Joint meeting of the Dutch and Belgian Societies for Parasitology

“Challenges for the control of parasites”

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Institute of Tropical Medicine

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50TH ANNIVERSARY OF BELGIAN SOCIETY FOR PARASITOLOGY (1962-2012)

It is the golden jubilee year meeting of the Belgian Society for Parasitology (BSP), which was established on February 10th 1962 at the Institute of Tropical Medicine, Antwerp. We thank our colleagues from the Dutch Society for Parasitology for joining us on this important occasion.

HISTORY

The initial impulse for the creation of BSP was provided by the pioneers of the time in tropical parasitology, many of whom were involved in Congo, Ruanda and Burundi. From its inception till now, the aim of the Society has been to promote the study of all aspects of human and veterinary parasitology (helminthology, protozoology and entomology), to foster cooperation between parasitologists at national and international level and to support young scientists in the development of their career. Since its modest start in 1962, the BSP has grown over time and counts about 80 members today.

INTERNATIONAL ORIENTATION

Until 1991, the statuary and scientific meetings of the BSP were conducted in two major national languages: French and Dutch. Based on a survey among members in 1991 it was decided to use English as the official language of the Society. Since then meetings and official communications are done in English, which underlines the international character and ambition of the BSP. Moreover, the BSP is a long standing member of the World Federation of Parasitologists and European Federation of Parasitologists, and many of BSP members are and have been active on the international scene.

MEETINGS

Until 2002 the BSP used to organize annual statuary meeting of 2-3 hours with the occasional one day meeting. On the initiative of the management board, the BSP decided to hold one full day annual scientific meeting from 2003 with lectures by invited speakers and oral and poster presentations, mainly by young researchers. This tradition was welcomed by all members and has since been further developed. The BSP has a tradition of organizing joint meetings with other societies: from 1991 to 1994, in 1996, 1999 and 2006 in Belgium with the closely related Belgian Society of Protozoology (BSProto); in 1993 and 1999 in the U.K with the British and Dutch Societies of Parasitology; in 1996 in Belgium with the Dutch, Czech and French Societies of Parasitology. Since 2004, the annual scientific meeting of the BSP has been organized jointly with the Dutch Society for Parasitology on a 2 yearly basis. The current 2012 meeting is thus the 5th meeting in that tradition.

SUPPORT TO YOUNG SCIENTISTS

To encourage the young scientists the BSP has created awards with the support of sponsors. The award for best presentation at the annual BSP annual meeting was created in 2005 and has been sponsored by Janssen Animal Health from 2006 and by Elanco from 2012. In 2012, the Pfizer Animal Health travel grant has been created to support young Ph.D. researchers to attend an international conference. The Merial award is granted jointly by the Dutch and Belgian Societies for Parasitology to young researchers from the Benelux who have completed the Ph.D. degree. Of the 14 awards granted since its inception in 1996, 7 have been granted to Belgian parasitologists.

A BRIGHT FUTURE

The BSP and BSProto have been exploring the possibility of a merger for quite some time. The first formal attempt was made in 1991, yet the minds were not ready to shape one unique Society that captured the spirit and scopes of both Societies. Since 2002, the BSP and the BSProto boards have re-iterated this idea, and as a result are proud to announce that the boards of both Societies have agreed to propose the merger to the members at the respective 2012 statuary meetings. The members of the BSProto have agreed to merge with the BSP earlier this year. The BSP members will be invited at the statuary meeting to discuss and vote on this unique opportunity to finally create a joint Society.

On the occasion of this golden jubilee year of the BSP we wish a very bright future to all our members and participants of the meeting.

The BSP committee

PROGRAMME

09:30	Registration and coffee	
Session 1 – Chair: Peter Geldhof		
10:00	BSP & NVP Presidents	Welcome address
10:15	Verschave S. et al.	THE PARASITIC PHASE OF *OSTERTAGIA OSTERTAGI*: QUANTIFICATION OF THE ESTABLISHMENT RATE USING SYSTEMATIC REVIEW AND META-ANALYSIS TECHNIQUES.
10:30	Van Coppernolle S. et al.	ANALYSIS OF THE VACCINE INDUCED IMMUNE RESPONSE AGAINST THE ABOMASAL PARASITE *OSTERTAGIA OSTERTAGI* IN CATTLE SUGGESTS A PIVOTAL ROLE FOR NATURAL KILLER CELLS
10:45	Van Meulder F. et al.	GRANULYSIN PRODUCED BY GLOBULE LEUKOCYTES AS A POTENTIAL KEY ELEMENT IN THE DEVELOPMENT OF VACCINE-INDUCED IMMUNITY AGAINST *OSTERTAGIA OSTERTAGI*.
11:00	Coffee break and poster viewing	
11:20	**Keynote: Prof. Matthew Baylis**, Department of Epidemiology and Population Health, University of Liverpool, UK	**MODELLING THE EFFECTS OF CLIMATE CHANGE ON VIRAL AND PARASITIC DISEASES**
12:00	Meurs L. et al.	MICRO-GEOGRAPHICAL VARIATION IN *SCHISTOSOMA HAEMATOBIUM* AND *S. MANSONI* INFECTION IN A COMMUNITY IN NORTHERN SENEGAL
12:15	Devleesschauwer B. et al.	UNRAVELLING THE BURDEN OF PARASITIC ZOONOSES IN NEPAL
12:30	Lunch and poster viewing	
13:30	BSP general meeting	
Session 2 – Chair: Jan Van den Abbeele		
14:00	**Keynote: Prof. Willem Takken**, Laboratory of Entomology, Wageningen University and Research Centre, The Netherlands	**INSECTICIDE RESISTANCE AND THE CONSEQUENCES FOR MALARIA CONTROL**
14:40	Odongo S. et al.	NANOBODY® IS A NEW TOOL FOR DIAGNOSIS OF ANIMAL AFRICAN TRYPANOSOMIASIS (NAGANA)
14:55	Haynes C. et al.	CHARACTERIZATION OF *T. VIVAX* (TRANS)-SIALIDASE AS A TOOL IN THE FIGHT AGAINST TRYPANOSOMIASIS
15:10	Munday J.C. et al.	PENTAMIDINE-MELARSOPROL RESISTANCE IN AFRICAN TRYPANOSOMES: FROM SIMPLICITY TO COMPLEXITY AND BACK AGAIN.
15:25	Coffee break and poster viewing	
Session 3 – Chair: Robert Sauerwein		
15.45	Vitouley H. et al.	CLINICAL EVOLUTION OF ANIMAL TRYPANOSOMOSIS (*T. VIVAX*) IN PRESENCE OF DRUG RESISTANCE
16:00	Fortin A. et al.	EFFICACY AND TOLERABILITY OF OLEYLPHOSPHOCHOLINE (OlPC) IN EXPERIMENTAL LEISHMANIASIS
16:15	Lempereur L. et al.	BIOINFORMATIC SCREENING FOR TRANSMISSION BLOCKING VACCINE CANDIDATES OF *THEILERIA ANNULATA*
16:30	Merial, Elanco & Pfizer awards	
17:30	Reception	
19:00	Dinner at Peerdestal restaurant	

POSTERS

Session 1	**Control of parasites**
Bosschaerts T. et al.	SHORT ORAL TREATMENT WITH OLEYLPHOSPHOCHOLINE (OLPC) IMPROVES CLINICAL CANINE LEISHMANIASIS DUE TO *LEISHMANIA INFANTUM* IN A NATURAL MODEL OF INFECTION
Mekonnen Z. et al.	POOLING STOOL SAMPLES: A COST-EFFECTIVE STRATEGY TO ASSESS INFECTION INTENSITY OF SOIL-TRANSMITTED HELMINTHS AND TO MONITOR DRUG EFFICACY?
Mul M.F. et al.	CONTROL OF POULTRY RED MITE (DERMANYSSUS GALLINAE) IN LAYER FARMS USING AN AUTOMATED MONITORING DEVICE
Noé L. et al.	ANTHELMINTIC RESISTANCE OF SHEEP GASTRO-INTESTINAL NEMATODES IN 4 CONTINENTAL EUROPEAN COUNTRIES
Ronsyn R. et al.	EVALUATION OF THE OVATEC PLUS FLOTATION METHOD AS A DECISION TOOL FOR SELECTIVE ANTHELMINTHIC TREATMENT AGAINST STRONGYLES IN HORSES
van Doorn D. et al.	ANTHELMINTIC RESISTANCE AND EGG RE-APPEARANCE PERIOD IN HORSES IN BELGIUM AND THE NETHERLANDS
Wilkinson K. et al.	ANTHELMINTIC RESISTANCE OF CATTLE GASTRO-INTESTINAL NEMATODES IN 5 EUROPEAN COUNTRIES
Session 2	**Molecular Parasitology and immunology**
Borloo J. et al.	X-RAY STRUCTURE OF *OSTERTAGIA OSTERTAGI* ASP-1 PROVIDES DETAILED INSIGHTS IN DIMERIZATION MECHANISM AND PROTEIN CYCLIZATION
Caron Y. et al.	ENCEPHALITOZOONOSIS IN A DWARF PET RABBIT
Grit G.H. et al.	THE EFFECT OF *GIARDIA DUODENALIS* ON BOVINE MONOCYTE-DERIVED DENDRITIC CELLS AND T-CELL RESPONSES IN VITRO.
Hendrickx S. et al.	EXPERIMENTAL INDUCTION OF MILTEFOSINE AND PAROMOMYCIN RESISTANCE ON INTRACELLULAR *LEISHMANIA* AMASTIGOTES
Huyse T. et al.	REGULAR TREATMENT OF PRAZIQUANTEL DO NOT IMPACT ON THE GENETIC MAKE-UP OF *S. MANSONI* IN NORTHERN SENEGAL: EVIDENCE FOR DRUG TOLERANCE?
La Greca F. et al.	ROLE OF TNF DURING *TRYPANOSOMA VIVAX* INFECTIONS
Roelfsema J.H. et al.	EVALUATION OF MITOCHONDRIAL GENES NAD1, COX1, 12S AND NAD5 IN MOLECULAR DIAGNOSIS OF CESTODES
Session 3	**Epidemiology of parasitic diseases**
Campos Ponce M. et al.	ARE INTESTINAL PARASITES FUELLING THE RISE IN DUAL BURDEN HOUSEHOLDS IN VENEZUELA?
Gilmoor A. et al.	INTESTINAL PARASITES IN HEALTHY CHILDREN OF THE GAMBIA: A PILOT STUDY
Hoek D. et al.	PREVALENCE OF INTESTINAL PARASITES IN A RURAL COMMUNITY OF VENEZUELA: COMPARISON OF MICROSCOPY AND PCR
Lempereur L. et al.	SPOTLIGHT ON BABESIOSIS IN BELGIUM
Santosh G. et al.	PILOT STUDY ON HUMAN AND ZOONOTIC INFECTIONS OF GASTROINTESTINAL PARASITES IN SOUTHERN INDIA
Soenen K. et al.	LIVER FLUKE INFECTION: CHARACTERIZATION AND DISEASE RISK OF SMALL WATER BODIES AS A COMPLEMENTARY TOOL FOR DISEASE CONTROL
van der Voort M. et al.	HELMINTH INFECTIONS: DO THEY AFFECT THE PRODUCTIVE EFFICIENCY OF SPECIALISED DAIRY FARMS?

INVITED SPEAKERS

MODELLING THE EFFECTS OF CLIMATE CHANGE ON VIRAL AND PARASITIC DISEASES OF LIVESTOCK

Matthew Baylis
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Climate change is widely expected to affect infectious diseases of humans, animals and plants – but which, when, where and how? The Liverpool University Climate and Infectious Diseases of Animals (LUCINDA) group focuses on developing improved methods to predict the future of infectious diseases given climate sand environmental change so that appropriate measures of mitigation or adaptation can be taken. This presentation will consider work on two diseases of animals: bluetongue and liver fluke. Bluetongue (BT) is a viral disease of ruminants spread by *Culicoides* biting midges. In the last 15 years BT has dramatically emerged in Europe, because of (i) spread of an old-world vector, *C. imicola*, in southern Europe; (ii) transmission of the causative virus (BTV) by indigenous midges, especially *C. obsoletus* group, in both southern and northern Europe. BT is 'climate-sensitive' – the distribution of the midge vectors and their ability to transmit BTV are both affected by climate, especially temperature. This leads to the hypothesis that BT's emergence is linked to recent climate change. We tested this hypothesis by developing a climate-sensitive model of the risk of BT outbreaks (given viral introduction). The risk of a disease outbreak is defined mathematically by the basic reproduction number (R0) – the number of secondary infections arising from a new infection in an entirely susceptible population. Key, climate-sensitive parameters are the ratio of vectors to hosts, for which we developed a new climate-driven model; and the biting rate, mortality rate and virus development rate of the vectors, for which associations with temperature were obtained from published literature. The model captures many aspects of BT's recent emergence: (i) it demonstrates an increasing risk of outbreaks in southern Europe from the 1980s, and in northern Europe from the 1990s; (ii) it accurately predicts areas of southern Europe to which *C. imicola* has spread; (iii) it shows that 2006, the year of BT's first occurrence in northern Europe, was the year of highest risk out of the previous fifty; (iv) it finds that increased risk in southern Europe is mostly associated with changing vector density (such as vector spread), while in northern Europe it is change to viral transmission parameters; (v) it suggests that previous outbreaks of BT, and the related African horse sickness, in Europe also occurred during periods of climatically-determined high disease risk. Having demonstrated that the model can successfully explain aspects of the BT's past, we use it to predict the disease's future by driving it with simulated climate data up to 2050. The simulations show that the risk of BT outbreaks is expected to continue increasing in Europe, but faster in the north than the south. There is a high level of agreement between different climate models in both the scale and direction of change in risk. Liver fluke causes an important disease of ruminants in many parts of the world, including the UK. Evidence suggests its prevalence is increasing in UK. Climate-sensitive statistical models have been developed for the distribution of liver fluke infection in the UK; and an F0 model (related to the BT R0 model) so that we can start to explore how the risk of disease may respond to climate change.

INSECTICIDE RESISTANCE AND THE CONSEQUENCES FOR MALARIA CONTROL

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Malaria control has been highly successful in effectively reducing cases of *Plasmodium falciparum* by combining early diagnosis and drug treatment with the mass distribution of insecticide-treated nets (LLIN) and indoor residual spraying (IRS) (1). This strategy is being threatened by the rapid development of insecticide resistance and drug resistance, both of which are occurring in many continents. In West Africa, mosquitoes have become resistant to four classes of insecticides and LLINs no longer provide protection to mosquito bites (2). Elsewhere in Africa, insecticide-resistance levels of mosquitoes have increased in recent years. The Innovative Vector Control Consortium aims for the development of novel insecticides, to overcome the threat of insecticide resistance. However, this process is slow, depends much on "old" insecticides used in agriculture, and a new class of insecticides has so far not emerged. Surprisingly, in this strategy the evolutionary principle of resistance development is rarely considered. It is obvious, though, that unless evolutionary aspects are being considered, vector (and parasite) control will continue to be following a race to stay ahead of resistance. It is proposed that this can be overcome by applying integrated vector management (IVM) strategies, in which the most effective and feasible tools available for larval and adult mosquito control are being applied without being dependent on the classical monotherapy that relies on insecticides (3). Successful examples of IVM for malaria control will be discussed, as well as future strategies in which vector control is likely to remain highly effective and sustainable.

REFERENCES

1. WHO. World Malaria Report 2011. Geneva: World Health Organization; 2011.
2. Edi CVA, Koudou BG, Jones CM, Weetman D, Ranson H. Multiple-Insecticide Resistance in *Anopheles gambiae* Mosquitoes, Southern Cote d'Ivoire. *Emerg Infect Dis.* 2012 Sep;18(9):1508-11.
3. Thomas MB, Godfray HCJ, Read AF, van den Berg H, Tabashnik BE, van Lenteren JC, et al. Lessons from Agriculture for the Sustainable Management of Malaria Vectors. *Plos Medicine*. 2012 Jul;9(7).

ORAL PRESENTATIONS

THE PARASITIC PHASE OF *OSTERTAGIA OSTERTAGI*: QUANTIFICATION OF THE ESTABLISHMENT RATE USING SYSTEMATIC REVIEW AND META-ANALYSIS TECHNIQUES.

Verschave S., Vercruysse J. , Claerebout E., Geldhof P. and Charlier J.
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Ostertagia ostertagi is the most common gastro-intestinal (GI) nematode of cattle in temperate climate regions and poses important constraints on animal productivity. This parasite has a direct life cycle, consisting of a free-living and a parasitic phase. During the parasitic phase, the three main parameters that determine the parasite density within the host are the establishment rate and mortality rate and fecundity rate of adult worms. Transmission models and nematode control will benefit from more accurate estimates of these life history traits and their variation.
The aim of this study is therefore to quantify one of the three main life history traits of the parasitic phase of *O. ostertagi*, namely the establishment rate and to assess factors affecting this rate.
A literature search was conducted using a systematic review protocol to find experimental trials in which naïve calves were infected with *O. ostertagi* without treating them with anthelmintic drugs and in which a necropsy was performed to assess the intestinal worm burden. Using general keywords a first search resulted in 5266 potential publications. Next, a title-based selection generated 404 infection trials. An article-based selection resulted finally in only those trials needed to calculate the worm burden/infection dose ratio, which serves as estimate for the establishment rate. An overall inverse variance weighted estimate was computed by using the summary data reported in the trials, or if needed, by using summary data calculated based on the provided info. The influence of host age, infection dose, infection mode and duration of infection on the establishment rate was analysed through a random effect model with these factors as moderator variables.

ANALYSIS OF THE VACCINE INDUCED IMMUNE RESPONSE AGAINST THE ABOMASAL PARASITE *OSTERTAGIA OSTERTAGI* IN CATTLE SUGGESTS A PIVOTAL ROLE FOR NATURAL KILLER CELLS

Stefanie Van Coppernolle, Frederik Van Meulder, Belgacem Mihi, Iris Peelaers, Jozef Vercruysse, Edwin Claerebout, Peter Geldhof
Department of Virology, Parasitology and Immunology, Faculty of Veterinary Medicine, Ghent University, Belgium

Vaccination against gastro-intestinal nematodes in cattle would offer a valuable alternative to the use of anthelminthic drugs. However, the development of such vaccines is largely hampered by a lack of knowledge on the protective immune responses against these parasites. The aim of this study was to compare the immune response induced by a host-protective experimental vaccine against *Ostertagia ostertagi* in cattle, based on native ASP antigens combined with QuilA adjuvant, with the responses induced by non-protective versions of the same vaccine, i.e. native ASPs combined with $Al(OH)_3$ and a *Pichia pastoris* expressed ASP combined with QuilA. Each animal was immunized three times intramuscularly with a three-week interval. After the final immunization, animals received a trickle infection of 1000 infective L3 larvae/day for 25 days. All results were compared to the results obtained from non-vaccinated, non-infected (naive) animals.
We found no significant vaccine induced changes in frequencies of lymphocyte subpopulations in peripheral blood during the course of the whole experiment. Surprisingly, *in vitro* exposure of peripheral blood mononuclear cells from nASP/QuilA vaccinated animals to nASP solely resulted in detectable proliferation of natural killer (NK) cells. This effect was not observed in animals vaccinated with the non-protective vaccines, nor in naive animals, showing that only the protective vaccine induced a systemic NK cell 'memory' to the nASP antigen.
Phenotypical analysis of abomasal and abomasal lymph node mononuclear cell (MC) fractions showed no vaccine induced changes in frequencies of lymphocyte subpopulations. Although MC isolated from the abomasal LN of all infected animals responded to nASP, proliferation was highest in

the nASP/QuilA vaccinated group, again with NK cells being the strongest responders. This response was not observed in naïve animals.
The outcome of this study suggests a previously unidentified role for systemic NK cell 'memory' in vaccine induced protective immune responses against *Ostertagia ostertagi*.

GRANULYSIN PRODUCED BY GLOBULE LEUKOCYTES AS A POTENTIAL KEY ELEMENT IN THE DEVELOPMENT OF VACCINE-INDUCED IMMUNITY AGAINST *OSTERTAGIA OSTERTAGI*.

Van Meulder F., Rinaldi M., Van Coppernolle S., Claerebout E., Vercruysse J., Geldhof P.
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Ostertagia ostertagi is considered the most economically important bovine parasite. An experimental host-protective vaccine against *Ostertagia ostertagi* was developed based on ASP-proteins derived from excretory-secretory material of the helminth. Further optimization and commercialization of this vaccine, however, requires a thorough understanding of the vaccine-induced immune response.
Previous studies in which immune cell counts and cytokine transcription levels were investigated, did not detect differences between vaccinated and susceptible animals. Therefore, a broader whole-transcriptomic approach using a micro-array was applied. Interesting targets discovered by the micro-array were further analysed on a protein level.
This approach revealed a significant upregulation in expression of the granule-proteins granulysin (GNLY) and granzyme B (GZMB) and the high affinity IgE-receptor 1 (FCER1A) in infected vaccinated animals compared to infected non-vaccinated animals. Moreover, these genes significantly correlated with faecal egg count reduction and worm counts. We have further shown that granulysin is produced by globule leukocytes in the abomasum and is secreted into the mucus, presumably through antibody-dependent triggering of the IgE receptor 1.
Correlations between globule leukocytes and protection, and the suggestion of a protective agent being present in the mucus, have been described previously. This is however the first time to our knowledge that such an agent is actually identified.
Our results, along with the knowledge that granulysin has recently been associated with immunity against a variety of helminths, render granulysin a very interesting topic for further research.

MICRO-GEOGRAPHICAL VARIATION IN *SCHISTOSOMA HAEMATOBIUM* AND *S. MANSONI* INFECTION IN A COMMUNITY IN NORTHERN SENEGAL

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The global distribution map shows a large overlap of *Schistosoma mansoni*- and *S. haematobium*-endemic areas in Africa. Both species are known for their heterogeneous spatial distribution on continental, national as well as district levels. Little is known however, about the distribution of schistosomiasis on a smaller scale, e.g. on community level, especially in co-endemic areas. We studied a co-endemic rural community in northern Senegal and looked at the spatial distribution of *Schistosoma* infections (by microscopy; n=599) and at behavioural patterns (by questionnaire; n=295).
Overall *S. mansoni* and *S. haematobium* prevalences were 55 and 44%, respectively. Mixed *Schistosoma* infections were observed in 32% of the population. *Schistosoma mansoni* and *S. haematobium* infection intensity showed significant hotspots in the north (p=0.001) and south (p=0.004) of the community, respectively. Single *Schistosoma* infections showed significant north-south gradients, with more single *S. mansoni* in the north (p=0.037) and *S. haematobium* in the south

(p=0.007). Mixed infections did not show spatial heterogeneities. The observed spatial distributions were related to known risk factors as well as the distance from and the reported use of five water contact sites which were located from the north-west to the south-west of the village.
This is the first study investigating the spatial distribution of *S. mansoni* and *S. haematobium* in a co-endemic community. Our results indicate that - even on such a small scale - significant clustering of *Schistosoma* infection occurs and that *S. mansoni* and *S. haematobium* do not necessarily cluster together. This might be explained by the locations of the different water contact sites, probably with contrasting transmission intensity of *S. mansoni* and *S. haematobium* (e.g. due to snail distributions). However, other factors (e.g. genetic, immunological, or behavioural) cannot be excluded. Obviously, these results warrant further investigation of micro-geographical variation of schistosomiasis in other settings and its underlying mechanisms.

UNRAVELLING THE BURDEN OF PARASITIC ZOONOSES IN NEPAL

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BACKGROUND
Parasitic zoonoses (PZ) pose a significant but often neglected threat to public health, especially in developing countries. In order to get a better understanding of their health impact, summary measures of population health may be calculated, such as the *disability-adjusted life year* or DALY-metric. However, the data required to calculate such measures are often not readily available for these diseases, which may lead to a vicious circle of underrecognition and underfunding.
METHODS
We reviewed the burden of PZ in Nepal, one of the poorest and least develop countries in the world. This review process took place in two phases: (1) a qualitative assessment to identify the endemic PZ and available data, and (2) a quantitative health impact assessment expressed in terms of DALYs. Since no PZ are included in the current lab-based surveillance systems, a comprehensive collection of online and offline data sources was conducted, and various statistical methods were applied to these data sources, including meta-regression, predictive modelling, stochastic simulation, and data extrapolation.
PRINCIPAL FINDINGS
It was found that the highest public health impact was imposed by toxoplasmosis, followed by neurocysticercosis, zoonotic intestinal protozoal and helminths infections, and cystic echinococcosis. Nepal is likely to be endemic for larva migrans, trichinellosis, foodborne trematodiasis and alveolar echinococcosis, but insufficient data were available to quantify their health impact, due to their low or focalized incidence. No evidence was found for the occurrence of anisakiasis, zoonotic schistosomiasis, or zoonotic trypanosomiasis.
CONCLUSIONS
In settings with limited surveillance capacity, it is possible to quantify the health impact of PZ and other neglected diseases by applying various statistical methods, thereby unravelling the burden of these diseases and interrupting the vicious circle of neglect. In Nepal, we found that several PZ are endemic and are imposing a not insignificant burden to public health. However, still several critical data gaps could be identified. As effective surveillance systems are key to any public health intervention, these systems should be further promoted in developing countries such as Nepal, as these countries are affected the most by PZ and other neglected diseases.

NANOBODY® IS A NEW TOOL FOR DIAGNOSIS OF ANIMAL AFRICAN TRYPANOSOMIASIS (NAGANA)

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Animal African trypanosomiasis (nagana), a disease of livestock caused by blood-borne protozoan parasite called trypanosomes, remains a big challenge to livestock industry in the Sub-Saharan Africa. One of the factors contributing to persistence of the disease is inadequacies of the disease surveillance system which is lack of effective disease monitoring tools, fund and sheer negligence.
Our study was undertaken to apply Nanobody® (Nb) as alternative diagnostic test for *T. congolense* (the most pathogenic and widely spread animal trypanosmiasis in Africa). The diagnostic Nb was obtained from bio-panning and selection of binders from immune cDNA library of Alpaca previously immunized with *T. congolense* lysate.
A proof-of-principle diagnostic test (antigen-capture sandwich test) using the Nanobody® (4741 and 4742) performed on sera of cattle experimentally infected with *T. congolense* gave promising result with a level of sensitivity comparable to buffy coat. More so, all strains of *T. congolense* obtained from different regions in Africa were detectable by the test. With its current level of sensitivity and specificity, the test would be very useful in diagnosis of *T. congolense* without necessarily relying on unaffordable and electric powered diagnostic gadgets in current use. Further, the Nb test format scored positive on sera of mice with active *T. congolense* infection and negative after Berenil® treatment meaning that it is able to distinguish active infection from convalescent state. With this result the test would be of clinical importance in veterinary practice for evaluation of treatment efficacy.

CHARACTERIZATION OF *T. VIVAX* (TRANS)-SIALIDASE AS A TOOL IN THE FIGHT AGAINST TRYPANOSOMIASIS

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Trypanosoma vivax is a protozoan extracellular parasite that infects animals and mostly affects cattle, in Africa and South America, leading to dramatic economic losses in the concerned countries. The major pathological feature is the high level of anemia that seriously affects the hosts.
T.vivax bloodstream form was shown to express sialidase (SD) activity, by which the SD enzyme is able to cut off terminal sialic acid (SA) residues from sugar entities. Knowing that red blood cells (RBCs) possess high amounts of this SA on their surface and that reduction in this SA level serves as a measure for the aging of the cell, we hypothesize that SD might be responsible for the drastic anemia levels observed during infection. The structure and function of this SD remains mostly unknown. Also its accessibility towards the immune system, its role in the induction of pathology and possible drug target applications are to be discovered.
In the study presented here there are two major goals: (I) the in depth characterization of the *T.vivax* SD, (II) the development of strategies for the inhibition of SD activity during infection.
From the available sequences of putative *T.vivax* SD genes, 3 were generated synthetically in order to produce recombinant SD protein (rSD) in yeast. Subsequently, activity of the rSD was assessed and confirmed. Furthermore, the generation of a nanobody® library against rSD is ongoing. Next steps include (I) crystallization and structure unraveling of the *T.vivax* rSD and (II) inhibition studies.

PENTAMIDINE-MELARSOPROL RESISTANCE IN AFRICAN TRYPANOSOMES: FROM SIMPLICITY TO COMPLEXITY AND BACK AGAIN.

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Given the paucity of new drugs for tropical parasitic diseases, resistance to the few old treatments available is a major concern in disease control. It has been known for decades that *Trypanosoma brucei* resistant to melarsoprol are often cross-resistant to pentamidine and *vice versa* (Melarsoprol-Pentamidine cross-resistance; MPXR). In the 1990s it was discovered that a purine transporter, P2 or TbAT1, was able to transport both the melaminophenyl arsenical and diamidine classes of trypanocides, and 10 years ago we constructed a *TbAT1* knockout that was moderately resistant to diminazene aceturate (Berenil) and was 2-3-fold less sensitive to pentamidine and the arsenical drugs. The low-level MPXR was in large part explained with the discovery of additional transporters for pentamidine and melaminophenyl arsenicals, especially the High Affinity Pentamidine Transporter (HAPT1), which has very low affinity for diminazene. Recently we have focussed on finding the gene encoding the HAPT1 drug transporter, and any other genetic changes underlying high level MPXR. The screening of a genome-wide RNAi library with pentamidine and melarsoprol identified a locus containing two aquaglyceroporin genes, bAQP2 and TbAQP3. Knockout of TbAQP2, but AQP3, produced a strong MPXR phenotype, and then TbAQP2 gene was disrupted in the multi-drug resistant line B48. Re-expression of TbAQP2 in B48 completely reversed the resistance to pentamidine and melarsoprol specifically. Various disruptions of TbAQP2 have now been found in additional trypanosome lines that were selected (either *in vivo* or *in vitro*) for resistance to either pentamidine or melarsoprol, and from *T. b. brucei*, *T. b. gambiense* and *T. b. rhodesiense*. Some of these strains are known to be transmissible through tsetse flies. The identification of this genetic marker is a major advance e in understanding drug resistance in pathogenic protozoa.

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CLINICAL EVOLUTION OF ANIMAL TRYPANOSOMOSIS (T. VIVAX) IN PRESENCE OF DRUG RESISTANCE

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To assess the impact of animal trypanosomosis and the effect of drug resistance on the health of small ruminants, twelve *T. vivax* isolates in Burkina Faso were injected into 12 groups of 5 Sahelian goats, two being treated with 3.5 mg/kg body weight diminazene aceturate (DA), two with 0.5 mg/kg body weight isometamidium chloride (ISM) and one left untreated as control. A monitoring was performed every 5 days for 100 days to evaluate the parasitaemia by buffy coat examination, the haematocrit and the body weight. Among the 12 groups, 6 were additionally monitored using a trypanosome specific 18S-PCR-RFLP every 5 days from day 30 to day 100 to verify the complete clearance of the parasites from the blood of the hosts. In six groups of goats, trypanosomes

disappeared completely after treatment, five groups showed relapses in at least one goat treated with ISM and one group showed relapses in one goat treated with DA and one with ISM. For the 6 groups that were screened both using microscopic examination and trypanosome specific 18S-PCR-RFLP, the following results were observed: for the groups treated with DA, no relapses by microscopic examination and 83.3% (10/12) using the 18S-PCR-RFLP. For the groups treated with ISM, 25% (3/12) relapses by microscopic examination and 83.3% with the 18S-PCR-RFLP (10/12). The evolution of the PCV and the weight during the observation period from relapsing (either by microscopical examination or by 18S-PCR-RFLP diagnosis) and non relapsing animals were compared. The relative average PCV in goats that relapsed microscopically, decreased significantly more than in non-relapsing goats. This difference was not significant when relapses were detected using the trypanosome specific 18S-PCR-RFLP. This indicates that only the animals with the highest parasitaemia suffered from the infection. Relapses after treatment where the host controls the parasitaemia to a level below the sensitivity of the microscopical examination do not affect body weight nor PCV.

EFFICACY AND TOLERABILITY OF OLEYLPHOSPHOCHOLINE (OlPC) IN EXPERIMENTAL LEISHMANIASIS

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The alkylphospholipid oleylphosphocholine (OlPC) is a structural analogue of miltefosine and represents a potential therapeutic alternative for the treatment of leishmaniasis. This study assessed the *in vitro* and *in vivo* activity profile of OlPC in a hamster model of visceral leishmaniasis and analyzed its non-clinical safety and pharmacokinetic profile.

The *in vitro* activity of OlPC against intracellular amastigotes of *L. donovani, L. infantum*, *L. tropica, L. mexicana* and *L. panamensis* showed mean IC_{50} values below 5 µM, while the IC_{50} values against *L. major* and *L. braziliensis* were 7.7 and 13.5 µM, respectively. The *in vitro* activity was similar to miltefosine. The *in vivo* efficacy was dose-titrated in the *L. infantum* hamster model using an aqueous (OlPC/H_2O) and liposomal OlPC formulation in a single and repeated (5-day) oral dosing regimen. Treatment with 20 and 40 mg/kg for 5 days showed that both OlPC formulations were equipotent and had a markedly higher efficacy compared to miltefosine. A single dose of 100 mg/kg of OlPC/H_2O or OlPC-liposomes reduced the amastigote parasite burdens by 96.2% and 99.3% in liver, 99.8% and 99.9% in spleen and 87.6% and 96.9% in bone marrow, respectively. OlPC was shown to be orally bioavailable (±80%) and can be considered a long-acting drug with a mean elimination half-life of 70 and 60 hrs in rats and dogs. Safety pharmacological and toxicological analysis did not reveal any major side effects or target organs for toxicity.

In summary, this study suggests that OlPC is a promising new candidate to shorten and simplify current case management of visceral leishmaniasis. Further studies are necessary to assess the full potential of OlPC as a drug candidate.

BIOINFORMATIC SCREENING FOR TRANSMISSION BLOCKING VACCINE CANDIDATES OF THEILERIA ANNULATA

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A transmission blocking vaccine strategy can be considered as a part of an "integrated program" designed to control vector borne parasitic diseases, such as tropical Theileriosis caused by *Theileria annulata*.

To select candidate antigens for a transmission blocking vaccine a bioinformatic screen was performed to identify *Theileria annulata* genes predicted to encode proteins potentially expressed by tick transmissible stages and located on the parasite surface. Fifteen candidates were selected, including Tams 1, a previously identified immunodominant polymorphic surface antigen of merozoites and piroplasm stages. Screening for orthologues in other vector borne Apicomplex parasites (*Babesia* and *Plasmodium)* was implemented using available databases. Orthologues in *Plasmodium* were found for 2 of the *Theileria* candidates: 1) a gamete surface antigen Pfs 230 containing 6Cyst motifs, known to be a potential transmission blocking candidate and 2) a SERA (Serine Repeat Antigen) gene, another potential malaria vaccine candidate.

To determine if the *Theileria* candidates display significant predicted amino acid sequence diversity or conservation, an allelic polymorphism study was performed on the 3 main candidates. This was performed using DNA representing different isolates from geographically distinct areas and from samples obtained within the same region. Conservation will demonstrate potential absence of selection from the bovine immune system and indicate that the candidate antigen may be hidden, whereas diversity may indicate a potential to evade a transmission blocking response. Sequencing analysis included computation of the ratio of non synonymous to synonymous (dn/ds) nucleotide substitutions was performed and compared to data available for the divergent Tams1 antigen.

Identification of genes expressed in tick transmissible stages could lead to generation of constructs to test as a recombinant vaccine. Thus sequence data generated by this study will provide information on potential difficulties that would need to be overcome due to antigenic diversity or a lack of natural boosting. For certain tick borne diseases, such as tropical Theileriosis where the carrier state is of economic importance, a vaccine that blocks parasite transmission, in addition to those that control disease, could have a major impact on livestock productivity and further research is required.

POSTERS

X-RAY STRUCTURE OF *OSTERTAGIA OSTERTAGI* ASP-1 PROVIDES DETAILED INSIGHTS IN DIMERIZATION MECHANISM AND PROTEIN CYCLIZATION

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CAP superfamily proteins (a.k.a. SCP/TAPS proteins) constitute an extremely diverse family of proteins in organisms spanning the entire animal kingdom. Even though CAP proteins such as the activation-associated secreted proteins (ASPs) are known to be involved in host-parasite interactions and are among the most abundant proteins in the excretome/secretome of numerous parasitic nematodes, their exact function(s) remain(s) elusive. Given the current issues caused by anthelmintic resistance, novel routes in parasite control are highly necessary, the most promising of which may be vaccination-based. ASPs are promising vaccine candidates in several parasitic nematode species, including *Ostertagia ostertagi*, one of the most prevalent and pathogenic gastro-intestinal parasites in cattle. However, the aforementioned lack of functional (and structural) information precludes the development of protective recombinant ASP vaccines. In this frame, we provide high-resolution crystallographic data of recombinantly produced ASP-1 from *O. ostertagi* (*Oo*-ASP-1).

Besides confirming the overall CAP superfamily topological hallmarks for *Oo*-ASP-1, i.e. the presence of the N-terminal CAP domain and the C-terminal cysteine-rich domain, we delve deeper in the 3D-structure of this abundantly secreted protein and elaborate on its highly peculiar traits. In agreement to its biologically relevant quaternary structure, i.e. exclusively as a dimer, inspection of the structure revealed the *Oo*-ASP-1 dimer as being maintained through a single intermolecular disulphide bridge, stabilizing an unusually small interaction surface. Moreover, unlike any other CAP superfamily member described to date, an additional intramolecular disulphide bridge links the N- and C-termini, thereby yielding a cyclic molecule. Whereas cyclization of the molecule may render it more resistant to proteolysis, *in vivo* dimerization of *Oo*-ASP-1 may be crucial in its function.

Apart from providing a brief overview on CAP superfamily proteins and ASPs in particular, the findings presented here and their importance in future developments in the field of vaccine-based helminth control will be discussed.

SHORT ORAL TREATMENT WITH OLEYLPHOSPHOCHOLINE (OlPC) IMPROVES CLINICAL CANINE LEISHMANIASIS DUE TO LEISHMANIA INFANTUM IN A NATURAL MODEL OF INFECTION

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The alkylphosphocholine OlPC is an orally bioavailable (80%) and long-acting drug, with a t_{max} of 4 hrs and a mean elimination half-life of about 60 hrs in healthy dogs. In the present study, oral doses of 1.5, 4 and 8 mg/kg were administered to healthy dogs for a 28-day period to assess general tolerance. Dosing of 1.5 mg/kg did not produce any adverse event, but dose-dependent vomiting was noted at the two highest doses. No sign of organ toxicity was detected at any dose, and all clinical signs disappeared upon treatment cessation.

We next selected the dose of 4 mg/kg/day to treat orally for 14 days a cohort of eight shelter dogs naturally infected with *L. infantum* and classified as clinically sick. Dogs were assessed at the clinical

and parasitological level at the beginning of treatment (day 0) and on days 15, 30 and 90. Regarding tolerance, two out of eight treated dogs experienced diarrhoea during the first week post-treatment, and a third one had diarrhoea and one episode of vomiting. The remaining five dogs tolerated the treatment without any side effect. On day 0, the average clinical score of the dogs was 18 (range 12-31, scale of 0-74). Clinical score was reduced to 11 (range 7-16) at the end of treatment (day 15), and went further down to 5 (range 1-10) and 2 (range 0-5) on days 30 and 90, respectively. In addition, all dogs gained weight after OlPC treatment, reflecting an excellent clinical improvement. PCR analysis failed to detect parasite DNA in the blood of any of the dogs on day 15, but detailed examination indicated that the bone marrow may act as a parasite reservoir.
These preliminary results show that short oral treatment with OlPC improves clinical signs of canine leishmaniasis. Additional studies are needed to find the optimal dosing regimen of OlPC in dogs and to assess its long-term treatment efficacy.

ARE INTESTINAL PARASITES FUELLING THE RISE IN DUAL BURDEN HOUSEHOLDS IN VENEZUELA?

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INTRODUCTION
In developing countries undergoing rapid economic development the prevalence of dual burden (co-existing overweight/obesity and stunting) households is increasing. While intestinal parasites are usually prevalent in these countries, their role in the dual burden phenomenon of overweight/obesity and stunting has so far been neglected. We studied the associations between dual burden households and intestinal parasite infection in a rural community of Venezuela.
METHODS
We examined data of 41 households (133 children and 92 adults). A dual burden household was defined as a household with at least one overweight/obese adult (BMI>25) and at least one stunted child (height -for-age z score <-2). Intestinal parasite infection (Giardia lamblia and geohelminths) was determined by direct and ferric haematoxylin stained smears of 2 faecal samples.
RESULTS
In this community, 47.3% of the individuals were infected with intestinal parasites. Among adults, 65.2% were overweight/obese, 13.8% of the children were stunted. More than one in four households (26.8%) were dual burden households. Being infected with intestinal parasites was significantly associated with being in a dual burden household (OR=2.11; 95% CI: 1.11-4.00.
CONCLUSIONS
This cross sectional study suggests that parasitic infection is strongly associated with adult overweight/obesity and childhood stunting, pointing to a triple burden of disease in this community in Venezuela. While the relationship between parasitic infection and stunting has been well established, the association of intestinal parasite infection with overweight/obesity in adults needs to be explored further.

ENCEPHALITOZOONOSIS IN A DWARF PET RABBIT

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INTRODUCTION
The microsporidian parasite (Protozoa) *Encephalitozoon cuniculi* commonly infects rabbits. Three current forms of this disease are recognized: ocular, neurological and renal.
CASE DESCRIPTION
An acute head tilt accompanied by nystagmus and a mild ataxia appeared suddenly. After its purchase, this rabbit regularly showed episodic nasal discharge and sneezing episodes resolving spontaneously. A treatment based on antibiotics (enrofloxacine) and corticosteroid (dexamethasone) was set up without resolution of the head tilt.
CLINICAL FINDINGS
A serology revealed the presence of *E. cuniculi* specific antibodies. This active infection was confirmed with the presence of spore in faeces because it was not possible to find spore in urine.
TREATMENT AND OUTCOME
The rabbit was treated with fenbendazole 20mg/kg BW (Panacur Puppy®) orally once a day during 28 days. The head tilt almost completely resolved after one week of treatment.
CLINICAL RELEVANCE
To the author knowledge, this is the first time encephalitozoonosis is described in Belgium.

INTESTINAL PARASITES IN HEALTHY CHILDREN OF THE GAMBIA: A PILOT STUDY

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Intestinal parasitic infections make up approximately 85% of neglected tropical diseases (NDTs), which affect over 500 million of Sub-Saharan Africa's (SSA) poorest. Infection with these parasites may result in mortality and high morbidity. Despite the high prevalence of intestinal parasites in surrounding West African countries, low prevalence has been reported in The Gambia. In this pilot study, we aimed at determining the proportion of children with intestinal parasites in Sukuta and Marakissa School, The Gambia, using state of the art molecular diagnostic tools. Stool samples were collected from a total of 280 healthy children, 20 per age group randomly selected ranging from 0 to 14 years of age. Using multiplex real-time PCR, each child was screened for 7 different intestinal parasites, the protozoa: *Dientamoeba fragilis, Giardia lamblia, Cryptosporidium parvum* and the helminths: *Ascaris lumbricoides, Necator americanus, Ancylostoma duodenale,* and *Strongyloides stercoralis*. Despite unexpected previous deworming attempts in the age groups 7 to 14, several parasites were detected. Results indicate that the most commonly present helminths were *Necator americanus* and *Ancylostoma duodenale* with a cumulative positive percentage of 12.1%, followed by *Strongyloides stercoralis* (2.9%) and *Ascaris lumbricoides* (1.4%). The most commonly detected protozoa was *Giardia lamblia* (36.4%) followed by *Dientamoeba fragilis* (17.5%) and *Cryptosporidium parvum (*6.1%). Ongoing studies include improving DNA extraction from helminth ova and microscopical examination of all fecal samples. These findings indicate that intestinal parasites are more common in The Gambia than previously reported. In light of the adverse effects that intestinal parasites have on child health and considering that helminths may induce immune suppression, it is

recommended that further studies in larger cohorts investigate the prevalence of different intestinal parasites and their effect on susceptibility to other infections and on vaccine efficiency.

THE EFFECT OF *GIARDIA DUODENALIS* ON BOVINE MONOCYTE-DERIVED DENDRITIC CELLS AND T-CELL RESPONSES *IN VITRO*.

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Giardia duodenalis is an important intestinal parasite in animals and humans. Knowledge on the immune response against *Giardia* is indispensible for the development of a vaccine. Although dendritic cells are important in the initial phase of the immune response, the role of dendritic cells in the immunity against *G. duodenalis* is poorly documented and has only been studied in the mouse, which is not a natural host for this parasite.
In this study we addressed the effect of *G. duodenalis* trophozoites and excretion/secretion (ES) material on bovine monocyte-derived dendritic cells (MoDCs) and we examined the ability of stimulated DC to induce a T-cell response *in vitro*. In three experiments with 4 calves each, MoDCs were incubated with different numbers of live *Giardia* trophozoites or concentrations of ES material. None of the MoDC maturation markers (CD40, CD80 and MHC-II) were upregulated and no significant production of IL-4, IL-6, IL-10, IL-12 or TNF-α was measured in MoDC cultures after stimulation with *Giardia.* However, a dose-dependent decrease of ovalbumin uptake was observed in MoDCs incubated with trophozoites, suggesting functional maturation.
MoDCs stimulated with *Giardia* trophozoites induced a dose-dependent proliferation of allogenic peripheral blood mononuclear cells, which were depleted for antigen presenting cells. Fluorescent labeling of the proliferating cells with PKH showed that mainly $CD3^+$ αβ-T-cells were expanding, including both $CD4^+$ and $CD8^+$ T-cells. When $CD4^+$ T-cells were incubated with *Giardia*-stimulated MoDC, higher levels of IFN-γ and lower levels of IL-10 were produced, compared to T-cells that were incubated with unstimulated MoDC.
Our data show that *G. duodenalis* trophozoites activate bovine MoDCs *in vitro* and cause a state of semi-maturation, capable of inducing T-cell proliferation. A broader panel of cytokines will be measured to determine the phenotype of the induced immune response.

EXPERIMENTAL INDUCTION OF MILTEFOSINE AND PAROMOMYCIN RESISTANCE ON INTRACELLULAR *LEISHMANIA* AMASTIGOTES

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The limited number of treatment options linked to the increasing rate of treatment failures related to antimony (Sb) resistance is the major challenge in current anti-leishmania therapy. Miltefosine (MIL) has already moved to first-line status while the use potential of paromomycin (PMM) is currently under investigation. Once routinely used in the field, both will also become at risk for resistance. The present laboratory study addressed the dynamics of *in vitro* PMM and MIL resistance induction in *Leishmania donovani* and *L. infantum* isolates/clones, adopting a novel approach of exerting drug pressure only at the intracellular amastigote level. Briefly, intracellular amastigotes surviving the highest drug pressure were collected for promastigote expansion in the absence of drug pressure and used to start a next selection cycle on intracellular amastigotes. Compared to 'standard'

induction protocols on promastigotes, this procedure adequately mimics the natural situation in the field.
Regardless of the used species/strain, PMM-resistant parasites could be selected within 2-3 cycles. There was no cross-resistance with MIL and the Sb-resistance background (if present) remained unchanged. Subsequent cloning of one of the strains revealed the polyclonal nature of the induced strain, with some clones still fully susceptible to PMM, while others were tolerating 10x higher levels of PMM compared to the parent clone. It is difficult to judge whether such a 'rapid' selection for PMM would indeed occur in the field, but these laboratory observations at least stress the need for close epidemiological monitoring and the implementation of strong treatment policies to ensure long term efficacy of PMM. Surprisingly, selection for MIL-resistance using the same selection protocol did not lead to resistant *L. donovani* parasites. Although recovery of promastigotes from exposed amastigotes was possible at increasing drug concentrations, no decrease in susceptibility could be observed after 8 successive selection cycles, both as amastigote or promastigote. These results in some way reflect the current field situation where isolates of MIL-treated relapse patients still prove to be susceptible for MIL in *in vitro* drug-susceptibility assays. Relevant to note is that *L. infantum* on the other hand showed increasing levels of MIL-resistance during successive selection cycles, suggesting that MIL-resistance selection mechanisms may at least include some species-specific factors.

PREVALENCE OF INTESTINAL PARASITES IN A RURAL COMMUNITY OF VENEZUELA: COMPARISON OF MICROSCOPY AND PCR

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Over one billion people are infected with intestinal parasites worldwide, causing significant morbidity and mortality, particularly in developing nations. In this cross-sectional study we aim to determine the prevalence of intestinal parasites in the rural community "El 25" situated in north-central Venezuela and to compare the performance of PCR vs microscopy for the determination of intestinal parasites in stools. This investigation is part of a larger study aimed at identifying the risk factors of (re)infection after mass helminth treatment.
A total of 226 members from this community were included and microscopical examination was performed on SAF-fixed stools. For molecular determination, DNA was isolated from stools preserved in ethanol and a real time multiplex PCR for determination of *Giardia lamblia*, *Cryptosporidum parvum* and *Dientamoeba fragilis* was used. A monoplex PCR for the determination of *Entamoeba histolytica* was also performed.
Results indicate that according to microscopy 14% of the population was *G. lamblia* positive and 18% *D. fragilis* positive. According to the PCR 34% was shown to be *G. lamblia* positive and 35% *D. fragilis* positive. Neither *C. parvum* nor *E. histolytica* were found by microscopy or PCR. The PCR technique has been shown by us and others to be a sensitive technique that can detect DNA from cysts and trophozoites. However, when using PCR also DNA from the disintegrated stages of these parasites could be detected, indicating previous exposure. In order to diagnose patients shedding infectious stages of these protozoan parasites, microscopy remains an essential tool and should not be excluded. Most prevalent intestinal helminths determined by microscopy were *Ascaris lumbricoides* (25%) and *Trichuris trichiura* (25%) followed by hookworms (9%). For the molecular determination, a procedure to improve the isolation of DNA from helminthic ova in stools is currently being implemented.

REGULAR TREATMENT OF PRAZIQUANTEL DO NOT IMPACT ON THE GENETIC MAKE-UP OF *S. MANSONI* IN NORTHERN SENEGAL: EVIDENCE FOR DRUG TOLERANCE?

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The Senegal River Basin (SRB) experienced a major epidemic of intestinal schistosomiasis in the early nineties, after the construction of a dam for irrigation purposes. Exceptionally low cure rates following praziquantel (PZQ) treatment at the onset of the epidemic raised concerns about PZQ tolerant strains of *Schistosoma mansoni*, although they could also be attributed to the intense transmission at that time. A field study in the same region more than 15 years later found cure rates for *Schistosoma mansoni* still to be low, whereas *S. haematobium* responded well to treatment. We collected *S. mansoni* miracidia from children at baseline (prior to treatment), six months after two PZQ treatments and two years after the start of the study when they had received a total of five PZQ treatments. In total, 434 miracidia from 12 children were successfully genotyped with at least six out of nine DNA microsatellite loci. We found no significant differences in the genetic diversity of, and genetic differentiation between parasite populations before and after repeated treatment. Rapid re-infection alone cannot explain the sustained high genetic diversity after repeated treatment, suggesting increased PZQ tolerance of these *S. mansoni* isolates. More extensive field studies with shorter follow-up times are needed to confirm these observations.

ROLE OF TNF DURING *TRYPANOSOMA VIVAX* INFECTIONS

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BACKGROUND:

Trypanosomes are parasitic flagellated protozoa that survive in blood and tissue fluids of their host. *T. vivax* is a geographically widespread causative agent of the animal trypanosomiasis or 'nagana', affecting livestock in Africa and South America where the disease represents a major economic burden. In contrast to the extensive research focused on *T. brucei*, little is known regarding the biology and the immunopathology of *T. vivax* infections. In the context of the former, tumor necrosis factor (TNF) has been shown to play a major negative role in disease development. Interestingly, the opposite holds true for *T. vivax* infections where TNF is mainly needed for proper parasitemia control.

AIM:

To unravel the mechanisms underlying the increased susceptibility of TNF-KO mice by studying: (i) the effect of the disease on various TNF-producing cell populations, (ii) the qualitative and quantitative antibody response in the presence and absence of TNF, (iii) the kinetics of infection-associated TNF production.

RESULTS AND CONCLUSION:

So far, our study has resulted in the following observations: (i) TNF-KO mice do not control the first parasitemia peak, (ii) *T. vivax* has a similar destructive effect on the cellular immune system both in wild type and TNF-KO mice, (iii) TNF secretion correlates with the parasite load in the host, (iv) the

observed difference in infection pattern in WT and TNF-KO mice cannot be explained by a defective antibody response in the latter, (v) monocytes and neutrophils are the main producers of the TNF.

SPOTLIGHT ON BABESIOSIS IN BELGIUM

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Infection of humans with species of *Babesia* can have serious implications for human health and blood transfusion. However the risk of human infection in many European countries is not known with accuracy. This study aimed to assess the general prevalence of *Babesia* parasites in Belgium, with particular emphasis on species with zoonotic potential. Investigation for evidence of *Babesia* spp. in ticks identified species previously unreported in Belgium, *Babesia* sp. EU1 and *Babesia capreoli*, and confirmed the presence of potential zoonotic species. Evaluation of infection rate in ticks collected from respective vertebrate hosts and the environment was found to be between 1.3 and 14.6%. A seroprevalence of 14.3% for *Babesia* spp. was estimated in bovines from Southern Belgium and a prevalence of between 9 and 40%, against three known zoonotic *Babesia* spp., was obtained using samples representing a human "at risk" population. Co-infections with *Babesia* and *Borrelia* spp. or *Anaplasma phagocytophilum* were identified.
We conclude that babesiosis should be considered as a threat for susceptible livestock and humans, especially the elderly and immunocompromised. Preventive action can be implemented to minimize the risk of acquiring tick-borne disease, but the most useful strategy remains dissemination of relevant risk information to the medical community and the general public.

POOLING STOOL SAMPLES: A COST-EFFECTIVE STRATEGY TO ASSESS INFECTION INTENSITY OF SOIL-TRANSMITTED HELMINTHS AND TO MONITOR DRUG EFFICACY?

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Background: Up to date cost-effective strategies to guide health care decision makers on how to optimize control of soil-transmitted helminths (STH) and on how to detect the development of anthelmintic resistance are scarce. In the present study, we developed and evaluated a novel pooling strategy to assess intensity of STH infections and to monitor drug efficacy.
Methods/Principal Findings: Stool samples from 840 children attending 14 primary schools in Jimma, Ethiopia were pooled (pool size of 10, 20 and 60) to evaluate the infection intensity of STH. In addition, the efficacy of a single dose mebendazole (500 mg) through reduction in fecal egg counts (FECR) was evaluated in two of these schools. Both individual and pooled samples were examined with McMaster egg counting method. For each of the three STH, we found a significant positive correlation between mean fecal egg counts (FEC) of individual and FEC based on pooled samples, ranging from 0.62 to 0.98. Compared to the FEC based on individual samples, there was no significant difference in FEC, except for *A. lumbricoides*. For this STH, pools of 60 samples resulted in significantly higher FEC. FECR for the different number of samples pooled was comparable for all pool sizes, except for hookworms. For this parasite, pools of 10 and 60 samples provided significant higher FECR results.

Conclusion: This study highlights that pooling stool samples holds promise as a cost-effective strategy to assess intensity of STH infection on a population level and to monitor preventive chemotherapy programs. When using the McMaster egg counting method, up to 10 samples can be pooled. However, further research is required to gain more insights into the impact of pool size, sample size, detection limit of the FEC method, intensity and aggregation of infections on the validity of pooling stool samples.

CONTROL OF POULTRY RED MITE (*DERMANYSSUS GALLINAE)* IN LAYER FARMS USING AN AUTOMATED MONITORING DEVICE

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The Poultry red mite *(Dermanyssus gallinae)* is a common ectoparasite in poultry farms worldwide, feeding on blood of poultry and sometimes humans in order to development to the adult stage and reproduce. A poultry red mite (PRM) infestation may result in high economic losses, veterinary risks and allergic reactions among farm workers. Control of PRM has become more difficult due to development of resistance to some acaricides (Chauve, 1998; Marangi et al., 2009; Nordenfors et al., 2001) and a ban on others. Therefore recent research has been focused on more environmentally friendly control methods such as the use of natural enemies (Lesna et al., 2009), attract and kill methods using fungi (Koenraadt and Dicke, 2010) and development of a vaccine (Bartley et al., 2009). For an effective, timely and place specific application of the control methods, monitoring of the size, place and the development of the PRM population is necessary. Currently, the monitoring methods for PRM are labor intensive, mostly applicable to one poultry housing system and only fit for research purposes. Therefore, we aim to develop an automated monitoring device for PRM in layer farms which is composed of an automated counter of PRM and a dynamic adaptive model. This monitoring device enables to assess the PRM population in 1) the actual situation, 2) after a treatment (effect) and 3) in future situations (necessary to indicate timely treatment). Recently, an experimental automated PRM counter has been developed using the approach of methodical design.

ANTHELMINTIC RESISTANCE OF SHEEP GASTRO-INTESTINAL NEMATODES IN 4 CONTINENTAL EUROPEAN COUNTRIES

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Anthelmintic resistance (AR) is widespread in gastro-intestinal nematodes in the main sheep-rearing countries, including the United Kingdom, Australia and New Zealand. This study aimed to gain a better understanding of the occurrence of AR in the following EU markets: Spain, Greece, France and Italy. In each country, ten sites were selected, eachhaving at least 50 sheep naturally infected with gastro-intestinal nematode parasites. Animals were allocated into 4 treatment groups (moxidectin, ivermectin, fenbendazole and levamisole/netobimin in France, all oral formulations) and one negative control group based on individual pre-treatment faecal egg counts (FEC). A modified McMaster technique, with a sensitivity of 50 eggs per gram of faeces was used to monitor faecal egg excretion. The assessment of efficacy was based on the difference between the mean (arithmetic and geometric) FEC of the treatment group and the control group 14 days after treatment. The preliminary results illustrate differences in the prevalence of AR between countries. In general, moxidectin and ivermectin showed the highest efficacy, whereas AR against fenbendazole and

against levamisole was diagnosed more frequently. More detailed results will be presented at the meeting.

EVALUATION OF MITOCHONDRIAL GENES NAD1, COX1, 12S AND NAD5 IN MOLECULAR DIAGNOSIS OF CESTODES

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Cestodes, or tapeworms, are a group of parasitic organisms. The adult tapeworms from various species such as *Taenia saginata*, *T. solium* or *Diphyllobothrium* sp. can infest the human gut. Several species can infect the human body in their larval stage, for example *T. solium*, and cause severe illness. Our laboratory receives different types of patient samples such as cyst fluid, pieces of proglottids and even fecal samples with cestode eggs. Molecular diagnostics for these parasites should be highly sensitive, able to detect a cestode infection for instance in cyst fluid from patients with a yet undiagnosed disease, but also be a means to specify the infecting cestode. We aim to perform molecular testing that is able to detect a wide variety of different cestodes, using sensitive PCRs and then to determine the species by sequencing the PCR products. We have tested PCRs described by others, as well as PCRs that we have developed ourselves. We currently use PCRs on mitochondrial genes Cox1, 12S rRNA and Nad5. Previously, we used PCRs on the Cox1 and Nad1 genes as published by Bowles and co-authors in 1992 and 1993. The sensitivity and specificity of the PCRs have been determined. Patient samples have been screened using all PCRs retrospectively and prospectively. Furthermore, a large collection of specimens from animal origin has been studied as well. The results show that 12S and Nad5 are suitable targets for highly sensitive PCR and harbour a large number of nucleotide polymorphisms that allow for typing and sub-typing cestode species.

EVALUATION OF THE OVATEC PLUS FLOTATION METHOD AS A DESISION TOOL FOR SELECTIVE ANTHELMINTHIC TREATMENT AGAINST STRONGYLES IN HORSES

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The objective of the present study was to evaluate the Ovatec Plus flotation method (OPFM; Pfizer Animal Health Diagnostics) as an easy-to-use fecal flotation system for the selection of those horses that do not need to be treated in the framework of a selective anthelminthic management program. In this study, the number of eggs was determined using the OPFM, however the egg counts were not expressed as Eggs Per Gram (epg), as the faecal sample used for diagnosis was not standardized. As such the OPFM was used as a qualitative diagnostic method. The OPFM outcome was evaluated, using contingency tables with the McMaster assay as gold standard (sensitivity of 50 epg). Two hundred equine fecal samples were selected as follows: 54 McMaster negative samples, 47 samples with 50-150 epg, 49 samples with 200-600 epg and 50 samples with > 600 epg, and all 200 samples were examined with the OPFM. Out of 71 OPFM negative samples, 50 (70%) had a negative McMaster result, 18 (26%) had a McMaster count between 50 and 150 epg and 3 (4%) had a McMaster count above 150 epg, resulting in a sensitivity of 97% for samples above and 62% for samples ≤150 epg. The OPFM specificity was 100% compared to the McMaster. When using cut-off values for selective anthelminthic treatment in horses, a threshold of 200 to 500 epg is commonly used. As such, the OPFM provides a cheap and easy-to-use tool to identify those horses in which treatment is not needed with a 100% sensitivity if the 500 epg threshold is used, and with a 97% sensitivity if the 200 epg threshold is used, and to pre-select those samples in which a lab-based McMaster is required to quantify the egg counts.

PILOT STUDY ON HUMAN AND ZOONOTIC INFECTIONS OF GASTROINTESTINAL PARASITES IN SOUTHERN INDIA

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South-Asia contributes substantially to the number of gastrointestinal (GI) infections occurring globally in humans, including *Ascaris*, *Trichuris* and hookworms, the so called soil-transmitted helminths (STH). There is presumptive evidence that animals such as dogs and pigs contribute to the epidemiology of these parasites. In southern India, the role of these animals as reservoir for most GI parasites, especially the human STH still remains unclear. The main objective of this pilot study was to assess parasitic infections in both humans and pigs in Vellore district (southern India).

The study was carried out in 5 villages of Jawadhi hills, which houses an aboriginal population, mostly heterogeneous set of ethnic and tribal groups. A total of 100 stool samples were collected from children, aged 2-10 years and 54 stool samples from pigs which were reared in close proximity to the study children. The samples were then screened for various intestinal parasites using a saline wet mount microscopy.

Of the total human stool samples collected, *Giardia* (20%) accounted for most of the parasitic infection. Hookworm was seen in 9% of the samples collected. A single case of *Enterobius vermicularis* and *Hymenolepis diminuta* were also observed. In the pig stool samples collected, *Balantidium coli* (37.5%) accounted for majority of the infection followed by *Giardia* (2%).There were 7 (13%) samples that showed hookworm-like eggs. None of the stool samples collected either from humans or pigs showed *trichuris* or *ascaris* infection.

In the present study, *Ascaris* and *Trichuris* infections were absent in both humans and pigs, and hence no conclusions could be drawn on the role of animals as reservoir for human STH. However, it indicates the presence of potential zoonotic protozoa, *B. coli* and *Giardia.* In the near future, molecular methods will be applied to identify the hookworm infections in humans and the hookworm-like eggs found in pigs.

LIVER FLUKE INFECTION: CHARACTERIZATION AND DISEASE RISK OF SMALL WATER BODIES AS A COMPLEMENTARY TOOL FOR DISEASE CONTROL

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Fasciolosis, caused by the trematode parasite *Fasciola hepatica*, causes serious production losses in dairy cattle worldwide. In Flanders, the cost for liver fluke infections at dairy farms was estimated at € 8.2 million a year. The presence of the intermediate host *Galba truncatula* is a key-factor for disease transmission. As part of the SATHELI project, aimed at detecting small water bodies (SWB) and their dynamics by means of very high-resolution imagery obtained from satellites and unmanned drones, field visits are performed to estimate the *F. hepatica* disease risk for different types of SWB's. The research methodology and preliminary results are presented.

In this study, four dairy farms in two geographically distinct areas (Bruges and Zoersel) and which had previous exposure to *F. hepatica* were selected. Different types of SWB's on the pastures were identified for monthly sampling by a transect analysis. This comprises a 15 minutes search for fresh water snails in a 10 m transect. The retrieved snails are morphologically identified. The population dynamics of *G. truncatula* are studied by measuring the shell length of the snails and cercarial

shedding is evaluated *in situ*. Infection status of the herd is determined by sampling ten animals of different age classes (1st, 2nd season grazers and adults) before and after grazing season and in winter. Blood and faecal samples are tested by complementary diagnostic techniques (sedimentation-flotation, antibody- and copro-antigen ELISA).
The number of habitats sampled at the farms varies between 11 and 18. Five main habitats types could be differentiated: pond, ditch, trench, furrow and moist trampled/transition areas. Both the serum *F. hepatica* ELISA and sedimentation flotation technique confirmed the infection of the farms at T0.

HELMINTH INFECTIONS: DO THEY AFFECR THE PRODUCTIVE EFFICIENCY OF SPEACIALISED DAIRY FARMS?

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Subclinical infections with gastrointestinal (GI) nematodes and liver fluke are an important cause of production losses in grazing dairy cattle. Attempts to evaluate the economic impact of these production losses were mainly based on partial analysis techniques, and few studies have looked more integrally at the impact on productive efficiency at farm level. Because the impact of helminth infections has become more subtle and is farm-specific, a more refined economic evaluations of actions is needed to increase or maintain the health of livestock on individual farms. The objective of this research is to analyse the effect of GI nematode and liver fluke infections on the technical efficiency in dairy farms. Farm-specific results from a parasitic monitoring campaign, expressed as an optical density ratio (ODR), are linked with individual farm data from the Belgian Farm Accountancy Data Network (FADN). As a result, a dataset of about 45 specialised dairy farms is obtained, combining economic and epidemiologic information. Their technical efficiency (TE) is calculated with non-parametric data envelopment (DEA) analysis. Multiple variants of the DEA approach are used, differing in the way they incorporate infection in the production model. Rank correlation, regression models, and cluster analysis are used to analyse the relationship between TE and the level of helminth infection. Preliminary data analysis shows an ODR mean and standard deviation of 0.82 ± 0.21 and 0.79 ± 0.34, for GI nematode and liver fluke infections, respectively. More than 80% of the farms have an ODR above 0.5, suggestive for a negative effect of helminth infections on milk production. The TE scores, which range between 0 (totally inefficient) and 1 (fully efficient), show a mean technical efficiency of 0.708. More than 90% of the farms are shown to produce at an inefficient level

ANTHELMINTIC RESISTANCE AND EGG RE-APPEARANCE PERIOD IN HORSES IN BELGIUM AND THE NETHERLANDS

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The objective of the present study was to examine the occurrence of anthelmintic resistance in horses in Belgium and The Netherlands using a Faecal Egg Count Reduction test and to investigate the egg reappearance period of strongyle type eggs after treatment with ivermectin and moxidectin in animals naturally infected with gastrointestinal nematode parasites. The study was conducted using a randomized complete block design for each study site, with the individual animal as the experimental unit. Horses were sampled in up to 10 different sites per country. At each site, suitable animals were initially ranked according to pre-treatment strongyle FEC, and randomly distributed over the 2 treatment groups. Each treatment group had a minimum of 5 animals. Efficacy of treatment was evaluated based on the reduction in faecal egg excretion after treatment (Day 14) compared to the faecal egg excretion before treatment (Day 0), and further up to 56 days for the ivermectin treated animals and up to 84 days for the moxidectin treated animals. The diagnostic technique used to monitor the faecal egg excretion was a modified McMaster technique, with a sensitivity of 25 eggs per gram of faeces. Preliminary results show overall good efficacy on Day 14 (FECRT), although for both compounds treatment failures after D14 were observed, indicating a shorter egg reappearance period than aniticipated. More detailed and final results will be presented at the meeting.

ANTHELMINTIC RESISTANCE OF CATTLE GASTRO-INTESTINAL NEMATODES IN 5 EUROPEAN COUNTRIES

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Anthelmintic resistance (AR) in gastro-intestinal nematodes of cattle (mainly *Cooperia* spp.) is emerging worldwide, This study aimed to gain a better understanding of the prevalence of AR in 5 major European markets: Spain, UK, France, Germany and Italy. In each country, ten sites were selected having at least 20 cattle naturally infected with gastro-intestinal nematode parasites. Animals were allocated into 2 treatment groups (moxidectin or ivermectin 1% injectable formulations) based on individual pre-treatment faecal egg counts (FEC). A modified McMaster technique with a sensitivity of 12.5 eggs per gram of faeces (epg) was used to monitor faecal egg excretion. Assessment of efficacy was based on the difference between the mean (arithmetic and geometric) FEC of the treatment group pre- and 14 days post treatment. The intermediate results illustrate differences in AR prevalence between countries and between compounds. In general, the preliminary results show a high efficacy of moxidectin. The final and more detailed results will be presented at the meeting.

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