

JOINT SPRING SYMPOSIUM 2008

Danish Society for Parasitology and
Danish Society for Tropical Medicine & International Health

Program

- Title: *“Parasite Vaccines and Immunomodulation”*
- Time: Friday 28 March, 2008,
- Venue: Faculty of Life Sciences (former KVL), 1870 Frederiksberg C, main building.
Lecture room 1-01 (same venue as previous two years)
- 8.00: Registration of participants
- 8.45: Welcome by Jørgen Kurtzhals, chairman of Danish Society for Parasitologists
- 8.50-9.40: **Joseph Urban**, Key-note lecture
- 9.40-10.10: 15 minute oral presentations, Chair: Stig Milan Thamsborg
16. **H.H. Petersen***, **P.Nejsum**, **A. Roepstorff**, **H. Kringel** and **S.M.Thamsborg**.
Effect of host genetics on antibody responses to *Ascaris suum* and *Trichuris suis* in pigs
13. **C. S. Sikasunge ***, **I. K. Phiri**, **M.V. Johansen**, **A. L.Willingham III** and **P. S. Leifsson**
Host-cell apoptosis in *Taenia solium* induced brain granulomas in naturally infected pigs
- 10.10-10.40: Coffee break and poster session
- 10.40-11.25: 15 minute oral presentations, Chair: Christen Rune Stensvold
3. **Poulsen, I.*; Nissen, S.*; Thamsborg, S. M.; Roepstorff, A.; Nejsum, P.; Akiiki, R.**
Prevalence of gastrointestinal nematodes in Kabale district in Uganda
12. **Ahmed U. N.*; Stensvold C. R.; Nielsen H. V**
Serological detections of immunoglobulin G (IgG) antibodies against *Neospora caninum* and *Toxoplasma gondii* in sera from African and Danish patients
- 11.10-12.00: **Jeff Bethony**, Key-note lecture: Type 1 hypersensitivity reactions in a phase 1 trial of the *Na*-ASP-2 hookworm vaccine in previously-infected Brazilian adults

12.00-12.45: Lunch

12.45-13.30: Poster session

13.30-14.30: 15 minute oral presentations, Chair: Lasse Skafte Vestergaard

1. **Tina Dobrilovic*, Lea Barfod, Lars Hviid**

Human monoclonal antibodies recognizing pregnancy-specific variant surface antigens can inhibit adhesion of *Plasmodium falciparum*-infected erythrocytes to chondroitin sulphate A

2. **Wang, C. W.*; Magistrado P. A.; Engström M.; Vasconcelos N. M.; Schou S.; Turner L.; Theander T. G.; Lavstsen T.**

Plasmodium falciparum expressing VSA associated with severe malaria is associated with transcription of a conserved subset of rifins

11. **Staalsoe, T.; Megnekou, R. and Hviid, L.**

Plasma antibodies from experimentally infected mice recognize parasite induced antigens on the surface of *Plasmodium berghei* infected erythrocytes in a partially strain specific manner

5. **Hempel C.* , Wiese L., Kurtzhals J. and Penkowa M.**

Demyelination in experimental cerebral malaria

15.00-15.50: **David Arnot**, Key-note lecture: Malaria Vaccines: Unanswered and unasked questions

15.50- 15.55: Announcement of the two recipients of the Young Scientist Award.

15.55-16.00: Closing remarks, Lasse Skafte Vestergaard

16.00- : Beer and beverages

Abstracts

Key-note Lectures:

TYPE 1 HYPERSENSITIVITY REACTIONS IN A PHASE 1 TRIAL OF THE *Na*-ASP-2 HOOKWORM VACCINE IN PREVIOUSLY-INFECTED BRAZILIAN ADULTS

Jeffrey M. Bethony^{1,2} David J. Diemert³, Antonio Germano Pinto², Helton Santiago^{2,3}, Rodrigo Correa-Oliveira², and Peter J. Hotez^{1,3}

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Necator americanus secretory protein-2 (*Na*-ASP-2) is secreted by hookworm larvae upon entry into the host and is thought to play a role in parasite development. Vaccination of laboratory animals with *Na*-ASP-2 reduces hookworm infection and antibodies to ASP-2 in naturally-infected humans are associated with reduced worm load. Recombinant *Na*-ASP-2 was expressed in *Pichia pastoris* and formulated with Alhydrogel. After no significant vaccine-related adverse events were seen in a Phase 1 study of the vaccine in hookworm unexposed adults living in the US, a double-blind, dose-escalation Phase 1 study was planned in which 48 healthy Brazilian adults who had been treated for hookworm infection within 3 months of study entry were to be enrolled into one of three dose cohorts and randomized to receive either *Na*-ASP-2 Hookworm Vaccine or recombinant hepatitis B vaccine. In June 2007, vaccinations were initiated for the first cohort, in which participants were randomized to receive either 10 mg *Na*-ASP-2 or hepatitis B vaccine. 9 participants were vaccinated on the first day of the study. Within 2 hours of vaccination, 3 of 7 volunteers injected with *Na*-ASP-2 developed generalized urticaria. The reactions were not associated with signs of anaphylaxis, bronchoconstriction or angioedema, and all responded to treatment with an antihistamine. Anti-*Na*-ASP-2 IgE antibody responses were measured by ELISA on sera collected pre-vaccination and revealed that the individuals who developed urticaria all had elevated levels of antigen-specific IgE compared to those who didn't develop urticaria. Other than the allergic reactions, the most common adverse events were mild-to-moderate injection site pain and swelling. The data so far demonstrate that the *Na*-ASP-2 vaccine can induce immediate-type hypersensitivity reactions in previously infected adults. Vaccinations with the *Na*-ASP-2 vaccine are currently on hold and further clinical testing of this vaccine will require development of assays to detect pre-existing anti-*Na*-ASP-2 IgE before vaccination so as to prevent hypersensitivity reactions.

MALARIA VACCINES: UNANSWERED AND UNASKED QUESTIONS

David Arnot

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Abstract: In the last 10 years malaria vaccine research has gone from being the relatively obscure obsession of a few research groups to a major global health objective with prestigious supporters and greatly enhanced budgets. However the field has found it difficult to increase its effective productivity despite the injection of money and the influx of external project coordinators and

research managers. The vaccine development 'pipeline' contains a thin stream of closely related candidates in trial, few of whom have a substantial body of research data supporting their candidate status and none of whom command broadly based support from the core group of experienced researchers in the field. I will argue that this is mainly a failing of the 'advocacy' approach and suggest that a return to more traditional 'objective' research models coupled with redressing the imbalance between neglected pre-clinical R&D and an over-funded 'race to clinical trials' would serve the field better.

Submitted abstracts:

O designates oral presentation

P designates poster presentation

* designates young scientists competing for the two young scientist awards

1. O HUMAN MONOCLONAL ANTIBODIES RECOGNIZING PREGNANCY-SPECIFIC VARIANT SURFACE ANTIGENS CAN INHIBIT ADHESION OF *PLASMODIUM FALCIPARUM*-INFECTED ERYTHROCYTES TO CHONDROITIN SULPHATE A

Tina Dobrilovic*¹, Lea Barfod¹, Lars Hviid¹

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Pregnancy-associated malaria (PAM) is an important health problem in *Plasmodium falciparum*-endemic areas. The accumulation of parasite-infected erythrocytes (IE) in the placenta is due to parasite-encoded variant surface antigens (VSA) binding to chondroitin sulfate A (CSA). Acquired immunity to PAM is mediated by IgG with specificity for VAR2CSA and possibly other VSA_{PAM}. We have previously reported the production of VSA_{PAM}-specific human monoclonal IgG1 antibodies (HumAbs). We now report results on the ability of eight HumAb supernatants to inhibit adhesion of IE to purified CSA in static adhesion inhibition assays.

Three HumAb supernatants inhibited IE adhesion in a concentration dependent manner, whereas five HumAb supernatants showed no blocking ability on the whole. Pooled HumAb supernatants were comparable to plasma from multigravidae and more effective than single HumAb supernatants.

Our data indicate that we have antibodies of functional importance in protection against placental IE sequestration. These HumAbs could have satisfactory impact for vaccine development

2. O *PLASMODIUM FALCIPARUM* EXPRESSING VSA ASSOCIATED WITH SEVERE MALARIA IS ASSOCIATED WITH TRANSCRIPTION OF A CONSERVED SUBSET OF RIFINs

Wang, C. W.*; Magistrado P. A.; Engström M.; Vasconcelos N. M.; Schou S.; Turner L.; Theander T. G.; Lavstsen T.

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Variant surface antigens (VSA) expressed on the surface of *P. falciparum* infected red blood cells are considered targets for anti malaria disease vaccines. The VSA, *Plasmodium falciparum* erythrocyte membrane protein 1 (PfEMP1) encoded by group A *var* genes have previously been associated with severe malaria (SM). Uniquely, the 5' regions of group A and not group B or C *var* genes merge with the 5' regions of a neighbouring *rif* gene belonging to another VSA gene family. In this study, a 3D7 parasite line was selected to express a VSA-SM phenotype by repeated rounds of panning on DynaBeads coated by IgG from a pool of hyper-immune serum. The transcript abundances of *var* and *rif* genes were investigated in quantitative real-time PCR, using specific primers to 59 *var* and 154 *rif* genes. High transcript abundances of group A *var* genes and their neighbouring *rif* genes were found in the selected line. Additionally, further *in silico* analysis of the sequence diversity and organization of the RIFIN repertoire of the 3D7, HB3, and DD2 parasite lines was conducted. This analysis showed that the high abundant *rif* transcripts found in the VSA-SM expressing 3D7 line belong to an inter-genomic highly conserved subgroup of *rif* genes (group A RIFIN) with 5' regions that merge with the 5' region of a *var* gene. In conclusion, a functional relationship between the encoded group A PfEMP1 and the relatively conserved RIFIN may exist, associating these group A RIFINs with severe malaria.

3. O PREVALENCE OF GASTROINTESTINAL NEMATODES IN PIGS IN KABALE DISTRICT IN UGANDA

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Pig production is becoming increasingly important in Uganda and other African countries. Parasites, and in particular gastrointestinal nematodes, remain a major constraint to production. A study was carried out in the rural communities in Kabale District in the South Western part of Uganda in order to estimate the prevalence of gastrointestinal nematode parasites based on coprological examination. A total of 54 households were randomly selected within 11 villages and visited in October 2007 during the end of the dry season. Housing system, sanitary conditions, recent deworming and type of feed were recorded. Faeces were sampled from rectum of 2-3 pigs (age: 3-12 months) per household. In total 107 faecal samples were collected and examined by means of a modified McMaster technique. 91% of the animals excreted parasite eggs. The following parasite eggs were identified: strongyles in 88.8 %, *Ascaris suum* in 39.3 %, *Trichuris suis* in 17.8 % and spiruid eggs in 48.6 % of the pigs. Out of the 107 pigs, 9 % harboured 0, 23 % harboured 1, 37% harboured 2, 23% harboured 3 and 8% harboured all 4 types of eggs. Total faecal egg counts ranged from 0-58.920. Among the 19 *T. suis* egg positive pigs, 15 pigs were selected for post mortem examination of the gastrointestinal tract in order to recover adult helminths by means of sieving of contents (mesh size 212/500 µm). The post mortem examinations showed 14/15 (93%) infected with *Oesophagostomum* spp. (range of worm burden: (10-2180)), 11/15 (73%) *Ascaris suum* (1-36), 10/15 (67%) *Trichuris suis* (6-58) and 3/15 (20%) *Hyostromylus rubidus* (worms not quantified). Nematode infections were widespread and polyparasitism very common. However, worm burden were generally low which may be related to the common practice of rearing pigs on slatted floors in wooden elevated pens.

4. P MOLECULAR CHARACTERISATION OF BLASTOCYSTIS ISOLATES FROM DANISH SWINE AND CATTLE HERDS

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Blastocystis is a protistan intestinal parasite of unsettled clinical significance often isolated from human and non-human hosts. So far, nine distinct subtypes (species) have been identified from man, mammals and birds on the basis of small subunit (SSU) rRNA gene analysis.

Attempts to describe host specificity are crucial to identify aspects regards transmission and zoonotic impact. The project involved the molecular characterisation (subtyping) of the protistan intestinal parasite *Blastocystis* sp. from Danish cows and pigs. DNA was extracted directly from faeces and submitted to PCR targeting the small subunit (SSU) rRNA gene amplifying a 310 bp product. PCR products were sequenced and sequences were submitted to phylogenetic analysis for subtype identification. The majority of the pigs clustered with subtype 5 and three separate pigs clustered with subtype 3. The cows clustered with subtype 4 and subtype 8 except three cows which clustered with subtype 5. It appears that the cows clustering with subtype 4 and 8 is a new *Blastocystis* subtype. This, however, needs further confirmation.

Previously, pigs have been shown to host subtype 1, 2, 3 and 5, and the present data confirm this trend. Isolates from humans comprise mainly subtype 1-4, and thus the role of pigs in zoonotic transmission calls for further investigation. Cattle, however, may not represent reservoirs of human *Blastocystis* infection.

5. O DEMYELINATION IN EXPERIMENTAL CEREBRAL MALARIA

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Cerebral malaria (CM) is a serious and often fatal complication of *Plasmodium falciparum* malaria. It is an acute encephalopathy with loss of consciousness, convulsions and fever. The underlying pathology includes dysregulated cytokine expression, increased permeability of the blood-brain barrier and sequestration of host cells.

We used the murine CM model (C57BL/6 mice infected with 10⁴ *P. berghei* infected erythrocytes) to study the possibility that CM results in demyelination. Experimental murine and human CM share several clinical and pathological characteristics. Brains from terminal ill mice and uninfected control mice were after transcatheter perfusion, immersion fixed in Zamboni's fixative and embedded in paraffin. Myelin was visualised immunohistochemically with antibodies specific for myelin basic protein.

Extensive demyelination was detected in terminally ill mice. Demyelination was particularly evident in the cerebellum and in the corpus callosum. Antibodies specific for the myelin producing cells, oligodendrocytes, showed less intense staining in terminal ill mice. However, the amount of apoptosis detected with TUNEL stain was not significantly increased in terminal ill mice. The underlying mechanisms remain to be elucidated.

6. P VALIDATION OF THE USE OF ACRIDINE ORANGE IN FLOW CYTOMETRIC PARASITIC ENUMERATION

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Since the introduction of the Giemsa stain of thin blood smears, it has been the golden standard for parasitic enumeration. This is however a very tedious and subjective method. An automated alternative for determining the parasitized fraction of red blood cells would greatly facilitate scientific work with malaria parasites.

Flow cytometry for determining the amount of malaria-infected RBCs is based on the fact that mature erythrocytes lack a nucleus and therefore do not normally contain nucleic acids. Infected RBCs contain DNA and RNA from *Plasmodium*, and they are therefore easily discriminated from the non-infected population using the great variety of nucleic acid staining dyes. Specificity of the dyes used and reliable consistent results have been a problem for using flow cytometry to determine parasitemia in experimental animal models, because of the high amount of reticulocyte RNA in the blood samples in particular when dealing with animals undergoing treatment.

Now a new method has been developed using the DNA/RNA discriminating dye acridine orange in a two-channel (FL1/FL3) method that would make it possible to clearly discriminate between infected and non-infected populations and we have been interested in optimizing and validating it for our lab for *P. berghei* in mice and rats.

Comparison between the AO-two channel method and microscopic counts to check for accuracy in 20 different samples showed an accurate correlation between the two, linear regression, $y=0,9547x - 0,0075$, $R^2=0,9342$. Intra-assay variation was shown to be minimal. Analysis further showed that it is possible to save the samples for up to 1 day after acquisition, and that this is especially critical when dealing with samples with large number of reticulocytes. Sample processing time is about 5min making it an rapid, easy and accurate method for parasitic enumeration

7. P DEVELOPMENT OF *ANGIOSTRONGYLUS VASORUM* IN DANISH SNAILS AND SLUGS

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A study was conducted to determine development of the nematode *Angiostrongylus vasorum* in Danish terrestrial snails and slugs under different temperature conditions.

A total of 240 snails and slugs of the species *Cepeae nemoralis*, *Cepeae hortensis* and *Limax maximus* were each infected with 100 first stage larvae and placed in incubators at constant temperatures of 10°C (3 groups) or 18°C (3 groups). Five snails from each group were sampled every third day and individually digested in pepsin-HCl solution to free the larvae from the tissue. Recovered *A. vasorum* larvae were counted and the developmental stage determined. Further 200 *C. nemoralis* divided in 2 groups placed at 10°C and 20°C. Ten snails from each group were sampled every seventh day and the larvae were recovered and their developmental stage determined as above.

Fully developed third stage larvae were found in all slugs (*L. maximus*) kept at 18°C from day 21 and 24 post infection and in 20% of the snails (*C. nemoralis*) kept at 20°C at day 35, increasing to

50% at the end of the experiment on day 63 post infection. None of the larvae in snails and slugs kept at 10°C developed into infective third stage. Furthermore no larvae in *C. hortensis* and *C. nemoralis* at any temperature developed into the infective third stage in the short term study. *A. vasorum* larvae are able to develop from first to the infective third stage in common Danish snails and slugs and the development is clearly temperature dependent. The slug *L. maximus* appears to be a better-suited host than the snails *C. nemoralis* and *C. hortensis* since the infective stage is reached earlier in this species.

8. P ANGIOSTRONGYLUS VASORUM IN EXPERIMENTALLY INFECTED FOXES; LUNG LESIONS AND DEVELOPMENT OF EGGS

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Fourteen adult and fourteen juvenile foxes were each orally infected with either a low or a high dose of *Angiostrongylus vasorum* third stage larvae. Six uninfected foxes served as controls. All foxes were euthanized ten weeks post infection and subsequently, tissue specimens of the lungs were sampled, fixed in 10% buffered formalin, and processed for histology. All samples were stained with haematoxylin and eosin and in selected cases with other histochemical stains and immunohistochemical methods. Granulomas, often in the shape of conglomerates, were found occupying major parts of the lung tissue in both age groups. They consisted of epithelioid cells, macrophages and giant cells. The granulomas were found to initiate from small arteries and from there expand into the lung tissue. The granulomas often contained parasite eggs with could be differentiated into 4 distinct developmental stages. Stage I eggs contained a bulky content of eosinophile granules and no defined cells. Stage II eggs had large well-defined cell borders and distinct basophilic nuclei and a few eosinophile granules (morula stage). Stage III eggs contained a cell mass organised in a larval shape with many small basophilic nuclei but without well-defined cell borders. Stage IV eggs contained well-defined L1 larvae (embryonated egg).

Comparable lung lesions developed in both age and dose groups. However, host age appeared to have a stimulating effect on the formation of fibrosis, while young foxes mainly mediate an arrangement of granulomas into a pattern of conglomerates. The infective dose had no significant impact on the development of lung lesions. All four developmental egg stages were observed concurrently in most samples indicating that production of larvae is continuous in foxes in contrast to dogs.

9. P DISTRIBUTION OF FRESHWATER SNAILS IN NAM DINH PROVINCE, VIETNAM WITH SPECIAL REFERENCE TO INTERMEDIATE HOSTS OF FISHBORNE ZONOTIC TREMATODES

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Fishborne zoonotic trematodes, such as *Clonorchis sinensis*, heterophyids and others, constitute a public health concern in parts of northern Vietnam and infections are often thought to be linked to fish culture. One common fish culture system is the VAC ponds where individual households have one or more ponds. Fish fry, of mainly various carp species, produced in hatcheries, is introduced into nursery ponds in February/March and after approximately 1 month, juvenile fishes are transferred to household ponds, referred to as grow-out ponds. Grow-out ponds are usually fertilized with organic debris, including animal excreta, to stimulate algal growth and subsequently fish growth. We studied the distribution of freshwater snails in relation to certain ecological factors in two communes, Nghia Lac and Nghia Phu, Nghia Hung District, Nam Dinh Province during July-September 2006. These areas are under intense rice cultivation with an extensive canal network supplying water to fields and household fish ponds. Sixteen snail species were found, the most widely distributed being *Angulyagra polyzonata*, *Melanoides tuberculata*, *Bithynia fuchsiana* and *Pomacea bridgesi*. Snail diversity and counts were higher in nursery ponds than in grow-out ponds. Also there was evidence that snail numbers were lower in grow-out ponds containing specimens of the Black Carp, *Mylopharyngodon piceus* than in ponds without. Species of the Thiaridae were more abundant than other species in ponds while species of the Bithyniidae, Stenothyridae and Planorbidae dominated in rice-fields and small canals. Ten morphological types of cercariae were reported. Trematode infections were common in the thiarid species but also occurred in species of the Bithyniidae, Stenothyridae, Planorbidae and Lymnaeidae. *Melanoides tuberculata* had the most diverse trematode fauna and the highest overall prevalence of infection (13.3%). No trematode infections were found in *Angulyagra polyzonata*, *Idiopoma umblicata*, *Pomacea bridgesi*, *P. canaliculata*, *Pila polita*, *Neritina violacea*, and *Lymnaea viridis*.

10. P VARIANT SURFACE ANTIGENS EXPRESSION BY ERYTHROCYTIC STAGES OF *P. falciparum*

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The variant surface antigens (VSA) are parasite proteins expressed on the surface of *P. falciparum* infected red blood cells, and are potential targets for naturally acquired immunity to malaria. Different families of clonally variant surface proteins exist, two of them are located on the outer surface of the infected red cell membrane, encoded by *var* and *rif* genes, while the other group of proteins located within Maurer's clefts of IRBC membrane, are encoded by *stevor* genes. The PfEMP1 encoded by group A *var* genes have been associated with severe malaria in children, while VAR2CSA is associated with pregnancy-associated malaria (PAM). The developing gametocytes have been shown to transcribe *var* and *rif* genes, but no study has tried to analyze if these transcripts are translated into proteins.

1. The objective of this project is to identify the PfEMP1 and RIFIN proteins that are expressed by gametocyte stages, and subsequently characterize their stage specific roles and involvement in host immune responses.

2. In addition, this study aims at addressing the question whether antibodies against different PfEMP1 domains and RIFINs are able to recognize the surface of infected erythrocytes from children and pregnant women.

Preparation of recombinant proteins and antibodies

Recombinant proteins of different PfEMP1 domains and RIFINs will be produced in a baculovirus expression system.

Specific antibodies will be generated by immunization of rabbits and by affinity purification of human plasma from malaria endemic areas.

Testing of VSA expression using Flow cytometry (FACS)

Testing of *var* and *rif* genes transcription profile using real time RT-PCR

11. O PLASMA ANTIBODIES FROM EXPERIMENTALLY INFECTED MICE RECOGNISE PARASITE INDUCED ANTIGENS ON THE SURFACE OF *PLASMODIUM BERGHEI* INFECTED ERYTHROCYTES IN A PARTIALLY STRAIN SPECIFIC MANNER

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Malaria parasites express clonally variant surface antigens (VSA) on the surface of infected erythrocytes. In *P. falciparum* certain types of VSA are expressed by parasites causing specific malaria syndromes, like pregnancy associated malaria (PAM) and severe malaria in children. Having antibodies against these types of VSA appear to confer protection not against malaria infection per se, but against these specific malaria induced syndromes. This offers the hope that a fairly limited subset of VSA-genes could be used for vaccines against specific malaria syndromes. The rodent malaria parasite *P. berghei* can cause syndromes very closely mimicking human cerebral malaria and PAM. However, very little is known about the VSA-expression of this parasite, though a gene family called *bir* is thought to be its major VSA-gene family.

In order to validate a flow cytometry based assay developed to measure VSA specific antibody binding to live *P. berghei* infected murine erythrocytes, 80 BALB/c mice were infected with either of two different strains of *P. berghei*, K173 and ANKA followed by parasite suppressive treatment. The levels of plasma IgG recognising the VSA of the two strains of parasites were measured. Both groups of mice produced antibodies recognising the VSA of both parasite lines. The levels of IgG recognising K173 infected erythrocytes is similar in the two groups of mice (P=0,81), whereas the ANKA parasite strain is recognised more strongly by the mice that was initially infected with this parasite (P<0,001). This finding is in concordance with the finding that the ANKA strain has a much larger repertoire of *bir* genes than K173.

12. O SEROLOGICAL DETECTIONS OF IMMUNOGLOBULIN G (IgG) ANTIBODIES AGAINST *NEOSPORA CANINUM* AND *TOXOPLASMA GONDII* IN SERA FROM AFRICAN AND DANISH PATIENTS

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Very little is known about the prevalence of the intracellular parasite *Neospora caninum* in humans in contrast to the related parasite, *Toxoplasma gondii*, which is zoonotic. The present study aimed to investigate the presence of IgG antibodies against *Neospora caninum* in human serum samples.

A total of 653 serum samples from African, Tanzania and Sierra Leone and Danish patients were screened by indirect enzyme-linked immunosorbent assay (ELISA). Most of these serum samples were shown to be seropositive for *Toxoplasma gondii*. By the use of ELISA there were 66 possible

seropositive serum samples (10.1 %) for *N. caninum*. Forty-four (6.7 %) of these were African serum samples.

A confirmatory method, Western Blot (WB), was conducted on 55/66 samples to verify the seroreactivity against *N. caninum*. No immunodominant bands were observed; the bands were located in a wide span from approximately 13 to 205 kDa. Cross-reactivity with *Toxoplasma gondii* was considered to be a factor to these findings.

Another attempt was conducted to detect *N. caninum* specific antibodies, in which it was sought to minimize the possible cross reactivity with *T. gondii*. A pre-absorption step in WB was incorporated to remove specific antibodies against *T. gondii*. This was done on 34/55 sera and 31/34 sera did react towards *N. caninum* specific proteins after the removal of *T. gondii* specific antibodies. Immunodominant bands were observed at approximately 13, 37 and 54 kDa after the pre-absorption. Whether these bands were truly *N. caninum* specific was not definitively determined in the present study, and future studies should aim at addressing this question further.

13. O HOST-CELL APOPTOSIS IN TAENIA SOLIUM INDUCED BRAIN GRANULOMAS IN NATURALLY INFECTED PIGS

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To assess whether apoptosis occurs in pig brain granulomas due to *T. solium* cysticerci, brain tissues from 30 pigs naturally infected with *T. solium* cysticercosis were evaluated by terminal deoxynucleotidyl transferase-end labeling (TUNEL) staining. In addition, tissues were stained with CD3 marker to identify T lymphocytes. Examination of TUNEL stained tissues showed apoptotic cells in early lesions that contained live or viable cysticerci. Apoptotic cells were primarily found interspersed with normal cell types, and were mostly located in the inflammatory infiltrate. Late or advanced granulomas with disintegrated scolices did not show TUNEL positive cells. CD3+ cells were found in both early and advanced lesions and apoptosis mainly colocalized with CD3+ T lymphocytes. This suggests that these cells are constantly undergoing apoptosis and thus die as soon as they arrive at site of infection. Apoptosis indeed may be one way by which *T. solium* cysticerci down-regulate the host's cellular immune response in early cysticercosis. We recommend that future research should focus on the mechanism by which *T. solium* cysticerci induce apoptosis including cell surface receptors, as these may be targets for production of a therapeutic drug or vaccine.

14. P A POSSIBLE RISK FACTOR FOR TRANSMISSION OF FISHBORNE ZONOTIC TREMATODES TO CULTURE FISH IN NORTH VIETNAM

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Fishborne zoonotic trematode (FZT) infections are reported to seriously affect human health in Southeast Asia. Control programs focusing mainly on treatment of patients or mass treatment have been conducted with limited successes in Thailand and Korea. Apart from human, domestic animals are definitive hosts for FZT but very little is known about the infections in the animals, especially in Vietnam. This study was conducted to assess the source of infections to fish in Nghe An province of Vietnam, an area where the prevalence of FZT was very low in humans but very high in fish. Faecal samples from dogs, cats and pigs were examined for small trematode eggs by the DBL method, worm identification was done on a subsample of animals and questionnaire survey relating to husbandry practices was carried out. Results showed that prevalence of FZT infections was highest in cats (50%), followed by dogs (37.3%) and lowest in pigs (14.2%). Collected worms were identified as *Haplorchis taichui*, *H. pumilio*, *Echinochasmus japonicum*, *E. perfoliatus*, *Centrocestus formosanus* and *Echinostoma* spp. that were reported in fish in the same areas. In addition, dogs and cats were 95% and 100% free-roaming, respectively and practiced indiscriminate defecation on the pond bank, or in the vegetable garden connected to the fish ponds. Pig faeces were released directly to the pond without incubation and used as fish fodder (82.8% of cases). Based on our findings, dogs, cats and pigs might play a central role in transmission of FZT to culture fish in Nghe An province. Future control strategies of FZT should include control of the infections in domestic animals.

15. P STUDIES ON THE EPIDEMIOLOGY AND CONTROL OF MANSONELLA PERSTANS INFECTIONS IN UGANDA

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Mansonella perstans is a human filarial parasite which is widely distributed in Africa as well as in parts of Central and South America and the Caribbean. It is transmitted by tiny biting midges of the genus *Culicoides*. The adult worms are rarely recovered but appear to mainly live in the peritoneal cavity, whereas the microfilariae circulate in the blood. Despite of the widespread occurrence of *M. perstans* infections, very few studies have been carried out on the epidemiology and morbidity in endemic populations, and no thorough drug trials have been conducted to identify an effective and suitable drug for treatment and control. Infections with *M. perstans* are very common in Uganda. Here we report on recent studies carried out on *M. perstans* in endemic communities in Uganda: The counting chamber technique is evaluated as a tool for diagnosis and quantification of *M. perstans* microfilaraemia, the pattern of *M. perstans* microfilarial periodicity is established, the distribution of *M. perstans* microfilaraemia in two endemic communities is analysed, and the effect of ivermectin alone or in combination with albendazole on *M. perstans* microfilaraemia is evaluated in a randomized double-blind field trial.

16. O EFFECT OF HOST GENETICS ON ANTIBODY RESPONSES TO ASCARIS SUUM AND TRICHURIS SUIS IN PIGS

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Previous studies in pigs concurrently infected with *Trichuris suis* and *Ascaris suum* have shown that infection levels are related to host genetics. The aim of the present study was to examine a possible effect of host genetics on *T. suis*- and *A. suum*-specific antibody responses in pigs infected with both parasites. A total of 195 piglets were produced after artificial insemination of 19 sows (Danish Landrace-Yorkshire crossbreds) with semen selected from 13 individual Duroc boars. At 10 weeks of age the piglets were trickle infected with *T. suis* and *A. suum* eggs (5 and 25 eggs/kg/day, respectively) in the feed for 14 weeks until necropsy and worm counts (*Ascaris* only). Serum samples were taken 0, 7 and 14 weeks post start of infection (p.i.) and examined for IgG1, IgG2 and IgA antibodies specific to excretory/secretory (E/S) antigens of adult *T. suis* and L3 *A. suum* by ELISA technique. The mean *A. suum*- and *T. suis*-specific antibody responses were higher week 7 p.i. compared to week 14 p.i. for all isotypes, except for the mean *T. suis*-specific IgG2 level, which was significantly higher at week 14 p.i. only. The effect of litter on *T. suis*-specific antibody responses was (highly= $p < 0.01$) significant for all isotypes at 7 and 14 weeks p.i. ($P < 0.05$), while the effect of litter on *A. suum*-specific antibodies was only significant for IgG2 week 7 p.i and IgG1 and IgA week 14 p.i. The heritabilities of the antibodies for *T. suis* and *A. suum* ranged from 0.18 to 0.45 and from 0.05 to 0.41, respectively. The data suggest that the antibody response partly can be explained by genetic factors.

17. P POPULATION DYNAMICS OF *TRICHURIS SUIS* IN TRICKLE INFECTED PIGS

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The population dynamics of *Trichuris suis* in trickle infected pigs was studied during long term exposure to infective eggs. 23 pigs 10 weeks of age were inoculated with 5 *Trichuris* eggs/kg/day. 7, 8, and 8 pigs were necropsied at weeks 4, 8, and 14 post start of infection (p.i.), respectively. Worms were isolated from the colon and caecum using an EDTA method and by sub-sampling 5% of the content of the intestine. Serum samples were taken at each slaughter day and analysed by ELISA for anti-*Trichuris* antibodies (IgG1, -IgG2 and -IgA). At the three necropsy days the mean numbers of worm were 478 (min-max: 277-618), 472 (14-1140) and 0.5 (0-4), respectively. The parameter k of the negative binominal distribution was 14.6, 1.1 and 0.2 at 4, 8 and 14 weeks p.i., respectively suggesting an increase in the aggregation of worm load with time. At week 4 p.i. the main population of worms (59%) was less than 1 cm long and the rest (41%) more than 1 cm but still immature. By week 8 p.i. the main population (56%) was mature, however still 23% was smaller than 1 cm and 21% longer than 1 cm but immature. Overall, despite that pigs were continuously reinfected, a dramatic decrease in numbers of worms was observed as a function of time leaving only one pig infected with four worms at day 14 p.i.