Key Note:

1. Prevention and chemotherapy of human and animal helminth diseases in an age of anthelmintic resistance

Prichard, R.K.
Institute of Parasitology, McGill University, 21111 Lakeshore Road, Ste-Anne-de-Bellevue, Quebec, Canada.

For the past 40 to 50 years, livestock production has relied heavily on the use of anthelmintics to reduce the impact of parasitic disease on animal production and to reduce morbidity in companion animals. In recent years, very large scale mass chemotherapy programs have been instituted for the control of human helminth diseases, such as onchocerciasis, lymphatic filariasis, soil transmitted helminthiases and schistosomiasis. Anthelmintic resistance is now a very serious problem for parasite control in livestock, becoming increasingly important in companion animals and data will be presented showing that it appears to be developing in some helminth infections of humans. In the latter case, the huge mass chemotherapy programs (which are the largest chemotherapy programs in the world) are likely to impose serious selection pressure for more resistance development. This situation calls for changes in our approaches to these infections in animals and humans. Greater attention needs to be given to preventing, or reducing infection, that does not rely primarily on the use of anthelmintics. At the same time, we need to better monitor the effectiveness of chemotherapeutic and chemoprophylactic interventions and we need improved tools to monitor the occurrence and spread of anthelmintic resistance. There needs to be an acceptance and expectation that anthelmintic resistance will become worse. A better understanding of the mechanisms and genetics of resistance will help us to develop monitoring tools that are more sensitive, cost effective and easier to use than current tools based on parasite morphology. Technology has advanced greatly and some of the possibilities to develop better monitoring tools will be presented.

Young scientists:

2. O. The effects of fruit derived cysteine proteinases on cestodes

Mansur, F.,[1,3] Luoga, W.P.,[1,4] Duce, I.R.,[1] Buttle, D.J.,[2] and Behnke, J.M.[1]
[1]*School of biology, University of Nottingham, University Park, Nottingham NG7 2RD*
[2]*Department of Infection and Immunity, University of Sheffield, S10 2RX, UK*
[3]*Faculty of Medicine and Health Sciences, Islamic Science University of Malaysia (USIM),Tingkat 13, Menara B , Pesiaran MPAJ, Jln. Pandan Utama, 55100 K. Lumpur, Malaysia*
[4] *Department of Life Sciences, Mkwawa University College of Education, Iringa, Tanzania.*

Gastrointestinal (GI) helminths pose a significant threat to livestock industry and are a recognized cause of global morbidity in humans. Control relies principally on chemotherapy but in the case of nematodes is rapidly losing efficacy through widespread development and spread of resistance to conventional anthelmintics and hence the urgent need for novel classes of anthelmintics. Cysteine proteinases (CPs) from plants such as papaya, pineapple and figs have been shown to be effective against three murine nematodes *H. bakeri*, *P. muricola* and *T. muris in vitro* and *in vivo*. Preliminary evidence suggests an even broader spectrum of activity with efficacy against the canine hookworm *A. ceylanicum*, juvenile stages of parasitic plant nematodes of the genera *Meloidogyne* and *Globodera* and a murine cestode *H. microstoma in vitro*. This project focused on cestodes, with *in vitro* experiments on 2 different rodent cestodes *H. diminuta* and *H. microstoma* confirming that cysteine proteinases from papaya latex and pineapple do indeed affect cestodes by causing a significant reduction in motility leading to death of the worms. Observation of damage by scanning electron microscopy revealed tegumental damage. These findings were also verified by pilot *in vivo* studies in which treatment with papaya latex on rodent hosts infected with *H. diminuta* and *H. microstoma* demonstrated significant reductions in worm burden as well as worm biomass. However, there were no differences seen in faecal egg counts in both rodent cestode models studied.

3. P. Effect of fasting and host genetics on the anthelmintic efficacy of plant derived Cysteine Proteinases

Luoga, W.P.,[1,5] Mansur, F.,[1,6] Stepek, G.,[2] Buttle, D.J., [3], Duce, I.R.,[1] Garnett, M.C.[4] and Behnke, J.M.[1]
[1]*School of Biology, University of Nottingham, NG7 2RD, U.K: Division of Infection and Immunity, Institute of Comparative Medicine, University of Glasgow, G611QH, UK:* [3]*Department of Infection and Immunity, University of Sheffield, S10 2RX, UK:* [4]*School of Pharmacy, University of Nottingham, NG7 2RD, U.K.* [5]*Department of Life Sciences, Mkwawa University College of Education, Iringa, Tanzania.* [6]*Faculty of Medicine and Health Sciences, Islamic Science University of Malaysia (USIM), Kuala Lumpur, Malaysia*

Plant Cysteine proteinases from papaya latex, pineapple fruit and stem extracts have been demonstrated to be substantially effective against gastrointestinal nematodes of rodents and sheep. The current study investigated the effect of fasting on the efficacy of papaya latex supernant (PLS), and compared efficacy in a range of inbred mouse strains of contrasting genotype. Two blocks of Mice (45 &72) were infected with a suspension of 150 L3 of *H. bakeri*. Each block divided into three groups, The first group of mice was fasted for about 5h prior to treatment with 0.2ml containing 240nmol of PLS on day 20 after infection, while the second group of animals was treated with the same dose of PLS but not fasted. The third group was the control group, with the

animals being orally gavaged with 0.2ml of ultra-filtered distilled water and as for group 1, fasted for 5h before oral dosing. Treatment was repeated daily for 5 days. Mice were killed for worm counts and faecal egg counts were conducted on days 14, 16 and 18 (pre-treatment) and on days 21, 23 and 25 (post-treatment).The results showed that fasting before treatment of mice did not significantly improve efficacy and by avoiding fasting the side effects of treatment will be minimized. Comparison of efficacy in a range of mouse strains indicated that efficacy varied between mice of different genotype, a factor that will have to be taken into account when developing these agents further for use in domestic animals.

4. O. DBL2β domains from *Plasmodium falciparum* malaria field isolates bind ICAM-1

Bengtsson, A.,[1] Joergensen, L.,[1] Wivel, M.,[1] Andersen M.A.,[1] Theander, T.G.,[1] Craig, A.,[2] and Jensen, A.T.R.[1]

[1]Centre for Medical Parasitology, Department of International Health, Immunology & Microbiology, Faculty of Health Sciences, University of Copenhagen and Department of Infectious Diseases, Copenhagen University Hospital (Rigshospitalet) CSS Oester Farimagsgade 5, Building 22 & 23, PO 2099, 1014 Copenhagen K, Denmark. [2]Liverpool School of Tropical Medicine, Liverpool L3 5QA, United Kingdom

The *Plasmodium falciparum* erythrocyte membrane protein 1 (PfEMP1) antigens play a major role in antigenic variation, cytoadhesion of infected erythrocytes (IE) and immunity to malaria. Previously, we have shown that the 3D7 PfEMP1 PFD1235w is associated with severe malaria and IE surface expressing PFD1235w to cytoadhere to ICAM-1 on CHO cells. In this study, we have identified a panel of Ghanian field isolates which have PFD1235w-like sequences with particularly high sequence identity to the DBL1α, CIDR1α and DBL2β domains of 3D7. Following specific antibody selection PFD1235w-like protein was detected on the surface of two isolates that also adhere to CHO-ICAM-1. Using recombinant domains in an Enzyme-Linked Immuno-Sorbent Assay (ELISA) we identified 3D7 and field isolate DBL2β domains to be responsible for ICAM-1 binding. The DBL2β domains all bound in a similar pattern to a panel of mutated ICAM-1 variants with the binding being similarly reduced by seven monoclonal ICAM-1 antibodies. In addition, heterologous DBL2β domains were able to inhibit the 3D7 DBL2β:ICAM-1 binding indicating that the field isolates and 3D7 share important binding epitopes. These findings support PFD1235w as a vaccine candidate against severe malaria as PFD1235w-like DBL2β domains can be found in field isolates with high sequence identity, share ICAM-1 binding and might induce cross-reactive anti-adhesion antibodies when used for immunization.

5. P. The *Plasmodium falciparum* erythrocyte membrane protein 1 (PfEMP1) DBL2β domain binds ICAM1

Andersen M.A.,[1] Bengtsson A.,[1] Wiwel M.,[1] Jørgensen L.,[1] Theander T.G.,[1] Craig A.,[2] and Jensen, A.T.R.[1]

[1]Centre for Medical Parasitology, Department of International Health, Immunology and Microbiology, Faculty of Health Sciences, University of Copenhagen and Department of Infectious Diseases, Copenhagen University Hospital (Rigshospitalet), Copenhagen, Denmark. [2]Liverpool School of Tropical Medicine, Liverpool, United Kingdom.

Plasmodium falciparum is by far the most virulent of the five different *Plasmodium* species known to infect humans and cause malaria. *P. falciparum* infected erythrocytes (IE) surface express a

family of polymorphic antigens (PfEMP1) that binds to various host endothelial receptors and is responsible for the antigenic variation and pathogenesis of this parasite. The PFD125w PfEMP1 of *P. falciparum* 3D7 has been associated with severe malaria and IE expressing this PfEMP1 is known to adhere to ICAM1 expressed on CHO-cells. To identify the PFD1235w domain(s) responsible for ICAM1 binding we used recombinant protein (NTS, CIDR1α, DBL1α-CIDR1α, DBL2β, DBL3β, DBL4γ, DBL5δ, DBL5δ-CIDR2β, CIDR2β) and ICAM1 in Enzyme-Linked Immuno-Sorbent Assay (ELISA). This assay identified the DBL2β domain as binding to ICAM1 in a concentration dependent manner and the binding could be inhibited by adding a panel of monoclonal ICAM1 antibodies.
Since ICAM1 binding has previously been associated with severe malaria, characterization of the DBL2β:ICAM1 interaction may provide important knowledge for the development of a vaccine against severe malaria.

6. O. Prevalence of single nucleotide polymorphisms in the *Plasmodium falciparum* multidrug resistance gene (*Pfmdr-1*) in Korogwe district in Tanzania before and after introduction of artemisinin-based combination therapy.

Thomsen, T.T.[1], Ishengoma, D.S.,[2] Mmbando, B.P.,[2] Lusingu, J.P.,[2] Vestergaard, L.S.,[1] Theander, T.G.[1] Lemnge, M.M.,[2] Bygbjerg, I.C.[1] and Alifrangis, M.[1]
[1]*Centre for Medical Parasitology, Department of International Health, Immunology and Microbiology. University of Copenhagen. Copenhagen Denmark; Department of Infectious Disease, Copenhagen University Hospital, Copenhagen, Denmark*
[2]*National Institute for Medical Research (NIMR), Tanga Centre, Tanga, Tanzania*

The antimalarial drugs chloroquine (CQ), sulfadoxine-pyrimethamine (SP) and amodiaquine (AQ) were previously the preferred first-line drugs for treatment of uncomplicated malaria. However, *Plasmodium falciparum* resistance to these drugs is now highly prevalent in most of the malaria endemic world and thus alternatively, the World Health Organization (WHO) has recommended the use of Artemisinin-based combination therapies (ACTs). In Tanzania, artemether-lumefantrine (AL) was implemented in November 2006. AL remains highly efficacious, but the widespread use may facilitate emergence of artemisinin tolerance/resistance in Africa as recently shown at the Thai-Cambodian border. Resistance development may initially be detected at molecular level, such as temporal changes in the prevalence of certain single nucleotide polymorphisms (SNPs) in the *Pfmdr-1* gene, suggested to be associated with AL resistance. In Tanzania, 830 *Plasmodium falciparum* positive samples collected between 2003 and 2010 were examined for SNPs of *Pfmdr-1* at codon 86, 184, and 1246 by the use of standard PCR-restriction fragment length polymorphism (PCR-RFLP). Both the N86 and the 184F increased from 2006 to 2010 (Linear regression, N86: (coef. and [95% c.i], 0.067 [0.017-0.12], P=0.009), 184F: (coef. and [95% c.i] 0.05 [0.01-0.09], P=0.013) while no difference was found for D1246 during the same period (coef. and [95% c.i], 0.003 [-0.055-0.06], P=0.9). The observed changes may be due to introduction of AL and if so gives cause for concern and calls for continued surveillance of these molecular markers.

7. P. Gastrointestinal parasites detected in faecal samples of alpacas from the Peruvian Andes

Hald, S.H.[1], Nedergaard, K.[1], Kyvsgaard, N.C.[1] and Monrad, J.[1].
[1]*Department of Veterinary Disease Biology, Faculty of Life Sciences, University of Copenhagen, Denmark.*

Peru has the largest population of alpacas in the world. Alpacas are primarily kept for their fleece but are also harvested for meat and leather. This study was conducted in order to determine the effect of ivermectin and levamisole on faecal parasite egg counts and the post-treatment strongyle reinfection rates in the transitional period between dry and rainy weather. One hundred and twenty one-year-old male alpacas were randomly divided into three groups and marked with earmarks as they were herded freely in the Andes. On day 0 faecal samples were collected from the rectum and animals from the two treated groups were injected subcutaneously with either ivermectin or levamisole while the control group was left untreated. Faecal samples were subsequently taken on days 17, 32 and 49 post treatment. Later on the number of animal was reduced to 90 due to lack of time. Faecal samples were processed with McMaster and sedimentation techniques, and larval cultures were made from every sample with a positive McMaster result. Samples from day 0 were also processed with a Baermann technique, but since these were all negative no further examinations for lungworms were performed. Initially 75 % [67;83] of the animals were found to be infected with strongyles (EPG ranging from 50 to 350, with a mean of 95). On day 17 we detected a significant reduction ($p<0.05$) in the number of strongyle eggs shed in faeces, for the two treated groups compared to the control group. For the ivermectin treated group we found the effect of treatment to be 96 % [84;99]. The corresponding value for the levamisole treated group was 92 % [78;97]. The relatively low efficiency of treatment could be caused by the relatively low egg counts found initially. On day 32 we detected 26 % [0;42] of the alpacas in the levamisole group and 6 % [0;13] of the ivermectin group to have a strongyle EPG $\geq$ 50. On day 49 it was 29 % [11;47] for the levamisole group and 19 % [0;33] for the ivermectin group.

8. P. Sedimentation of helminth eggs in water

Sengupta, M.E.[1], Thamsborg, S.M.[1], Andersen, T.J.[2], Olsen, A.[1] and Dalsgaard, A.[1]
[1]*Department of Veterinary Disease Biology, Faculty of Life Sciences, University of Copenhagen, DK-1870, Frederiksberg C, Denmark,* [2]*Department of Geography and Geology, University of Copenhagen, DK-1350 Copenhagen C, Denmark.*

Helminth parasite eggs in the environment are of public health concern when using wastewater for irrigation of crops. The settling velocity of eggs from three different helminth species (*Ascaris suum*, *Trichuris suis* and *Oesophagostomum* spp.) was experimentally determined in tap water and in wastewater. The experimental settling velocities of eggs in tap water was compared with calculations of the settling velocities described by Stoke's law using measurements of size and density of eggs and density and viscosity of tap water. The mean settling velocity in tap water of 0.0612 mm s^{-1} for *A. suum* eggs was significantly lower than 0.1487 mm s^{-1} for *T. suis* eggs and 0.1262 mm s^{-1} for eggs of *Oesophagostomum* spp. The theoretical settling velocity for *T. suis* and *Oesophagostomum* spp. eggs were comparable with the experimentally observed velocities, while it was three times higher for *A. suum* eggs. The mean settling velocity in wastewater was 0.1582 mm s^{-1} for *A. suum* eggs, 0.0870 mm s^{-1} for *T. suis* eggs and 0.1051 mm s^{-1} for *Oesophagostomum* spp. eggs which was not different from each other and from the mean settling velocity of particles, 0.0474 mm s^{-1}. The similar settling velocity of wastewater particles and helminth eggs strongly indicates that the eggs are incorporated into the particle flocs and that the settling velocity of eggs is determined by the settling behaviour of particles in wastewater. The observed velocities of eggs settling as single particles are low and will probably not result in significant sedimentation in natural aquatic habitats. The findings of this study has implications for conceptual and numerical models dealing with removal of helminth eggs from natural water as previous models have relied on measurements of settling velocities of eggs settling as single particles in clean water.

9. P. Prevalence of Molecular Markers of Antimalarial Drug Resistance in *Plasmodium vivax* and *Plasmodium falciparum* in two districts of Nepal

Ranjitkar, S.[1], Schousboe, M.L.,[1] Thygesen, T.T.,[1] Adhikari, M.,[2] Bygbjerg, I.C.[1] and Alifrangis, M.[1]

[1]*Centre for Medical Parasitology, Department of International Health, Immunology and Microbiology, Faculty of Health Sciences, University of Copenhagen, Copenhagen, Denmark*
[2]*Central Department of Microbiology, Tribhuvan University, Kathmandu, Nepal*

Sulphadoxine-pyrimethamine (SP) and chloroquine (CQ) have been used in treatment of *falciparum* and *vivax* malaria in Nepal, respectively. Recently, resistance to both drugs have necessitated a change towards artemisinin-based combination therapy (ACT) against *Plasmodium falciparum* in highly endemic areas of Nepal while SP is still used against *P. falciparum* infections in low endemic areas and CQ is used in suspected malaria cases in areas with lack of diagnostic facilities. The present study determined the prevalence of molecular markers of *P. falciparum* and *P. vivax* CQ and SP resistance from malaria patients to explore if high levels of *in vivo* resistance were reflected at molecular level as well. Single nucleotide polymorphisms (SNPs) in the resistance related genes of *P. falciparum* and *P. vivax* were analysed in 92 and 41 blood samples, respectively; for CQ (*Pfcrt, Pvmdr1*) and SP (*Pfdhfr, Pfdhps, Pvdhfr*) using various PCR-based methods. Based on the *P. falciparum* samples, the molecular level of CQ resistance in *P. falciparum* was high since nearly all parasites expressed *Pfcrt* mutant haplotypes CVIET (55%) or SVMNT (42%). Likewise regarding *P. falciparum* SP resistance; the most prevalent *Pfdhfr* haplotype was double mutant CN**RN**I (91%) while frequency of *Pfdhps* double mutant S**GE**AA and A**GE**AA were 38% and 33% respectively. Combined, the frequency of quadruple mutations (CN**RN**I S**GE**AA/A**GE**AA) was 63% and thus, the molecular level of SP resistance was high. Based on the *P. vivax* samples, CQ and SP resistance are most likely low due to low prevalence of *Pvmdr1* Y976F mutation (5%) and absence of triple/quadruple mutations in *Pvdhfr*, respectively. Therefore, CQ may still be used in the treatment of *P. vivax* infections while the change to ACT for *P. falciparum* seems justified.

10. O. Transport and survival of *Cryptosporidium parvum* oocysts in soil columns following applications of raw and separated liquid slurry

Petersen, H.H.,[1] Enemark, H.L.,[2] Olsen, A.,[3] Amin, M.M.G.[4] and Dalsgaard, A.[1]

[1]*Department of Veterinary Disease Biology, University of Copenhagen, Stigbøjlen 4, 1870 Frederiksberg, Denmark.* [2]*Section for Adaptive Immunology and Parasitology, National Veterinary Institute, Bülowsvej 27, 1790 Copenhagen V, Denmark.* [3]*Department of Veterinary Disease Biology, University of Copenhagen, Thorvaldsensvej 57, 1871 Frederiksberg, Denmark*
[4]*Department of Agroecology and Environment, Faculty of Agricultural Sciences, Aarhus University, Blichers Allé 20, 8830 Tjele, Denmark*

The widespread waterborne pathogen *Cryptosporidium parvum* is primarily transmitted to humans via contaminated drinking and recreational water. Nearly all drinking water in Denmark is groundwater, but this can be contaminated with oocysts from application of contaminated manure to the field. Oocysts transport to groundwater requires that the oocysts are transported through soil and bedrock to the water table. The purpose of this study was to determine the potential transport of the protozoan pathogen *C. parvum* through soil to land drains and, subsequently water courses in a

laboratory setup using simulated rainfall and six 20 cm long replicate intact soil columns. Two types of contaminated slurry, namely raw slurry and the separated liquid fraction of the slurry were applied ten cm into the soil, following irrigation once a week over a four week period. *C. parvum* oocysts were detected in the leachates from soil columns to which *Cryptosporidium* positive slurry had been injected. Although recovery rates were low, regardless of slurry type, *C. parvum* oocysts were detected from all soil columns. Variations in the leachate patterns were recorded between soil columns added raw and liquid slurry respectively with significantly more oocysts in leachate from the latter. At the end of the study soil columns were destructively sampled to establish the location of remaining oocysts within the soil. Distribution within the soil was almost similar in all the soil columns, with the majority of oocysts found in the first section were the slurry was applied and with numbers decreasing with increasing depth.

11. P. AFM analysis of *Plasmodium falciparum*-infected erythrocytes involved in pregnancy associated malaria.

Quadt, K., Andersen, D., Nielsen, M., Hviid, L. and Barfod, L.
Centre for Medical Parasitology at Department of International Health, Immunology and Microbiology, University of Copenhagen and at Department of Infectious Diseases, Copenhagen University Hospital (Rigshospitalet), Copenhagen, Denmark

People living in malaria-endemic areas acquire protective immunity over several years. This naturally acquired immunity is mediated by antibodies to asexual blood-stage antigens, in particular to members of the *P. falciparum* erythrocyte membrane protein 1 (PfEMP1) family of adhesins. PfEMP1 variants are expressed on the surface of infected erythrocytes (IEs), where they mediate adhesion to several different host vascular receptors. During the growth of the *P. falciparum* parasites, they export PfEMP1, to the surface of the erythrocytes. Each parasite expresses a wide range of different VSAs and is even able to change them to escape the immune response. The PfEMP1 variant VAR2CSA is exclusively expressed on IEs adhering to chondroitin sulphate A (CSA) in the placenta of pregnant women. Primigravidae are consequently susceptible to infection by VAR2CSA-expressing parasites. The main consequences are maternal anemia and low birth weight babies. According to former investigation, PfEMP1 are concentrated in electron-dense knob protrusions, which are considered to play a role in the pathogenesis of malaria.
Atomic force microscopy is a very powerful tool, which allows high-resolution visualization of a sample's morphology on nano-scale. This method was used to investigate the knobs on the surface of *Plasmodium falciparum*-infected erythrocytes.
The aim was to analyze the knob-density of different parasite isolates and compare them to their binding affinity to CSA. The parasites were either laboratory lines or field isolates selected for binding to CSA. The isolates show both low and high affinity for CSA.

12. P. Application of the Symplex™ technology to production of recombinant, PfEMP1-specific antibodies

Jeppesen, A.E., Barfod, L., Hviid, L. and Dalgaard, M.B.
Centre for Medical Parasitology at Department of International Health, Immunology and Microbiology, University of Copenhagen and at Department of Infectious Diseases, Copenhagen University Hospital (Rigshospitalet), Copenhagen, Denmark

The antigen family PfEMP1 is an important target of naturally acquired protective immunity to *Plasmodium falciparum* malaria. PfEMP1-specific antibodies are therefore a powerful tool in malaria vaccine research. At Centre for Medical Parasitology (CMP), we have used Epstein-Barr virus (EBV)-immortalized memory B cells from donors who are clinically immune to malaria to produce human monoclonal IgG antibodies with specificity for clinically important PfEMP1 antigens. However, the technology is low-throughput, relatively low-yield, and susceptible to cell culture contamination. Production of recombinant versions of human monoclonal antibodies would alleviate these drawbacks and open possibilities for genetic engineering, such as isotype and subclass manipulation. To this end, the Symplex™ technology has several advantages over other recombinant antibody technologies. Most significantly, it ensures correct linking of heavy and light chain variable regions. The cloning process is also simplified, and high yields of antibody can be obtained after transient transfection of HEK293 cells with a vector containing linked antibody genes. We have now established The Symplex™ technology at CMP, and used it to generate recombinant versions of several human IgG antibodies secreted by EBV-immortalized B cell clones. The application of such an antibody in analysis of cross-reactive epitopes in distinct PfEMP1 antigens will be presented.

13. P. Optimisation of methods for hatching of A*scaris suum* eggs

Katakam, K.K.,[1] Dalsgaard, A.,[2] Thamsborg, S.M.,[1] and Roepstorff, A.K.[1]
[1]*Danish Centre for Experimental Parasitology, Department of Veterinary Disease Biology, University of Copenhagen,* [2]*Section for Microbiology, Department of Veterinary Disease Biology, University of Copenhagen, Denmark*

Many helminth eggs are highly resistant to environmental factors and eggs of the large round worm *Ascaris* sp. are often used as indicator organisms in determining the efficacy of sewage plants and manure handling in inactivating pathogens. For non-embryonated eggs, *in vitro* development of larvae inside the eggs is a useful criterion for egg viability, but this measure does not indicate whether the larvae are 'fit for fight' and it cannot be used for already embryonated eggs. Hatching of larvae from the eggs upon chemical stimulation may therefore be a better measure for the viability of *Ascaris* eggs, however, a validated, 'golden standard' hatching method does not exist. Therefore, a number of previously described methods have been tested, using different batches of fully embryonated eggs, which were all considered to be of high quality. Incubation of eggs in a physiological culture medium with 5% bile in a CO_2 incubator (5% CO_2, 38ºC) for 16 h has been described to be highly effective (Han et al., 2000), and this method resulted in almost 90% hatching of one batch of embryonated eggs, while there was no hatching in the other egg batches. Modifications of Han et al's method by including continuous shaking resulted in high hatchability of all the batches. Hatching in 100% CO_2 at 38°C (Jaskoski & Colucci, 1964) also resulted in variable hatching success of the test batches (0-90% hatching), while addition of continuous shaking reduced this variability to 50-90%. The results are not yet fully conclusive and have to be confirmed with a larger set of egg batches and compared to infectivity of the egg batches in sentinel pigs.

14. O. Erythropoietin prevents cerebral malaria and demyelination in experimental *Plasmodium berghei* ANKA infection

Jendresen, C.*[1], Hempel, C.,[1] Hyttel, P.,[2] Staalsø, T., [1], Nyengaard, J.R.[3], and Kurtzhals, J.A.L.[1]
[1]*Centre for Medical Parasitology at ISIM, University of Copenhagen and Department of Clinical Microbiology, Rigshospitalet,, Copenhagen, Denmark.* [2]*Department of Basic Animal and Veterinary Science, University of Copenhagen, Denmark.* [3]*Stereological Research Laboratory, Aarhus University, Denmark.*

Cerebral malaria (CM) is a severe condition caused by *Plasmodium falciparum* infection. Neurological sequelae, which are frequent in survivors, are suggested to be partly due to CNS demyelination and axonal damage. We have previously found that erythropoietin (EPO) increases survival and prevents clinical signs in murine experimental CM (ECM). We assessed the degree of demyelination in ECM and studied the effects of EPO treatment on the myelin content. C57BL/6 mice were infected with *P. berghei* ANKA and treated with EPO or placebo on days 4-7 post infection and euthanized on day 8 when terminally ill. Uninfected control mice were treated in a similar manner. ECM was characterized by low body temperature, high parasitemia, and poor motor coordination as well as changed behaviour. Brains were saline perfused and assessed with immunohistochemistry (IHC), transmission electron microscopy (TEM), and histological stainings. Scoring of myelin content was performed blinded by two investigators. IHC and luxol fast blue staining for myelin showed a profound loss of myelin in brains from infected, placebo-treated mice. Particularly in the cerebellum a loss of myelin was observed, in accordance with the impairment of motor coordination. TEM showed no decrease of myelin thickness in terminally ill mice in the corpus callosum, but axonal myelination was irregular and the sheaths were focally disrupted. EPO-treatment significantly reduced axonal damage and loss of myelin in infected mice. We are presently quantifying differences between treatment groups by western blots. This study suggests an association between clinical signs of CM and demyelination, and adds to the promising use of EPO in the treatment of CM patients.

15. P. Isolation of minute *Ascaridia galli* larvae from chickens

Ferdushy, T., [1] Kyvsgaard, N.C.,[2] Roepstorff, A.[1]
[1]*Section for Parasitology, Health and Development, Department of Veterinary Disease Biology, University of Copenhagen, Denmark.* [2]*Section for Microbiology, Department of Veterinary Disease Biology, University of Copenhagen, Denmark.*

When studying population dynamics and transmission rates for the common round worm *Ascaridia galli* in chickens, it is necessary to have a reliable method for isolation of minute larvae from the intestinal wall. In the present study, three different isolation methods were compared, viz. (1) incubation in 10 mM EDTA in 0.9% NaCl at 38°C overnight (used for isolation of *Trichuris suis* larvae; Kringel et al., 2002), (2) embedment of the intestinal wall in 1% Agar-gel incubated in 0.9% NaCl (38°C) overnight (a modification of a technique used for small *Ascaris suum*; Slotved et al., 1997), and (3) digestion with Pepsin-HCl (1.2% HCl (30%) and 3% pepsin (1:30 000 IU) in 1 L 42ºC tap water, P^H 1.2) for 90 min at 38°C (a modification of a technique used for isolation of *Trichinella* larvae; Kapel and Gamble, 2000). In each of two replicate trials, 15 seven-weeks old Lohman Silver chickens were inoculated orally with 500 *A. galli* eggs and necropsied 3 days later. *A. galli* larvae were isolated from the small intestinal wall by one of the 3 abovementioned methods

(n=5 for each method in each of the 2 trials), while larvae were recovered from the intestinal contents of all animals by means of the Agar-gel method. The total mean recoveries (intestinal wall + contents) of larvae by the EDTA, Agar-gel and Digestion methods were (19.9%), (23.9%) and (28.3%), respectively in 2 trials. More larvae were recovered from the intestinal wall (63-78%) than from the intestinal contents, regardless of the experimental group ($P<0.0001$). The mean percentages of larvae isolated from the intestinal wall were 13.5% (EDTA), 18.7% (Agar-gel) and 20.5% (Digestion). Although these figures were not statistically different ($P=0.15$), the larval recoveries were highest in the Digestion group of both trials. Therefore, the Pepsin-HCl digestion method will be the method of choice in future experiments.

16. O. A combinatorial vaccine against placental malaria and cervical cancer

Agerbæk, M.Ø., Andersen, G., Theander, T. and Salanti, A.
Department of International Health, Immunology and Microbiology, University of Copenhagen, Denmark

Placental malaria (PM) causes severe consequences for the 200.000 women infected every year. At Centre of Medical Parasitology it has previously been found that the accumulation of infected erythrocytes in the placenta is caused by VAR2CSA, a unique malaria parasite protein. One domain of VAR2CSA, DBL4, shows promising results as a vaccine candidate. We aim to optimise the response towards this antigen, and various DBL4 constructs have been designed and tested for inhibition of parasite adhesion.
A PM vaccine should be administered to women before they become pregnant, and the target group for vaccination should be pre-puberty girls. Thus, the PM vaccine would have to induce a long lasting immune response. We aim at making a vaccine combining a response against malaria parasites and human papillomavirus (HPV), which are known to cause cervical cancer. Cervical cancer is the most common cancer affecting women in developing countries. We have produced the vaccine against HPV, which consists of a virus-like particle. The particles have been purified by ultracentrifugation and verified by transmission electron microscopy. Virus-like particles are great inducers of immunity and stimulate long-term protection. By coupling the malaria DBL4 to the HPV particle, using streptavidin-biotin, we hope to induce long lasting immunity to both HPV and placental malaria. The coupling is confirmed by SDS-PAGE and Western blot. If the two vaccines can be successfully combined, it will be an advantage for fighting both HPV and PM in Africa and could save million of lives.

17. O. Whipworm (*Trichuris* spp.) infections in animals and man: Genetics of β-tubulin and susceptibility to benzimidazoles

Hansen, T.V.A., Nejsum, P., Olsen, A., Thamsborg, S.M.
Department of Veterinary Disease Biology, Faculty of LIFE, University of Copenhagen.

A persistent problem in the control of whipworm (*Trichuris* spp.) infections in man and a range of animals is the relatively low efficacy of treatment with a single application of benzimidazoles (BZ). This study focused on the presence of single nucleotide polymorphisms (SNPs) in the β-tubulin gene. SNPs in codon 167, 198 and 200 in this gene have been associated with BZ anthelmintic resistance in intestinal nematodes of veterinary importance. Recently it has been shown that SNPs at codons 200 are present in *Trichuris* populations of humans previously unexposed to BZ and it was hypothesised this species/genus therefore might be tolerant to BZ by nature.

DNA was extracted from a total of 180 *Trichuris* spp. adult worm specimens obtained from 7 different host species (pig: 24, deer: 21, lamb: 19, mouse: 15, dog: 19, baboon: 50, human: 29). In order to amplify the β-tubulin fragments which covered codons 167, 198 and 200 of the gene, degenerate primers were designed. The sequences obtained were used to design species specific primers and used to amplify a 479 bp fragment of the β-tubulin gene.
The PCR products were sequenced, analysed and evaluated. We did not identify SNPs associated with BZ resistance in codon 167, 198 or 200 in any of the studied *Trichuris* spp.
The phylogenetic analysis showed close evolutionary relationship between *Trichuris* spp. from pig, baboon and human; from lamb and deer; and from mouse and dog.

18. O. Pigs co-infected with *Trichuris suis* and *Oesophagostomum dentatum*: Possible immunological interactions elucidated by gene expression and histopathology

Andreasen, A.,[1] Petersen, H.H.,[1] Iburg, T.,[1] Skovgaard, K.,[2] Dawson, H.,[3] Urban, J.,[3] Kringel, H.,[1] Thamsborg, S.M.[1] and Roepstorff, A.[1]
[1]*Department of Veterinary Disease Biology, Faculty of Life Sciences, University of Copenhagen, Denmark.* [2]*National Veterinary Institute, Technical University of Denmark.* [3]*ARS, USDA, Beltsville Human Nutrition Research Center, Beltsville, MD 20705, USA.*

The overall aim of this study was to identify possible interactions between *Trichuris suis* and *Oesophagostomum dentatum* in pigs with regard to worm population dynamics and host immune responses. Forty-eight helminth naïve pigs were allocated into 4 groups in a 2x2 factorial design. Two groups were trickle inoculated with either 10 *T. suis* eggs/kg/day or 20 *O. dentatum* L3/kg/day. One group was trickle infected with both 10 *T. suis* eggs/kg/day and 20 *O. dentatum* L3/kg/day. The remaining group served as an uninfected control. All trickle infections continued until necropsy. In each group, six pigs were necropsied 5 weeks post first inoculation (wpi) and the remaining 6 pigs per group were necropsied 10 wpi. Previously presented worm data demonstrated that *T. suis* significantly reduced the *O. dentatum* numbers in co-infected pigs, while *O. dentatum* tended to enhance the *T. suis* burdens. Regarding immunity, *T. suis* is known to elicit a strong immune response, while *O. dentatum* induces relatively low levels of protective immunity. The response in co-infected pigs needs to be elucidated. Quantitative Real-Time PCR was used to measure the expression of immune function related cytokine, antibody and receptor genes in tunica mucosa of the proximal colon (PCM) and from the ileo-caecal lymph node (CLN) of each pig. Similarly, eosinophils in lamina propria and crypt lengths in the proximal colon were measured. The gene expression data in both locations and at both necropsies confirmed the presence of interactions between the two nematodes with regard to IL-13 ($p<0.06$, PCM, 5 and 10 wpi), ARG1 ($p<0.01$, CLN, 10 wpi), CCL11 ($p<0.05$, PCM, 10 wpi), IgE ($p<0.05$, PCM, 10 wpi), and IL-17A ($p<0.05$, CLN, 5 wpi). An interaction between the worm species was also found for eosinophils in lamina propria of the proximal colon 5 wpi. It is expected that some of these interactions may support, or even explain, the observed interactions between the two worm populations in co-infected animals.

19. P. Population dynamics of the minute intestinal trematode *Haplorchis pumilio* following experimental infection of young dogs

Sofie Nissen[1], Lan Anh Nguyen[2], Stig Milan Thamsborg[1], Anders Dalsgaard[1] and Maria Vang Johansen[1,]
[1] *Department of Veterinary Disease Biology, Faculty of Life Science, University of Copenhagen, Denmark,* [2]*National Institute for Veterinary Research, Hanoi, Vietnam*

Fishborne zoonotic trematodes (FZT) are highly prevalent in Southeast Asia and recent studies found that the most prevalent FZT was *Haplorchis pumilio*. Dogs were found to have the highest intensity of infection and contribute the most to the contamination of the environment with FZT eggs. Controlling the infection in dogs represents a major challenge. Knowledge is needed to make evidence-based recommendations for control of FZT. On this background, we conducted an experimental infection in dogs with *H. pumilio* to elucidate population dynamics and host reactions. Eight household-reared dogs (3-6 months old), were each infected with 500 metacercariae. Another eight dogs were included as uninfected controls. Faecal examination for eggs was performed twice weekly using a sieving and sedimentation technique, temperature and weight of the dogs were measured as was total white blood cells, eosinophils and microhaemotocrit values. Subsets of dogs were examined post-mortem for presence of adult FZT on day 17, 27-29 and 57 p.i. The worm establishment ranged from 3 – 24% (mean 12%). All eight infected dogs became faecal positive for small trematode eggs but no more than 2 epg were found at any time. Adult flukes were present on all days of necropsy. Following sectioning of the small intestine and caecum the predilection site of the flukes was identified as the lower part of the jejunum. The results of the hematological tests did not differ between the infected and control group. Further, no clinical symptoms were observed in the infected group and no macroscopic pathological changes could be assigned to the trematode infections, neither did histo-pathological examination of the intestine reveal any differences between the two groups. This pilot study provides basic knowledge on the establishment, duration and location of *H. pumilio* infection in the intestine. Further population dynamic studies are needed before recommendations for prevention and control can be provided.

20. O. Detection of water borne protozoa

Al-Sabi, M.N.S.[1,2], Enemark, H.L.[1], Kurtzhals, J.A.L.[2]
[1]*Department of Veterinary Diagnostics and Research, Danish Technical University, DK-1790 Copenhagen V, Denmark.* [2]*Centre for Medical Parasitology, Rigshospitalet and University of Copenhagen, Copenhagen, Denmark*

Protozoa of several species play a key role in water borne outbreaks of diarrhea worldwide. Identification of such protozoa depends mainly on parasite detection. However, water contains several hundreds of thousands of microorganisms belonging to different taxa. The exact identification of pathogenic protozoa relies on selective isolation and detection that is conducted by experienced technicians. Advanced techniques such as immuno-fluorescent dyes, polymerase chain reaction, and many other techniques, may be used for species-specific identification of pathogenic protozoa. Each diagnostic technique has defined sensitivity and specificity and the decision to use certain techniques depends on several practicalities such as reason for analysis, sample size, time, cost, and logistic requirements. In this work we present and discuss existing criteria for the differentiation of waterborne parasitic protozoa from other organisms in the aquatic environment.

21. P. Clinical and hematological study on crossbred cattle and water buffaloes (*Bubalus bubalis*) naturally infected with *Theileria annulata* in Sharkia province, Egypt

[1,2] Mahmmod, Y. S.; [2] Elbalkemy F. A.; [1] Klaas, I.C.; [2] Elmekkawy, M. F. and [2] Monazie, A. M.
[1] *Department of Large Animal Sciences, Faculty of Life Sciences, Copenhagen University, Grønnegårdsvej 2, Frederiksberg C, Copenhagen, Denmark.*

[2] *Department of Animal Medicine, Faculty of Veterinary Medicine, Zagazig University, Zagazig City, Sharkia Province, Egypt.*

The aim of the study was to investigate the clinical and hematological findings in crossbred cattle and water buffaloes naturally infected with *Theileria annulata* with special reference to the clinical picture of tropical theileriosis in Egyptian buffaloes. A total 50 field cases of cattle and buffaloes delivered to Veterinary Medicine Hospital, Zagazig University during the period from March to June 2008 and were investigated clinically and laboratory at arrival. Forty four (88%) cattle and buffaloes were naturally infected with *T. annulata* and showed typical signs of infection: fever, enlargement of the superficial lymph nodes, severe lacrimation, bilateral conjunctivitis, photophobia, corneal opacity and respiratory manifestations. Six animals (12%) showed no clinical signs and were free from external, internal and blood parasites. It was clear that the severity of clinical signs in infected buffaloes was more prominent than in infected cattle with persistence of some lesions after recovery as corneal opacity and pulmonary lesions. Hematological analysis revealed a significant decrease in RBCS count, PCV%, hemoglobin amount and WBCs in the infected animals comparing to the healthy ones ($P \leq 0.05$). In conclusion *T. annulata* infection is associated with impairment and alteration of blood parameters in both cattle and water buffaloes. Theileriosis in water buffaloes might cause irreversible ocular changes that could lead to complete blindness. Data obtained in this study might put the basis for the subsequent studies under natural and experimental field conditions.

Senior scientists:

22. O. A novel immuno-evasive mechanism in *Plasmodium falciparum* malaria: Non-specific IgM-masking of PfEMP1-specific IgG epitopes

Barfod, L.,[1] Pleman, S.T.,[1] Pleass, R.J.[2] and Hviid, L.[1]

[1] *Centre for Medical Parasitology at Department of International Health, Immunology and Microbiology, University of Copenhagen and at Department of Infectious Diseases, Copenhagen University Hospital (Rigshospitalet), Copenhagen, Denmark;* [2]*Liverpool School of Tropical Medicine, Liverpool L35QA, United Kingdom*

Plasmodium falciparum malaria is a major cause of mortality and severe morbidity. Its virulence is related to the parasite's ability to evade host immunity through clonal antigenic variation and tissue-specific adhesion of infected erythrocytes (IEs). The *P. falciparum* erythrocyte membrane protein 1 (PfEMP1) family is central to both. Here we present evidence of a novel *P. falciparum* evasion mechanism: the masking of PfEMP1-specific IgG epitopes by non-specific (natural) IgM. Natural IgM binding to erythrocytes infected by parasites expressing the PfEMP1 protein VAR2CSA blocked subsequent specific binding of human monoclonal IgG, except for one antibody recognizing an epitope distant from the VAR2CSA region interacting with the Cμ4 domain of natural IgM. Natural IgM binding protected the parasites from FcγR-dependent phagocytosis of IEs, but did neither affect VAR2CSA-dependent IE adhesion to CSA nor increased C1q deposition on IEs. Taken together, our results indicate that the VAR2CSA affinity for natural IgM has evolved to allow placenta-sequestering *P. falciparum* to evade acquired protective immunity without compromising VAR2CSA function or increasing susceptibility to complement-mediated lysis. Furthermore, functionally important PfEMP1 epitopes not prone to IgM masking are likely to be particularly important targets of acquired protective immunity to *P. falciparum* malaria.

23. P. The developmental stage of strongyle eggs affects the outcome of Real-Time PCR analysis

Roust, T., Haakansson, I.T., Rhod, M., Nielsen, M.K.
Department of Large Animal Sciences, Faculty of Life Sciences, University of Copenhagen, Denmark.

Several molecular diagnostic tests are based upon measuring and quantifying DNA obtained from parasite eggs. It is well-known that such eggs undergo development during storage, but it remains unknown to which extent the stage of development can affect the diagnostic test result. This project investigated whether the developmental stage of strongyle eggs affects real-time polymerase chain reaction (PCR) results. Mixed species strongyle eggs were obtained from the faeces of a naturally infected horse. Eggs were isolated and placed in microtiter plates with demineralised water. A total of 25 wells containing 100 eggs each were set up and kept refrigerated for up to five days. Once daily, five wells were microscopied on an inverted microscope, the developmental stages of the eggs were noted, and the eggs harvested for DNA extraction. The protocol was repeated three times. Genomic DNA was extracted using a commercial kit previously validated for strongyle type eggs. PCR reactions were performed with a primer set specific for the ribosomal DNA region for all strongyle type parasites (NC1, NC2). PCRs were performed in triplicates using SYBR Green as fluorescent dye. PCR results were registered as cycle of threshold (Ct) values. Statistical analysis revealed significant differences between days. Results illustrated a significant increase in PCR yield after three days, which was associated with beginning embryonation of the eggs. In conclusion, storage time and developmental stage of strongyle egg are significant sources of error in studies based on quantitative real-time PCR analysis performed. For storage more than three days, eggs should be killed and kept on ethanol for further analysis.

24. O. Variability in faecal egg counts – a statistical model to achieve reliable determination of anthelmintic resistance in livestock

Nielsen, M.K.[1], Vidyashankar, A.N.[2], Hanlon, B.[3], Petersen, S.L.[4], Kaplan, R.M.[5]
[1] Department of Large Animal Sciences, Faculty of Life Sciences, University of Copenhagen, Denmark. [2] Department of Statistics, George Mason University, Fairfax, Virginia, USA. [3] Department of Statistics, University of Wisconsin, Madison, Wisconsin, USA. [4] EquiLab Laboratory, Slangerup, Denmark. [5] Department of Infectious Diseases, College of Veterinary Medicine, University of Georgia, Athens, Georgia, USA

Anthelmintic resistance is an increasing challenge for the control of parasites in livestock. The faecal egg count reduction test (FECRT) is the practical gold standard method for evaluating resistance, but the interpretation is complicated due to high levels of variability. A hierarchical statistical model was therefore developed for analysis of FECRT data from multiple farms. Horse age, gender, zip code and pre-treatment egg count were incorporated into the model. Horses and farms were kept as random effects. Resistance classifications were based on model-based 95% lower confidence limit (LCL) values of predicted mean efficacies, and cutoff values were justified statistically. The model was used to evaluate the efficacy of pyrantel embonate paste on 64 Danish horse farms. Of 1644 horses, 614 had egg counts > 200 eggs per gram (EPG) and were treated. The cutoff LCL values used for classifying pyrantel resistance were: >92%: no resistance, 88-92%: suspect resistance, and <88%: resistance. Using model-adjusted LCLs, we classified seven (10.9 %) farms as pyrantel resistant, five (7.8 %) as suspect resistant, and the remainder of farms (81.3 %) as not resistant. In comparison, traditional arithmetic calculations classified nine farms (14.1 %) as resistant and 11 farms (17.2 %) as suspect resistant. Using 10000 Monte Carlo simulated data sets,

our methodology provides a reliable classification of farms into different resistance categories with a false discovery rate of 1.02 %. The methodology was shown to be unaffected by single outlier horses on the farms, while traditional calculations were strongly biased. The statistical model combines information between farms to distinguish between variability and genuine reduction in efficacy and can be adapted to handle FECRT data obtained from other livestock species, drug types, and parasite species.

25. P. Potassium channels as drug targets in *Plasmodium* parasites.

Ellekvist, P.[1], Mlambo, G.[2], Maciel, J.[2], Luciani, M.H.[1], Klaerke, D.A.[1], and Kumar, N.[2]
[1]Dept. of Physiology & Biochemistry, LIFE faculty, University of Copenhagen, DK1870-Frederiksberg; [2]Malaria Research Institute, Johns Hopkins Bloomberg School of Public Health, MD-21205 Baltimore.

Potassium channels are integral membrane proteins, which contribute to maintain vital parameters such as the cellular membrane potential and cell volume. Malaria parasites encode two K^+ channel homologues, Kch1 and Kch2, which are well-conserved among members of the *Plasmodium* genus. In the rodent malaria parasite *P. berghei*, the two K^+ channel homologues, PbKch1 and PbKch2, were studied using targeted gene knock-out. The transgenic parasites were characterized in a mouse model in terms of growth-kinetics and transmission potential. Second, using a tracer-uptake technique and $^{86}Rb^+$ as a K^+ congener, the K^+ transporting properties of the transgenic parasites were assessed. Third, the impact on parasite membrane potential of the two K^+ channels was investigated using a potential-dependent fluorophore DiBAC4 bis-oxinol.
Results: Knock-out of either K^+ channel did not grossly affect the phenotypes in terms of asexual replication and pathogenicity in a mouse model. However, *P. berghei* parasites deficient in PbKch1 (PbKch1-null parasites), but not PbKch2-null parasites, were unable to form oocysts in female *Anopheles stephensi* mosquitoes. PbKch1-null parasites, but not PbKch2-null parasites, had a low $^{86}Rb^+$ uptake, when compared to wild-type (WT) parasites. The Kch1-mediated $^{86}Rb^+$ uptake was inhibited by K^+ channel blockers; the residual, non-Kch1-mediated, $^{86}Rb^+$ uptake was not sensitive to further inhibition by K^+ channel blockers. Finally, Kch1, but not Kch2, apparently influenced the membrane potential of the parasites. Conclusion: Our studies suggest unequivocally that *Plasmodium* K^+ channel 1 homologue is a functioning K^+ channel, which contributes to the K^+ permeability of the parasites plasma membrane. The channel is, for yet unknown reasons, necessary for sexual replication of *P. berghei parasites* in the mosquito midgut. These studies provide a rationale for pharmacological inhibition of the Kch1 orthologue in human parasites as a novel strategy to disrupt malaria transmission.