Key-note lectures

Global Health in the 21st Century: Are Parasites Still Hot?

Bygbjerg I.
Department of International Health, Immunology and Microbiology (ISIM), Department of Health Sciences, University of Copenhagen, Denmark.

True parasitism is where parasites live at the expense of their hosts, e.g. by depriving them of essential material, destroying their tissues or damaging them by liberation of toxins.
According to *Wahlgren & Akuffo* (2003), there are no living organisms (besides maybe virus) that do not carry their own parasitic organism. So, unless we manage to harm mother Earth to the extent of extinction of life, by using up natural resources, degrading environment and liberating CO_2 and CH_4 , parasites will remain with us in the 21.th Century. However, present distribution and diversity may change significantly, among others by climate change and natural disasters, as elaborated on by the next speaker, *Màrius V. Fuentes*. I shall concentrate on other environmental changes and man-made disasters, although these are intimately interwoven with the 'natural'. Examples will be given from each of the main groups of parasites: protozoa, helminths and arthropods, and from the tropics as well as from colder climates.

Climate change, natural disasters and parasitic diseases with special emphasis on fasciolosis

Fuentes, M. V.
Departament de Parasitologia, Facultat de Farmàcia, Universitat de València. Av. Vicent Andrés Estellés s/n, 46100 Burjassot, València, Spain.

The environment plays an important role in the epidemiology of infectious diseases in general, and of parasitic diseases in particular. Each parasite species requires particular conditions, biotic as well as abiotic, for its geographical expansion and propagation. The most important abiotic factors are temperature, relative humidity, rainfall, sun irradiation, edafology, hydrology, wind speed, etc. Parasite species with a direct or an indirect life cycle, those with free environmental stages and those with intermediate hosts, mainly vector-borne, are strongly influenced by the variations of these abiotic factors, some of which determine the climate of a particular geographic area. Thus, climate plays an important role in determining the seasonal and geographic distribution and frequency of many parasite species. The relationship between parasite species and climate has been widely studied, especially in those parasites important in animal and human health, which has made it possible to predict the periods of transmission risk for humans and animals, as well as to develop models of parasite population dynamics.
Climate change, mainly perceived as an alteration of rainfall patterns, an increase of temperatures and rise of the sea level, will not only alter the environment in which the biological cycle of each parasite develops, but will also have a strong influence on the epidemiology and transmission of each species. Climate change effects have to be

considered particularly in terms of the various transmission cycles of infectious agents, those transmitted directly and those transmitted by other living beings, especially invertebrates. In the same manner, natural disasters change the conditions of an affected area. In this case, the changes will have a temporary –more or less lasting– effect, which in turn might affect the global transmission patterns as well.

Studies, including prediction models, concerning the epidemiology and transmission of animal parasites have to incorporate the analysis of climatic alterations in the short as well as in the long run.

To shed more light on the effects of climate change and natural disasters on the epidemiology of parasitic diseases, the main results of a multidisciplinary research group of Universitat de València concerning these topics are presented.

Fasciolosis has been well studied in some parts of the world, especially in human and animal endemic areas. As an example, the epidemiology of human and animal fasciolosis in the Andean mountain range has been well characterized, and the effects of the climate on transmission patterns have been modelled as well. Moreover, this parasitic disease influenced by El Niño-Southern Oscillation and other cyclic climatic events has also been studied.

Effects of repercussive environmental changes, particularly those caused by natural disasters, on the parasite community of wild animals, has also been analysed and a model, which can explain behavioural changes produced in parasites and their hosts, has been created. This model will be extrapolated to other animal species, domestic and wild, as well as to humans, thus, making the prevention and control of parasitic diseases possible, especially those which will be more important on a worldwide scale as a consequence of climate change or natural disasters.

As climate change and natural disasters are expected to influence the epidemiology of animal and human parasites, modifying their transmission patterns and geographical distribution, parasitic diseases have to be included in future research on human and animal health effects expected by new trends of global climate.

References

Diaz, J.H., 2006.- Global climate changes, natural disasters and travel health risks. *Journal of Travel Medicine*, 13, 361-372.

Epstein, P.R., 2005.- Climate change and human health. *New England Medical Journal of Medicine*, 353, 1433-1436.

Fuentes, M.V., 2006.- Remote sensing and climate data as a key for understanding fasciolosis transmission in the Andes: review and update of an ongoing interdisciplinary project. *Geospatial Health*, 1, 59-70.

Fuentes, M.V., 2007.- Is the El Niño-Southern Oscillation likely to increase the risk of *Fasciola* transmission? *Annals of Tropical Medicine and Parasitology*, 101, 555-557.

Fuentes, M.V., Valero, M.A., Bargues, M.D., Esteban, J.G., Anglés, R. & Mas-Coma, S., 1999.- Analysis of climatic data and forecast índices for human fascioliasis at very high altitude. *Annals of Tropical Medicine and Parasitology*, 93, 835-850.

Githeko, A.K., Lindsay, S.W., Confalonieri, U.E. & Patz, J.A., 2000.- Climate change and vector-borne diseases: a regional analysis. *Butlletin of the World Health Organization*, 78, 1136-1143.

Huntingford, C., Hemming, D., Gash, J.H.C., Gedney, H. & Nuttall, P.A., 2007.- Impact of climate change on health: what is required of climate modelers? *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 101, 97-103.

Mas-Coma, S., Valero, M.A. & Bargues, M.D., 2008.- Effects of climate change on animal and zoonotic helminthiases. *Revue Scientifique et Technique de l'Office International des Épizooties*, 27, 443-452.

Patz, J.A. & Kovats, R.S., 2002.- Hotspots in climate change and human health. *British Medical Journal*, 325, 1094-1098.

Patz, J.A., Graczyk, T.K., Geller, N. & Vittor, A.Y., 2000.- Effects of environmental change on emerging parasitic diseases. *International Journal for Parasitology*, 30, 1395-1405.

Submitted abstracts

P designates poster presentation
O designates short oral presentation
*** designates young scientist competing for this year's award. However, previous recipients cannot win the award again.**

1-P. Albendazole and mebendazole have low efficacy against *Trichuris trichiura* in school-age children in Kabale district, Uganda

Annette Olsen [a], Harriet Namwanje [b], Peter Nejsum [c], Allan Roepstorff [c], Stig M. Thamsborg [c]
[a] DBL-Centre for Health Research and Development, Department of Disease Biology, Faculty of Life Sciences, University of Copenhagen, Denmark. [b] Vector Control Division, Ministry of Health, Kampala, Uganda. [c] Danish Centre for Experimental Parasitology, Department of Disease Biology, Faculty of Life Sciences, University of Copenhagen, Denmark

Three groups of *Trichuris trichiura*-infected school-age children were treated with one dose 400 mg albendazole, 100 mg mebendazole twice daily for 3 d, or 100 mg mebendazole twice daily for 5 d. The albendazole study investigated cure and egg reduction rates and found that only 5 of 66 infected children were egg-negative 7 d post-treatment, giving a cure rate of 8% and a geometric mean egg reduction rate of 89%. However, at day 14 post-treatment, all children were again egg-positive with significantly higher egg counts than at day 7 (P <0.001). The two mebendazole studies aimed for the recovery of adult *T. trichiura* worms. After the 3 d course of mebendazole treatment, only four worms were recovered on days 3-5 after start of treatment from 2 of 34 infected children. With the 5 d course of mebendazole treatment, 10 of 21 infected children expelled a total of 27 worms. In the last case the first worm appeared on day 4 post-treatment, and the highest number of worms was recovered when the study ended at day 7. In conclusion, even with the longest treatment regimen and collecting stool samples over seven consecutive days, only very few worms were recovered. The results of this study suggest that alternative drugs and/or alternative regimens in current control programmes against *T. trichiura* need renewed attention.

2-O*. Differences in transmission intensities of *falciparum* malaria affect the frequency of human complement receptor 1 (CR1) polymorphisms in North-Eastern Tanzania

Helle H. Hansson[1], Lasse S. Vestergaard[2], Martha M. Lemnge[3], Bruno P. Mmbando[3], Anders Enevold[1], Mette L. Schousboe[1], John P. Lusingu[3], Thor G. Theander[1], Ib C. Bygbjerg[4], Michael Alifrangis[1]. [1]Center for Medical Parasitology, University of Copenhagen and Rigshospitalet, Copenhagen, Denmark, [2]Department of Infectious Diseases, Rigshospitalet, and Institute of International Health, Immunology & Microbiology, University of Copenhagen, Copenhagen, Denmark, [3]National Institute for Medical Research, Tanga, Tanzania, United Republic of, [4]Institute for International Health, Immunology and Microbiology, University of Copenhagen and Rigshospitalet,

Copenhagen, Denmark

The complement receptor one (CR1) expressed on human erythrocytes is involved in rosetting and impaired or altered function of this receptor might confer some protection against cerebral malaria. It has been hypothesized that two altered alleles observed in Africa, Swain-Langley (Sl) 2 and McCoy (McC) ^{b}, may be responsible for the advantageous effect. The objective of this study was to determine and compare the genetic frequency of the CR1 polymorphisms of the Sl and McC alleles in populations living in two villages with high and low malaria transmission intensities in Korogwe District, Tanzania. Single nucleotide polymorphisms (SNPs) in the CR1 gene were detected by PCR followed by a sequence specific oligonucleotide probe (SSOP) – ELISA method. High frequencies of the Sl2 allele were seen in both villages compared to Caucasian populations ($P \leq 0.001$). Furthermore, significantly higher frequencies of the Sl2 allele was found in the village with high transmission compared to village with low transmission ($P=0.003$), though, only significantly in individuals above five years of age ($P \leq 0.001$). For the McC^{b} allele, no differences between the villages were found. The results indicate that the high frequency of the Sl2 allele in Tanzania may have been selected for by malaria. This supports previous findings suggesting a protective role of this polymorphism. CR1 is an important rosetting receptor and the Sl2 allele can possibly diminish disease severity. Further studies of CR1 in the pathogenesis of cerebral malaria, appear to be justified.

3-O*. Expression of microRNAs involved in inflammation and endothelial activation in experimental cerebral malaria

Casper Hempel[1,3], Fatima El-Assaad[1], Valéry Combes[1], Angeles Sanchez-Perez[1], Jørgen Kurtzhals[3], Jean-Marie Mathys[2] and Georges E. R. Grau[1]
(1) Vascular Immunology Unit, Faculty of Medicine & Bosch Institute, University of Sydney, Australia (2) Dept. of Molecular and Clinical Genetics, Royal Prince Alfred Hospital, The University of Sydney, Australia (3) Centre for Medical Parasitology at Dept. of Clinical Microbiology and Dept. of Infectious Diseases, Copenhagen University Hospital and Dept. of International Health, Immunology, and Microbiology, University of Copenhagen, Denmark.

Cerebral malaria (CM) is a serious complication to untreated malaria and accounts for substantial mortality worldwide. CM is characterised by dysregulated immune responses but its pathogenesis remains incompletely understood. Recently, small non-coding RNAs, named microRNAs (miRNAs), have emerged as gene expression regulators that play an important role in immune functions and inflammation. The expression of miRNAs has not been studied in CM, therefore we aimed to elucidate the expression of selected miRNAs.
Using quantitative PCR, we analysed the relative expression of selected miRNAs in the brain and in the heart. Organs were harvested from *Plasmodium berghei* ANKA infected CBA mice at the time of clinical CM onset. let-7, miR-24, miR-26a, miR-126, miR-146, miR-150 and miR-155 were significantly down-regulated in brains of infected mice, compared to those of uninfected animals. In contrast, no significant change in the relative

expression levels of these miRNAs was detected in the heart tissue; an organ with no pathology during acute malaria.
Taken together, our findings suggest a role for miRNAs in the immunopathogenesis of murine CM. These results prompt further exploration of miRNAs and their therapeutic potential for improving CM outcome.

4-O. An analysis of nuclear mitosis during the cell division cycle of intra-erythrocytic *Plasmodium falciparum*.

David E. Arnot* [1] & Dominique Bengtsson[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 Øster Farimagsgade 5, Bygning 22 & 23, Postboks2099, 1014 Copenhagen K, Denmark.

The nuclear divisions occurring during the malaria parasite's intra-erythrocytic schizogony remain a poorly understood part of the cell cycle of this Apicomplexan protozoan. The problems essentially revolve around the uncertain fate of the nuclear envelope during schizogony and the relative timing of the S and M phases during this process. One puzzling feature of this process is that these divisions often result in numbers of daughter shizonts (6, 18, 20 etc) that are difficult to explain as the results of the geometric progression of a simple binary division (1, 2, 4, 8, 16 etc). By using laser scanning confocal microscopy and improved nuclear preservation techniques for *in situ* hybridization with fluorescently labeled oligonucleotide probes for chromosome telomeres, we have been able to analyze the behavior of *P.falciparum* chromosomes throughout the mitotic nuclear divisions of schizogony. A framework for a new model of the cell cycle during the multiplication of bloodstage *Plasmodium falciparum* is presented.

5-P*. I*n vivo* significance of neuroglobin in cerebral malaria

[1]DellaValle, B; [1]Hempel, C.; [2]Kurtzhals, J.; and [1]Penkowa, M.
[1]Section of Neuroprotection, University of Copenhagen, Panum Institute, Blegdamsvej 3, Byg. 18.1.37, Copenhagen N, Denmark. [2]Department of Clinical Microbiology, Rigshospitalet 7602, Blegdamsvej 9, Copenhagen E, Denmark

Cerebral malaria (CM) is a life-threatening complication of Plasmodium falciparum malaria and is presently without treatment apart from anti-parasitic drugs and intensive care. The pathogenesis of CM is associated with the sequestration of red blood cells in the brain microvasculature, blood brain barrier dysfunction, and damaging inflammatory mediation. Neuroglobin (Ngb) is a recently discovered globin thought to function as an endogenous neuroprotective protein in the brain. This study was designed to investigate the *in vivo* significance of Ngb in the pathogenesis of CM.
C57BL/6j mice were infected with parasitized red blood cells of the *Plasmodium berghei* ANKA strain. Half of the infected mice received an i.p. injection of erythropoietin alpha (EPO) and did not show clinical signs of CM. Half of the control mice also received EPO. In CM mice and EPO treated infected mice the expression profile of Ngb was

different than control mice. Additionally, neuronal expression of Ngb was lower in uninfected mice that were administered EPO.
To date this is the first study on the role of Ngb in CM. The results of this study suggest that Ngb responds to the pathology associated with experimental CM in a novel way not yet reported in other *in vivo* Ngb studies. Further characterization of the mediators involved in this response is necessary and may reveal potential therapeutic targets for the treatment of CM.
To date this is the first study on the role of Ngb in CM. The results of this study suggest that Ngb responds to the pathology associated with experimental CM in a novel way not yet reported in other *in vivo* Ngb studies. Further characterization of the mediators involved in this response is necessary and may reveal potential therapeutic targets for the treatment of CM.

6-O*. Real-time PCR for detection of low intensity *Schistosoma japonicum* infections in stool samples from China

Lier, T.[1]; Simonsen, G.S.[1]; Wang, T.[2]; Lu, D. [2]; Haukland, H.H.[1]; Vennervald, B.J.[3]; Hegstad, J.[1]; Johansen, M.V[3]
[1]Department of Microbiology and Infection Control, University Hospital of North Norway, Tromsø, Norway, [2]Anhui Institute of Parasitic Diseases, Wuhu, China, **5** DBL-Centre for Health Research and Development, Faculty of Life Sciences, University of Copenhagen, Copenhagen, Denmark

After decades of screening and treatment the prevalence and average infection intensity for *Schistosoma japonicum* are now low in most of the endemic areas. This represents a diagnostic challenge. Real-time PCR for detection of *Schistosoma japonicum* in stool samples was compared to conventional diagnostic methods in a study of 1727 individuals from the Anhui Province, China. The seroprevalence using an indirect hemagglutination assay (IHA) was much higher (26.1%) than the prevalence in stool-based tests which were 5.3%, 3.2% and 3.0% for PCR, hatching test and Kato-Katz thick smear, respectively. A large proportion of the positive stool samples were only positive in one or two tests. PCR displayed better agreement with IHA than the other two stool-based tests. A commonly used diagnostic algorithm is to screen with IHA and test the seropositive with a stool-based test. Such an algorithm would in the present study result in treatment of 22 versus 50 people, respectively, if Kato-Katz or PCR had been used as the stool-based test. The benefit of increased sensitivity using PCR in schistosomiasis diagnosis should be weighed against additional costs.

7-O*. The use of an experimental murine malaria model to elucidate the immunological mechanisms of DTP vaccine and Vitamin A

[1,2]Hein-Kristensen, L.; [1]Jørgensen, M.J.; [2,3]Wiese, L.; [2]Kurtzhals, J. and [1]Benn, C.
[1]Bandim Health Project, Indepth Network, Statens Serum Institut, Artillerivej 5, 2300 Copenhagen S, Denmark. [2]Centre for Medical Parasitology, Department of Clinical Microbiology, Copenhagen University Hospital (Rigshospitalet), 2100 Copenhagen,

Denmark [3]Department of Infection and Diseases, Copenhagen University Hospital (Hvidovre), Denmark.

The World Health Organisation recommends vitamin A supplementation (VAS) to all children aged 6 months to 5 years in low-income countries and for logistic reasons this has been linked to the routine immunizations of the Expanded Programme of Immunization. Worryingly, recent observational studies suggest that VAS may increase mortality from non-targeted diseases when given with diphtheria-tetanus-pertussis (DTP) vaccine, especially in girls.
We investigated the non-specific effects of pre-treatment with VAS and DTP vaccine in an established rodent model of experimental cerebral malaria. Outcome measures were mortality, parasitemia, and plasma cytokine concentrations before and during the infection. Our a priori hypothesis was that VAS/DTP would aggravate the outcomes. The effect of VAS and DTP was evaluated in 6 separate experiments. During the experiments it became clear that not all mice developed cerebral malaria. Furthermore, the dose of inoculated iRBC and parasite strain may have modified the effect of VAS/DTP. Overall, we found a negative effect of VAS/DTP on parasitemia, but it may have been modified by inoculum dose and virulence of the parasite variant. The effect depended on pathogenesis; VAS/DTP tended to have a greater negative effect on parasitemia and significantly depressed cytokine responses in mice developing cerebral malaria compared with mice dying of anaemia. The results support the hypothesis that VAS/DTP may have detrimental effects on disease outcomes. The divergent effect according to pathogenesis may help elucidate why VAS has divergent effects on different diseases in humans.

8-P*. Multiplex PCR on single unembryonated A*scaris suum* eggs

Carlsgart, J.; Roepstorff, A. and Nejsum, P.
Danish Centre for Experimental Parasitology, Department of Veterinary Disease Biology, Faculty of Life Sciences, University of Copenhagen, Dyrlægevej 100, DK-1870 Frederiksberg C, Denmark

A sensitive and inexpensive method for DNA isolation and amplification by PCR from single unembryonated *Ascaris* sp. eggs is described. The resistant shell of single eggs was crushed mechanically and PCR applied to the crude egg contents without any further purification steps. The ITS1 region of the rDNA and three regions of the mtDNA could be successfully amplified. Using two primer sets, it was possible to amplify the rDNA and mtDNA simultaneously in one single reaction. The ability to perform PCR on single unembryonated eggs may result in better and more precise species identification of eggs recovered from faecal material, environmental samples, and possibly archaeological samples and in this way e.g. explore transmissions routes of human and pig *Ascaris* and identify zoonotic infections. In addition, single egg PCR makes it possible to perform population genetic studies without having to recover adult worms by deworming or autopsy.

9-O. Potential impact of and adaptation to climate change on schistosomiasis transmission in China and Sub-Saharan Africa.

Xiao-Nong Zhou, Kun Yang, Guo-Jing Yang, Xiao-Hua Wu, Robert Bergquist, Jürg Utzinger, Anna-Sofie Stensgaard, Flemming Møhlenberg, Carsten Rahbek and Thomas K. Kristensen

[1] National Institute of Parasitic Diseases, Chinese Center for Disease Control and Prevention, Shanghai 200025, People's Republic of China;
[2] Department of Schistosomiasis Control, Jiangsu Institute of Parasitic Diseases, Wuxi 214064, People's Republic of China;
[3] DBL, Department for Veterinary Disease Biology, Faculty of Life Science, University of Copenhagen, Denmark
[4] Ingerod 407, Brastad, Sweden
[5] Department of Public Health and Epidemiology, Swiss Tropical Institute, Basel, Switzerland

Appraisal of the present and future impact of climate change and variability on the transmission of infectious diseases is a complex but pressing public-health issue. In order to take adaptation to the climate impact to schistosomiasis transmission, we developed a biology-driven model to assess the potential impact of rising temperature on the transmission of schistosomiasis in China, and then take different adaptation approaches based on the prediction and local settings. We find a temperature threshold of 15.4°C for development of *Schistosoma japonicum* within *Oncomelania hupensis*, and a temperature of 5.8°C at which half the snail sample investigated was in hibernation. Historical data suggest that the occurrence of *O. hupensis* is restricted by the mean January temperature of 0°C. Then we forecast an expansion of schistosomiasis transmission into currently non-endemic areas in the north, with an additional risk area of 783,883 km^2 by 2050. By taking these results considerations, 3 pilots on adaption to climate change was set up in the sensitive areas where potential expansion may occurred, in order to prevent the *Oncomelania hupensis* from migration northforward where is non-endemic area for *Schistosoma japonicum*. Two models of adaption, including building sinking pound for *Oncomelania hupensis* in the water courses, surveillance for new infectious sources, were performed and economic benefit was assessed. The assessment for acceptation of the adaption strategy was performed to be better adopted by local stakeholders. It is concluded that schistosomiasis is able to be controlled once adaptation intervention implemented to reduce the impact raised by climate changes.

10-O*. Genetic variation in whipworm (*trichuris* spp.) populations

[1]Nissen, S.; Nejsum, P.; Christensen, H., Olsen, A. and Thamsborg, S. M.
[1]Department of Veterinary Disease Biology, Faculty of Life Science, University of Copenhagen.

The two nematodes, *Trichuris suis* and *Trichuris trichiura* are believed to be two separate but closely related species, i.e. being reproductive isolated but difficult to distinguish by morphological characters. In areas where humans and pigs live in close proximity a zoonotic transmission might be a hazard. Furthermore, eggs of *T. suis* populations have

potential in treating patients with autoimmune-related diseases in the western part of the world. In both cases there is a strong need for knowledge on the genetic diversity within and between *Trichuris* spp. in the two hosts, which was the aim of the present study. Sympatric worm material isolated from 10 humans and 5 pigs in Uganda supplemented with *T. suis* from Tanzania, Denmark and USA and *T. trichiura* from England, was obtained. Based on morphology, worms from the two hosts could only be discriminated by the length of the male spicule (student t-test, $p<0.001$). The second internal transcribed spacer (ITS-2) in the ribosomal DNA was amplified by PCR and cloned. Between 1 and 6 clones from 20 worm were sequenced, which resulted in a total of 94 sequences (49 human-derived and 45 pig-derived) that could be allocated into as many as 56 different haplotypes. A very large intra-individual variation was found within the human-derived sequences (0.2 – 45.0%) compared to the pig derived sequences (0.2 – 1.4%). This was due to the fact that the human-derived worms consisted of two main ITS-2 sequence types; one of them being unique (69% of the human-derived sequences, consensus sequence 481 nucleotides long) and the other being identical to the sequence type found in pig-derived worms (31% of the human-derived worms, consensus sequence 531 nucleotides long). The results indicated that the nematodes found in pigs belong to a genetically distinct species (*T. suis*) whereas the nematodes in humans showed a considerably larger variability either related to ancestral polymorphism or more recent cross-breeding between *T. trichiura* and *T. suis*.

11-P*. Different plasma antibody recognition patterns of primary infections and later recrudescense infections of *Plasmodium berghei anka* infected b6 mice

[1]Boeje, S.; [1]Kurtzhals, J.A.L.; [2,3]Hviid, L and [1,2]Staalsoe, T.
Centre for Medical Parasitology at [1]Department of Clinical Microbiology and [2]Department of Infectious Diseases, Copenhagen University Hospital (Rigshospitalet) and at [3]Department of International Health, Immunology and Microbiology, University of Copenhagen, Denmark

In the human malaria parasite *Plasmodium falciparum* expression of variant surface antigens (VSA) on the host erythrocyte is modified by acquired immunity. Parasites from non-immune malaria patients with life threatening malaria express VSA that are serologically distinct from parasites isolated from non-severe or asymptomatic cases, in that they are frequently recognised by antibodies in plasma of semi-immune individuals. It is thus crucial to characterise the VSA expression in severe malaria to discover future vaccine targets. However, these investigations are not easily carried out in humans because of ethical reasons. The genomes of *Plasmodium sp* infectious to laboratory rodents also contain VSA-encoding multigene families. In the present study we have investigated whether there is a similar pattern of different VSA expression in naïve and semi-immune hosts in mice infected with *Plasmodium berghei*. Using a panel of plasma samples from 50 animals with different immune status we compared the serological characteristics of parasites obtained from 9 primary and 7 recrudescent infections. From this analysis it appeared that the VSA of parasites from primary infection were recognised strongly by plasma donors already after approximately 40 days after their initial infection, whereas parasites from recrudescent infections expressed VSA that were most strongly recognised by plasma obtained from animals exposed for more than 60

days, suggesting that like in *P. falciparum* severe malaria in naïve hosts are caused by parasites expressing frequently recognised VSA, while less frequently recognised variants cause recrudescent infections.

12-O*. Helminth-induced immunomodulation of an autoimmune disease (multiple sclerosis) in rats

Poulsen, I.H[1], Hasseldam, H.[2], Roepstorff, A.[1], Thamsborg, S.M.[1], Iburg, T.[1], Kringel, H.[1]

[1] Department of Veterinary Disease Biology, Faculty of Life Science, University of Copenhagen
[2] Department of Biomedical Sciences, BRIC, University of Copenhagen

Helminth infections are known to have a potent systemic immunomodulatory effect on the host immune response, reducing the impact of autoimmune diseases. The swine whipworm, *Trichuris suis*, is known to alleviate symptoms in Crohns Disease and Ulcerative Colitis patients, and several other helminths have successfully been used to improve signs of disease in experimental models of e.g. multiple sclerosis and type-1 diabetes. With the purpose of enabling studies on the immunomodulatory effect of *T. suis*, we set out to investigate whether *T. suis* can establish in different strains of experimental rats. The rats were inoculated with infective *T. suis* eggs and subsequently euthanized after different time spans. The intestines were examined for visible larvae and faeces were examined for hatched and unhatched eggs. Only a few larvae were found in the large intestine within the first 3 days after inoculation. To verify this observation, one rat was infected with a very large dose of *T. suis* eggs and euthanized after 24 hours. By histology it was demonstrated that *T. suis* eggs are able to hatch in the rat and that larvae invade the epithelial cells of the caecum and colon. No clinical symptoms were observed in the rats. The aim of the second part of the study was to investigate whether inoculations with *T. suis* eggs would affect the progress of Experimental Autoimmune Encephalomyelitis (EAE) in rats, a model for multiple sclerosis. An EAE response was induced in 16 rats of the Dark Agouti strain and the rats were scored daily to monitor the progress of disease. From the time the first symptoms (score 1) of disease appeared, 8 rats were inoculated with 7.000 *T. suis* eggs 3 times a week, while the other 8 rats were kept as non-infected controls. The treatment with *T. suis* continued until the rats were euthanized, either at 14 days after symptom debut or until their clinical score reached 4. There was a significant ($P = 0.0011$) improvement of the clinical scores in the group of rats treated with *T. suis* compared to the untreated rats. The results from this preliminary study indicate that EAE rats may be a suitable model for helminth-induced immunomodulation.

13-O*. Metastrongyloid parasites in the red panda (*Ailurus fulgens*) -a coprological survey

[1,2]Meyland-Smith, F.; [1]Bertelsen, M. F.; [3]Willesen, J. L. and [2]Monrad, J

[1]Center for Zoo and Wild Animal Health, Copenhagen Zoo, Roskildevej 38, DK-2000 Frederiksberg, DENMARK. [2]Danish Center for Experimental Parasitology, Faculty of Life Sciences, University of Copenhagen, DK-1870 Frederiksberg, DENMARK.

[3]Department of Small Animal Clinical Sciences, Faculty of Life Sciences, University of Copenhagen, DK-1870 Frederiksberg, DENMARK

During a survey in 1982 metastrongyloid parasites were reported in red pandas of all North American zoos using the Baermann method for coprological surveillance. In red pandas of European zoos *Angiostrongylus vasorum* and *Crenosoma striatum* had been diagnosed up till 2008, and *A. vasorum* had been identified as a potential cause of death. Hence, a prevalence study of metastrongyloid parasites in the red panda population of zoos within the European Association of Zoos and Aquariums was conducted during the winter of 2008/2009. A total of 113 red pandas from 53 zoos were included in the study. An overall prevalence for metastrongyloid parasites of 34.5 % (39/113) was found. Three categories of metastrongyloid first stage larvae were found. *A. vasorum* was diagnosed in 2.65 % (3/113), *Crenosoma spp.* was diagnosed in 4.42 % (5/113), and an unidentified metastrongyloid parasite was diagnosed in 27.4 % (31/113) of the animals. The isolated first stage larvae of each of the parasite categories were morphologically described. The significance of the parasitological findings in the present study was unclear and further investigations are warranted as well as further description of the unidentified metastrongyloid found. Experimental infections of laboratory animals to describe the life cycle and adult morphology of the parasite as well as genetic sequencing are suggested.

14-O*. A robust and quantitative *in vitro* assay of CSA-specific adhesion of *Plasmodium falciparum*-infected erythrocytes

Tina Dobrilovic & Lars Hviid. Centre for Medical Parasitology, University of Copenhagen and Rigshospitalet, Copenhagen, Denmark

Parasite-encoded, clonally variant surface antigens (VSA) on the surface of *P. falciparum*-infected erythrocytes (IEs) allow their adhesion to a number of vascular host receptors.
Pregnancy-associated malaria (PAM), which is a major cause of mother-offspring morbidity and mortality in areas with stable transmission of *P. falciparum* parasites, is caused by IEs that can sequester selectively in the placental intervillous space because they express particular pregnancy-associated VSA (VSAPAM) with specificity for chondroitin sulphate A (CSA).
Antibody-mediated interference with CSA-specific IE adhesion in the placenta appears central to acquired immunological protection from PAM. A standardised and reproducible *in vitro* assay of specific IE adhesion to CSA is therefore a research priority, in particular if the assay is quantitative and can be implemented in laboratories with limited facilities.
We have developed a modified version of the standard, manual Petri dish assay used in most studies so far. Our assay overcomes problems with patchy IE adhesion and low reproducibility caused by low affinity of CSA for plastic surfaces and bias-prone manual assay read-out. It does not require advanced equipment for removal of non-adhering erythrocytes, and it does not quantify adhesion by using radio-labelled IEs. The assay is characterised by uniform CSA coating that allows non-patchy IE adhesion, which can be

easily and objectively quantified and documented using a microscope-mounted digital camera and object recognition software.

15-P*. Risk of viable fish-borne zoonotic metacercariae in "*goí cá*" a northern vietnamese raw fish dish

[1*]Aduhire, M. A.; [2]Dalsgaard, A.; [1]Johansen, M. V.

[1]DBL-Center for Health Research and Development, Department of Veterinary Disease Biology, Thorvaldsensvej 57, 1871 Frederiksberg C, Danmark (* Msc student)

[2]Department of Veterinary Disease Biology, Stigbøjlen 4, 1870, Frederiksberg C, Danmark

The aim of the study was to assess the potential risk of fish-borne zoonotic trematode (FZT) infections when eating popular raw fish dishes in Northern Vietnam. To identify the most popular raw fish dish, a questionnaire (N=40) was conducted in Nghia Lac and Nghia Phu communes in the province of Nam Dinh,Vietnam. The most popular raw fish dish identified in the area was "*Goí Cá*". An experimental fish dish study on the "*Goí Cá*" was conducted to assess the viability of FZT metacercariae (MC) using 18 silver carp (*Hypophtalmichthys sp*.) caught in the same locality. Fish filets were dipped in 2% NaCl for 15 minutes, left to dry at room temperature (26ºC) and lastly incubated in spices (garlic, Thai ginger, peppermint and chili). Times of incubation were 30 minutes (26ºC), 2 hours (26ºC) and 24 hours (4ºC). To further assess the MC viability, 20 encysted *H. pumilio* MC were incubated in increasing salt and acetic acid solutions (5%, 10% and 20%) at different time points (0h, 2h, 4h, 6h, 8h, 24h, 48h, 72h and 168h at 4ºC) and finally exposed to 2% trypsin + PBS (pH 7.2; 37ºC) for 30 minutes. Of the 1429 MC recovered, which were mainly *Haplorchis sp*. from the 18 carps, 1048 (73.3%) were found viable. Exposure of *H. pumilio* MC to increasing salt concentrations did not have any significant effect on their viability. Nonetheless, a decrease in the survival (S) of *H. pumilio* MC incubated in 10% acetic acid from 2 h (S=75%) to 168 h (S=25%) was noticeable. The number of live *H. pumilio* exposed to 20% acetic acid from 2h (S=60%) to 72h (S=40%) demonstrated that acetic acid had an effect on the viability of *H. pumilio* MC. Our data suggest that the spices used in the popular raw fish dish "*Goi Cá*" were insufficient to inactivate FZT MC.

16-P*. Determination of ivermectin strongyle efficacy and egg reappearance period (ERP) on Danish horse farms

Mette L. Larsen[1], Christian Ritz[2], Stig L. Petersen[3], Martin K. Nielsen[1] [1]Department of Large Animal Sciences, Faculty of Life Sciences, University of Copenhagen, Denmark [2]Department of Basic Sciences and Environment, Faculty of Life Sciences, University of Copenhagen, Denmark [3]EquiLab Laboratory, Slangerup, Denmark.

Anthelmintic resistance is widespread in the world. Cyathostomins have developed resistance to several groups of anthelmintics and a number of studies have shown shortened cyathomstomin Egg Reappearance Period (ERP) after treatment with

ivermectin. In Denmark, usage of anthelmintics has been restricted by prescription-only for a decade, but current levels of anthelmintic resistance are not known. Efficacy of ivermectin (0.2 mg/kg) was evaluated on a total of 196 horses from farms using selective therapy. Fecal Egg Count Reduction Tests (FECRT) were performed 14 days post treatment. 79 of these horses were infected with *Parascaris equorum* and 117 horses were infected with strongyles only. Overall efficacy of ivermectin was 96.9% against *P. equorum* and 100% against strongyles. ERP was investigated on 9 farms with a total of 96 horses, using a selective treatment strategy. 65 untreated horses were investigated from the same farms as controls. All egg counts were determined with the McMaster method. Horses were dosed and treated by the owners, but investigators verified weight estimations by girth tape measurements. Weekly FECRTs were performed from 2 to 6 weeks post treatment. Average efficacy of ivermectin was 96.9% 6 weeks post treatment. One farm had 90% efficacy of treatment at 6 weeks. The large majority of horses were dosed correctly or slightly overdosed, while only 8 horses were marginally underdosed. The untreated group showed a notably increase in Fecal Egg Count (FEC) during the study period. In conclusion this study did not provide evidence of shortened ERP or reduced efficacy of ivermectin.

17-P*. The effect of sample handling and storage on the accuracy and repeatability of equine strongyle fecal egg counts

Kathrine Pilegaard[1], Ulla V. Andersen[1], Christian Ritz[2], Ray M. Kaplan[3], Martin K. Nielsen[1] [1]Department of Large Animal Sciences, Faculty of Life Sciences, University of Copenhagen, Denmark [2]Department of Basic Sciences and Environment, Faculty of Life Sciences, University of Copenhagen, Denmark [3]Department of Infectious Diseases, College of Veterinary Medicine, University of Georgia, USA.

Faecal Egg Counts (FEC) are increasingly used to diagnose and monitor equine strongyle burdens and evaluate anthelmintic efficacy. In horses, the Faecal Egg Count Reduction Test (FECRT) is the golden standard used to investigate anthelmintic resistance. The purpose was to examine the effect of a combination of storage time and storage temperature as well as airtight versus uncovered storage on the number of strongyle eggs and their degree of embryonation. Faeces were stored airtight at ÷18°C, 4°C, 20°C, and 37°C for a total of 120 hours. Sub-samples were removed for FEC analysis at 0, 3, 6, 12, 24, 48 and 120 hours, using a Stoll technique. In another set of experiments, a pile of faeces was kept uncovered and another airtight on the stall floor for 24 hours. Statistical analyses were performed using linear models. In both studies, each FEC was measured 5 times and experiments were repeated 3 times. Temperature had no influence on the FEC after 12 hours of storage, and storage at 4°C did not reduce FEC for the entire 120 hours. Significant reductions were found after 24 hours at ÷18°C and 37°C, respectively and after 48 hours at 20°C. Availability of air had no significant influence on the FEC or degree of embryonation after 24 hours of storage at 11.8-16.9 °C. Airtight storage of faeces at 4°C preserves FEC for up to 120 hours. Further, faeces may be collected from the stall floor at temperatures in the investigated range for up to 24 hours after defaecation.

18-P*. Comparison of three flotation methods for counting eggs in faeces

*Al-Jubery, A; Mohamed, A; Ranjitkar, S.; Nejsum, P. and Roepstorff, A.
Danish Centre for Experimental Parasitology, Department of Veterinary Disease Biology, Faculty of Life Sciences, University of Copenhagen, Denmark

Quantitative assessment of intestinal helminth infections by flotation of eggs in faeces (faecal egg counts; FEC) is a commonly used method in veterinary parasitology. However, the precision and accuracy in the results depend of the method. We here compare three methods for FEC estimation, namely Simple McMaster, Concentration McMaster and the newly developed FLOTAC method. Faecal material was obtained from an *Ascaris suum* negative pig (as determined by the Concentration McMaster Technique) and 722,000 *A. suum* eggs were added. The faeces was mixed manually for 3 h to give a homogeneous sample with 639 eggs per gram. All three methods were evaluated by examination of 25 subsamples using the same flotation fluid (saturated NaCl with 500 g glucose per liter, specific gravity 1.27 g/ml). The FEC was found to be 184 (SD 77), 456 (SD 67) and 327 (SD 54) by Simple McMaster, Concentration McMaster, and FLOTAC method, respectively. Analysis of variance (ANOVA) showed that the FECs were all significantly different ($P<0.001$) from each other. The recovery of the eggs was highest in concentration McMaster (71.4 %), lowest in simple McMaster (28.8%) and intermediate for FLOTAC (51.2%). The Concentration McMaster therefore seems to be the method of choice for FEC estimation of *A. suum* eggs. However, in cases of low FEC the FLOTAC technique may be preferred due to a lower detection limit (1 egg per gram of faeces) where it is 20 for the Concentration McMaster method.

19-P*. Concurrent experimental infection with *Trichuris suis* and *Oesophagostomum dentatum* in pigs: Effects on parasite population dynamics

Andreasen, A ; Petersen, H.H.; Kringel, H.; Thamsborg, S.M. and Roepstorff, A.
Department of Veterinary Disease Biology, Faculty of Life Sciences, University of Copenhagen.

The aim of this study was to examine possible interactions between *Trichuris suis* and *Oesophagostomum dentatum* infections in pigs with regard to the population dynamics of the worms such as faecal egg excretion, worm burden, location and length. 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 (Group T) or 20 *O. dentatum* L3/kg/day (Group O). One group (OT) was trickle infected with both 10 *T. suis* eggs/kg/day and 20 *O. dentatum* L3/kg/day. The remaining group C 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 were necropsied 10 wpi. A significantly higher faecal *O. dentatum* egg excretion was seen in Group O compared with Group OT ($p<0.05$). Faecal egg counts remained high in Group O while a marked decrease was seen in Group OT from 4 wpi. The faecal *T. suis* egg excretion was generally higher in Group OT compared to Group T. The *Oesophagostomum* worm burden was significantly higher in Group O compared to

Group OT both at 5 weeks ($p<0.01$) and 10 weeks ($p<0.05$). At both necropsies the mean *T. suis* worm burden was higher in Group OT compared to Group T, although not significantly so. The weighted mean location for *O. dentatum* was more posterioly in the large intestine in Group O compared to Group OT, while *T. suis* was located in the proximal part of the large intestine in both Groups T and OT. The length of adult *O. dentatum* females seemed to be slightly shorter in group OT. The results clearly indicate that *Trichuris* may negatively influence *Oesophagostomum* populations in co-infected individuals – we observed an almost ten fold reduction in *Oesophagostomum* worm burdens in these animals. In contrast the presence of *Oesophagostomum* may potentially enhance populations of *Trichuris.* We conclude that co-infections may result in altered parasite population dynamics compared to mono-species infections.

20-P. Differentiation between *Entamoeba histolytica, E. dispar* and *E. moshkovskii* using pyrosequencing and prevalence analysis of *E. moshkovskii* in patients positive for the '*Entamoeba* complex' diagnosed in Northwestern Europe.

Christen R. Stensvold[1*], Marianne Lebbad[2], Jaco J. Verweij[3], Venkata R. S. Geedi[1], Jørgen S. Jensen[1], Cathrine Jespersgaard[4], Georg von Samson-Himmelstjerna[5], Henrik V. Nielsen[1].

[1]Department of Bacteriology, Mycology and Parasitology, Statens Serum Institut, Artillerivej 5, DK-2300 Copenhagen S, Denmark. [2]Department of Parasitology, Mycology and Environmental Microbiology, Swedish Institute for Infectious Disease Control, Solna, Sweden. [3]Department of Parasitology, Leiden University Medical Center, P.O. Box 9600, 2300 RC Leiden, The Netherlands. [4]Department of Biochemistry, Statens Serum Institut, Artillerivej 5, DK-2300 Copenhagen S, Denmark. [5]Stiftung Tierärztliche Hochschule, Hannover Institut für Parasitologie, Bünteweg 17, D-30559 Hannover, Germany

Entamoeba histolytica, *E. dispar* and *E. moshkovskii* are morphologically similar, quadrinucleate cyst producing protozoa, all of which are known to infect humans. *E. histolytica* is the cause of dysentery and invasive amoebiasis, whereas *E. dispar* is considered apathogenic; the clinical significance of *E. moshkovskii* remains unsettled. Prevalence rates of *E. moshkovskii* among patients positive for quadrinucleate cysts are scarce, but recently a prevalence of up to 50% in Australia was reported by nested PCR. We developed a method based on single-round PCR and pyrosequencing for the detection and differentiation of *E. histolytica*, *E. dispar* and *E. moshkovskii* in DNA extracted directly from faecal specimens. We compared the diagnostic performance of this new method with a duplex real-time PCR method targeting *E. histolytica* and *E. dispar* and a conventional, single-round PCR for *E. moshkovskii*.

Hence, quadrinucleate cysts in 100 faecal specimens from Swedish, Dutch and Danish patients positive for the *Entamoeba* complex by microscopy were identified to species level using the three methods. One patient had a mixed infection of *E. moshkovskii* and *E. dispar*, 12 patients had *E. histolytica*, 86 had *E. dispar* and one patient was positive for both *E. histolytica* and *E. dispar*.

A 100% concordance between pyrosequencing results and results obtained by the two other methods were obtained for all 89 samples analysed by pyrosequencing.

The study showed PCR and pyrosequencing could be used as an appropriate tool for rapid and high throughput species identification of *Entamoeba*. Moreover, it was seen that *E. moshkovskii* was detected in only one of the 100 patients by non-nested PCR methods.