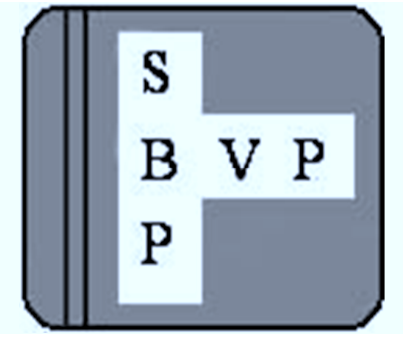

Scientific meeting of
the Belgian Society for Parasitology

Friday 10 June 2011

Veterinary Faculty

University of Liège

PROGRAMME

09:00	Coffee and registration	
10:00	BSP President	Welcome address

Session 1 - Chair: Bertrand Losson

10:15	Keynote 1: J.-C. Dujardin	IN THE SHADOW OF THE GENOME: A CHALLENGING JOURNEY TO THE DIVERSITY OF *LEISHMANIA DONOVANI*
10:50	Inocêncio da Luz et al.	*IN VITRO* TOOLS TO MONITOR DRUG SUSCEPTIBILITY IN FIELD ISOLATES OF *LEISHMANIA* SPP.
11:10	Hendrickx et al.	EXPERIMENTAL PAROMOMYCIN RESISTANCE IN A *LEISHMANIA DONOVANI* FIELD ISOLATE
11:30	Alves-Ferreira et al.	THE ENZYME RIBOSE-5-PHOSPHATE ISOMERASE FROM *LEISHMANIA MAJOR*: MOLECULAR AND STRUCTURAL ANALYSIS IN COMPARISON WITH THE ANALOGOUS HUMAN ENZYME
11:50	Ehsan et al.	*CRYPTOSPORIDIUM* AND *GIARDIA* IN DIFFERENT WATER MATRICES IN BELGIUM
12:10	Lempereur et al.	*DERMACENTOR RETICULATUS* POPULATIONS IN BELGIUM AND PRELIMINARY INVESTIGATIONS OF ASSOCIATED *BABESIA* SPP. PATHOGENS
12:30	Lunch	
13:30	BSP general meeting and election of committee members	

Session 2 - Chair: Edwin Clarebout

14:00	Keynote 2: Paul Michels	THE USE OF COMPARATIVE SYSTEMS BIOLOGY FOR THE SELECTION OF ENZYMES AS DRUG TARGETS IN TRYPANOSOMATID PARASITES
14:35	Chitanga et al.	LOW LEVEL DIMINAZENE ACETURATE RESISTANCE IN AFRICAN TRYPANOSOMES IN THE ABSENCE OF DRUG PRESSURE
14:55	Lempereur et al.	WILD RUMINANTS AND POTENTIALLY ZOONOTIC *BABESIA* SPP. IN BELGIUM
15:15	Keynote 3: C.H. Hokke	HELMINTH GLYCOMICS: SCHISTOSOME GLYCOSYLATION AS A TARGET FOR IMMUNE INTERVENTION
15:50	Tea break	

Session 3 - Chair: Cornelis H. Hokke

16:20	Caron et al.	NEW INSIGHT IN THE LYMNAEIDS INTERMEDIATE HOST OF *FASCIOLA HEPATICA* IN BELGIUM
16:40	Vlaminck et al.	EVALUATION OF A NEW SERODIAGNOSTIC TEST FOR ASCARIOSIS IN PIGS
17:00	Masure et al.	GASTRO-INTESTINAL IMMUNE RESPONSE DURING THE EXPULSION PHASE OF *ASCARIS SUUM* IN PIGS
17:20	Belgacem et al.	EVALUATION OF THE MECHANISMS INVOLVED IN THE PATHO-PHYSIOLOGICAL ALTERATIONS OF THE ABOMASAL MUCOSA IN CATTLE INFECTED WITH *OSTERTAGIA OSTERTAGI*
17:40	Janssen Award and Closing session	
19:00	Dinner at Colonster castel	

IN MEMORIAM

PETER VAN DEN BOSSCHE

16 MARCH 1962 – 11 NOVEMBER 2011

Peter Van den Bossche was the secretary of the Belgian Society for Parasitology (BSP) since 2002. Peter tragically died when his car was hit in a car accident in Antwerp early in the morning of 11 November 2011. He was 48 years old and left a beloved wife and three children. Early in the morning of the armistice day, Peter was on his way to the Institute of Tropical Medicine (ITM) where he was working since 2000. This tragedy created a huge loss for his family and friends but also among his colleagues at the ITM and various professional organisations, including BSP.

Peter started his career in Eastern Zambia where he worked on the epidemiology and the control of tsetse-transmitted trypanosomiasis. He developed, with his Belgian and Zambian colleagues, basic but successful tsetse and trypanosome control programmes based on a drug delivery scheme and rudimentary insecticide impregnated targets made of cheap black cloth and bamboo sticks. He was then invited to work for a regional tsetse and trypanosomiasis control project, which allowed him to operate in the whole SADC region. Peter produced a PhD thesis based on his field experience entitled "The development of a new strategy for the sustainable control of bovine trypanosomosis in Southern Africa".

Due to his large field and research experience, Peter acquired an international reputation as an expert in the control and epidemiology of vector transmitted pathogens. With his lucid vision, his pragmatic approach and his communicative enthusiasm, he undertook many field studies in collaboration with many colleagues from the North as well as from the South. At work, Peter manifested enormous zeal and enthusiasm. His pleasant character and eternal optimism caused many of his colleagues within and outside the ITM to become real friends of his.
His commitment in multiple projects and missions abroad did not prevent him from enjoying social life as an actual Burgundian.

We are very grateful for the opportunity we had to know Peter and be his friend. He will remain for us a very inspiring leader.

Tanguy Marcotty on behalf of the BSP committee

INVITED SPEAKERS

IN THE SHADOW OF THE GENOME: A CHALLENGING JOURNEY TO THE DIVERSITY OF *LEISHMANIA DONOVANI*

Jean-Claude Dujardin

Molecular Parasitology, Institute of Tropical Medicine, Antwerp

Visceral leishmaniasis is a potentially fatal disease endemic to large parts of Asia and Africa primarily caused by the protozoan parasite *Leishmania donovani*. Here, we report a high-quality reference genome sequence for a strain of *L. donovani* from Nepal, and use this sequence to study variation in a set of 16 related clinical lines, isolated from visceral leishmaniasis patients from the same region, that also differ in their response to in vitro drug susceptibility. We show that whole-genome sequence data reveals genetic structure within these lines not shown by multilocus typing, and suggests that drug-resistance has emerged multiple times in this closely related set of lines. Sequence comparisons with other *Leishmania* species, and analysis of single-nucleotide diversity within our sample showed evidence of selection acting in a range of surface- and transport-related genes, including genes associated with drug resistance. Against a background of relative genetic homogeneity, we found extensive variation in chromosome copy number between our lines. Other forms of structural variation were significantly associated with drug-resistance, notably including gene dosage and the copy number of an experimentally-verified circular episome present in all lines and described here for the first time. This study provides a basis for more powerful molecular profiling of visceral leishmaniasis, providing additional power to track the drug resistance and epidemiology of an important human pathogen.

THE USE OF COMPARATIVE SYSTEMS BIOLOGY FOR THE SELECTION OF ENZYMES AS DRUG TARGETS IN TRYPANOSOMATID PARASITES

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Trypanosoma brucei, when living in the blood of its human host, is for its ATP supply entirely dependent on the conversion of glucose into pyruvate by glycolysis. Its glycolytic pathway is organized in a unique manner; the majority of its enzymes are sequestered in peroxisome-like organelles called glycosomes. The glycolytic enzymes of trypanosomes have been perceived as promising drug targets because of their essentiality for the parasites' viability and their peculiar structural and functional properties related to the pathway's organization. Indeed, experimental data support this notion.

In order to optimize the selectivity of glycolytic enzyme-inhibitors to be used as drugs, we embarked on a systems biology approach in which the contribution of each *T. brucei* enzyme to the glycolytic-flux control was determined. A mathematical model of glycolysis of bloodstream-form of this organism was developed on the basis of experimentally determined kinetic data of all enzymes. Simulations using this model reproduced correctly the steady-state process, and predicted fluxes and metabolite concentrations as were determined under various conditions. Calculated flux control coefficients for all steps showed that the process is not controlled by ATP utilization of the cell but by ATP supply, with most control exerted by glucose uptake into the cell and the remainder shared by several enzymes. The predictions made with this model were compared with those obtained using an available kinetic model for glycolysis in human erythrocytes. In these latter cells, glycolysis appears largely controlled by ATP utilization rather than supply and the flux control coefficient of the glucose transporter is negligible. By a differential flux control analysis, we calculated what extent of parasite- versus host-enzyme selectivity of inhibitors should be achieved for different enzymes to guarantee that trypanosome glycolysis will be inhibited by at least 95% with less than 5% effect on the flux in human erythrocytes.

HELMINTH GLYCOMICS: SCHISTOSOME GLYCOSYLATION AS A TARGET FOR IMMUNE INTERVENTION

Cornelis H. Hokke

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Schistosomes, blood-dwelling parasitic flatworms with a complex life cycle, infect about 200 million people in (sub-)tropical areas worldwide and cause an enormous burden of disease. Similar as for other human and animal helminth infections, glycans and glycoconjugates expressed by schistosomes play a prominent role in the parasite's biology and the interaction with the host. A significant amount of structural data regarding glycosylation of different schistosome life stages and glycoconjugate subsets has been collected in the past decades, but large gaps in our knowledge of the schistosomal glycome remain. While previously schistosome glycoconjugates have been studied and used as diagnostic targets, our current research focuses on schistosome glycans associated with immunity. On the one hand, we study the glycosylation of individual immunomodulatory schistosome glycoproteins and their differential interaction with lectins of the immune system such as DC-SIGN, MR, MGL and galectins. These studies contribute to our understanding of innate immune mechanisms directing the immune response mounted to parasitic worms. On the other hand we perform large scale glycomics studies with the aim of identifying novel glycosylated vaccine targets. In humans, a large portion of the antibody response to schistosomes is directed against the numerous different glycan epitopes expressed by the different life stages of the parasite. At present it is unclear if such anti-glycan antibodies can confer protection to (re-)infection or not. To address this problem, we have first recorded N- and O-glycan profiles of all developmental stages of *S. mansoni*, including the invasive larvae, juvenile and adult worms, eggs and miracidia. These profiles indicated that the expression of many glycans and glycan elements gradually shifts between the different stages, but clearly also glycans unique for particular stages were found. Subsequently, we have printed the hundreds of glycans and glycan fractions purified during the structural investigations on a so-called shotgun glycan microarray. We have screened the glycan microarray with sera from different cohorts of schistosome-infected individuals collected in countries endemic for *S. mansoni* and *S. haematobium*, to determine serum antibody response profiles in relation to variables such as infection intensity, duration, treatment and resistance to infection. The generated data and the possibilities for the development of a glycan-based vaccine and other intervention methods for schistosomiasis and other helminth infections will be discussed.

ORAL PRESENTATIONS

IN VITRO TOOLS TO MONITOR DRUG SUSCEPTIBILITY IN FIELD ISOLATES OF *LEISHMANIA* SPP.

Inocêncio da Luz R.[1], Hendrickx S.[1], Dujardin J.C.[2], Cos P.[1], Maes L.[1]

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Visceral leishmaniasis (VL) is a life-threatening vector-borne disease caused by intracellular protozoa's of the genus *Leishmania (L. donovani, L. infantum)*. Curing VL is challenging and current treatment options are limited. For more than seventy years, pentavalent antimonials (Sb^V) have been the first-line therapy. Unfortunately, their use in disease-endemic areas is now threatened by the emergence of drug-resistance. Since the incidence of both VL and Sb^V resistance is increasing, a better understanding of the factors involved in the selection of drug-resistant mutants is important. In view of the lack of standardized laboratory tests, our research specifically focused on the development and validation of *in vitro* tools to phenotypically monitor drug resistance. The only valid method to assess drug susceptibility is the intracellular amastigote assay on macrophages. Since clinical isolates frequently show very variable *in vitro* growth, a method for enhanced metacyclogenesis and infectivity of promastigotes first needed to be developed. The standardized assay was subsequently used to define the *in vitro* susceptibility of field isolates originating from different sources and clinical settings. Monitoring sequential *L. infantum* isolates from Sb-treated dogs in Brazil provided a clear indication that resistance can indeed develop quickly after only a single Sb^V course. In *L. infantum* strains obtained from HIV-co-infected patients in South-America and France, pre-treatment isolates were already highly resistant to Sb^V but still susceptible to Sb^{III}, possibly limiting successful cure on the long term and a possible initial step towards full resistance. Hence, continued monitoring of drug resistance remains a priority and current treatment options in man and dogs need to be tailored to conserve all therapeutic options for VL in the future.

EXPERIMENTAL PAROMOMYCIN RESISTANCE IN A *LEISHMANIA DONOVANI* FIELD ISOLATE

Inocêncio da Luz R., Hendrickx S., Kuypers K., Van Assche T., Cos P., Maes L.
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New treatments still need to be explored due to the limited anti-leishmania therapeutic options and the increasing problem of treatment failure caused by antimony (Sb) resistance. The aminoglycoside antibiotic paromomycin (PMM) has been shown to be effective against *Leishmania* and its clinical potential as a first-line drug is currently under investigation. Once routinely used in the field, PMM will frequently be used in patients unresponsive to Sb with the real risk of rapid development of drug resistance. This study addressed the dynamics of *in vitro* PMM resistance induction.

Continuous PMM drug pressure was exerted on a Sb-resistant *L. donovani* clone whereby the novel approach was adopted to select for drug resistance at the intracellular amastigote level. Up till now, similar work in literature has always used axenic promastigotes. Briefly, the highest drug pressure was maintained at the intracellular stage and surviving amastigotes were collected for promastigote expansion and metacyclogenesis to initiate a next selection cycle. The added value of this selection protocol is that it fully mimics the situation as it would happen in the field, namely drug pressure in the mammalian host stage and disruption of drug pressure in the vector stage.

PMM-resistant parasites could already be selected within two selection cycles and subsequent cloning revealed the polyclonal nature of the induced strain, with some clones still fully susceptible to PMM (IC_{50} < 85 μM), while others were highly resistant tolerating 10x higher levels of PMM (IC_{50} > 300 μM) compared to the parent strain (IC_{50} = 44.5 ± 5.6 μM). Cross-resistance with the other anti-leishmania drug miltefosine was not present while the Sb-resistance profile remained unchanged, creating a multi-drug (Sb + PMM) resistant strain.

It is unknown whether such ‘rapid’ selection would occur in the field, but these laboratory observations at least endorse the need to set-up close epidemiological monitoring and adopt strong treatment policies to ensure long term efficacy of PMM.

THE ENZYME RIBOSE-5-PHOSPHATE ISOMERASE FROM *LEISMANIA MAJOR* : MOLECULAR AND STRUCTURAL ANALYSIS IN COMPARISON WITH THE ANALOGOUS HUMAN ENZYME

Alves-Ferreira M.[1,2], Baptista L.P.[3], Schwarz M.A.[1], Capriles P.V.[3], Guimarães A.C.[1], Aloise R.[1,4], Custódio F.L.[1], Miranda A.B.[5], Da-Silva S.A.[6], Degrave W.M.[1], Dardenne L.E.[2]

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Leishmaniasis is an infectious disease caused by protozoa of the genus *Leishmania*, and is endemic in several parts of the world. There is no vaccine against these parasites, so all control of this disease relies on therapeutic treatment. However the drugs currently used are toxic and there are reports of strains being resistant. The Pentose Phosphate Pathway serves two very important purposes: it is responsible for maintenance of the redox level in the parasite, by production of NADPH, and for the synthesis of ribose-5-phosphate (R5P), used for nucleotide production. The enzyme ribose-5-phosphate isomerase (Rpi) catalyses the reversible isomerization between ribulose-5-phosphate (Ru5P) and R5P. Two types of Rpi exist: RpiA, that is broadly distributed among eukaryotic organisms, in metazoa as well as fungi, and in some bacteria; and RpiB found in most prokaryotic organisms. Previous studies showed that trypanosomatids possess the isoform RpiB that is analogous to the RpiA in humans. We performed some molecular and structural studies of the RpiB from *L. major* (LmRpiB). The gene of *L. major* has been cloned in the pBadThio/TOPO vector (Invitrogen), and the protein expressed in insoluble form, purified and produced for raising a polyclonal antiserum. For identification of the native enzyme we performed a 2D-PAGE with soluble proteins from *L. major* promastigotes, followed by western-blot analysis. We identified a spot with MW = 18.27 kDa and pI = 6.52, values very close to the predicted ones (18.61 and 6.40, respectively). This expression corroborates with the RT-PCR result obtained using mRNA from promastigotes. The three-dimensional structures of LmRpiB and *Homo sapiens* enzymes (HsRpiA) were determined, using comparative modeling and *ab initio* methods. The structural characterization of the active site occupation of LmRpiB and HsRpiA was performed by docking studies with the substrates R5P (ring-closed and ring-opened forms) and Ru5P. Supported by: CNPq, FAPERJ, dDI.

CRYPTOSPORIDIUM AND *GIARDIA* IN DIFFERENT WATER MATRICES IN BELGIUM

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The protozoan parasites *Cryptosporidium* and *Giardia* are worldwide considered as an important cause of gastrointestinal disease. Drinking water is a main infection source, especially when produced from surface water. Next to human wastewater, livestock is considered to contribute to surface water contamination. Recent studies indicated that zoonotic sub-genotypes of both parasites are highly prevalent in ruminants in Belgium. Nevertheless, in most reported outbreaks the source of contamination remained uncertain because morphological identification or molecular genotyping up to species level could not discriminate between (oo)cysts from human or animal sources.

In this study, a protocol was optimised using FiltaMax Xpress filtration, immunomagnetic separation and immunofluorescence for the detection of *Cryptosporidium* and *Giardia* in three different water matrices from four catchment sites in Belgium: raw surface water (RW), water after sedimentation and prior to treatment (SW), and purified drinking water (PW). The average recovery rates for *Cryptosporidium* were 44 ± 10 % in RW, 41 ± 12 % in SW and 45 ± 16 % in PW. For *Giardia* the recovery rates were 28 ± 22%, 34 ± 15% and 45 ± 20%, respectively.

Monthly monitoring of 3 water matrices at the Blankaart catchment site revealed that raw water samples were frequently contaminated with *Giardia* and *Cryptosporidium*. Peak values up to 35 *Giardia* cysts/l and 51 *Cryptosporidium* oocysts/l were obtained in April 2010 and February 2011, respectively. (Oo)cyst contamination of sediment water followed a similar seasonal pattern, but (oo)cyst numbers were lower compared to raw water, indicating that the sedimentation procedure decreases the parasite load. Only low numbers of (oo)cysts were recovered from raw water samples (< 1 /liter) in Zillebeke, Dikkebus and Gavers sites. No natural infection was found in pure water in all sites. The sub-genotype of the recovered (oo)cysts is currently determined to identify potential sources of contamination.

DERMACENTOR RETICULATUS POPULATIONS IN BELGIUM AND PRELIMINARY INVESTIGATIONS OF ASSOCIATED *BABESIA* SPP. PATHOGENS

Cochez C.[1*] and Lempereur L.[2*], Madder M.[3], Claerebout E.[4], Simons L.[1], De Wilde N.[4], Linden A.[5], Saegerman C.[6], Heyman P.[1], Losson B.[2]

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*equal contribution

The occurrence of indigenous clinical cases of canine (Losson *et al.,* 1999) and equine (Mantran *et al.,* 2004) babesiosis in Belgium during the last two decades suggested that the vector of the pathogens responsible for these diseases, *Dermacentor reticulatus,* could be present in Belgium. Recent reports indicated an expanding geographical distribution of *Dermacentor reticulatus* in Western Europe but until now it was uncertain that indigenous *Dermacentor* populations were established in Belgium. Four different locations throughout Belgium, identified as potential *Dermacentor reticulatus* sites, were monitored by flagging during 2010. Ticks were stored in 100% ethanol immediately after trapping and morphologically identified. Tick DNA extraction was performed according to the proteinase K protocol (20mg/ml) and to avoid false-negative results, an additional PCR targeting the tick 16S rRNA gene was performed. Only tick-DNA positive samples were further analyzed for the presence of *Babesia* spp. A *Babesia* spp. genus-specific PCR was applied based on the amplification of the 18S rRNA gene. Two different tick species were identified, *Ixodes ricinus* and *Dermacentor reticulatus*. A total of 236 *D. reticulatus* adult ticks were collected from the 4 sites. Ticks were found mainly from early March until the end of May with a peak of activity in April. Four out of 234 tick extracts remained negative for the 16S rRNA gene PCR even after diluting the samples 10 and 100 X. The remaining 230 DNA extracts yielded negative results for *Babesia*. This is the first record of indigenous questing populations of *D. reticulatus* in several areas of Belgium. The low number of ticks examined for the presence of *Babesia* spp. specific DNA did not allow us to conclude about the carrier status of *B. canis canis* by this tick species. Additional studies should be carried out in order to define more accurately the distribution and vectorial capacity of this tick species in Belgium.

LOW LEVEL DIMINAZENE ACETURATE (DA) RESISTANCE IN AFRICAN TRYPANOSOMES, IN THE ABSENCE OF DRUG PRESSURE

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Animal trypanosomosis is a major constraint to animal health and production in Africa. Communities rely often exclusively on trypanocidal drugs for controlling the parasites in their livestock. Unfortunately, this approach is seriously threatened by the increasing number of reports of drug resistance. It has been shown that resistance to diminazene aceturate (DA) in *T. congolense* is linked to a single mutation modifying the normal functioning of a P2-type purine transporter responsible for the uptake of the drug. An important question that remains unanswered is whether this mutation is forced by the drug pressure or whether other non-drug related properties of such mutation could result in its widespread dissemination. Twelve *T. congolense* isolates originating from tsetse or wildlife in game reserves in Zambia and South Africa were screened for the mutation inducing DA resistance using *Bcl*I-PCR-RFLP. A routine screening method in mice was used at three different doses of the drug (5, 10 and 20mg/kg) for the *in vivo* characterization of the sensitivity of the isolates to DA. To verify the complete absence of trypanosomes in animals, a trypanosome-specific 18S-PCR was performed on the mouse blood at the end of the 2 months observation period. The blood of four groups of experimental animals was also examined weekly for the presence of parasites using the 18S-PCR to monitor the dynamics of the parasite densities during and after treatment. The results of the *Bcl*I-PCR-RFLP shows that seven of the 12 *T. congolense* isolates have a resistant PCR-RFLP profile whilst the remaining five presented a mixed profile. This means that the mutation linked to DA resistance was found to be present on at least one allele of each of the 12 isolates. The results for the drug sensitivity tests in mice showed that 100% (n=24) of the isolates relapsed at 5 mg/kg, 0.08% (n=72) and 0% (n=72) at the dose of 10 and 20 mg/kg respectively. However, results of a trypanosome-specific PCR demonstrated a 100% (n=24) isolates that relapsed at 5 mg/kg, 51.4 % (n=72) and 38.9 % (n=72) at the dose of 10 and 20 mg/kg respectively. These results indicate that relapsed trypanosome infections are at very low density.

WILD RUMINANTS AND POTENTIALLY ZOONOTIC *BABESIA* SPP. IN BELGIUM

Lempereur L.[1-4], Wirtgen M.[2], Nahayo A.[2], Caron Y.[1], Shiels B.[4], Saegerman C.[3], Losson B.[1], Linden A.[2]

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Different *Babesia* species are described as potentially zoonotic and cause a malaria-like disease. New species have recently been described and in Europe, *Babesia* sp. EU1, reclassified as *B. venatorum* is of potential zoonotic importance. The role of cervids to maintain tick populations and act as a reservoir host for some of these zoonotic pathogens is suspected. Because of the recent first report of potentially zoonotic *Babesia* species in *Ixodes ricinus* in Belgium and combined with sparse information regarding the prevalence of the different *Babesia* species and their potential zoonotic impact, this study was performed to investigate the range of *Babesia* species found in ticks collected from wild cervids in Southern Belgium. In 2008, ticks were collected from wild cervids found dead, shot down during the hunting period or killed for sanitary reasons in the frame of the activities of the WildScreen Network in Southern Belgium. DNA extraction was performed from individual tick and each extracted sample was evaluated for absence of inhibition with a PCR test based on the tick 16S rRNA gene. The *Babesia* spp. genus-specific PCR based on the amplification of a part of the 18S rRNA gene was applied on the validated DNA extracts. Sequencing and blast analysis were performed to identify *Babesia* species. A total of 1044 *Ixodes ricinus* ticks were collected originating form 13 roe deer and 34 red deer and 1023 were validated and subsequently screened for *Babesia* sp. DNA. Twenty eight samples were found to be positive: *Babesia divergens* (8), *B. venatorum* (14), *B. capreoli* (2) or *Babesia* sp. (4), respectively. The infection rate was estimated at 2.7%. This study confirms the presence of potentially zoonotic species and *Babesia capreoli* in Belgium, with an infection rate that is in agreement with other previous publications. *B. venatorum* and *B. capreoli* are known to have cervids as host; however, the importance of deer as a reservoir for *B. divergens* is not fully understood.

NEW INSIGHT IN THE LYMNAEIDS INTERMEDIATE HOST OF *FASCIOLA HEPATICA* IN BELGIUM

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The present work was carried out in the frame of a Belgian Science Policy project (PONDSCAPE -"Towards a sustainable management of pond diversity at the landscape level"). During the summer 2008, 7103 lymnaeids snails were collected from 125 ponds distributed in 5 clusters of 25 ponds each. Each cluster was located in a different geological region of Belgium. These snails belonged to the following species or genus: 2474 *Galba truncatula* (the main intermediate host of *Fasciola hepatica* in Belgium) and 4629 *Radix* sp. Moreover, several biological (presence of trampling and dungs, snail size …) and non biological factors (pH, depth, fences …) were also registered from the different biotopes. DNA was extracted based on Chelex® technique. Then the snail DNAs were screened through a multiplex PCR that amplifies the lymnaeid internal transcribed spacer 2 sequence (500-600 bp) (acting as an internal control) and a 124 bp *Fasciola* sp. sequence. Lymnaeid snails were found in 93 biotopes (66%). Thirty *Galba truncatula* (1.31%) and 7 *Radix* sp. (0.16%) were found positive for *Fasciola* sp. A classification and regression tree (CART) analysis was performed in an attempt to better understand the relative importance and relationships among the different recorded factors. For example, the geographic localization is one of the best explanatory variables for the abundance of the different snail species and the presence/absence of faecal material is the best explanatory variable for the presence of specific *F. hepatica* DNA material in the different screened snails.

EVALUATION OF A NEW SERODIAGNOSTIC TEST FOR ASCARIOSIS IN PIGS

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Ascaris suum is the most important and most prevalent helminth parasite on modern pig farms and causes substantial economic losses in today's pig industry, despite the availability of highly effective anthelmintics. Because infections with *A. suum* show no clear pathological signs, many farmers underestimate the presence and impact of this parasite on their farm. Current diagnosis of ascariasis relies on the detection of parasite eggs in the faeces and/or the presence of white spots on the livers. However, these diagnostic tests are not performed routinely and generally they don't provide a correct estimate of the level of exposure to this parasite. Therefore, a more accurate and simpler way to detect parasite infection is required to correctly assess and/or adjust current management practices.

An experimental ELISA was developed to detect serum antibodies against *A. suum* haemoglobin (AsHb), a purified protein from the pseudocoelomic fluid of adult worms. Sera from 182 pigs, infected daily with *A. suum* for a period of 14 weeks, were used to validate the ELISA. AsHb-specific serum IgG levels on week 7 (1.256 ± 0.512) and week 14 (1.219 ± 0.403) post infection were significantly higher in comparison with day 0 (0.115 ± 0.113). The cut-off value of negative sera was set at 0.566 ODR (mean + 4*SD). On a ROC analysis this cut-off corresponded to a sensitivity of 98.9% and a specificity of 98.9%. Currently we are screening 1,356 serum samples from 113 Flemish pig farms and 360 serum samples from 30 Dutch pig farms in order to evaluate whether this experimental ELISA could be used as an improved diagnostic mean for the detection of *Ascaris* infestations on pig farms.

GASTRO-INTESTINAL IMMUNE RESPONSE DURING THE EXPULSION PHASE OF *ASCARIS SUUM* IN PIGS.

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During a primary infection with *Ascaris suum*, animals are able to eliminate the 4th stage larvae (L4) from the small intestine between 10 and 28 days post infection (DPI), during what is known as the "expulsion phase". The objective of the current study was to identify effector mechanisms that may play a key role during this phase of the infection. Pigs were infected with 2000 *A. suum* eggs and samples were collected from the small intestine at 10, 17 and 28 DPI. During the expulsion phase real time RT-PCR showed an increase in transcription level of genes encoding for Th1 type cytokines such as INFγ, IL12 and T-box 21, while no increase was observed for Th2 type cytokines. Furthermore, a clear increase was observed in NOS2A, the gene encoding for inducible nitric oxide synthase (iNOS). Immunofluorescence confirmed this finding at cellular level, showing that there is an increase in iNOS positive cells in the small intestinal mucosa as L4 are being expelled ($p<0,05$ at 17 DPI, $p<0,01$ at 28 DPI). To investigate *in vitro* the possible effects of nitric oxide on *A. suum*, L4 larvae were collected from a donor animal at 13 DPI and cultured in the presence of NO-donors (e.g. SNAP). About 20% fewer larvae were actively moving after 4 and 6 hours of incubation compared to larvae incubated in absence of NO ($p<0,05$). However, the number of actively moving larvae returned to normal levels after 24 hours of incubation, indicating that NO only has a transient paralytic effect on the parasite *in vitro*. Taken together, these findings show for the first time that the expulsion phase during an infection with *A. suum* occurs without the induction of a Th2 type response and toxic radicals produced in the intestinal mucosa may play a key effector role.

EVALUATION OF THE MECHANISMS INVOLVED IN THE PATHO-PHYSIOLOGICAL ALTERATIONS OF THE ABOMASAL MUCOSA IN CATTLE INFECTED WITH *OSTERTAGIA OSTERTAGI*

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Infections with the gastrointestinal nematode *Ostertagia ostertagi* remain in cattle an important economic problem in the temperate regions of the world, causing loss in animal production. The infection results in profound physio-morphological changes of the abomasal mucosa including mucus cell hyperplasia and cell de-dedifferentiation, accompanied by increased gastric pH and hypergastrinemia. The causes and the mechanisms behind these alterations are still unclear. Therefore the aim of the current study was to determine the pathways involved in the observed cell hyperplasia and dedifferentiation during an infection with *O. ostertagi*. Further, an attempt was made to evaluate if increase in gastric pH may be due to a decrease number of mature acid-producing parietal cells in the mucosa and if this can be linked to the alteration in the gastric cell population during infection. Transcription levels of several molecules belonging to the EGFs, WNT, and FGF signaling pathways, involved in the onset of cell hyperplasia and cell dedifferentiation, were found to be up-regulated at 24 days post-infection (dpi). On the other hand, gene transcription levels of ATP4A and AQ4, two specific markers for parietal cells activity, showed a significant down-regulation only after 60 days of exposure to *Ostertagia*. In consistent with these findings, immunostaining showed that cell hyperplasia in the abomasal mucosa occurs earlier compare to the loss of mature acid-producing parietal cell, suggesting that alteration in cell population leads to a decrease in number of parietal cells in the infected mucosa. This may explain the increase in gastric pH measured after infection. Additionally several pro-inflammatory factors such as IL8, IL1β, IL6 and COX-2 were found to be up-regulated during the infection starting at 9 dpi. These cytokines may have a direct inhibitory effect on parietal cells and they may also play a role in cell migration and maturation.

POSTERS

AN OPTIMIZED DNA EXTRACTION AND MULTIPLEX PCR FOR THE DETECTION OF *FASCIOLA* SP. IN LYMNAEID SNAILS.

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This study deals with the development and validation of an original PCR protocol to assess the presence of *Fasciola hepatica* in *Galba truncatula* its main intermediate host in Western Europe. In the present study two DNA extraction techniques are compared and a new multiplex PCR is described. The Chelex® DNA extraction technique showed to be more appropriate than the classical Phenol/Chloroform/Proteinase K based method because of the absence of toxic organic solvent, shorter duration and lower cost, and a higher reproducibility regarding DNA concentrations and wavelength ratios. The multiplex PCR was set up to amplify the lymnaeid internal transcribed spacer 2 sequence (500-600 bp) that act as an internal control and a 124 bp *Fasciola* sp. sequence that is repeated more than 300,000 times in fluke whole genome. Ninety six snails were pooled and 6 snails (6.25%) found positive for *Fasciola* sp. The limit of detection is lower than the minimal biological infestation unit (one miracidium). DNA extracts from *Paramphistomum daubneyi*, *Dicrocoelium lanceolatum*, and *Fascioloides magna* didn't cross react.

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