pregnant women in Nanoro, Burkina Faso. (p. 23)
14. Trappeniers Katrien *et al.* Control of the Endosymbiont *Sodalis glossinidius* by the Tsetse Fly Innate Immunity (p. 24)
15. Yang Y.Y. Michelle *et al.* Dynamics of anti-glycan antibody responses in *Schistosoma japonicum*-infected rhesus macaques studied by schistosome glycan microarray (p. 24)
16. Zavala Gerardo *et al.* Is intestinal parasite infection associated with obesity? An ecological analysis in Mexico (p. 25)

SESSION 1: INSECT-BORNE PARASITES

INVITED SPEAKER

Teun Bousema is an infectious disease epidemiologist with an interest in the transmission of *P. falciparum* from man to mosquito and the development of transmission-blocking interventions. Teun was trained as a biomedical scientist in Nijmegen. After graduation, he worked in the Volta Region in Ghana for one year before starting a PhD project on the effects of antimalarial drugs and immunity on the transmissibility of malaria in Kenya. He subsequently lived and worked in Tanzania before joining the London School of Hygiene & Tropical Medicine where he has a research group that focuses on public health interventions for malaria elimination. Since 2012, he is also affiliated to the Radboud university medical center as Associate Professor. His major scientific contributions include a better understanding of the epidemiology and transmissibility of submicroscopic malaria infections and the optimisation of transmission blocking drugs primaquine and ivermectin.

Teun is an elected member of the Young Academy of the Royal Netherlands Academy of Arts and Sciences (KNAW) and was shortlisted for the New Scientist Talent 2015.

WHERE ARE WE WITH MALARIA TRANSMISSION CONTROL?

Teun Bousema

In the last decade, the success of malaria control has been spectacular in many African and non-African settings. Malaria-attributed mortality has been halved in recent years and many countries where malaria used to be highly prevalent are currently involved in malaria elimination programmes. The enthusiasm for malaria elimination has been fuelled by a considerable increase in (research) funding, an ambitious malaria eradication agenda launched by Bill and Melinda Gates and the pilot implementation of the first-ever malaria vaccine in endemic settings. However, positive trends are not universal and insecticide and antimalarial resistance may threaten the recent gains in malaria control. Inspired by both the gains in malaria control and the threat of antimalarial resistance, community treatment campaigns now form a cornerstone intervention. This strategy has been described as fighting fire with fire where large-scale distributions of antimalarial drugs are used in the hope of eliminating drug resistant parasites. At present, the optimal drug combination and treatment strategy for community treatment campaigns are unknown. In general, there have been very few formal assessments of the impact of community treatment campaigns for sustained malaria control and even fewer success stories. In the lecture, an overview of recent achievements, opportunities and stumbling blocks for malaria elimination will be presented. There will be a special focus on the relevance or irrelevance of currently available malaria vaccines, spatially targeted interventions, transmission-blocking antimalarial drugs and novel strategies for integrated malaria control.

FREE ORAL PRESENTATIONS

FEASIBILITY OF ELIMINATING VISCERAL LEISHMANIASIS FROM THE INDIAN SUBCONTINENT

Epke A. Le Rutte¹, Luc E. Coffeng¹, Daniel M. Bontje¹, Epcó C. Hasker², José A. Ruiz Postigo³, Daniel Argaw³, Marleen C. Boelaert² and Sake J. De Vlas¹

¹Department of Public Health, Erasmus MC, University Medical Center; Rotterdam, PO Box 2040, 3000 CA Rotterdam, The Netherlands; ²Institute of Tropical Medicine, Nationalestraat 155, 2000 Antwerp, Belgium; ³World Health Organization, Avenue Appia 20, 1211 Geneva, Switzerland.

Background: Visceral leishmaniasis (VL), the deadliest parasitic infection in the world after malaria, is a neglected tropical disease transmitted by sandflies. About 300 million people are at risk globally, mainly affecting the poorest of the poor in rural areas. VL is targeted for elimination as a public health problem by 2017. In the context of VL, the elimination target is defined as an annual VL

incidence of <1 per 10,000 capita at (sub-)district level. Interventions focus on vector control (indoor-residual spraying (IRS)), surveillance and on diagnosing and treating VL cases. We used mathematical modelling to quantify VL transmission dynamics and predict the feasibility of achieving the VL elimination target with current control strategies under varying assumptions about the reservoir of infection in humans.

Methods: We developed three deterministic age-structured transmission models with different main reservoirs of infection in humans: asymptomatic infections (model 1), reactivation of infection after initial infection (model 2), and post kala-azar dermal leishmaniasis (PKDL; model 3), and fitted these to the KalaNet data from Bihar India and Nepal. Predictions were made for optimal and sub-optimal IRS effectiveness for three different levels of VL endemicity.

Results: Structurally different models explained the KalaNet data equally well. However, the predicted impact of IRS varied substantially between models, such that a conclusion about reaching the VL elimination targets for the ISC heavily depends on assumptions about the main reservoir of infection in humans: asymptomatic cases, recovered (immune) individuals that reactivate, or PKDL cases.

Conclusions: Available data on the impact of IRS so far suggest one model is probably closest to reality (model 1). According to this model, elimination of VL by 2017 is only feasible in low and medium endemic settings with optimal IRS. In highly endemic settings and settings with sub-optimal IRS, additional interventions will be required.

MOLECULAR DETECTION OF INFECTION HOMOGENEITY AND IMPACT OF MILTEFOSINE TREATMENT IN A SYRIAN GOLDEN HAMSTER MODEL OF LEISHMANIA DONOVANI AND L. INFANTUM VISCERAL LEISHMANIASIS.

Eline Eberhardt, Annelies Mondelaers, Sarah Hendrickx, Magali Van den Kerkhof, Louis Maes, Guy Caljon

Laboratory of Microbiology, Parasitology and Hygiene (LMPH), University of Antwerp, Wilrijk, Belgium

Control of visceral leishmaniasis, a fatal tropical protozoal disease caused by *Leishmania infantum* and *L. donovani*, primarily relies on chemotherapy using an increasingly compromised repertoire of anti-leishmanial compounds. For evaluation of novel drug candidates, the Syrian golden hamster can be considered as a clinically relevant laboratory model. In this study, two molecular parasite detection assays were developed targeting *cathepsin-like cysteine protease B (CPB)* DNA and *18S rRNA* to achieve absolute amastigote quantification in the major target organs liver and spleen, and to evaluate the impact of sub-curative treatment using miltefosine at 40 mg/kg for 5 days as a paradigm. Both qPCR techniques showed excellent agreement and a strong correlation to the conventional microscopic detection on Giemsa-stained tissue smears. Using multiple single tissue pieces and all three detection methods, we also evaluated the robustness of extrapolating whole organ parasite burdens from single tissue pieces by confirming homogeneity of infection throughout infected livers and spleens. Comparison of pre- and post-treatment *L. infantum* and *L. donovani* burdens in infected hamsters using the three detection methods consistently revealed a stronger parasite reduction in the spleen compared to the liver, indicating an organ-dependent clearance efficacy for miltefosine. In conclusion, this study demonstrated high homogeneity of infection in liver and spleen in the hamster model and advocates the use of molecular detection methods for assessment of low tissue burdens and for evaluation of drug efficacy.

EFFECT OF PLASMODIUM FALCIPARUM EXPOSURE ON URINARY METABOLOMICS PROFILING

M.E. Betouke Ongwe^{1,2}, Isabelle Kohler¹, Benjamin Mordmueeler³, Jacqueline Janse¹, Peter Kremsner³, Bertrand Lell², Oleg Mayboroda¹, Maria Yazdanbakhsh¹

1. Leiden University Medical Centre, Leiden, The Netherlands; 2. Centre de Recherches Médicales de Lambaréné (CERMEL), Lambaréné, Gabon ; 3. University of Tuebingen, Institute of Tropical Medicine, Tuebingen, Germany

Background - Metabolomics is a post-genomic discipline which has become an integral part of modern clinical research, allowing for an improved understanding of disease mechanisms via assessment of the metabolites present in body fluids. Metabolomics is an essential tool in personalized medicine and appears promising in malaria research to better understand the molecular mechanisms underlying naturally acquired immunity against *P. falciparum*.

This study consisted in the longitudinal urinary profiling of healthy individuals before and after intravenous administration of *P. falciparum* sporozoites controlled challenge, aiming at deciphering the metabolic changes observed under malaria infection.

Methods - 20 healthy Gabonese and 5 Europeans were voluntarily challenged by *P. falciparum* sporozoites (3200 PfSPZ) and followed up until development of symptoms and/or positive thick blood smear test. Urine samples were regularly collected prior to the challenge and during the development of symptoms until treatment. Samples were analyzed in an untargeted approach using state-of-the-art analytical platforms, namely liquid chromatography-mass spectrometry (reversed-phase and hydrophilic interaction chromatography) and nuclear magnetic resonance (NMR) spectroscopy. Multivariate data analysis was used to investigate the difference between classes and highlight the metabolites possibly responsible for sample classification.

Results - In opposition to Europeans, Gabonese individuals did not all become parasitemic. Supervised data analysis methods highlighted relevant differences in the Gabonese subgroup, with a significant sample discrimination between individuals that became parasitemic and Gabonese that did not develop symptoms. This group separation was predominant in the urine profiles before the challenge, and linked to different urinary levels of a specific metabolite observed in both hydrophilic interaction chromatography and NMR data.

Discussion - This metabolomics study highlighted significant differences in the urinary metabolite profiles between parasitemic and non-parasitemic individuals. These differences were mainly observed before the challenge with *P. falciparum*, suggesting that molecular mechanisms underlying parasitemia may be already perceptible at an earlier stage, even before the infection.

SESSION 2: TICK-BORNE PARASITES

INVITED SPEAKER

Michalis Kotsyfakis is a molecular biologist with a PhD from a Joint Graduate Program of the Departments of Medicine and Biology, University of Crete, and the Institute of Molecular Biology and Biotechnology, FORTH in Heraklion. Michalis performed his post-doctoral Research at the Laboratory of Insect Molecular Genetics in Heraklion and at the Laboratory of Malaria and Vector Research (NIAID/NIH) in Maryland, USA. He uses a systems biology approach to study arthropod disease vectors with a focus on ticks and tick-borne diseases, and has been studying the regulation of vertebrate host proteolysis by tick salivary proteins which underlies blood meal acquisition processes and vertebrate immunological reactions to arthropods.

Michalis is currently the head of the Laboratory of Genomics and Proteomics of Disease Vectors. His current research focuses on using and understanding arthropod vectors as models to address two fundamental practical problems: how to combat the public health threat posed by the disease agents carried by these vectors and how to translate the mechanisms adopted by disease vectors into treatments for human and animal diseases. Michalis has been awarded with an NIH Fellows Award for Research Excellence in Biomedical Research and the President's award from the Grant Agency of the Czech Republic for excellent research performance.

HIGH-THROUGHPUT DISEASE VECTOR BIOLOGY: THE EXAMPLE OF THE TICK *IXODES RICINUS*

Michalis Kotsyfakis

Laboratory of Genomics and Proteomics of Disease Vectors, Institute of Parasitology, Biology Center of the Czech Academy of Sciences, Branisovska 31, 37005 Budweis, Czech Republic; mich_kotsyfakis@yahoo.com

The *Ixodes ricinus* tick is an excellent model organism to explore the host modulatory mechanisms employed by arthropod ectoparasites upon hematophagy. This is because the specific tick species feeds on its vertebrate hosts for over a week. During this period of time, it successfully overcomes major host homeostatic responses to tick feeding such as the vertebrate immune response. As a consequence, many individuals bitten by ticks do not sense the tick bite and consequently do not seek timely treatment for the diseases that ticks transmit. As a result, *I. ricinus* serves as a vector for Neglected Diseases in Europe such as Lyme Disease and Tick-Borne Encephalitis.

We emphasize in the exploitation of *I. ricinus* tick as a model organism:

A) to apply a systems-based and multimodal approach to discover tick effectors that determine blood feeding success

B) to understand the vertebrate host proteolytic cascades that are regulated by tick salivary secretions at sites of tick infestation and that facilitate blood meal uptake and the successful completion of the tick lifecycle.

Accordingly, our research aims:

A) to explore the basic mechanisms of tick blood feeding success as the conceptual basis for future development of novel methods/tools to control tick populations

B) to demonstrate the pharmacological action of tick salivary proteins in the regulation of vertebrate host homeostasis (haemostasis, vascular biology, immunity, neuromodulation) and their potential for drug development in the near future.

FREE ORAL PRESENTATIONS

SUCCESSFUL VACCINATION AGAINST BABESIA PARASITES AND THEIR TICK VECTORS USING RECOMBINANT ANTIGENS

Theo Schetters

ProtActivity, Cuijk, The Netherlands

Ticks and tick borne diseases are a major impediment for the growth and productivity of livestock, which affects the livelihood of rural communities in Africa that depend on small holder farming. For decades, tick infestation is being controlled by using acaricides, which has led to the selection of tick strains that are resistant to such treatment. In several areas, multi-drug resistant strains have emerged that require other methods of control. As a consequence, tick infestation is increasing, causing direct effects on productivity and growth, and also increases the transmission of tick borne diseases such as babesiosis and anaplasmosis. Vaccination against ticks is considered an alternative strategy to acaricide treatment, and a commercial vaccine that provides partial protection against *Rhipicephalus microplus* is available. This vaccine is based on the immunoprotective activity of an antigen derived from the surface of epithelial cells from the midgut of the ticks (Bm86). The antigen is produced in *Pichia pastoris*, and formulated in a water-in-oil suspension. In order to improve efficacy, we have evaluated vaccine formulations containing Bm86 and additional tick antigens. It appeared that vaccination of cattle against Bm86 and subolesin induced more than 95% reduction in fully engorged female ticks upon larval infestation. Newly developed in vitro feeding techniques corroborated the synergetic effect of antibodies against Bm86 and antibodies against subolesin on tick feeding. Ideally, this vaccine also comprises antigens that induce protection against the parasites that are transmitted by ticks. Mammals can be protected against *Babesia* infection by vaccination with antigens from supernatants of in vitro cultures of the parasite. We identified a major protective *Babesia* antigen in supernatants of *B. divergens* cultures and *B. canis* cultures. The antigens are GPI-anchored merozoite surface proteins. Vaccination with recombinantly produced protein induced protection against *B. divergens* and *B. canis* in cattle and dogs respectively (>90% reduction of parasitemia). These results provide the basis for the development of combined tick-*Babesia* vaccines.

RELATIONSHIP BETWEEN THEILERIA PARVA ENTOMOLOGICAL INOCULATION RATE AND PREVALENCE IN TREE AGROECOLOGICAL ZONES OF THE DEMOCRATIC REPUBLIC OF CONGO

Amzati Sefu Gaston^{1,2}, Jean-Berckmans Muhigwa² Roger Pelle³, Appolinaire Djikeng³, Eunice Machuka³, Maxime Madder⁴, Nathalie Kirschvink¹, Tanguy Marcotty¹

1. University of Namur, Belgium; 2. Université Evangélique en Afrique, Bukavu, D.R. Congo; 3. Biosciences eastern and central Africa - International Livestock Research Institute (BecA-ILRI) hub, Nairobi, Kenya; 4. University of Pretoria, South Africa

Theileria parva is a protozoan parasite which causes East Coast fever (ECF), a lethal lymphoproliferative disease of cattle. ECF kills over 1 million cattle/year with economic losses of ~USD300 million in eastern, central and southern Africa.

The study was carried out to assess the prevalence of *T. parva* in the bovine host as well as in pooled free-living *Rhipicephalus appendiculatus* tick vectors by nested-PCR based on three antigens genes (p104, Tp1 and Tp2) and to estimate the entomological inoculation rate (EIR). Three cross-sectional studies was conducted in three different seasons (two wet seasons and one dry season) and three agroecological zones (AEZ) namely low, medium and high altitude of the Democratic Republic of Congo. The farms were selected by first stratifying the study area by AEZ and implementing a random sampling in administrative localities. A total of 1230 blood samples and 6060 ticks were collected from cattle and 1740 ticks from pasture.

The overall prevalence of *T. parva* in cattle was respectively 56%, with Variations observed between AEZ and seasons. *R. appendiculatus* was the most abundant tick specie with a burden of 26 ticks/animal corresponding to 71.5% of the tick load. The tick burden was higher in low and

intermediate altitude lands than highlands. The overall *T. parva* prevalence in free-living *R. appendiculatus* was 3.5% and the resulting mean EIR was 0.13 *T. parva* inoculations per day.animal. The prevalence was higher in the lowland and intermediate altitude than high altitude. Prevalence of infection in cattle and ticks and tick infestation levels were analysed for association with seasonal and agroecological factors.

The results on *R. appendiculatus* abundance, *T. parva* prevalence and calculating entomological inoculation rate at different places and times should have important implication in the selection of the most appropriate *T. parva* control strategies and integrated vector management in the region.

SESSION 3: OPEN SESSION

PROGRESS TOWARDS ONCHOCERCIASIS ELIMINATION IN THE PARTICIPATING COUNTRIES OF THE AFRICAN PROGRAMME FOR ONCHOCERCIASIS CONTROL: EPIDEMIOLOGICAL EVALUATION RESULTS

Afework H. Tekle¹, Honorath G.M. Zouré¹, Mounkaila Noma¹, Michel Boussinesq², Luc E. Coffeng³, Wilma A. Stolk^{3*}, Jan H.F. Remme⁴

1. African Programme for Onchocerciasis Control, Ouagadougou, Burkina Faso; 2. Institut de Recherche pour le Développement (IRD), Montpellier, France; 3. Department of Public Health, Erasmus MC, University Medical Center Rotterdam, Rotterdam, the Netherlands; 4. Consultant, 120 rue des Campanules, Ornex, France; * Presenting author

Background: The African Programme for Onchocerciasis Control (APOC) was created in 1995 in order to control onchocerciasis as a public health problem by implementing sustainable mass ivermectin treatment. When research showed that mass treatment can lead to complete elimination of the infection, APOC shifted its target to elimination of transmission. Epidemiological evaluations have been undertaken from 2008 to 2014 to measure the decline in infection prevalence. We analyzed this unique database, to assess progress towards elimination in areas with ≥ 6 years treatment.

Methods: Progress towards elimination was evaluated in 54 areas, covering a total population of 53 million people. First, parasitological surveys were done in about 10 selected high risk communities per area. By comparing observed prevalence levels to expected trends (as predicted by the ONCHOSIM model, developed at Erasmus MC), using Bayesian methods and Monte Carlo simulation, we classified the progress towards elimination as “faster than predicted”, “on track”, or “delayed”. Second, in areas close to elimination, additional parasitological surveys were done to assess whether mass ivermectin treatment can safely be stopped.

Results: Initial parasitological surveys were done in 54 areas, covering 639 villages and 127,665 people. The decline in prevalence was faster than predicted in 23 areas, on track in another 23 and delayed in 8 areas. Additional surveys were done in 22 areas and 13 of these met the epidemiological criteria for stopping treatment. Overall, 32 areas (25.4 million people) had reached or were close to elimination, 18 areas (17.4 million) were on track but required more years treatment, and in 8 areas (10.4 million) progress was unsatisfactory.

Conclusions: Onchocerciasis has been largely controlled as a public health problem. Great progress has been made towards elimination which already appears to have been achieved for millions of people. For most APOC countries, nationwide onchocerciasis elimination is within reach.

EFFECT OF ANTHELMINTHIC TREATMENT ON ADIPOSITY : A CLUSTER-RANDOMIZED PLACEBO CONTROLLED TRIAL IN NANGAPANDA, FLORES, INDONESIA

Dicky L. Tahapary^{1,2*}, Karin de Ruiter^{2*}, Ivonne Martin^{3,4}, Lisette van Lieshout², Bruno Guigas^{2,5}, Christa M Cobbaert⁶, Pradana Soewondo¹, Yenny Djuardi⁷, Apriyanto E Wiria^{2,7}, Jeanine J Houwing-Duistermaat³, Erliyani Sartono², Jan Smit^{8,9#}, Maria Yazdanbakhsh^{2#}, Taniawati Supali^{7#}

1. Department of Internal Medicine, Faculty of Medicine, Universitas Indonesia, Jakarta, Indonesia; 2. Department of Parasitology, Leiden University Medical Center, Leiden, The Netherlands; 3. Department of Medical Statistics and Bioinformatics, Leiden University Medical Center, Leiden, The Netherlands; 4. Department of Mathematics, Parahyangan Catholic University, Bandung, Indonesia; 5. Department of Molecular Cell Biology, Leiden University Medical Center, Leiden, The Netherlands; 6. Department of Clinical Chemistry and Laboratory Medicine, Leiden University Medical Center, Leiden, The Netherlands; 7. Department of Parasitology, Faculty of Medicine, Universitas Indonesia, Jakarta, Indonesia; 8. Department of Internal Medicine, Radboud University Medical Centre, Nijmegen, The Netherlands; 9. Department of Internal Medicine, Leiden University Medical Center, Leiden, The Netherlands; * These authors contribute equally; # These authors contribute equally

Abstract not provided

HELMINTH-DRIVEN TYPE 2 INFLAMMATION ENHANCES CD8⁺ T CELL-MEDIATED CONTROL OF ACUTE GAMMAHERPESVIRUS INFECTION

Marion Rolot^{1¶}, Annette Dougall^{1¶}, Chetty Alisha², Selkirk Murray³, Brombacher Frank², Bureau Fabrice⁴, Gillet Laurent¹, Vanderplasschen Alain¹, Horsnell William G.², Dewals Benjamin G.^{1,*}

1. Fundamental and Applied Research in Animals and Health (FARAH), Immunology-Vaccinology, Faculty of Veterinary Medicine, University of Liege, Belgium; 2. Institute for Infectious Disease and Molecular Medicine, University of Cape Town, Cape Town, South Africa; 3. Imperial College, Department of Life Sciences, London, UK; 4. GIGA, Molecular and Cellular Immunology, University of Liege, Belgium; ¶ contributed equally

The first stages of host colonisation with pathogens often determine the efficacy of their control through priming and maintenance of effective adaptive immune responses, which are essential for such control. Infection with different pathogens often occurs concurrently and a better understanding of how our immune system faces multiple aggressions with different sorts of pathogens is therefore important. In this study, we have investigated how helminth-driven Th2-type inflammation affects the control of host colonization by a gammaherpesvirus. We used *in vivo* imaging of murine herpesvirus 4 (MuHV-4) live infection to investigate viral replication in mice and observed that pre-exposed mice to helminth (*Schistosoma mansoni* or *Nippostrongylus brasiliensis*) better controlled lung acute infection and weight loss. The improved control of acute infection was associated with increased virus-specific effector CD8⁺ T cell responses in the bronchoalveolar lavage, lung, draining LN (dLN) and spleen; whereas depletion of CD8⁺ cells caused a loss of viral infection control irrespective of helminth exposure. Exposure to *S. mansoni* eggs caused increased numbers of dendritic cells (DCs), predominantly CD11b⁺ conventional DCs in the lung, and in the dLN, which was associated with higher numbers of antigen-loaded lung DCs migrating to the dLN after MuHV-4 infection. These results suggested that *S. mansoni* egg-induced inflammation might result in an improved priming of virus-specific CD8⁺ T cells. To address the role of type 2 inflammation, we next used interleukin 4 receptor α -chain (IL-4R α) deficient mice exposed to *S. mansoni* eggs and observed the absence of both the enhanced control of viral acute infection and the increased CD8⁺ T cell response, suggesting that IL-4R α signalling is involved. Collectively, we present data indicating that IL-4R α -dependent type 2 inflammation induced in the lung by helminth exposure improves host control of acute viral infection through the induction of an increased virus-specific cytotoxic T cell effector response.

UNRAVELING THE ROLE OF IL-17A IN THE INTESTINAL IMMUNE RESPONSE AGAINST THE PROTOZOAN PARASITE *GIARDIA MURIS* BY AN RNA SEQUENCING APPROACH

O. Paerewijck¹, L. Dreesen¹, F. Van Meulder¹, D. Ratman², K. De Bosscher², R.W. Li³, P. Geldhof¹

¹Department of Virology, Parasitology and Immunology, Laboratory of Parasitology, Faculty of Veterinary Medicine, Ghent University, Merelbeke, Belgium; ²VIB Department of Medical Protein Research, Ghent University, Ghent, Belgium;

³Agriculture Research Service, Animal Genomics and Improvement Laboratory, Beltsville, Maryland, United States of America

Background - The intestinal protozoan parasite *Giardia duodenalis* has a wide vertebrate host range. An infection with *Giardia* can lead to gastro-intestinal complaints. Although in most hosts these symptoms rapidly disappear, a chronic situation can develop. Recent data obtained in mice, cattle and humans indicates that development of immunity is dependent on IL-17A. The aim of this study was to further unravel the IL-17A induced effector mechanisms in a *Giardia*-mouse infection model.

Methods - C57Bl/6 wildtype (WT) and IL-17RA-KO mice were orally infected with 10³ *G. muris* cysts. Three weeks post-infection, intestinal tissue samples were collected for RNA purification and subsequent transcriptome analysis. Samples collected from non-infected WT and KO animals served as negative controls. Genes of interest were further subjected to transcription and expression analysis and immunolocalisation. Their functional role was investigated by performing infection experiments in KO mice, pending availability.

Results - Transcriptome analysis indicated that 844 genes were differentially expressed between WT infected and WT control mice, many of which have never been linked to *Giardia* infection before, including genes associated with circadian rhythm. Comparison of WT infected with IL-17RA-KO infected mice resulted in the identification of 287 differentially transcribed genes. One of these, mannose-binding lectin 2, is particularly interesting as it is involved in complement activation. Further work indicated that the expression and secretion of MBL2 following a *Giardia* infection is IL-17A dependent and infection studies in Mbl2-KO mice showed a higher load of *Giardia* trophozoites in comparison to WT mice.

Conclusions - The transcriptome approach that was followed resulted in the identification of a number of genes that seem to play a role in the IL-17A response following a *Giardia* infection. Additional research is now ongoing to further unravel the involvement of these genes in the development of intestinal immunity.

DISTINCT METABOLIC REQUIREMENTS FOR T HELPER 1 AND 2 POLARIZATION BY DENDRITIC CELLS

Leonard Pelgrom¹, Alwin van der Ham¹, Leonie Husaarts¹, Bruno Guigas¹, Maria Yazdanbakhsh¹ & Bart Everts¹

1. Department of Parasitology, Leiden University Medical Center, Leiden, The Netherlands

Background - Dendritic cells (DCs) play a central role in the activation and polarization of T cell responses. We recently found that toll-like receptor signalling promotes a shift to glycolysis to support the anabolic demands of murine DC activation and effective priming of T cell responses. However, the metabolic requirements for polarization of distinct T helper cell (Th) responses by DCs remain poorly defined.

Methods - Human monocyte-derived DCs were stimulated with helminth-derived antigens (*Schistosoma mansoni* soluble egg antigens [SEA] and omega-1) or pharmacological compounds that are known to prime Th2 responses (rapamycin) after which they were co-cultured with allogenic naïve CD4⁺ T cells. DC cellular metabolism was investigated using RT-qPCR, western blotting and extracellular flux analysis.

Results - Through genome wide expression analysis we identified suppression of genes involved in glycolysis as a key characteristic of Th2-polarizing DCs. Conversely, stimuli that promote Th1 responses enhance glycolysis in DCs. Furthermore, consistent with these observations, when glycolysis is blocked, DCs fail to induce Th1 responses and instead favour Th2 polarization. On the other hand, DCs that have been conditioned with SEA rely on mitochondrial fatty acid oxidation to promote effective differentiation of Th2 cells.

Discussion - These findings suggest that DC-driven polarization of different T cell responses is dependent on the activation of distinct metabolic programs in DCs and highlights that metabolic manipulation of DCs could hold promise as a novel therapeutic approach to control immune-polarization in disease settings.

POSTERS

SWIMMER'S ITCH IN BELGIUM: FIRST RECORDED OUTBREAKS, IDENTIFICATION OF THE PARASITE SPECIES AND INTERMEDIATE HOSTS.

Yannick CARON¹, Ariane CABARAUX², Françoise MARECHAL¹, Bertrand LOSSON¹

¹Research Unit in Parasitology and Parasitic Diseases, Fundamental and Applied Research for Animals & Health (FARAH), Faculty of Veterinary Medicine, University of Liège, Belgium; ²Environment and security unit, Les Lacs de l'Eau d'Heure (Non-Profit Making Association), Boussu-Lez-Walcourt, Belgium; Corresponding author: ycaaron@ulg.ac.be

Cercarial dermatitis or swimmer's itch is a skin condition in humans due to the larval forms of bird schistosomes of the genus *Trichobilharzia*. The life cycle of these schistosomes requires freshwater snails and waterfowls. Repeated exposures to cercariae can lead to skin sensitization with the induction of pruritic skin lesions. We describe here several outbreaks of human cercarial dermatitis at the Eau d'Heure lakes, Belgium. In July and August 2012, a total of respectively, 78 and 10 people reported a sudden skin rash accompanied by pruritus following recreational activities in the Plate Taille lake. However no ocellate furcocercariae were detected following light exposure of the collected snails from September 2012 to September 2013 (n= 402). No outbreaks were recorded in 2013 and 2014. In August 2015, about 30 new cases were recorded at the same place. Snails were collected (n= 270) in different locations around the lake. After light exposure, seven *Radix* spp. (2.6%) shed ocellate furcocercariae. Molecular identification based on the rDNA ITS-2 sequence ascribed the infected snails to *R. balthica* (= *R. peregra* = *R. ovata*) (6/7) and to *R. auricularia* (1/7). Based on the amplification of the D2 domain of the 28S rDNA the cercariae, were shown to belong to two different haplotypes of *Trichobilharzia franki*. This is the first record in Belgium of *T. franki* and associated skin condition.

CD4 T CELL AND $\gamma\delta$ T CELL RESPONSES DURING MALARIA IN INDONESIAN CHILDREN

Vera Asscher¹, Sanne E. de Jong¹, Linda Wammes¹, Firdaus Hamid^{1,2}, Aprilianto E. Wiria^{1,3}, Erliyani Sartono¹, Taniawati Supali³, Maria Yazdanbakhsh¹

1. Leiden Immunoparasitology Group, Department of Parasitology, Leiden University Medical Center, Leiden, the Netherlands; 2. Department of Microbiology, Faculty of Medicine, Hasanuddin University, Makassar, Indonesia; 3. Department of Parasitology, Faculty of Medicine, University of Indonesia, Jakarta, Indonesia.

Malaria remains a major global health problem, despite the range of interventions being deployed to prevent and control it. Vaccine and drug development efforts could benefit from a better understanding of the immune response to malaria and of the immunological factors responsible for control of infection. To gain more insight in T-cell responses to malaria, Indonesian school children with and without malaria were assessed at baseline and after 9 and 21 months. Peripheral blood mononuclear cells were analysed *ex vivo* by flow cytometry and stimulated with *Plasmodium falciparum*-infected red blood cells (PfRBC) for 96 hours. Cytokine levels in supernatants were analysed by Luminex. Subsequently, malaria-positive and -negative children were compared cross-sectionally and longitudinally. $\gamma\delta$ T cells levels were higher in malaria-positive children and decreased over time when the children became negative. Levels of total CD4 T cells and of CD4 T cell subsets did not change with infection. However, PD-1 expression levels on CD4 T cells were higher in malaria-positive children. TNF, IFN- γ and IL-17 responses after PfRBC stimulation did not differ between malaria-positive and -negative children. These data show that $\gamma\delta$ T cells and PD-1 expression on CD4 T cells are associated with malaria and are therefore interesting targets for future research.

RANDOMIZED, PLACEBO-CONTROLLED TRIAL OF THREE-MONTHLY ALBENDAZOLE TREATMENT ON TH2 RESPONSES: DIFFERENT EFFECTS SEEN ON IGE AND IL-5

Karin de Ruiter^{1*}, Dicky L. Tahapary^{1,2*}, Linda Wammes^{1#}, Aprilianto E. Wiria^{1,3}, Firdaus Hamid^{1,4}, Johannes W.A. Smit^{5,6}, Jeanine J. Houwing-Duistermaat⁷, Erliyani Sartono¹, Taniawati Supali³, Maria Yazdanbakhsh¹

1 Department of Parasitology, Leiden University Medical Center, Leiden, The Netherlands; 2 Department of Internal Medicine, Faculty of Medicine, Universitas Indonesia, Jakarta, Indonesia; 3 Department of Parasitology, Faculty of Medicine, Universitas Indonesia, Jakarta, Indonesia; 4 Department of Microbiology, Faculty of Medicine, Hasanudding University, Makassar, Indonesia; 5 Department of Internal Medicine, Radboud University Medical Centre, Nijmegen, The Netherlands; 6 Department of Internal Medicine, Leiden University Medical Center, Leiden, The Netherlands; 7 Department of Medical Statistics and Bioinformatics, Leiden University Medical Center, Leiden, The Netherlands; #Current address: Department of Medical Microbiology, Erasmus MC, Rotterdam, The Netherlands; *These authors have contributed equally to this work.

Background - Helminth parasites induce a strong Th2 response, characterized by high levels of IgE antibodies and elevated signature cytokines such as IL-5. As many global deworming programs are underway, there is concern that this might lead to emergence of Th1-mediated pathologies when its counterbalancing helminth-induced Th2 response is absent. It is therefore important to measure Th2 mediated responses after deworming. To this end, total IgE levels and IL-5 response to a polyclonal stimulus have been measured in a household-clustered RCT in Indonesia.

Methods - Total plasma IgE was measured in 1494 subjects and whole-blood IL-5 responses to mitogen phytohaemagglutinin (PHA) were assessed in 682 subjects at baseline, 9 and 21 months after three-monthly treatment with albendazole or placebo. Stool samples were collected to determine parasite infection by microscopy and qPCR.

Results - Anthelmintic treatment did not result in complete removal of helminth infections in the community (39.9% vs. 75.4% for placebo after 21 months). However, treatment led to a significant decrease in IgE levels in albendazole-treated subjects compared to placebo-treated subjects (estimates [95% CI] at 9 months -0.073 [-0.114 – -0.032], at 21 months -0.060 [-0.101 – -0.020]; overall p-value: $p_{\text{time}} < 0.001$). IL-5 responses to PHA were not significantly affected by anthelmintic treatment and rather seemed to be increased in albendazole-treated subjects (estimates [95% CI] at 9 months 0.037 [-0.052 – 0.127], at 21 months 0.107 [0.000 – 0.214]; $p_{\text{time}} = 0.146$).

Discussion - Intensive treatment of helminth parasites has different outcomes on B-cell related IgE levels and T-cell related IL-5 responses. This indicates that two years of deworming can have differential effects on responses typified as Th2 mediated which needs to be taken into account when considering the effects of helminths on non-communicable diseases.

PROGRESS TOWARDS LYMPHATIC FILARIASIS ELIMINATION IN GHANA: A MODEL-BASED ANALYSIS OF TRENDS IN INFECTION PREVALENCE DURING 15 YEARS OF MASS DRUG ADMINISTRATION

Kwadwo K. Frempong^{1, 2}, Daniel A. Boakye^{1, 4}, Mike Y. Osei-Atweneboana⁵, Samuel Odoom³, Nana Kwadwo Biritwum³, Luc E. Coffeng², Sake J. de Vlas² & Wilma Stolk²

1. Parasitology Department, Noguchi Memorial Institute for Medical Research, University of Ghana, Legon, Ghana; 2. Department of Public Health, Erasmus MC, Rotterdam, Netherlands; 3. Neglected Tropical Disease Programme, Ghana Health Services, Accra, Ghana; 4. African Programme for Onchocerciasis Control (APOC), Ouagadougou, Burkina Faso; 5. Council for Scientific and Industrial Research (CSIR)-Water Research Institute, Accra, Ghana

Background - Lymphatic filariasis (LF) is a devastating disease endemic in Ghana. Since 2000 there has been mass treatment (MDA) with albendazole and ivermectin which has driven prevalence very low. More than 50% of the 98 endemic districts passed the transmission assessment survey between 2010-2015 and have currently stopped MDA. Nonetheless, there are some areas recording infections and undergoing treatment. WHO aims at eliminating LF by 2020. Whether Ghana is on the verge of elimination, it is currently uncertain. This research is to ascertain the possibility of LF elimination in Ghana by the set target.

Method - Mf prevalence and coverage data on 480 communities (2000-2015) across the country were obtained from the Ghana Health Services and individual research works. We analyzed observed trends in infection prevalence during 15 years of mass drug administration using the simulation

model LYMFASIM, a individual-based stochastic model. The model was fitted to all datapoints jointly, to mimic average trends; it was also fitted to data from individual communities for which we had baseline endemicity data and at least one measurement later in time.

Results - The model predicted general trends in infection as observed. A slower-than-expected decline in mf prevalence can be explained by high baseline endemicity (reflecting unfavourable transmission conditions) in combination with low coverage.

Discussion - LF elimination is possible by 2020 in some communities based on the current strategy of MDA. However, this may require remedial actions such as measures to improve coverage & compliance and use of more effective drugs. Timelines for elimination may differ from community to community due to local differences in transmission dynamics and control measures.

KINETIC MODELLING OF THE TRYPANOTHIONE SYNTHESIS PATHWAY IN *TRYPANOSOMA BRUCEI* TO IDENTIFY THE MOST PROMISING DRUG TARGETS

Dimitri V. Lindijer¹, Alejandro E. Leroux², Barbara M. Bakker³, R. Luise Krauth-Siegel², Jurgen R. Haanstra¹

¹Systems Bioinformatics, Vrije Universiteit Amsterdam, Amsterdam, the Netherlands; ²Biochemie-Zentrum der Universität Heidelberg, Heidelberg, Germany; ³University of Groningen, University Medical Center Groningen, Department of Pediatrics, Center for Liver, Digestive and Metabolic Diseases, Groningen, The Netherlands

Background - Trypanothione (T(SH)₂) is the main low molecular mass thiol used by *Trypanosoma brucei*. As T(SH)₂ is essential for survival of the parasite, the pathway that synthesizes this antioxidant is of interest for drug design. But drugs against metabolic enzymes often do not fully block an enzymatic step and it is therefore important to identify which enzyme is in control of this pathway, i.e. which enzyme has to be inhibited the least to have a strong effect on pathway output. Control of an enzyme is a property of the entire system and hence computational modelling of the pathway may yield important insights.

Methods - To analyse the control of the trypanothione synthesis flux in *T. brucei* we have created a kinetic computer model of this pathway. It consists of (i) the three enzymes needed to synthesize trypanothione from glutamate, glycine, cysteine and spermidine, (ii) a redox reaction that detoxifies reactive oxygen species through oxidation of T(SH)₂, and (iii) a reductase to regain the T(SH)₂. The parameters for each enzyme are mostly based on novel measurements in an in vivo-like assay buffer.

Results - A steady-state analysis of our model yields intermediate concentrations for the thiols that are higher than measured. Intermediates are already diluted through growth, but the addition of a degradation reaction for the thiols (based on half-life data for glutathione in yeast) brings most intermediate concentrations within the range measured in trypanosomes.

Discussion - We are currently validating our model based on published RNAi knockdown studies and will do a metabolic control analysis to rank drug targets for their potential to, when inhibited, lower the T(SH)₂ concentration and kill the parasite. Once validated, our model can be connected to the previously constructed model of glycolysis and the pentose phosphate pathway, moving further towards the creation of an *in silico* trypanosome.

GUIDANCE IN DESIGNING SURVEYS FOR MONITORING THE PROGRESS OF SCHOOL-BASED DEWORMING PROGRAMMES TO CONTROL SOIL-TRANSMITTED HELMINTHIASIS

Aklilu Habteab Ghebretinsae¹, Jozef Vercruyse¹, Bruno Levecke¹
1. Faculty of Veterinary Medicine, Ghent University, Ghent, Belgium

Background - There is a worldwide upscale in school-based deworming programmes to control the morbidity caused by soil-transmitted helminths (STH). However, there is a lack of guidance in designing surveys to verify whether these programmes progress as anticipated.

Methods and Results - We expanded an existing 2-level hierarchical model that accounts for variation in egg counts within individuals due to the egg counting procedure (level 1; Poisson

distribution) and between individuals within a school due to host-parasite interactions (level 2; negative binomial distribution (NB)), to a 3-level model that also accounts for clustering of STH infections between schools (level 3; NB or zero-inflated NB distribution). In addition, we adapted the model for variation in the efficiency of deworming programmes at both the individual and the school level. To maximize the flexibility in survey design, the framework was worked out for the examination of both individual and pooled stool samples.

Results - From the derived formulae to estimate the variance, we updated the methodology to calculate the number of schools and the number of individuals per school required for assessing (i) the intensity of infections, (ii) the efficiency of programmes and (iii) the absence of infections for any scenario of disease epidemiology, diagnostic strategy and programmatic efficiency. To give these three applications the widest relevance as possible we illustrated each of them using available data on the efficiency of deworming and the related costs to diagnosis STH infections in epidemiological surveys in Eastern Africa.

Discussion - This updated framework reflects more the evident variation in both the egg counts across school children from different schools and the programmatic efficiency, now allowing managers to design and budget surveys for monitoring the progress of their deworming programmes according to the local objectives, epidemiology, and resources.

EVIDENCE OF A DRUG-SPECIFIC IMPACT OF EXPERIMENTALLY SELECTED PAROMOMYCINE AND MILTEFOSINE RESISTANCE ON PARASITE FITNESS IN *LEISHMANIA INFANTUM*

Hendrickx Sarah¹, Beyers Jolien, Mondelaers Annelies¹, Eberhardt Eline¹, Lachaud Laurence², Delputte Peter¹, Cos Paul¹, Maes Louis¹

1. Laboratory of Microbiology, Parasitology and Hygiene (LMPH), University of Antwerp, Antwerp, Belgium; 2. Laboratoire de Parasitologie-Mycologie et Centre National de Référence des Leishmanioses, Centre Hospitalier Universitaire et Université de Montpellier, France

Background - Miltefosine (MIL) and paromomycin (PMM) are used to treat visceral leishmaniasis for only one decade and increasing numbers of MIL-treatment failures and primary resistance against both drugs have already been reported. Since enhanced parasite fitness has been described for antimony resistant strains, former research by our group explored possible modifications in parasite fitness in *Leishmania donovani* related to PMM-resistance; however, poor promastigote infectivity hampered assessment of fitness of the intracellular amastigote stage. The present study explored the influence of both MIL- and PMM-resistance in a *L. infantum* patient isolate that had been experimentally selected for drug resistance on intracellular amastigote level and that maintained full infectivity of both parasite stages.

Methods - *In vitro* and *in vivo* growth, metacyclogenesis, infectivity and macrophage stress responses were evaluated to compare parasite fitness between the parent wild-type and the derived PMM- and MIL-resistant strains.

Results - No significant differences were observed between the parent wild-type and PMM-resistant strain on promastigote level, while clear fitness benefits could be demonstrated for PMM-resistant amastigotes in terms of *in vitro* and *in vivo* growth and intracellular stress response. MIL-resistant promastigotes showed decreased *in vitro* growth and incomplete metacyclogenesis. At intracellular amastigote level, a lesser growth and weakened stress response suggest a markedly reduced parasite fitness compared to wild-type parasites.

Discussion - The use of PMM should be restricted to combination therapy and closely monitored given the rapid selection towards resistance and the fitness advantages of PMM-resistant amastigotes. Although the observed reduced fitness of MIL-resistant strains may explain the challenging nature of MIL-resistance selection *in vitro*, the growing number of MIL-treatment failures certainly requires further exploratory research.

SCHISTOSOMA EGG ANTIGENS PRIME DENDRITIC CELLS FOR TH2 POLARIZATION VIA A PROSTAGLANDIN E2-DEPENDENT MECHANISM

Maria M. M. Kaisar¹, Martin Giera², Hulda Jónasdóttir², Maria Yazdanbakhsh^{1*} and Bart Everts^{1*}

Leiden University Medical Center, The Netherlands: ¹Department of Parasitology; ²Center of Proteomics and Metabolomics;

*contributed equally

Helminth-derived molecules (HDMs) are well known for their ability to induce T helper 2 (Th2) polarization via functional modulation of Dendritic Cells (DCs). Yet the molecular mechanisms through which HDMs condition DCs for Th2 polarization are still incompletely understood. To this end, we used human monocyte-derived DCs that were stimulated with *Schistosoma* soluble egg antigen (SEA), a potent Th2-polarizing antigen mixture. We found that DCs stimulated with SEA rapidly produced Prostaglandin E2 (PGE2). This effect was not driven by omega-1, a major glycoprotein present in SEA known to prime Th2 responses, as omega -1 did not promote PGE2 synthesis and SEA from which omega-1 had been depleted (SEA $\Delta\omega$ 1) retained its ability to prime DCs for PGE2 synthesis. Importantly, neutralization of PGE2 during stimulation of DCs with SEA $\Delta\omega$ 1 abrogated their capacity to prime a Th2 response, while stimulation of DCs with exogenously added PGE2 was sufficient to recapitulate the Th2-priming effect of SEA $\Delta\omega$ 1. Mechanistically, we found PGE2 synthesis to be dependent on signalling via syk, suggesting a role for Dectin in this process. Moreover, SEA and PGE2 induced OX40L expression in these DCs, a costimulatory molecule that has been linked to priming of Th2 responses. In summary, we identified a novel pathway in DCs involving syk-PGE2-OX40L through which *Schistosoma* egg derived antigens induce Th2 responses. As this pathway appears to be distinct from the earlier described mode of action through which omega -1 primes Th2 responses, it highlights the complexity of mechanisms involved in Th2 polarization by helminths such as schistosomes. Currently, studies are underway to identify the molecules and/or molecular structures present in the schistosome eggs that drive the PGE2-dependent Th2 polarization.

TRANSCRIPTIONAL AND IMMUNE PROFILING OF *SCHISTOSOMA HAEMATOBIIUM*-INFECTED GABONESE SCHOOLCHILDREN

Łucja Labuda^{1,3}, Bruce Rosa², John Martin², Ulysse Ateba Ngoa^{1,3}, Ayola Adegnika^{1,3}, Makedonka Mitreva², Maria Yazdanbakhsh¹

1. Leiden University Medical Center, Leiden, The Netherlands; 2. Centre de Recherches Médicales de Lambaréné, Lambaréné, Gabon; 3. McDonnell Genome Institute, Washington University in St. Louis, St. Louis, MO, USA

Background - *Schistosoma haematobium* infection results in alterations in immune function, yet the concurrent interplay between various arms of the immune system and the effect of parasite removal following anthelmintic treatment has not been studied extensively.

Methods - Cytokine responses of whole-blood samples in *S. haematobium*-infected and uninfected Gabonese schoolchildren at pre- and 7 months post-praziquantel (PZQ) treatment were measured using Luminex, and flow cytometry was used to characterize the memory T cell compartment and Treg frequency. Genome-wide transcriptional survey of PBMC was performed by RNA-seq.

Results - Schistosome-specific cytokine responses increased post-PZQ treatment and were inversely associated with Treg frequency. Effector memory T cells also increased post-treatment. Conversely, TLR responses decreased with treatment. Schistosome infection was associated with alterations in 634 genes while parasite removal resulted in expression changes of 218 genes. Infection was associated with an activated immune phenotype while treatment led to down-regulated pathways including TLR signalling. Although measured cytokines and cell subsets were in part in-line with gene expression data, changes in genes associated with immune cell trafficking and nuclear receptor signalling are novel.

Discussion - These results provide novel insights into the effect of *Schistosoma* parasites on human host responses and suggest new areas to be investigated.

PREVALENCE OF GASTROINTESTINAL NEMATODES IN ORGANIC DAIRY GOATS IN FLANDERS

Karen Malrait¹, Johannes Charlier², Edwin Claerebout³, Luc Decombel¹, Els Goossens¹

1. University College Ghent, Faculty of Science and Technology, Melle, Belgium; 2. Avia-Gis, Zoersel, Belgium; 3. Ghent University, Faculty of Veterinary Medicine, Merelbeke, Belgium

Problem - Gastrointestinal parasites affect animal health and lead to production losses in ruminants. Prevailing methods to control the infection level in dairy goats are preventive treatment with anthelmintics or applying a zero-grazing strategy. Organic goats, however, are obliged to graze outside, whilst preventive treatment is not allowed, thus increasing the risk of disease after ingesting infectious larvae on the grass.

Objective - The objectives of this study were (i) to quantify the magnitude of worm infections in organic dairy goat farms in Flanders, (ii) to monitor the infection throughout the grazing season as well as (iii) to identify the dominant worm species.

Methods - Individual faecal samples of 10% of the dairy goats were taken at random every four weeks on ten farms from February until September 2015. The samples were pooled and the faecal egg count was determined using the McMaster method and expressed in eggs per gram (EPG). A mean faecal egg count was also determined per farm. Positive samples were used to start a coproculture. The Baermann technique was used to obtain L3 larvae for identification.

Results - Infection levels of gastrointestinal nematodes varied considerably between farms: 40 % of the farms did not have any infection, while in the infected farms the mean faecal egg count exceeded 1120 EPG. Pasture and feed management could explain the differences in infection level. As expected, the most abundant worm species found in the coprocultures was *Haemonchus contortus*. *Teladorsagia circumcincta*/*Trichostrongylus* spp. were also found quite regularly.

HAEMOSTATIC CHANGES OCCUR IN VIVO ALREADY IN THE EARLY NON-HEPATOSPLENIC PHASE OF SCHISTOSOMIASIS

Mirjam M. Mebius^{1,2}, Akim A. Adegnika^{3,4,5}, Jeannot F. Zinsou^{3,4}, Jean-Claude Dejon Agobe^{3,4}, Claudia J. de Dood⁵, Maria Yazdanbakhsh⁵, Govert J. van Dam⁵, Paul L. A. M. Corstjens⁵, Aloysius G. M. Tielens^{1,6}, Philip G. de Groot², Rolf T. Urbanus², Jaap J. van Hellemond¹

1. Dept. Medical Microbiology & Infectious Diseases, Erasmus University Medical Center, Rotterdam, The Netherlands; 2. Dept. Clinical Chemistry & Haematology, University Medical Center, Utrecht, The Netherlands; 3. Centre de Recherches Médicales de Lambaréné (CERMEL), BP118 Lambaréné, Gabon; 4. Institut für Tropenmedizin, Universität Tübingen, Tübingen, Germany; 5. Dept. Parasitology, Leiden University Medical Center, Leiden, The Netherlands; 6. Dept. Biochemistry & Cell Biology, Fac. Veterinary Medicine, Utrecht University, The Netherlands

Background - Schistosomiasis, caused by parasites of the *Schistosoma* genus, is the second major parasitic disease after malaria. Haemostatic abnormalities, such as thrombocytopenia, increased von Willebrand Factor antigen (VWF:ag) levels, decreased levels of coagulation factors, and increased fibrinolysis, are observed in hepatosplenic schistosomiasis. It is known that in the non-hepatosplenic early phase of the disease platelet counts are normal. However, it is unclear whether other haemostatic changes observed in the hepatosplenic form of the disease are also absent in early schistosomiasis.

Methods - Citrate plasma was obtained from ten individuals with non-hepatosplenic schistosomiasis haematobium and four healthy controls recruited from the Lambaréné area in Gabon. *Schistosoma* urine egg count and circulating anodic antigen (CAA) levels were used to confirm an ongoing infection. Levels of VWF:ag, active VWF, ADAMTS13 antigen (ADAMTS13:ag) and osteoprotegerin (OPG) were measured in plasma with ELISA. ADAMTS13 activity was determined with FRETs-VWF73 substrate and ristocetin co-factor activity (VWF:RCo) was assessed with BC-VWF reagent.

Results - VWF:ag and active VWF levels were elevated in individuals with non-hepatosplenic schistosomiasis haematobium compared to healthy controls ($p=0.002$ and $p=0.004$, respectively). The percentage of active VWF was slightly decreased in patients compared to controls ($p=0.024$). No abnormalities in the VWF degrading protease ADAMTS13 were observed: ADAMTS13:ag and

ADAMTS13 activity were normal in both patients and healthy controls. VWF:RCo was similar between patients and healthy controls and platelet counts were normal in all individuals. Increased VWF:ag levels could be caused by inflammation-mediated endothelial activation as OPG levels, a marker of inflammation-mediated endothelial activation, were elevated in patients versus healthy controls.

Discussion - In plasma of patients in the early, non-hepatosplenic phase of schistosomiasis haematobium VWF:ag, active VWF and OPG levels are elevated, whereas platelet counts, and ADAMTS13:ag and activity are normal. This indicates that *in vivo* inflammation-mediated endothelial activation and haemostatic changes occur already early in infection.

MOLECULAR MALARIA DIAGNOSTICS FOR FIELD CONDITIONS: DEVELOPMENT AND VALIDATION OF A MULTIPLEX DIRECT ON BLOOD PCR-NUCLEIC ACID LATERAL FLOW IMMUNOASSAY FOR THE DETECTION AND DIFFERENTIATION OF PLASMODIUM SPECIES

Johanna M. Roth, Laura M. de Bes, Pètra F. Mens, Henk D.F.H. Schallig
Royal Tropical Institute (KIT), KIT Biomedical Research, Amsterdam, The Netherlands

Accuracy of malaria microscopy and rapid diagnostic tests (RDTs) decreases at low parasite densities, which is challenging especially in areas with declining prevalence and warrants the search for highly sensitive tests. Molecular tools may be a suitable alternative, although costs and technical requirements currently hamper their implementation in resource limited settings.

To overcome these limitations, a multiplex direct on blood PCR was developed, which uses a nucleic acid lateral flow immunoassay as a simple read-out system (db-PCR-NALFIA). Multiplex db-PCR-NALFIA does not require DNA extraction and can differentiate between pan-Plasmodium, *P. falciparum* and *P. vivax*. Previous work on a similar but monoplex test format showed excellent results in field evaluations.

The multiplex db-PCR-NALFIA was laboratory-validated on PCR-confirmed patient samples, including UK returning travelers (n=128) and negative controls from the Dutch blood bank (n=41). Sensitivity was 100% and specificity 98% against reference standard nested PCR. Species included *P. falciparum*, *P. vivax*, *P. malariae*, *P. ovale* and mixed infections. Five discrepancies in species composition were observed between db-PCR-NALFIA and the reference standard. Of these discrepancies, multiplex db-PCR-NALFIA identified three *P. malariae* or *P. ovale* samples as *P. falciparum*, one *P. falciparum* infection as *P. falciparum*/*P. vivax* mixed infection and one *P. falciparum*/*P. malariae* mixed infection as pan-Plasmodium only. The limit of detection (LoD) was determined to be 1 parasite/ μ l, by real-time PCR.

In conclusion, multiplex db-PCR-NALFIA has excellent analytical accuracy and is ready for further field evaluations.

PREVALENCE OF MUTATIONS ASSOCIATED WITH SULPHADOXINE-PYRIMETHAMINE (SP) RESISTANCE IN *PLASMODIUM FALCIPARUM* SAMPLES FROM THE GENERAL POPULATION AND PREGNANT WOMEN IN NANORO, BURKINA FASO

E Ruizendaal¹, HDFH Schallig¹, PF Mens^{1,2}

1. KIT Biomedical Research, Royal Tropical Institute, Amsterdam, The Netherlands; 2. Centre of Tropical Medicine and Travel Medicine, Academic Medical Centre, University of Amsterdam, the Netherlands

Pregnant women are at increased risk of *Plasmodium falciparum* infection, which can result in maternal anemia and low birth weight babies. Most Sub-Saharan African countries have therefore implemented intermittent preventive treatment during pregnancy using sulphadoxine-pyrimethamine (SP). However, concerns are rising about its efficacy because of increasing SP resistance. Combinations of point mutations in the *Dhps* and *Dhfr* genes of *P. falciparum* are associated with resistance, especially the triple *Dhfr* (51I, 59R, 108N), the double *Dhps* (437G, 540E) and moreover the quintuple mutant. Our study aim was to estimate the current levels of SP resistance in Nanoro, Burkina Faso and to test whether mutation rates increase during pregnancy.

Filter paper samples from pregnant women at first antenatal care visit (ANC1) and at delivery were collected as part of an intervention trial (COSMIC). Samples from the general population (GP) were collected in a cross-sectional survey. After DNA extraction nested PCR was used to amplify the *Dhps/Dhfr* genes and products were sent for sequencing. We found a high prevalence of *Dhfr*-51, -59 and -108, with a trend of higher levels in GP and delivery samples compared with ANC1 samples (70.7%, 74% and 61.5% triple *Dhfr* mutants respectively). Statistical analyses will follow, but this trend could possibly indicate selection of resistant parasites during pregnancy. However, the concurrent higher mutation rate in GP samples needs to be explored. *Dhps*-437 also showed high mutation rates (89.4%, 84% and 83.4% respectively) without a clear trend. The *Dhps*-540 mutation was found in one GP sample and two delivery samples, of which one was a quintuple mutant. To our knowledge this is the first time the *Dhps*-540 mutation is found in Burkina Faso. This finding plus the high prevalence of the other mutations raises concerns about the efficacy of IPTp-SP in the future. Other drug combinations to tackle malaria in pregnancy should therefore be explored.

CONTROL OF THE ENDOSYMBIONT *SODALIS GLOSSINIDIUS* BY THE TSETSE FLY INNATE IMMUNITY

Katrien Trappeniers¹, Linda De Vooght¹, Irina Matetovici¹, Jan Van Den Abbeele¹

1. Institute of Tropical Medicine, Antwerp, Belgium

Background - Many pathogens go through an obligatory developmental cycle within blood feeding arthropod vectors. Here, successful transmission depends on encountering the host's innate immunity. Studies demonstrate that symbiotic microorganisms stimulate insect immunity, to prevent outgrowth of the microbiota. Interestingly, this immune activation can also affect vector competence and pathogen transmission. Tsetse flies, the main vector of African trypanosomes, harbor a recently established bacterial endosymbiont, *Sodalis glossinidius*. However, its biological role as well as its control by the tsetse innate immunity represent a true black box.

Methods - In order to gain insight in the innate immune response of the tsetse fly towards *Sodalis*, we used a whole transcriptome approach by RNA-sequencing (RNA-Seq). Here, we compared transcriptomes of different experimental series of *G.morsitans morsitans* flies: wild-type versus *Sodalis*-cleared flies; experimentally exposed to *Sodalis* or *E.coli*. Moreover, we established a *Sodalis*-free tsetse fly colony allowing us to determine the impact of the bacterial absence on the fly longevity and reproductive capacity.

Results - RNA-Seq analysis indicated strong immune activation to *E.coli*, 134 genes differentially expressed compared to the sterile saline control. Mainly the immune deficiency pathway (IMD) pathway, key pathway to Gram-negative bacteria, was induced. Indeed, peptidoglycan recognition protein LC (PGRP-LC) and two antimicrobial peptides, attacin and cecropin, were significantly expressed. On the other hand, experimental exposure to *Sodalis* showed no significantly expressed tsetse innate immunity genes. Moreover, a significant better survival and reproductive capacity was observed in *Sodalis*-free flies.

Discussion - As expected, tsetse flies react strongly upon *E.coli*. However, flies exposed to *Sodalis* show less immune stimulation. This suggests that *Sodalis* is less recognized by the tsetse immune system than the exogenous *E.coli*. Further functional experiments will clarify this hypothesis. In addition, our preliminary data suggest that the presence of *Sodalis* has a negative impact on the fly longevity and reproduction.

DYNAMICS OF ANTI-GLYCAN ANTIBODY RESPONSES IN *SCHISTOSOMA JAPONICUM*-INFECTED RHESUS MACAQUES STUDIED BY SCHISTOSOME GLYCAN MICROARRAY

Y.Y. Michelle Yang¹, Xiao Hong Li², Niels-Christian Reichardt³, R. Alan Wilson⁴, Angela van Diepen¹ and Cornelis H. Hokke¹

1. Department of Parasitology, Leiden University Medical Center, Leiden, Netherlands; 2. MOH National Institute of Parasitic Diseases, Chinese Center for Disease Control and Prevention, Shanghai, China; 3. CIC biomaGUNE, San Sebastian, Spain; 4. Centre for Immunology & Infection, University of York, York, UK

Background - Human immunity to the parasitic disease schistosomiasis requires many years of exposure to develop. Unlike humans, rhesus macaques clear an established infection naturally and become immune towards reinfection. In macaques, egg production decreases at 8 weeks post-infection and by week 22, physiological impairment of the worm caused by undefined antibody-mediated processes is observed. Since strong responses are observed against schistosome glycan antigens in human and animal infections, anti-glycan antibodies were studied.

Methods - Serum IgG and IgM of *S. japonicum*-infected macaques from in a longitudinal study of 22 weeks were analyzed on a glycan microarray containing a large repertoire of glycans from various life-stages of schistosomes. Additionally, an in vitro schistosomula killing assay was used to investigate the killing potential of infected macaque sera and glycan-directed monoclonal antibodies.

Results - Profound increase in IgG was observed 8 weeks post-infection mainly towards glycans that expressed (multi-)fucosylated terminal LDN and LeX motifs. The extent of glycan fucosylation was proportional to its antigenicity. Interestingly, even though many IgG and IgM responses have declined 22 weeks post-infection, IgG towards cercarial O-glycans with highly fucosylated LDN motifs remained. Moreover, macaque serum taken at later infection time points caused more rapid schistosomulae death in vitro than serum from earlier time points. To consolidate this schistosomula killing observed is contributed by anti-glycan antibodies in macaque serum. We showed that monoclonal antibodies against highly fucosylated LDN motifs were able to kill schistosomula in vitro in a concentration dependent manner, while mAbs against LeX motifs could not.

Discussion - Our observations indicate that schistosome infected macaques develop protective antibodies, possibly against schistosomula surface glycans. Our data also suggests that IgG against highly fucosylated LDN motifs that are sustained when worms deteriorate may be associated with infection clearance and resistance to re-infection in macaques.

IS INTESTINAL PARASITE INFECTION ASSOCIATED WITH OBESITY ? AN ECOLOGICAL ANALYSIS IN MEXICO

Zavala GA^{1,2}, Doak CM¹, Portrait F¹, García OP², Rosado JL², Jarquín T², Polman K^{1,3}, Campos-Ponce M¹

¹ Faculty of Earth and Life Sciences, VU Amsterdam University; ² Facultad de Ciencias Naturales, Universidad Autónoma de Querétaro; ³ Department of Biomedical Sciences, Unit of Medical Helminthology, Institute of Tropical Medicine, Antwerp

Background - Obesity is a worldwide healthcare challenge. Recent studies have shown an association between both viral and bacterial infection with obesity. However, studies on the association between intestinal parasites and obesity are scarce. The aim of this ecological study is to evaluate the association between the approximated probability of infection with *Ascaris lumbricoides* (*A. Lumbricoides*) or intestinal protozoa (all reported intestinal protozoa excluding *Entamoeba histolytica* or *Giardia lamblia*) in 2000, 2006 and 2012 with BMI for age z-score (BMIz) in 2012 in Mexico.

Methods - For this purpose, we used publicly available individual-level data for BMIz in 2012 and state-level data on the incidence of infection with *A. Lumbricoides* or intestinal protozoa in 2000, 2006 and 2012 as a proxy for probability of infection.

Results - A higher proximate probability of infection in 2012 with *A. Lumbricoides* was associated with a lower BMIz in 2012. In contrast a higher proximate probability of infection in 2012 with intestinal protozoa was associated with a higher BMIz in 2012. A higher proximate probability of infection with *A. lumbricoides* and intestinal protozoa in 2000 and 2006 were associated with an increased BMIz in 2012.

Discussion - Our results suggest that there may be species specific effects of intestinal parasitic infection that may have both, short and long term consequences on health. Further research is needed to confirm these ecological associations and study the possible mechanisms. These findings have important implications for Mexico, given the context of a high incidence of parasitic infection and emerging obesity epidemic.