

## **ABSTRACTS - KEYNOTE LECTURES:**

### **K1 - OPPOSING ROLES FOR IL-13 AND ITS DECOY RECEPTOR IN THE PATHOGENESIS OF SCHISTOSOMIASIS**

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IL-13 is a key inducer of several type-2 cytokine-dependent pathologies. It regulates inflammation, mucus production, tissue remodeling, and fibrosis. Consequently, it has become an important therapeutic target for a number of debilitating illnesses, including asthma, idiopathic pulmonary fibrosis, ulcerative colitis, liver fibrosis, as well as several other diseases in which IL-13 is believed to be over-produced. In the murine model of schistosomiasis, IL-13 has emerged as central mediator of chronic infection-induced liver pathology. Thus, elucidating the mechanisms that regulate the production and function of IL-13 has become an intensive area of research. IL-13 signaling is mediated by the type II IL-4 receptor, which consists of the IL-4R $\alpha$  and IL-13R $\alpha$ 1 chains.

However, another IL-13 binding chain, IL-13R $\alpha$ 2, appears to strongly inhibit the activity of IL-13. Animals deficient in IL-13R $\alpha$ 2 fail to downmodulate granuloma formation in the chronic phase of schistosome infection. They also develop severe IL-13-dependent fibrosis, portal hypertension and succumb to the infection at an accelerated rate. Together, these findings demonstrate opposing activities for IL-13 and IL-13R $\alpha$ 2 in health and disease and emphasize the utility of the soluble IL-13R $\alpha$ 2 as a potent antagonist of IL-13-driven fibrosis.

### **K2 - IS *TRICHURIS SUIS* A DOUBLE EDGED SWORD: DOES THIS WORM INDUCE ANTI-INFLAMMATORY RESPONSES OR ENHANCE PATHOLOGICAL LESIONS?**

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Inflammatory Bowel Disease (IBD) is a serious chronic intestinal inflammatory disorder afflicting 6/100,000 adults in the US. Two forms are recognized: ulcerative colitis and Crohn's disease. Published information suggests that multiple factors may interact to produce IBD: host genetics, adaptive cellular host immune responses, and pathogens and normal flora of the intestinal tract that stimulate innate mucosal immune responses through pattern-recognition signaling pathways. Current treatments are aimed at reducing inflammation and postponing surgery, which is needed in ~75% of cases. These treatments are seldom effective over the long term. Recently, deliberate infection with the helminth *Trichuris suis* has worked successfully to treat IBD patients whose disease was refractory to standard treatments (Summers et al., 2003). The mechanisms by which *Trichuris* infection ameliorates IBD are unknown. However, there is evidence that parasites such as *Trichuris* act to moderate the inflammatory responses involved in the development of IBD by triggering anti-inflammatory mechanisms. These mechanisms may include 1) the release of specific anti-inflammatory cytokines, 2) induction of specific anti-inflammatory responses in dendritic (DC) cells, and 3) induction of specific responses in T regulatory cells that shift the Th1/Th2 cytokine balance toward an anti-inflammatory milieu. However, in earlier studies we have shown that *Trichuris* and the common food borne bacterium *Campylobacter jejuni* synergize to produce disease and colonic pathology in immunologically naïve swine (Mansfield et al., 2003). We experimentally induced infections in *C. jejuni*-naïve conventionally reared (CR) and gnotobiotic (GN) pigs. Pigs were given *C. jejuni* alone, *Trichuris* alone, *C. jejuni* and *Trichuris* together, or no infection. Clinical signs were more severe in pigs with dual infections, with mucohemorrhagic diarrhea as the most prominent sign. In either CR or GN pigs, severe pathology was only present in the colon of pigs that had both *Trichuris* and *C. jejuni*. Current studies in my laboratory examine the underlying mechanisms by which *Trichuris* may function to enhance pathology or cure disease using in vitro systems and a murine model of IBD.

### **K3 - THE ROLE OF ERYTHROPOIETIN IN MALARIA PATHOGENESIS**

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*Plasmodium falciparum* infection is a major cause of infant mortality primarily due to severe and complicated malaria. There is little evidence of any direct toxic effects of substances released from the parasites. Instead, the pathology appears to be primarily caused by dysregulation of normal processes in the host such as excess inflammatory responses, bone marrow suppression, apoptosis and anaerobic metabolism. Many of these factors may be triggered by adherence of infected erythrocytes to endothelial cells resulting in impaired microcirculation and activation of cellular receptors. One of the important complications of malaria is severe anaemia. This condition is due to the combined effect of erythrocyte destruction and bone marrow suppression. The bone marrow suppression is a general feature of all *P. falciparum* infections irrespective of severity and is rapidly reversible as soon as total parasite eradication has been achieved.

Erythropoietin (EPO) is a glycoprotein that is released by cells in the kidneys as well as a range of other organs in response to hypoxia. The most studied stimulus for EPO production is anaemia, which causes a systemic reduction in oxygen tension. Most studies find appropriately elevated EPO levels in anaemic malaria patients. The normal effect of EPO is induction of erythropoiesis in the bone marrow resulting in raised numbers of reticulocytes in the peripheral blood. In malaria patients, however, this response is suppressed. EPO acts on several cells in the bone marrow through binding to the EPO receptor (EPO-R), most importantly on erythroid colony forming units (CFU-E) and pro-erythroblasts. The suppression of erythropoiesis in malaria appears to be downstream from these cells.

EPO production can also be triggered in other tissues in response to localised hypoxia, such as that seen in cerebral ischaemia. In this context, the effect of EPO is cytoprotective through inhibition of apoptosis. As shown by Wiese et al. (abstract #O4), administration of EPO to mice with experimental cerebral malaria can reduce neuronal apoptosis. This novel function of EPO may explain the surprisingly low mortality that results from severe malarial anaemia as compared to other complications of malaria. However, the data are preliminary and need to be studied further in both experimental and human malaria.

## ABSTRACTS – ORAL PRESENTATIONS:

### O1 - IMMUNO-EPIDEMIOLOGY OF PORTAL FIBROSIS ASSOCIATED WITH *SCHISTOSOMA MANSONI* IN UGANDA

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*Schistosoma mansoni* infection is highly endemic in parts of Uganda, and periportal fibrosis is common in communities along the shore of Lake Albert. In this study, we have identified cellular immune responses associated with fibrosis. A cohort of 199 individuals aged 6-50, resident in the village for at least 10 years or since birth, were examined for evidence of periportal fibrosis by ultrasound. Whole-blood samples were assayed for levels of nine cellular immune molecules (IL-3, IL-4, IL-5, IL-10, IL-13, TNF-alpha, IFN-gamma, IL-1beta, and RANTES) after stimulation with egg and worm Ags. A lack of Ag specificity allowed the number of variables in the analysis to be reduced by factor analysis. The resulting factor scores were then entered into a risk analysis using a classification tree algorithm. Children, adult males, and adult females had different factors associated with fibrosis. Most cases of fibrosis in children (eight of nine) were associated with low (<47th percentile) IL-10 factor scores. Adult females at lowest risk had relatively high IFN-gamma factor scores (>83rd percentile), whereas those at highest risk had a combination of intermediate (32nd to 83rd percentile) IFN-gamma and relatively high (>60th percentile) TNF-alpha factor scores. Adult males at lowest risk of fibrosis had moderate TNF-alpha factor scores (55th to 82nd percentile), and a high risk was associated with either high TNF-alpha factor scores (>82nd percentile), or intermediate TNF-alpha combined with low RANTES factor scores (<58th percentile). These results demonstrate that periportal fibrosis is associated with cytokine production profiles that vary with both age and gender.

### O2 - ♦ DOES HELMINTH INFECTION INCREASE THE RISK OF OTHER ENTERIC PATHOGENS? A COMMUNITY STUDY OF SCHOOL CHILDREN IN PERI-URBAN GUINEA-BISSAU

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Concomitant helminths and bacteria may affect the course, and the resulting disease outcome, of the individual infections. The aim of the present epidemiological study from Guinea-Bissau was to explore correlations between intestinal helminth infections and other gastrointestinal pathogens in schoolchildren from a poor semi-rural area of the capital Bissau, while adjusting for certain socioeconomic risk factors. A total of 705 children were examined and helminthes were detected in 312 children (44.2%), enteropathogenic bacteria in 97 (13.7%), protozoans in 361 (51.2%), and rotavirus in 42 (6%). In all 537 (76.1%) had an infection of some sort, and 41.6% were concomitantly infected with more than one and up to five gastrointestinal pathogens. In the final model for helminth risk factors, only an association to *E. histolytica* remained significant. No associations were seen between egg counts and infection with any of the other pathogens. Other risk factors in relation to helminths included increasing age, male sex, and chicken husbandry in the neighbouring family. Maternal school attendance, belonging to a Muslim family, or having electricity decreased the risk of helminth infection. It cannot be ruled out that a higher intensity of hookworm or more infections with more pathogenic helminths, may have revealed associations with other pathogens, as has been shown in other studies.

### O3 - MORBIDITY IN SCHISTOSOMIASIS MANSONI: A SHIFT IN PARADIGM?

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Morbidity in *S. mansoni* and *S. japonicum* may present as hepatosplenic schistosomiasis (HS), characterised by enlargement of the liver and spleen and accompanied by increased firmness of the organs. Many of the eggs of these species pass, via the portal vein, to the liver, where they give rise to vascular and inflammatory granulomatous changes that can lead to periportal fibrosis, portal hypertension with the development of oesophageal varices and the risk of life-threatening haematemesis. Though classical autopsy studies in Brazil linked the development of *S. mansoni* HS, portal hypertension and its severe sequelae with gross periportal fibrosis, recent ultrasound and clinical studies in Africa suggest that HS can also be found in *S. mansoni*-infected children in the absence of gross hepatic fibrosis. Studies among fishermen in Uganda have shown that the severe consequences of HS such as oesophageal varices and haematemesis can exist in the absence of severe fibrosis. These observations are contrasting with the previous view that schistosomiasis morbidity was less severe in sub-Saharan Africa, due to the relative lack of severe peri-portal fibrosis and may have implications for assessment of the level of disease in control programmes.

### O4 - ♦ ERYTHROPOIETIN TREATMENT REDUCES APOPTOTIC CELL DEATH DURING MURINE CEREBRAL MALARIA

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Cerebral malaria (CM) is an acute encephalopathy with increased proinflammatory cytokines, sequestration of parasitised erythrocytes and localised ischaemia. In addition to its erythropoietic functions, erythropoietin (EPO) is considered a novel neuroprotectant in the central nervous system as EPO confers neuroprotection in humans and animals with various brain diseases.

Neuro-cognitive impairment following CM occurs in about 10% of the survivors and is associated with apoptotic cell death. We examined the effect of exogenous EPO on apoptosis of brain cells during CM in mice.

**Methods:** C57BL/6j mice, infected with *P. berghei* ANKA and treated with recombinant human EPO i.p. (5000U/kg/day) were studied on day 7, day 9, and when presenting signs of CM on day 10-13 respectively. Control mice received normal saline. Brain sections were investigated by immunohistochemistry, immunofluorescence and TUNEL\* for apoptosis.

**Results:** A. Localised neuronal apoptosis indicating irreversible pathology  
B. EPO treated mice had significant reduction of apoptotic cell death

**Interpretation:** This is the first report of the neuroprotective effect of EPO in CM. This neuroprotective role and its possible potential as treatment need to be further examined.

Endogenous EPO levels in severe malaria anaemia (SA) are elevated and could play a protective role against CM. This could contribute to the apparent dichotomy between CM and SA in African children.

\* TUNEL: Terminal deoxynucleotidyl transferase (TdT)-mediated deoxyuridine triphosphate (dUTP)-digoxigenin nick end labelling

## ABSTRACTS - POSTERS:

### **P1 - ♦ EQUINE LARVAL CULTURE WITH FOCUS ON *STRONGYLUS VULGARIS*: QUALITY EVALUATION AND THE INFLUENCE OF VARIOUS FACTORS**

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The prevalence of *Strongylus vulgaris* was examined in 105 horse farms on Zealand, Denmark, all of which participated in a worm control program based on selective treatment.

Two larval culture procedures based on examination of individual and pooled samples, respectively, were compared. The individual procedure revealed *S. vulgaris* prevalences of 5.7% and 26.7% at individual and herd levels, respectively, whereas the pooled-sample procedure revealed a herd prevalence of 15.3%.

The relationship between EPG (strongyle eggs) and the occurrence of *S. vulgaris* was evaluated, showing that the likelihood of diagnosing *S. vulgaris* infection was generally very low, but increasing at high EPG levels, yet only 50% at 7,000 EPG.

The results of the prevalence study showed no systematic correlation between the occurrence of *S. vulgaris* and herd size.

The effect of light conditions during incubation on the strongyle larval recovery was studied by subjecting the larval cultures from three horses to different light intensities. In comparison with shady conditions, incubation in direct sunlight resulted in significantly decreased larval recovery, for total counts as well as for specific *S. vulgaris* larval counts.

A study on the role of presence/absence of soil nematodes in otherwise identical faecal cultures showed that presence of soil nematodes cause a significant decrease of larval detection.

### **P2 - EXPERIENCES FROM DANISH FIELD TRIALS TO EVALUATE THE EFFECT OF THE BIOLOGICAL CONTROL AGENT, *Duddingtonia flagrans*, FED TO EWES WITH LAMBS AT FOOT.**

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In conventional small ruminant production systems, use of drugs has led to development of anthelmintic resistant worm populations. In organic production the use of drugs is only allowed for salvage treatment and there is a need to develop new, alternative tools to be used in sustainable control strategies against parasitic nematodes. One such alternative method is biological control using the nematode destroying fungus *Duddingtonia flagrans*. Four groups of ewes with lambs at foot were given supplement with fungal spores (½ mill. per kg live weight) daily or just supplement for 10 (2003) or 12 weeks (2004). Every fortnight faeces, blood (2003) and herbage were collected and all animals weighed. Eggs per gram of faeces were determined and group faecal cultures set-up (ewes and lambs separately) to determine number of developing infective larvae. The herbage was washed to extract and count the number of infective L3 larvae. To monitor the infectivity of the pasture at the end of the fungus feeding period, tracers were introduced for a limited period and after housing for a couple of weeks, animals were killed to determine the worm burden. There seemed to be more eggs excreted from animals on the control pastures except with respect to *Nematodirus* spp. Herbage infectivity also seem to be higher on the two control pastures. The most pronounced feature of both years was a significantly higher weight gain found in the groups fed fungus daily compared to the non-fungus fed control groups (p<0.001, Repeated Measures Analysis of Variance, SAS GLMP). The positive effect of fungal treatment on the lamb production is in line with what has been reported earlier from other similar trials, and it opens up for implementation of BC in future integrated control strategies.

### **P3 - ♦ NOVEL REAL-TIME PCR FOR THE DETECTION OF *SCHISTOSOMA JAPONICUM* IN STOOL**

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In countries where the blood fluke *Schistosoma japonicum* is endemic, chemotherapy has been used on a large scale. This leads to a lower intensity of infections and consequently lower diagnostic values of commonly used diagnostic tests like serology and Kato-Katz stool smear.

We designed a novel real-time PCR method for detection of *S. japonicum* in stool samples. Further, we evaluated an inexpensive, non-commercial extraction method for stool samples.

PCR primer sequences were designed targeting the mitochondrial NADH dehydrogenase I gene.

Extraction with ROSE-buffer, as described by Pontes et al (2003), was tested in different modifications. SYBR<sup>®</sup> Green was used for detection.

The PCR showed high amplification efficacy on miracidial DNA. The best yield was obtained with ROSE extraction with ethanol precipitation and subsequent dilution (1:100) of the resuspended DNA pellet. Bovine serum albumin was added. Using this method, 26/27 (96%) stool samples, spiked with 1-100 *S. japonicum* eggs per gram, gave positive results. The single negative sample contained 1 egg per gram.

Our PCR method may be modified for detection with agarose gel or probes.

We conclude that this novel real-time PCR, in combination with a non-commercial ROSE extraction, is a sensitive tool for diagnosing *S. japonicum* in stool samples.

### **P4 - PREVALENCE OF *CRYPTOSPORIDIUM* AND *GIARDIA* IN DANISH LIVESTOCK**

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Until now, little was known about the prevalence of *Giardia* and *Cryptosporidium* in Danish livestock, except that both parasites occurred in Danish cattle, whereas minimal information was available regarding pigs. Similarly, little knowledge existed concerning possible influence of different management systems on the occurrence and intensity of infection. Considering the increasing tendency in Danish husbandry of allowing the animals to graze marginal areas near streams, we wanted to increase the knowledge of the prevalence of these potentially zoonotic agents, which might contaminate our environment. Hence, in 2003 an epidemiological survey was initiated including 50 dairy farms and 50 sow herds, which were each visited once for the collection of faecal samples and for registration of basic management parameters as well as hygiene levels in the surroundings of the examined animals. The farms were selected at random via a central farm registration system and are located throughout Denmark. On each farm faecal samples were collected from different age groups of animals, i.e. 5 sows/cows, 10 calves/piglets less than 1 month and 10 calves 3-9 months or 10 pigs 8-30 kg. The faecal samples were purified by a flotation/centrifugation technique and the number of cysts/oocysts quantified microscopically following staining with Crypto-Giardia CEL (Waterborne, Australia) immunofluorescence kit. The results show a herd prevalence of 98-100% and individual prevalence of approx. 33% for *Cryptosporidium* in both animal species and for *Giardia* in cattle, but 85% herd prevalence and 20% individual prevalence of *Giardia* in swine. Management parameters, e.g. in relation to intensity of parasite excretion and occurrence of diarrhoea, are being analysed statistically while still awaiting the final results of genotyping. However, initial analyses imply that individual factors have great influence on the excretion from each animal, whereas herd related factors may have less influence. The widespread occurrence of both parasites in both livestock species gives rise to further questions regarding importance in relation both to animal health and zoonotic potential, which we hope to examine in the future.

#### **P5- ♦ MUCOSAL ANTIBODY SECRETING CELLS IN ASCARIS SUUM INFECTED PIGS**

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*Ascaris lumbricoides* is the most prevalent human helminth. Studies of the human immune response to ascariasis have only been conducted in the peripheral blood and not at the site of the adult worm - the small intestine (SI). *A. lumbricoides* of humans and *Ascaris suum* of the pig are very closely related and to this date it has not been conclusively established whether they are one or two species. The similarities between the human and the porcine intestinal tracts and the fact that *A. suum* and *A. lumbricoides* are so closely related make pigs very attractive for comparative studies of local immune responses to this parasite.

Ten-12 weeks old pigs were inoculated with a single dose of 10,000 infective *A. suum* eggs. Pigs were necropsied days 10, 14, 21 and 42 post inoculation (p.i.) and the specific anti-*Ascaris* IgA, IgG and IgM antibody secreting cells (ASCs) in the lamina propria of the proximal and distal jejunum of inoculated pigs were assessed by the enzyme-linked immunospot assay (ELISPOT). Higher concentrations of specific IgA and IgM ASCs were found in the proximal jejunum compared to the distal. Specific anti-*Ascaris* IgG ASCs were almost absent from both sites. Specific IgA ASCs in the proximal jejunum increased significantly compared to the non-inoculated control pigs days 10, 14 and 21 p.i. and decreased to levels compared to the control pigs on day 42 p.i. We conclude that the host adaptive immune system of the SI is participating in recognizing and, potentially, in regulating *Ascaris* infections.

#### **P6 – OUTBREAK OF TRICHINELLOSIS ASSOCIATED WITH CONSUMPTION OF GAME MEAT IN WEST GREENLAND**

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Trichinellosis is a problem in humans in the Arctic as well as in other climates. The Inuit population of the Arctic has always been at risk of acquiring infection with *Trichinella*, and severe outbreaks have been recorded in Alaska and Canada. In West Greenland, a number of large outbreaks took place during the 1940'ies and 1950'ies when in total 420 cases of trichinellosis were registered of which 37 people died. Since then only sporadic cases have been registered. Here we describe an outbreak of infection with *Trichinella* spp. after consumption of presumably infected walrus meat from the west coast of Greenland. Design. Six persons had eaten of the meat, two males and four females, age range 6-47. All six exposed persons were followed 15-16 months after the onset of the outbreak with ELISA and Western blot analysis of *Trichinella*-specific IgG antibodies against E/S antigens and a synthetic glycan antigen  $\beta$ -tyvelose. Results. Four persons were found sero-positive for *Trichinella* IgG antibodies, three of them having clinical symptoms of trichinellosis. In a subsequent test one year later, one of two sero-negative persons had sero-converted, probably due to a new, unrelated infection. Discussion. Our study confirms that consumption of marine mammals in Greenland still constitutes a risk of acquiring trichinellosis but can be prevented by public health measurements. We recommend a surveillance program like that in Nunavik, Canada, should be started in Greenland to prevent more cases of trichinellosis.

## **P7 - DEVELOPMENT OF A TECHNIQUE TO IDENTIFY NEMATODE COHORTS IN PIGS**

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An ability to trace and follow different cohorts of parasites in the host makes it possible to answer basic questions like, when parasites establish during a repeated infection, and thus achieve a better understanding of host-parasite interactions. The objective of the present study was to develop a technique for identifying different cohorts of parasites using *Ascaris suum* as the infective agent and pig as the host. A polymerase chain reaction linked restriction fragment length polymorphism (PCR-RFLP) technique on mt-DNA was used to establish unique genotypes of four gravid female *A. suum*. Eggs were recovered from each of the worms. Each of four pigs was inoculated with 2000 embryonated eggs originating from one of the four identified *Ascaris* genotypes, respectively. *Ascaris* larvae were isolated from the small intestine at day 14 after infection using an agar technique. Single larvae from each pig were transferred to 96-well PCR plates and DNA was extracted using a worm lyses buffer followed by PCR-RFLP using the protocol as above. More than 100 larvae from each of the four pigs were analysed and all were found to have the same genotype as the parental female. We conclude that unique genotypes of female *A. suum* and offspring can be identified by means of PCR-RFLP. This technique will be used to follow and trace the fate of different cohorts of parasites during their life cycle in the host.

## **P8 - THE GEOGRAPHICAL DISTRIBUTION OF *WUCHERERIA BANCROFTI* AND *MANSONELLA PERSTANS* FILARIASIS IN UGANDA**

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The geographical distribution of *Wuchereria bancrofti* and *Mansonella perstans* infections in Uganda was assessed by day-time screening of school-aged children for specific circulating filarial antigen (CFA) and microfilariae (mf), respectively. Overall 17,533 and 12,207 children from 76 sites representing the various topographical and ecological zones in the country were examined for the infections. *W. bancrofti* was mainly confined to the north of the country (north of the Victoria Nile), while *M. perstans* was highly prevalent in a broad east-west oriented belt across the central part of the country. Geostatistical interpolation was used to create a map showing countrywide prevalence zones for the infections (by ordinary kriging), and to assess the population exposed to transmission with the two filarial species (by performing an overlay of the Landscan digital population model with the prevalence zone layer). For *W. bancrofti* infection, estimates based on population data from 2002 indicated that of the total population of 24.7 million people, 8.7 million (35.3%) lived in areas with > 1% CFA prevalence, and 4.2 million (17.0%) lived in areas with > 5% CFA prevalence, in the school-aged children. Similar estimates for *M. perstans* indicated that 20.4 million people (82.6% of the national population) and 6.8 million people (27.5%) lived in areas with > 1% and > 10% prevalence, respectively, of microfilaraemia in the school aged children. Further GIS based analyses of the data will be used to determine the role of climatic and environmental factors in the distribution of these infections in Uganda.

## **P9 - DEVELOPMENT AND SURVIVAL OF *TRICHURIS SUIS* EGGS ON PASTURE PLOTS**

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Eggs of the pig whipworm, *Trichuris suis*, are excreted with faeces, and their fate therefore depend on environmental factors. The present study was designed to measure development, survival, and infectivity of *T.suis* eggs quantitatively.

Swine faeces with known numbers of *T.suis* eggs were deposited on pasture plots with bare soil, short herbage or tall herbage. Eight depositions were made from April to February. Plots and sampling techniques were designed to minimize any loss of eggs due to vertical and horizontal transport. The eggs were quantified and differentiated in faeces and soil 1, 4, and 16 weeks post deposition (wpd), as well as in the autumn of year 1 and in the spring and autumn of year 2. At the two latter samplings, eggs from selected plots were administered to pigs to test for infectivity. Most eggs died within the faeces during the first 4 wpd. More *T.suis* eggs were in general recovered from plots with herbage than from bare soil plots, and the eggs seemed to survive better when deposited in the spring or autumn compared to the summer and winter depositions. Only in a few cases did eggs reach the morphologically fully developed stage within 1 year post deposition, and development was slower on tall herbage plots than on bare soil. Very few eggs had become infective to pigs in the spring of year 2 (max. 0.1% of the deposited eggs), while the infectivity had increased in the autumn of year 2 (max. 2.1%).

The results indicated that *T.suis* eggs face a huge mortality, especially within the first month on the pasture, that the survival rate depends on environmental factors and that the majority of the surviving eggs needs 1 year or more to become infective to pigs. The present results indicate that pasture rotation systems, in which pigs are returning to contaminated pastures after 1 or 2 years have little controlling effect of *T.suis* and this may explain why 'old' organic swine herds seems to have much more problems with this parasite than newly established herds.

## **P10 - THE POPULATION BIOLOGY AND EPIDEMIOLOGY OF *TOXOCARA CANIS* IN DANISH RED FOXES**

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From 1040 red foxes (*Vulpes vulpes*) collected from all Danish provinces (1997-2002), *Toxocara canis* was found in 618 (59%). The prevalence and average worm burden were significantly higher for cubs than older foxes and in male than in female. A multi-factorial logistic regression demonstrated that prevalence was influenced significantly by sex and age of foxes in addition to location, and year of collection. The highest prevalence and worm burdens were found in rural area. Both the length and the number of female worms were found to be positively correlated to the faecal egg excretion at a significant level. The sex of foxes influenced both the length and fecundity of the worms, and a general crowding effect was observed; worm numbers were negatively correlated to worm lengths. Somatic larvae of *Toxocara* were found in 20% of digested tissues samples, and faecal eggs in 41% of examined faeces.

## **P11 - ♦ *BLASTOCYSTIS HOMINIS*: DIAGNOSIS, EPIDEMIOLOGY AND CLINICAL IMPACT**

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*Blastocystis hominis* is a common intestinal parasite of world-wide distribution. The parasite is present in patients with gastrointestinal symptoms as well as in healthy individuals. Its pathogenetic potential has not yet been established, but numerous case reports suggest that *B. hominis* may cause

the development of various gastrointestinal symptoms and disorders including intermittent diarrhoea, nausea and general malaise. Diagnosis is conventionally done by microscopy after standard parasitological processing of faecal specimens; however, the parasite exhibits pronounced polymorphism and all forms or stages may not yet have been identified. Culture is possible and sensitive but time consuming and not recommended for routine use. A diagnostic PCR for the detection of *B. hominis* infections has yet not been established. Restriction fragment length polymorphism analyses have demonstrated the presence of at least 7 genotypes.

The aim of the present study is 1) to demonstrate any possible association between genotype and pathogenicity, 2) to elucidate epidemiological issues related to blastocystosis, 3) to develop and evaluate a diagnostic PCR for the detection of *B. hominis* infection and 4) to compare the sensitivity of different diagnostic methods.

## **P12 - DOGS AND CHICKENS MAY ACT AS TRANSPORT HOSTS FOR *SCHISTOSOMA JAPONICUM***

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The present study sought to elucidate whether dogs or chickens could act as transport hosts for *Schistosoma japonicum*. Three dogs and three chickens were used for the experiment. The dogs were allowed to eat goat faeces containing 80 *S. japonicum* eggs per gram. The chickens were given 2 ml of water containing more than 100 *S. japonicum* eggs per ml. The hatchability of the administered eggs was 51.8%. All faeces were collected for three days from the dogs and two days from the chickens and egg excretion and hatchability were measured. The results showed that nearly 50% of the eggs administered to the dogs were recovered in the faeces during the first two days and during the first 24 hours 36% of the recovered eggs were viable and able to hatch. In the chickens 9% of the administered eggs were recovered within 27 hours and within the first 3.5 hours after administration 19% of the recovered eggs were viable and able to hatch. The results suggest that both dogs and chickens may act as transport hosts for *S. japonicum* in endemic areas.

## **P13 - ♦ INTESTINAL ESTABLISHMENT AND REPRODUCTION OF ADULT *TRICHINELLA* SPP. IN SINGLE SPECIES AND MIXED SPECIES INFECTIONS IN FOXES (*VULPES VULPES*)**

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Intestinal establishment and reproduction of adult *Trichinella spiralis*, *Trichinella nativa*, *Trichinella britovi* and *Trichinella pseudospiralis* were examined as single species or mixed species infections in foxes. This is the first study of intestinal dynamics of *Trichinella* spp. in a carnivore model and the results suggest that the intestinal phase is relatively short as only very few worms were recovered 10 dpi. In mixed species infection with equal doses of *T. nativa* and *T. spiralis*, molecular typing demonstrated that 64% of the intestinal worms and 78% of the muscle larvae were *T. nativa*. Conversely, *T. spiralis* dominated in the mixed species infections with *T. pseudospiralis*, constituting 66% of the intestinal worms and 94% of the muscle larvae. Although, the individual recoveries of intestinal worms were only up to 5.6% on day 1, and up to 1.5% on day 4 post infection, the muscle larvae establishment was comparable to other fox studies. Infectivity, measured as muscle larvae burden did not differ among the four species of *Trichinella*, which is in contrast to other models with mice, rats, pigs, or herbivores. Although statistically significant differences in intestinal worm burdens were found for some days, no distinct species were recovered in consistently higher numbers than the others.

In conclusion, this study has shown that the intestinal phase of *Trichinella* in foxes is relatively short and that simultaneous infection with two species leads to establishment of muscle larvae from both species. The recovery of all *Trichinella* species in both mono and mixed infections from the same intestinal location suggests reproductive isolation rather than a physical intestinal separation as a mating barrier.

**P14 - ♦ STUDIES ON VERTICAL TRANSMISSION OF *TRICHINELLA* IN FERRETS, FOXES, PIGS, GUINEA PIGS, AND MICE**

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Vertical transmission of *Trichinella spiralis* was evaluated in ferrets (n=21), foxes (n=11), pigs (n=12), guinea pigs (n=16), and mice (n=41). The placental barrier to be crossed by migratory *Trichinella* larvae varies structurally in different animal species. Ferrets and foxes have an endotheliochorial placenta structure, guinea pigs and mice a haemochorial, and pigs an epitheliochorial placenta. The non-encapsulating *Trichinella pseudospiralis* larvae have an extended muscle migration prior to entering a muscle cell. To evaluate if *T. pseudospiralis* was more likely to be transmitted to offspring, an additional group of foxes (n=11) infected with *T. pseudospiralis* was included. Two different dose levels were used for ferrets, pigs, guinea pigs, and mice. In pigs and guinea pigs, infection was given at different times of the gestation period. Vertical transmission, measured as recovery of muscle larvae in the offspring, was demonstrated in both ferrets groups, in all four guinea pig groups, and in the high dose mouse group, but not in any fox or pig groups. In conclusion, the studies demonstrated the first vertical transmission of *Trichinella* in a carnivorous host, and confirm the phenomenon in rodents. Although vertical transmission may be important for the survival of the offspring, the low frequency of vertical transmission and the insignificant number of larvae transmitted to the offspring most likely reflect a limited importance in natural transmission.