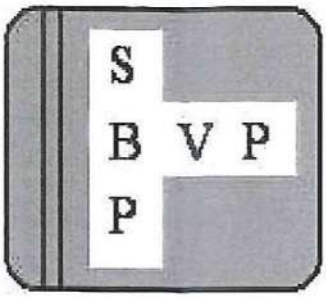


"One Health"

Joint Spring Meeting 2010

Belgian Society for Parasitology
Dutch Society for Infectious Diseases
Dutch Society for Parasitology

Thursday, June 3, 2010

Conference center Koningshof
Locht 117, 5504 RM Veldhoven, the Netherlands

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Program Spring meeting 2010

"ONE HEALTH"

DUTCH SOCIETY FOR PARASITOLOGY (NVP)
BELGIAN SOCIETY FOR PARASITOLOGY (BSP)
DUTCH SOCIETY FOR INFECTIOUS DISEASES (VIZ)

9.30 Welcome, coffee and registration

Parkzaal

10.15 Keynote lecture 1. The One Health Concept
Jakob Zinsstag (Swiss TPH, Basel, Switzerland)

10.45 Keynote lecture 2. Control of cysticercosis,
Pierre Dorny (ITG, Antwerp, Belgium)

11.15 Coffee break

11.45 Keynote lecture 3. The Q fever outbreak in the Netherlands
Yvonne van Duynhoven (RIVM, Bilthoven, NL)

12.15 Keynote lecture 4. Current status of anthelmintic resistance in parasitic nematodes of veterinary and medical importance.
Peter Geldhof (Univ. Gent, Belgium)

12.45 Janssen Animal Health award of the BSP

12.50 Lunch break + poster session + commercial activities

13.30 BSP and NVP annual member meetings (BSP in Parkzaal, NVP in zaal 83)

14.00 Parallel sessions
Parkzaal: session `Infectious diseases and clinical parasitology`
Zaal 83: session `General parasitology`

15.30 Tea break

16.00 Continuation of parallel sessions

16.40 Merial Award ceremony and lecture

17.10 Drinks

17.45 Dinner (optional)

20.00 Farewell

Program parallel afternoon sessions (12 min. presentations)

Parkzaal Infectious Diseases and clinical parasitology

14.15 Gijs Baaten (GGD Amsterdam, the Netherlands)
Fecal-orally transmitted diseases among travelers are decreasing due to better hygienic standards at travel destination.

14.30 Gini van Rijckevorsel (GGD Amsterdam, the Netherlands)
Incidence and trends of imported malaria in the Netherlands: 2000-2007.

14.45 Meta Roestenberg (Radboud University Nijmegen, the Netherlands)
Long lasting immunity and protection against *Plasmodium falciparum* malaria in human volunteers

15.00 Laetitia Lempereur (Univ. Liege, Belgium)
First molecular evidence of potentially zoonotic *Babesia microti* and *Babesia sp.* EU1 in *Ixodes ricinus* ticks in Belgium.

15.15 Kim Vereecken (ITG, Antwerpen, Belgium)
The influence of atopy and helminth infection on total IgE in Cuban schoolchildren.

15.30 Emmanuel Assana (ITG, Antwerp, Belgium)
Elimination of *Taenia solium* transmission to pigs in a field trial of the TSOL18 vaccine in Cameroon.

15.45 Tea break

16.15 Johnny Vlaminck (Ghent Univ., Belgium)
Immunization of pigs with *Ascaris suum* hemoglobin increases the immunological reaction against liver stage larvae but fails to induce a protective immunity.

16.30 Gijs Baaten (GGD Amsterdam, the Netherlands)
Travel-related schistosomiasis, strongyloidiasis, filariasis and toxocariasis: the risk of infection and the diagnostic relevance of blood eosinophilia.

Zaal 83 General parasitology

14.15 Ron Hokke (LUMC, Leiden, the Netherlands)
Antigenic glycans of schistosomes.

14.30 Lynn Meurs (ITG, Antwerp, Belgium)
Pro- and anti-inflammatory consequences of TOLL-like receptor ligation in Gabonese *Schistosoma haematobium* infected schoolchildren.

14.45 Carmen Aranzamendi (RIVM, Bilthoven, the Netherlands)
Time course of *Trichinella spiralis* infection and its effect on experimental allergic airway inflammation.

15.00 Michiel E. Janssens (ITG, Antwerp, Belgium)
FRET in RT-PCR: a fascinating way to diagnose.

15.15 Ely Bénéré (Univ. Antwerp, Belgium)
Novel laboratory tools for virulence studies of different assemblages of *Giardia duodenalis*.

15.30 Viki Bockstal (VIB, Brussels)
Trypanosoma brucei infection induces Fas (CD95)-dependent apoptosis of transitional B cells.

15.45 Tea break

16.15 Krystelle Nganou Makamdop (Radboud University Nijmegen, the Netherlands)
Immunization by both chloroquine and radiation attenuated *Plasmodium berghei* malaria parasites induce strong memory CD8+ T-cell responses.

16.30 Mayke Oesterholt (Radboud University Nijmegen, the Netherlands)
Cellular immunological responses in pregnancy associated malaria.

KEYNOTE LECTURES

THE ONE HEALTH CONCEPT

Jakob Zinsstag
Swiss Tropical and Public Health Institute, Socinstr. 57, PO Box, 4002 Basel, Switzerland.

CONTROL OF CYSTICERCOSIS

Pierre Dorny
Inst. Tropical medicine, Antwerp, Belgium

THE Q FEVER OUTBREAK IN THE NETHERLANDS

Yvonne van Duynhoven
RIVM, Bilthoven, the Netherlands

CURRENT STATUS OF ANTHELMINTIC RESISTANCE IN PARASITIC NEMATODES OF VETERINARY AND MEDICAL IMPORTANCE

Geldhof P., A. El-Abdellati, De Graef J., Levecke B., Claerebout E., Vercruysse J.
Laboratory for Parasitology, Faculty of Veterinary Medicine, Ghent University, Salisburylaan 133, 9820 Merelbeke, Belgium

Parasitic nematodes are worldwide, both in animals and humans, among the most common pathogens. Control of these parasites relies almost completely on the use of anthelmintic drugs. However, the intensive and frequent use of these drugs during the last decades has resulted in the development of resistance in many different nematode species. Cases of multiple drug resistance in nematodes of small ruminants have been reported globally and recent studies show that resistance in cattle and horses is also emerging. It is possible that a similar situation might develop for human parasites, although currently there is still no conclusive data that resistance alleles have been selected and that these alleles have spread in human parasite populations. Nevertheless, there are already worrying signs that anthelmintic efficacy may be declining and unless the problem is taken seriously and appropriate measures are implemented to prevent further decline in efficacy, medical doctors in the next decade may face similar problems to those faced by veterinarians at this time. At present the lack of simple and reliable tests for the detection of resistant nematodes seriously compromises our ability to monitor the spread of resistance. We are currently dependent on biological methods which are not sufficiently sensitive to detect low levels of drug resistance. Moreover, the only test that is currently available to detect resistance against all anthelmintic classes (faecal egg count reduction test) is labour-intensive, which may explain why only few large scale surveys have been conducted. The development of more sensitive and user-friendly diagnostic tests would be greatly aided by an understanding of the genetic and/or biochemical basis of resistance. An overview of the research recently performed in this area will be presented and discussed.

ORAL PRESENTATIONS

AFTERNOON SESSIONS

FECAL-ORALLY TRANSMITTED DISEASES AMONG TRAVELERS ARE DECREASING DUE TO BETTER HYGIENIC STANDARDS AT TRAVEL DESTINATION

Baaten G.G.G. [1,2,3,*], Sonder G.J.B.[1,2,3], Schim van der Loeff M.F.[1,2], Coutinho R.A.[1,2,4], Van den Hoek, J.A.R.[1,2]

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2 Department of Internal Medicine, Division of Infectious Diseases, Tropical Medicine and AIDS, Academic Medical Centre, Meibergdreef 9, 1105 AZ Amsterdam, The Netherlands
3 National Coordination Centre for Traveler's Health Advice (LCR), Nieuwe Achtergracht 100, PO Box 1008, 1000 BA Amsterdam, The Netherlands
4 Centre for Infectious Disease Control, National Institute for Public Health and the Environment, Antonie van Leeuwenhoeklaan 9, 3721 MA Bilthoven, The Netherlands
* Correspondence to: G.G.G. Baaten, Dept. Infectious Diseases, Public Health Service (GGD) Amsterdam, PO Box 2200, 1000 CE Amsterdam, The Netherlands. E-mail: gbaaten@ggd.amsterdam.nl

Objective
To evaluate whether changes in attack rates of fecal-orally transmitted diseases among travelers are related to changes in pre-travel vaccination practices or better hygienic standards at travel destination.

Methods
National surveillance data on all laboratory-confirmed cases of travel-related hepatitis A, shigellosis, and typhoid fever diagnosed in The Netherlands from 1995 through 2006 were matched with the numbers of Dutch travelers to developing countries to calculate region-specific annual attack rates. Trends in attack rates of non-vaccine-preventable shigellosis were compared with those of vaccine-preventable hepatitis A and typhoid fever. Trends were also compared with three markers for hygienic standards of the local population at travel destinations, drawn from the United Nations Development Programma database: the human development index, the sanitation index, and the water source index.

Results
Attack rates among Dutch travelers to developing regions declined for both hepatitis A, shigellosis, and typhoid fever. Region-specific trends in attack rates of shigellosis resembled trends of hepatitis A and typhoid fever.
Declining attack rates of the three fecal-orally transmitted diseases correlated with improvements in socioeconomic, sanitary, and water supply conditions of the local population at travel destination.

Conclusions
These findings suggest that improved hygienic standards at travel destination strongly contributed to the overall decline in attack rates of fecal-orally transmitted diseases among visiting travelers.

INCIDENCE AND TRENDS OF IMPORTED MALARIA IN THE NETHERLANDS: 2000-2007

Van Rijckevorsel G.[1,2], Sonder G.[1,2,3], Geskus R.[1,4], Van Genderen P.[5], Keuter M..[6], Ligthelm R.[7], Visser L.[8], Wetsteyn J.[3], Van den Hoek A.[1,3]

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5) Department of Internal Medicine, Harbour Hospital and Institute for Tropical Diseases, Haringvliet 2, 3011 TD Rotterdam, the Netherlands
6) Radboud University Nijmegen Medical Center, Department of Medicine, Division of General Internal Medicine, P.O Box 9101, 6500 HB Nijmegen, The Netherlands
7) Tropvacc BV, 's Gravenweg 53, 3062 ZB Rotterdam, The Netherlands. Chairman Malaria Board.
8) Leiden University Medical Centre, Department of Infectious Disease, Section Travel Medicine, P.O Box 9600, 2300 RC Leiden, The Netherlands.

Background:
To describe the epidemiology and trends of imported malaria in the Netherlands from 2000 through 2007.

Methods:
Based on national surveillance data regarding all reported infections of imported malaria, diagnosed 2000 through 2007, incidence and trends were estimated, using as denominator the number of Dutch travellers visiting malaria-endemic countries. The annual number of prescriptions for malaria chemoprophylaxis, collected from pharmacies, was used to estimate the number of unprotected travellers.

Results:
The number of imported malaria infections fell from 535 in 2000 to 197 in 2007. Most infections (72%) were acquired in Sub-Saharan Africa, and 75% were caused by *Plasmodium falciparum*. Meanwhile, travellers to malaria-endemic countries increased from 247,000 to 384,000, and chemoprophylaxis prescriptions increased from 131,400 to 186,300 per year (53% and 48% of all travellers, respectively). The number of unprotected travellers rose from 115,600 to 197,700, yet cases of *falciparum* malaria fell from 21.5 to 6.6/10,000 of unprotected travellers. These infections came mainly from Middle and West Africa but have decreased from 121.3 to 36.5/10,000 travelers. Importation of malaria from this region by immigrants visiting friends and relatives (VFR) decreased from 138 to 69 from 2000 to 2007.

Conclusion:
Importation of malaria to the Netherlands is declining even as more travellers visit malaria-endemic countries. This decline is not readily explained by increased use of chemoprophylaxis and may reflect a reduced risk of infection due to decreasing local malaria transmission as observed in some malaria endemic areas. Nevertheless, the increasing number of travellers not using malaria chemoprophylaxis remains worrisome.

LONG LASTING IMMUNITY AND PROTECTION AGAINST PLASMODIUM FALCIPARUM MALARIA IN HUMAN VOLUNTEERS

M. Roestenberg[1], A. Teirlinck[1], M. McCall[1], K. NganouMakamdop[1], K. Teelen[1], T. Arens[1], P. Beckers[1], J. Wiersma[1], G.J. van Gemert[1], M. van de Vegte-Bolmer[1], A. van der Ven[2], A.J.F. Luty[1], C. Hermsen[1], R. Sauerwein[1]
1. Department of Medical Microbiology, Radboud University Nijmegen Medical Center,
2. Department of Internal Medicine, Radboud University Nijmegen Medical Center
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The induction of long-term persisting immune responses that convey protection against *Plasmodium falciparum* malaria is a major hurdle in malaria vaccine development. Previously, we showed that sterile immunity can be induced in a well-controlled setting by exposing human volunteers to *P. falciparum* sporozoites whilst taking chloroquine prophylaxis. The bites of only 15 infectious mosquitoes on three occasions were sufficient to achieve this protection. The exposure to liver- and, briefly, blood-stage antigens under these conditions lead to the induction of a parasite-specific effector memory T-cell response, as measured by ex vivo parasite stimulation assays.

Six of ten previously protected volunteers were followed for a period of 28 months post-infection and were then re-challenged by the bites of five *P. falciparum*-infected mosquitoes. We found that specific effector memory T-cell responses to both pre-erythrocytic and asexual stage *P. falciparum* parasites persisted over a period of more than 2 years in the protected volunteers. In parallel, humoral immune responses initially detectable in only some volunteers, had waned. Four of the six previously immunized volunteers were fully protected against the re-challenge, whilst the two susceptible volunteers showed markedly delayed patency.

The persistence of sterile immunity in human volunteers over a period of more than two years is unprecedented. It reveals that the maintenance of protection, at least in this controlled experimental setting, is not dependent on full-blown or persisting blood stage infection. These results thus provide a glimpse into the promising future for whole-parasite vaccines for malaria.

FIRST MOLECULAR EVIDENCE OF POTENTIALLY ZOONOTIC BABESIA MICROTI AND BABESIA SP. EU1 IN IXODES RICINUS TICKS IN BELGIUM.

Laetitia Lempereur[1*], Ann De Cat[2], Yannick Caron[1], Maxime Madder[3], Edwin Claerebout[2], Claude Saegerman[4], Bertrand Losson[1].
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[2] Laboratory of Parasitology, Faculty of Veterinary Medicine, Ghent University, B-9820 Merelbeke (Belgium).
[3] Department of Animal Health, Institute of Tropical Medicine, B-2000 Antwerp (Belgium).
[4] Epidemiology and Risk analysis applied to the Veterinary Sciences, Department of Infectious and Parasitic Diseases, Faculty of Veterinary Medicine, University of Liège, B-4000 Liège (Belgium).

We report the first molecular evidence of the presence of *Babesia* sp. EU1 and *Babesia microti* in *Ixodes ricinus* ticks in Belgium. A one-year national survey collected 1005 ticks from cats and dogs. A PCR technique amplifying a part of the 18S rRNA gene detected *Babesia* spp. in eleven out of 841 selected and validated tick extracts. Subsequent sequencing identified *B. microti* (n=3) and *Babesia* sp. EU1 (n=6). This study has demonstrated a low infection rate (1.31% with 95% CI: 0.65-2.33) of *Babesia* spp. carriage in *I. ricinus* ticks in Belgium but reports for the first time two potentially zoonotic species belonging to this genus. Co-infection with *B. microti* and *Borrelia burgdorferi* sensu stricto also was demonstrated. In addition, this study clearly demonstrates that inhibitors of PCR amplification are present in engorged ticks.

THE INFLUENCE OF ATOPY AND HELMINTH INFECTION ON TOTAL IGE IN CUBAN SCHOOLCHILDREN

KIM VEREECKEN [1], KIREZI KANOBANA [1], MEIKE WÖRDEMANN[1], LENINA T MENOCAL HEREDIA[2], RAQUEL JUNO DIAZ[2], ANIRAN RUIZ ESPINOSA[3], LAZARA ROJAS RIVERO[3], MARIANO BONET GORBEA[2], KATJA POLMAN[1, 4]

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[3] Institute Pedro Kouri, Havana, Cuba (IPK)
[4] VU University Amsterdam, Amsterdam, the Netherlands

Background:
Total IgE levels are usually elevated in atopic individuals, and have been used as a diagnostic test for atopy/atopic disease. Total IgE is also raised in parasite infection. There are recent concerns about the validity of using total serum IgE to define atopy in helminth endemic countries. Here, we investigate the usefulness of total IgE in the evaluation of atopy in Cuban schoolchildren.

Objectives:
The aim of the study was to determine the prevalence of (different measures of) atopy and intestinal helminth infections in Cuban schoolchildren, and to study the influence of atopy and helminths on total IgE levels.

Methods:
A cross-sectional study in Cuban school aged children was performed. A total of 1049 children of 4-14 years old were included. We determined serum total IgE levels, atopy by skin prick testing (SPT - against *Dermatophagoides pteronyssinus* (Dpt), *D. farinae* (Df), cat dander, mixed tree, mixed grass, *Alternaria alternate* and cockroach allergen) and allergen-specific IgE antibodies (sIgE - against *Dpt*, *Blomia tropicalis* and cockroach allergen), and intestinal helminth infection by stool examination (*Ascaris lumbricoides*, *Trichuris trichiura*, *Ancylostoma duodenale*).

Results:
Of the 1049 children, 20.4% had intestinal helminth infections, 88.6% were positive for total IgE, 44,2% for sIgE, and 21.4% were SPT positive. Atopic sensitization was found predominantly for cockroach and house dust mites. The median value of total IgE in our study population was 372 kU/L. Total IgE correlated well with atopy as based on positive SPT (P=0.000) and sIgE (P=0.000), and median total IgE levels were significantly higher in atopic children than in non-atopic children (P=0.000), irrespective of the helminth infection status. Likewise, total IgE was positively correlated with helminth infections (P=0.000), and median total IgE levels were significantly higher in helminth positive than in helminth negative children (P=0.000), irrespective of their atopic status. Looking at the influence if atopy and helminth infections on total IgE more closely, the decrease in median total IgE levels in atopic children as compared to non-atopic children, was much more pronounced for sIgE than for SPT, and for helminth positives than for helminth negatives.

Conclusion:
Our study results demonstrate that total IgE levels in Cuban children are influenced by their atopic as well as their helminth infection status. These data confirm other observations on the limited value of total IgE for the diagnosis of atopy in helminth endemic areas; testing for sIgE or SPT against individual allergens would be preferred.

ELIMINATION OF *TAENIA SOLIUM* TRANSMISSION TO PIGS IN A FIELD TRIAL OF THE TSOL18 VACCINE IN CAMEROON

Emmanuel Assana[a], Craig T Kyngdon[b], Charles G. Gauci[b], Stanny Geerts[a], Pierre Dorny[a], Redgi De Deken[a], Garry A. Anderson[b], André P. Zoli[c], Marshall W. Lightowlers[b,*]

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[c]University of Dschang, Faculty of Agronomy and Agricultural Sciences P.O. Box 222, Dschang, Cameroon.

A pilot field trial of the TSOL18 vaccine was undertaken in free-roaming pigs in the Mayo-Danay district of Far North Cameroon. Two hundred and forty, 2-3 month old piglets were distributed to 114 individual households in pairs, with one animal of each pair being vaccinated and the other acting as a non-vaccinated control. Vaccinated animals received two initial immunizations intramuscularly in the neck one month apart with 200 μg TSOL18 plus 5 mg Quil A. At the time of the second immunization both vaccinated and control animals received an oral dose of 30 mg/kg oxfendazole. Vaccinated animals received a third immunization approximately 3 months after the first immunization. Antibody responses to the vaccine were assessed in serum samples by ELISA. Necropsies were undertaken when the pigs were approximately 12 months of age. Parasites were counted in half the body musculature and in the brain. Two hundred and twelve animals were available for necropsy at the end of the trial (110 vaccinated; 102 controls). Viable *T. solium* cysticerci were identified in 20 control pigs (prevalence 19.6%), including 14 animals that had estimated total body burdens of >1000 cysticerci. No cysticerci were found in any of the vaccinated animals indicating that the vaccine provided a very high level of protection ($P<0.0001$) against naturally acquired infection with *T. solium* in pigs. Combined application of TSOL18 vaccination and a single oxfendazole treatment in pigs is a simple and relatively sustainable procedure that has the potential to control *T. solium* transmission in endemic areas and, indirectly, reduce the number of new cases of neurocysticercosis in humans.

IMMUNISATION OF PIGS WITH *ASCARIS SUUM* HEMOGLOBIN INCREASES THE IMMUNOLOGICAL REACTION AGAINST THE LIVER STAGE LARVAE BUT FAILS TO INDUCE A PROTECTIVE IMMUNITY.

Johnny Vlaminck[1], Maria Martinez-Valladares[1], Sylvia Dewilde[2], Luc Moens[2], Kelly Tilleman[3], Joseph Urban[4], Edwin Claerebout[1], Jozef Vercruysse[1], Peter Geldhof[1]

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[3] Department of Biomedical Sciences, Antwerp University, Universiteitsplein 1, B-2610 Antwerp, Belgium.
[4] Laboratory of Pharmaceutical Biotechnology, Faculty of Pharmaceutical Sciences, Ghent University, Harelbekestraat 72, B-9000 Ghent, Belgium.
[5] Diet, Genomics, and Immunology Lab, Beltsville Human Nutrition Research Center, USDA/ARS, 10300 Baltimore Avenue, Beltsville, MD 20705.

In order to determine whether purified *Ascaris suum* hemoglobin (AsHb) is a suitable vaccine candidate for the control of *Ascaris* infections, pigs were vaccinated with AsHb in combination with QuilA as adjuvant and challenged with 3 x 333 infective *A. suum* eggs. The number of liver lesions and worms in the intestine was assessed on day 14, 28. and 56 post-infection (p.i.). No significant differences were found in the number of worms recovered between vaccinated and control pigs on any of these days p.i.. However, significantly more white spots were counted on the livers of vaccinated pigs on day 14 (+86%) and day 28 (+118%) p.i. compared with non-vaccinated controls. In order to investigate whether this increased immuno-reactivity in the liver was triggered by and directed against AsHb, the transcription and expression of AsHb in *Ascaris* larval stages were analysed. RT-PCR analysis showed that the AsHb transcript was barely detectable in freshly egg hatched third stage larvae (L3) and liver stage L3 in comparison with lung stage L3, fourth-stage larvae (L4) in the intestine or adults. In addition, AsHb protein could not be detected in extracts of freshly hatched L3 by immunoblot using AsHb-specific antibodies. Nevertheless, further analysis indicated that immunisation with AsHb induced a cross-reactive antibody response against several larval antigens. This could indicate that the increased white spot development in the vaccinated pigs was caused by L3 antigens released by migratory or dying worms.

TRAVEL-RELATED SCHISTOSOMIASIS, STRONGYLOIDIASIS, FILARIASIS, AND TOXOCARIASIS: THE RISK OF INFECTION AND THE DIAGNOSTIC RELEVANCE OF BLOOD EOSINOPHILIA

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Objective
The risk and extent of helminth infection and the predictive properties of blood eosinophilia in travellers are unknown. This study prospectively assessed the occurrence of clinical and subclinical schistosomiasis, strongyloidiasis, filariasis, and toxocariasis, and the screening value of eosinophilia in travellers from helminth-endemic countries.

Methods
Adult short-term travellers attending a travel health clinic for pre-travel health advice donated blood samples for serology and blood cell count before and after travel. They recorded data on itinerary and symptoms of parasitic diseases in a structured diary. Blood samples were tested for eosinophilia, and for antibodies against *Schistosoma species*, *S. stercoralis*, filarial species, and *Toxocara species*. Previous infection was defined as seropositivity in pre- and post-travel samples. Recent infection was defined as a seropositive post-travel sample with a seronegative pre-travel sample.

Results
Previous infection was found in 112 of 1207 subjects: schistosomiasis in 2.7%, strongyloidiasis in 2.4%, filariasis in 3.4%, and toxocariasis in 1.8%. Eleven of these travellers had evidence for more than one previous infection. Recent schistosomiasis was found in 0.51% of susceptible subjects at risk, strongyloidiasis in 0.25%, filariasis in 0.09%, and toxocariasis in 0.08%. The incidence rate per 1000 person-months was 6.4, 3.2, 1.1, and 1.1, respectively. Recent infections were largely contracted in Asia.
None of the symptoms studied had any positive predictive value (PPV). The PPV of eosinophilia for diagnosis was 15% for previous infection and 0% for recent infection.

Conclusion
The chance of infection with schistosomiasis, strongyloidiasis, filariasis, and toxocariasis during one short-term journey to an endemic area is low. However, previous stay or repeated travel lead to a cumulative risk of infection. Determining the blood eosinophil count appeared to be of no value in routine screening of asymptomatic travellers for the four helminthic infections. Findings need to be replicated in larger prospective studies.

ANTIGENIC GLYCANS OF SCHISTOSOMES

Smit C.H., Meevissen M.H.J., Van Diepen A., Wuhrer M., Deelder A.M. and Hokke C.H.

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Schistosomes infect about 200 million people in (sub-)tropical areas worldwide and cause an enormous burden of disease. Glycans and glycoconjugates expressed by schistosomes play a prominent role in the parasite's biology and the interaction with the human host. Structural data regarding glycosylation of different schistosome life stages and glycoconjugate subsets has been collected in the past decade, but many significant gaps in our knowledge of the schistosomal glycome remain. While previously schistosome glycoconjugates have been identified as diagnostic targets, we currently focus our research on glycans associated with immunity. On the one hand, we study the structure-function relation of glycans of individual immunomodulatory schistosome egg glycoproteins, while on the other hand we perform global glycomics studies of different developmental stages of the schistosome with the aim of identifying novel glycosylated intervention targets.
We have recorded glycoprotein-derived glycan profiles of a range of developmental stages of *S. mansoni*, including cercariae, early/late schistosomula, juvenile/adult worms, eggs and miracidia. These profiles indicated that the expression of many glycans and glycan elements gradually shifts between the different stages, but also glycans that are uniquely expressed in a particular stage were found. It has been established that a large portion of the antibody response to schistosomes is directed against numerous glycan elements. Such elements include *e.g.* multifucosylated LDN-type glycans unique for schistosomes, but also glycans common among helminths and sometimes also the mammalian host occur. It is unclear whether such antibodies may confer any protective mechanisms or not. Therefore, we have printed hundreds of purified glycans and glycan fractions on a glycan micro array. Preliminary studies in which we have screened the glycan array with sera from schistosome-infected individuals to determine the serum antibody response profiles in relation to variables such as infection intensity, duration, and treatment will be presented.

PRO- AND ANTI-INFLAMMATORY CONSEQUENCES OF TOLL-LIKE-RECEPTOR LIGATION IN GABONESE *S. HAEMATOBIUM*-INFECTED SCHOOLCHILDREN

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It is assumed that schistosome infection suppresses the host's immune system by inducing anti-inflammatory or regulatory responses. This has been demonstrated for antigenic stimuli but evidence that schistosomiasis also alters innate immunity is still scarce. We investigated the role of *S. haematobium* infection on cytokine responses to a number of TLR ligands as well as to schistosomal antigens SEA and AWA.

S. haematobium-infected and uninfected schoolchildren from a rural area near Lambaréné, Gabon, were studied. Whole blood as well as peripheral blood mononuclear cells (PBMCs) were incubated for 24h and 72h with TLR ligands and SEA and AWA. Concentrations of the pro-inflammatory cytokine TNF-α and of the anti-inflammatory/regulatory cytokine IL-10 were determined by ELISA. SEA and AWA induced a higher IL-10 response in infected children than in uninfected children. On the other hand, PBMC cultures of infected children produced more TNF-α and significantly higher TNF-α to IL-10 ratios than that of uninfected children in response to LPS, FSL-1 and Pam3, ligands of TLR4, TLR2/6 and TLR2/1 respectively.

As expected, infected children produced more anti-inflammatory cytokines than uninfected children in response to schistosomal antigens. Responses to TLR4, TLR2/6 and TLR2/1 ligands on the other hand, were more pro-inflammatory in infected than in *S. haematobium*-free children. This is the first human study that shows a more pro-inflammatory response to TLR ligands in the face of a more anti-inflammatory adaptive immune response in *Schistosoma*-infected children, which is in concordance with results from murine models. Hence, the universally accepted idea that schistosomiasis infection suppresses the host's immune system does not appear to be true for single TLR ligation. Considering the fact that a whole schistosome is a complex mix of ligands that would stimulate the innate immune system, it is important to further investigate innate cross-talk.

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TIME COURSE OF *TRICHINELLA SPIRALIS* INFECTION AND ITS EFFECT ON EXPERIMENTAL ALLERGIC AIRWAY INFLAMMATION

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Trichinella spiralis is a zoonotic pathogen that infects a wide range of mammalian hosts. The life cycle of this nematode is completed in a single host and resides in two distinct habitats, muscle (parenteral phase) and small intestine (enteral phase). The larvae are released in the stomach, migrate to the small intestine where they maturate into adult worms and release newborn larvae that rapidly disseminate throughout the host, and eventually enter skeletal muscle to remain for many years. Several studies have reported that certain helminth infections down-modulate the immune response in order to survive in the host and by doing so other immunopathologies such as allergy are also suppressed. Since we have previously shown that excretory/ secretory pr0oducts of *T. spiralis* suppress DC activation in-vitro, we aimed at studying the effect of *T.* spiralis infection on experimental allergic asthma. For this purpose, mice were infected orally with 500-muscle larva of *T. spiralis* at two different time-points, followed by ovalbumin (OVA) sensitization and challenge to induce allergic airway inflammation. The two time-points of infection simulating the two phases of *T. spiralis* infection were confirmed microscopically by determining the presence of the parasite in the intestine or muscles. We have found that during the parenteral phase of infection anti-OVA IgE in serum is significantly suppressed while during the enteral phase no changes were observed. However, no effect of *T. spiralis* infection on allergic airway inflammation was observed at any time point. Our results indicate that although systemic suppression of the immune response to a heterologous antigen (OVA) was observed there was no local suppression of allergic airway inflammation. Several factors such as the helminth species, infection of a definitive or accidental host, parasite load, chronic or acute infection and also the habitat in the host where the parasite resides may influence the outcome of helminth infection on other immunopathologies.

FRET IN RT-PCR: A FASCINATING WAY TO DIAGNOSE

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Some members of the *Theileria* spp. are important bovine pathogens in Africa. More specifically, *Theileria parva* and *Theileria annulata* cause the diseases East Coast fever and tropical theileriosis respectively. Correct characterization of these parasites has been a goal for many researchers to increase the knowledge on their epidemiology. Several other very similar parasites exist but their geographical distribution is not well known. Although benign, these pathogens often interfere with successful diagnosis of the pathogenic theilerial species because of their close relatedness. This paper describes the development of a molecular assay based on fluorescence resonance energy transfer and real time PCR to determine the presence of *T. parva* in bovine carrier animals with a piroplasm parasitaemia as low as 4×10^{-5}%. Additionally, this assay can at least differentiate between five other *Theileria* spp. including *T. annulata*. Here we discribe a fast, user-friendly and cost effective assay when compared to the existing diagnostic tests. This assay is a first step to the improvement of the current knowledge of the range of all the theilerial species in cattle and could replace the current labour intensive and time-consuming assays for the detection and differentiation between minimum six theilerial species including *T. parva* and *T. annulata*.

NOVEL LABORATORY TOOLS FOR VIRULENCE STUDIES OF DIFFERENT ASSEMBLAGES OF *GIARDIA DUODENALIS*

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Research on *G. duodenalis* pathogenicity and drug sensitivity depends on establishing *in vitro* trophozoite cultures, which mainly have been obtained for Assemblages A and B. Establishing cultures from faecal cysts remains difficult due to poor *in vitro* excystation and bacterial contamination. An alternative system for isolation has been established in which duodenal trophozoites are obtained from infected gerbils. The study aim was to investigate whether field strains from humans (Assemblages A and B), cats (Assemblage F), dogs (Assemblage D), and cattle (Assemblage E or mixed E/A) were infective for the gerbil and could be established *in vitro* as axenic cultures. Gerbil infection was successful for Assemblages A (1/1) and B (1/3) from humans, and for E (1/2) and mixed E/A (6/6) from cattle. No infections could be obtained with cat (0/3) and dog (0/6) isolates possibly due to low cyst quality. Despite successful isolation of trophozoites from gerbils, some strains subsequently failed or were difficult to establish *in vitro*. Therefore, several medium supplements such as L-glutathione, L-cysteine, *E.coli* lysate and fresh bovine bile were investigated for promoting growth. L-cysteine and ascorbic acid supported cloning of Assemblage A isolates, while L-glutathione promoted Assemblage E. Using this method, several pure cultures of Assemblage A (human and cattle) and E (cattle) were obtained and are now used for *in vitro* drug-sensitivity profiling and detailed studies on *in vitro* and *in vivo* virulence. Isolation of Assemblage B (human), D (dog) and F (cat) failed up till now, but further studies on a larger number of freshly collected isolates should provide more evidence about their infectivity for the gerbil and *in vitro* growth characteristics.

TRYPANOSOMA BRUCEI INFECTION INDUCES FAS (CD95)-DEPENDENT APOPTOSIS OF TRANSITIONAL B CELLS.

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African trypanosomes of the *Trypanosoma brucei* species are extracellular protozoan parasites that cause the deadly disease African trypanosomiasis in humans and contribute to the animal counterpart, Nagana. Trypanosome clearance from the bloodstream is mediated by antibodies specific for their Variant Surface Glycoprotein (VSG) coat antigens. However, *T. brucei* infection severely compromises the humoral immune defense system. It induces polyclonal B cell activation, B cell clonal exhaustion, sustained depletion of mature splenic Marginal Zone B (MZB) and Follicular B (FoB) cells and destruction of the B-cell memory compartment, leading to an inability to control parasitemia. We aim to demonstrate that *T. brucei* infection also affects B lymphopoiesis at different levels and through the induction of massive transitional B cell apoptosis in the spleen the parasite prevents replenishment of the mature B2 B cell pool and hence facilitates a sustained infection. Using a C57Bl/6 mouse model for *T. brucei,* we investigated B cell development and maturation at different time points of infection by flowcytometry and real-time PCR. Our results show a more than 95% reduction in B cell precursor numbers from the CLP, pre-pro-B, pro-B, pre-B and immature B cell stages in the bone marrow, together with a significant reduction in CXCL12 retention signals. In the spleen, *T. brucei* infection induces extramedullary B lymphopoiesis, as evidenced by a significant increase in HSC-LMPP, CLP, pre-pro-B, pro-B and pre-B cell populations. However, final B cell maturation is limited by infection-induced apoptosis of transitional B cells of both the T1 and T2 populations. In conclusion our results suggest that (i) disruption of B cell development in the bone marrow results from a loss of CXCL12 retention signals and (ii) infection-induced apoptosis of T1 and T2 transitional B cells is mediated by the death receptor Fas (CD95), and occurs independently from the TNF/TNF-R1 apoptosis pathway.

IMMUNIZATION BY BOTH CHLOROQUINE AND RADIATION ATTENUATED *Plasmodium berghei* MALARIA PARASITES INDUCE STRONG MEMORY CD8+ T-CELL RESPONSES.

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Introduction:
At present, a malaria vaccine is not available but is urgently needed. A key condition in the development of such a vaccine is a demonstrated capacity to induce effective, sustained immunological memory. Recent studies have revealed that attenuated parasites can induce protective immunity. Successful immunization studies, performed either with radiation attenuated sporozoites (RAS), or by sporozoite infection concomitant with chloroquine chemoprophylaxis (CPS), have been conducted in both mouse and man. Although the attenuated infections arising from the two approaches exhibit different degrees of pre-erythrocytic development, they both induce complete protection. Therefore, both approaches provide powerful tools with which to study immunological memory in malaria.

Methods:
Mice were immunized via RAS and CPS. Induction of both cellular memory and regulatory T cells (Tregs) was assessed in blood, liver and spleen collected from immunized and non-immunized mice before and after challenge infection. The phenotypic composition of the CD4+ and CD8+ T-cell memory pool was determined by flow cytometry. The functionality of the established memory was addressed by *ex vivo* stimulation assays and cytokine production. *P. berghei*-specific antibody levels as a result of immunization were measured by ELISA.

Results:
Although the total number of memory CD4+ or CD8+ T cells did not appear to be correlated with protection, liver effector memory CD8+ T cells were prominent in both RAS and CPS immunized groups as compared to the control group. In agreement with these results, CD8+ T cells but not CD4+ T cells from immunized mice produced significantly higher IFN□ levels as compared to control mice. Unexpectedly, Tregs induction as a result of challenge in the immunized group was observed in both blood and spleen but not in the liver. As expected, high antibody levels were measured in both immunized groups.

Conclusion:
Despite differences in pre-erythrocytic parasite development upon immunization with RAS or CPS, both methods induce a strong memory CD8+ T cell response.

CELLULAR IMMUNOLOGICAL RESPONSES IN PREGNANCY-ASSOCIATED MALARIA

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Introduction

Pregnancy-associated malaria (PAM) due to *Plasmodium falciparum* is detrimental to both mother and child. Ongoing anti-PAM vaccine development focuses on the induction of antibodies targeting VAR2CSA, a parasite-derived protein expressed on the surface of infected erythrocytes that sequester in the placenta, since naturally-acquired anti-VAR2CSA IgG titres increase in a gender-specific and parity-related way, and PAM shows a concomitant parity-related decrease in incidence. These findings imply a protective function for antibody responses. In contrast, a defined role for VAR2CSA-specific T cell responses is unclear and remains largely unexplored.

Methods

We are conducting a longitudinal, prospective study of 1000 pregnant mothers in Korogwe, north-eastern Tanzania. For a subgroup of mothers with and without evidence of *P. falciparum* infection, *ex vivo* frequencies of the T cell, B cell, monocyte, regulatory T cell and dendritic cell populations are being measured at inclusion and at delivery. Cytokine activity of isolated peripheral blood mononuclear cells is assessed following short-term stimulation *in vitro* with either VAR2CSA-specific reagents or *P. falciparum*-infected red blood cells. Cord blood mononuclear cells isolated at delivery are assessed in a similar way in order to determine the extent of sensitization to *P. falciparum* antigens *in utero*. For comparative purposes, *P. falciparum*-infected women are matched to uninfected women based on age, gestational age and gravidity.

Results

We have completed assays on samples collected at inclusion and data analysis is ongoing. The collection of samples at delivery is still ongoing. The focus of the results presented will be on the *ex vivo* phenotyping of T regulatory cells and the *in vitro* T cell responses to VAR2CSA-specific reagents.

Discussion

We will compare and contrast our cellular immunological findings with those from an identical study that is being conducted in parallel in southern Benin, in an area where malaria transmission is both more intense and perennial rather than seasonal.

POSTERS

IMPROVEMENT OF RAPID DIAGNOSTIC TESTS FOR MALARIA: *IN VITRO* DETECTION AND STABILITY OF NOVEL ANTIGEN TARGETS

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Currently available RDTs show a large variation in sensitivity/specificity and there are concerns about their stability under harsh conditions. RDTs detecting HRPII are generally less expensive, more stable and have a lower detection threshold than pLDH tests, but have their limitations as well: they detect only *Plasmodium falciparum,* the antigen can persist in the blood after parasite death and there can be false-negatives due to antigenic variation. To meet the need to improve RDTs, monoclonal antibodies for novel malaria antigens are being developed and screened for their possible utility in new RDTs, in a collaborative effort with the Foundation for Innovative New Diagnostics (FIND).

Three antigens have been selected based on literature searches; Glutamate Rich Protein (*Pf* only), Dihydrofolate Reductase-Thymidylate Synthase and Heme Detoxification Protein (both all human species). Recombinant antigens were produced and used to immunise mice, from which antibody producing cells were subsequently isolated. These antibodies were screened for specificity against *P.falciparum* and *P.vivax* and 30 were selected based on this specificity. To select the most optimal antibody couples, detection of a serial dilution of *P.falciparum* NF54 culture was measured and compared to commercial HRPII antibodies in a sandwich ELISA. Furthermore these antibodies are being tested in a lateral flow immunochromatographic set up for their detection of recombinant antigen and NF54 culture material.

The selected antibodies on lateral flow will be evaluated with a panel used by the WHO/FIND to test the performance of RDTs. And the next step will be to test the prototypes on patient samples from an endemic area in a field setting.

VISCERAL LARVA MIGRANS IN THE NETHERLANDS: TRENDS OF *TOXOCARA* AND *ASCARIS* SEROPOSITIVITY FROM 1998 TO 2009

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Toxocara canis, *Toxocara cati* and *Ascaris suum* are roundworms of dogs, cats and pigs respectively that can also infect humans. These zoonotic helminths have a worldwide distribution and are also endemic in The Netherlands. The feces of these infected animals contain eggs produced by these roundworms which contaminate the environment such as sandpits, playgrounds and backyards. Transmission to humans usually takes place after ingestion of embryonated eggs present in contaminated soil. Although usually asymptomatic infection with these zoonotic helminths can cause in humans a syndrome known as visceral larva migrans (VLM) characterized among others by fever, eosinophilia, hepatomegaly, respiratory and neurological distress. Since humans are accidental hosts, the larvae do not reach the adult stage, therefore the eggs of these helminths are not found in the human feces and diagnosis relies mainly on serology. For more than a decade we have been using the same home made ELISA and the ES antigen derived from *Toxocara canis* or *Ascaris suum* larvae. Here we analyzed the results from the *Toxocara* and *Ascaris* IgG-ELISA from a total of 2523 serum samples send to our laboratory at the RIVM from 1998 to 2009. Results indicate clear differences in the percentage of *Toxocara* and *Ascaris* seropositivity for the past 12 years. Meanwhile, the *Toxocara* seropositivity is 7 % in average the *Ascaris* seropositivity is 5 times higher. Only 4% of the tested serum samples were found to be both *Toxocara* and *Ascaris* positive. Furthermore, the trends of seropositivity were also different. For the past twelve years a decrease in the *Toxocara* but not in the *Ascaris* seropositivity was observed. The age and seasonal distribution of the *Ascaris* and *Toxocara* seropositivity and the consequences of these findings for public health will be presented.

DEVELOPMENT OF A GENETICALLY ATTENUATED WHOLE ORGANISM MALARIA VACCINE; FLP RECOMBINASE BASED REMOVAL OF FOREIGN DNA.

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Difficulties with inducing sterile and long lasting protective immunity against malaria with subunit vaccines has renewed interest in vaccination using attenuated Plasmodium parasites. Immunizations with sporozoites that are attenuated by radiation can induce strong protective immunity both in humans and rodent models of malaria. Recently, in P. berghei rodent parasites it has been shown that through the deletion of specific genes, sporozoites can also become attenuated in liver stage development and, importantly, immunization with these sporozoites results in protection against a wild-type challenge. The promise of vaccination using these genetically attenuated parasites (GAP) depends on translating the results in rodent malaria models to human malaria. In order to generate a P. falciparum vaccine consisting of GAPs a number of criteria must be met. First, any gene deletion must be permanent and the genome must not be able to revert back to the original, wild-type, state. Second, a number of genes governing independent biological processes critical for liver stage development need to be removed to ensure complete attenuation. Last, the safe formulation of a whole organism vaccine for use in humans, requires the removal of heterologous DNA sequences (i.e. selectable markers) which are often introduced when generating gene deletion parasites. The equivalent genes as identified in P. berghei have now been deleted in P. falciparum and these parasites have been analyzed in primary human hepatocytes and show a similar degree of attenuation in liver stage development. We now also report the development of an FLP recombinase based system which allows the removal of foreign DNA from P. falciparum and thereby we are able to generate GAPs that constitute a safer vaccine for use in humans.

THE EFFECT OF ECOLOGICAL AND CLIMATIC PARAMETERS ON APPARENT FLIGHT ACTIVITY OF BELGIAN *CULICOIDES*.

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Since the introduction of bluetongue virus serotype 8 (BTV-8) in Belgium in 2006, a vector monitoring programme was set-up in 2007 to study the population dynamics of the BTV vector, *Culicoides*. From April 2007 till April 2008 about 150000 midges comprising 41 species were caught with OVI light traps at 20 dairy farms spread over the different provinces of Belgium. This study examined the influence of environmental (land cover, vegetation and soil type) and climatic (temperature, wind and rain) factors on apparent flight activity of each of the 8 most frequently captured species, including possible BTV vectors (*C. obsoletus s.l.*, *C. dewulfi*, *C. chiopterus*, *C. punctatus*, *C. pulicaris*, *C. nubeculosus*, *C. festivipennis*, *C. achrayi*). Non parametric classification and regression trees (CART) were used to identify the possibly complex relationship between the spatio-temporal apparent activity of *Culicoides* species to the trap and the aforementioned factors. Furthermore parametric poisson and negative binomial regression analyses were used to confirm the influence of each variable on *Culicoides* apparent activity statistically. Overall, temperature emerged as a predominant factor influencing flight activity. Other important ecological parameters and their complex interplay will be presented and discussed for the 8 *Culicoides* species.

MODULATIONS OF NK AND NKT CELL POPULATIONS IN WILDTYPE *T. BRUCEI* INFECTION VERSUS IN PLCKO *T. BRUCEI* INFECTION.

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The C57Bl/6 mouse model for African Trypanosomiasis (AnTat 1.1E infection) exhibits a Th1 cytokine profile. High levels of INFγ, a major Th1 cytokine, in the Wildtype (WT) infection lead to the production of high amounts of TNFα, the latter known to be a measure for pathology. In contrast mice infected with a GPI-phospholipase C knockout (PLCKO) *T. brucei* parasite exhibit lower levels of INFγ, diminished pathology and prolonged survival, as determined by switching to a Th2 cytokine profile during the chronic stage of infection. The preservation of IgM^+ $CD1d^+$ marginal zone B cells (MZB's) during PLCKO infection, the confirmed role in parasite control of IgM^+ B cells and the capacity to present glycolipids to the environment through CD1d (an MHC like molecule) sets the link with Natural Killer T cells. Moreover NKT cells (like NK cells) have the capacity to produce high levels of not only INFγ but also IL-10 and IL-4 quickly after activation, it is therefore of interest to study the occurrence of these cells during the early stage (<15 days p.i.) of a WT versus a PLCKO infection.

The occurrence of high levels of VSG-GPI complexes available for uptake by immune cells during PLCKO infection brings us to hypothesize that the signaling through CD1d (for example on MZB IgM^+ $CD1d^+$ cells) of glycolipid fractions derived from this VSG-GPI complexes could generate an elevated beneficial stimulation of NKT cells (towards Th2 cytokine production). To assess the CD1d dependence of NKT activation, a CD1dKO mouse model was introduced. Comparing both mouse models (WT C57Bl/6 and CD1dKO C57Bl/6 mice), a CD1d dependence of the variant NKTs (vNKTs; NKT cell subset possessing a restricted pool of TcRs) could be detected. Indeed the expansion of vNKT cells occurring during PLCKO infection in the WT mouse model disappears in the CD1dKO mouse model. Future determination of specific cytokine production of the vNKT cells will elucidate/validate their role in modelling pathology and survival during trypanosome infection.

INDUCTION OF DRUG RESISTANCE IN *LEISHMANIA* USING THE *IN VITRO* INTRACELLULAR AMASTIGOTE MODEL.

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First-line treatment failure for all clinical forms of leishmaniasis due to antimony (Sb^V) resistance is now well documented. New treatment regimens and novel drugs are currently being adopted; however, it remains crucially important to monitor closely the development of drug resistance both in the laboratory and within the frame of epidemiological post-treatment follow-up studies. To better understand mechanisms of resistance, drug-susceptible and drug-resistant phenotypes need to be comparatively studied using cell-based, molecular-biological and genetic tools. This laboratory study specifically focused on the artificial induction of resistance against different drugs (Sb, miltefosine, paromomycin) using the *in vitro* amastigote model in macrophages. Amastigotes offer a significant advantage over the commonly used techniques with promastigotes.

The basic principle of the method was to maintain the highest possible drug pressure during the alternate cycles of promastigotes used to infect macrophages and the intracellular amastigote. Amastigotes surviving the highest drug concentration are allowed to transform back to promastigotes to allow expansion of the population, either adopting continued drug pressure (at half the IC_{50}) or not. These next generation promastigotes is then used for infection of macrophages under higher drug pressure. These selection cycles are repeated until the maximum level of resistance is reached. This way, quick induction of resistance to paromomycin was obtained with IC_{50} values showing a >2-fold increase (from 44 μM to 103 μM) after one selection cycle.

THE IMPORTANCE OF MANAGEMENT FACTORS OVER METEOROLOGICAL AND ENVIRONMENTAL FACTORS IN THE SPATIAL DISTRIBUTION OF *FASCIOLA HEPATICA* IN CATTLE IN A TEMPERATE CLIMATE ZONE

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Fasciola hepatica is a trematode parasite with a wide distribution in cattle in temperate climates and an important economical impact on the dairy sector. This study describes the associations between management, climate and environment with infection levels of *F. hepatica* in dairy herds in Flanders. A bulk milk antibody-ELISA was used to measure these infection levels in a random sample of 1762 dairy herds in Flanders in the autumn of 2006, 2007 and 2008. The infection levels were included in a Geographic Information System database next to meteorological (e.g. precipitation, land surface temperature), environmental (e.g. soil type, distance to water) and management parameters (e.g. mowing, length of grazing season). A descriptive analysis was conducted studying inter-annual changes in herd prevalence and spatial distribution and in meteorological circumstances. Logistic regression was used to determine associations between possible risk factors and infection levels. It was concluded that the prevalence and spatial distribution of *F. hepatica* was relatively stable, with small inter-annual differences in prevalence and location of clusters. Management practices (mowing, grass proportion, length of grazing season) were markedly better predictors of the spatial distribution of *F. hepatica* than climate and environment. However, a possible link was noted between inter-annual changes in prevalence and differences in meteorological circumstances (onset of spring, temperature and rainfall during summer). These differences in meteorological circumstances might be useful to predict inter-annual differences in infection levels.